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Application of chemical herders do not increase acute crude oil toxicity to cold-water marine species

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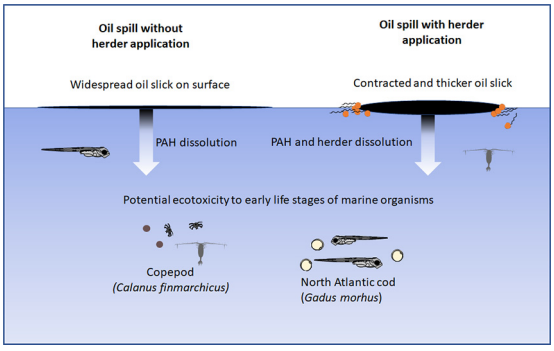
HIGHLIGHTS

- Acute toxicity of chemical herders was tested on sensitive life stages of marine organisms.
- Chemical herders caused a temporary delay in PAH dissolution into seawater.
- Low-energy WAFs prepared with and without herders displayed comparable ecotoxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

Chemical herders may be used to sequester and thicken surface oil slicks to increase the time window for performing in situ burning of spilled oil on the sea surface. For herder use to be an environmentally safe oil spill response option, information regarding their potential ecotoxicity both alone and in combination with oil is needed. This study aimed at assessing if using herders can cause toxicity to cold-water marine organisms. Our objective was to test the two chemical herders Siltech OP-40 (OP-40) and ThickSlick-6535 (TS-6535) with and without oil for toxicity using sensitive life stages of cold-water marine copepod (*Calanus finmarchicus*) and fish (*Gadus morhua*). For herders alone, OP-40 was consistently more toxic than TS-6535. To test herders in combination with oil, low-energy water accommodated fractions (LE-WAFs, without vortex) with Alaskan North Slope crude oils were prepared with and without herders. Dissolution of oil components from surface oil was somewhat delayed following herder application, due to herder-induced reduction in contact area between water and oil. The LE-WAFs were also used for toxicity testing, and we observed no significant differences in toxicity thresholds between treatments to LE-WAFs generated with oil alone and oil treated with herders. The operational herder-to-oil ratio is very low (1:500), and the herders tested in the present work displayed acute toxicity at concentrations well above what would be expected following in situ application. Application of chemical herders to oil slicks is not expected to add significant effects to that of the oil for cold-water marine species exposed to herder-treated oil slicks.

1. Introduction

The use of chemicals (dispersants, herders, and shoreline cleaning agents) as response options to minimize environmental effects of acute oil spills has received increased attention in recent years. Substantial amount

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of research have been conducted on the toxicity of chemical dispersants and oil and dispersant mixtures on cold-water organisms (Adams et al., 2014; Frantzen et al., 2015; Gardiner et al., 2013; Hansen et al., 2014; Hansen et al., 2017a; Hansen et al., 2012; Hansen et al., 2016; Hansen et al., 2018b; Hansen et al., 2015; Hansen et al., 2019a; Hansen et al., 2017b; Nordtug et al., 2011; Nordtug et al., 2015; Olsen et al., 2013; Olsvik et al., 2011; Olsvik et al., 2012; Prince, 2015). Yet very few have assessed ecotoxicity of chemical herders that are being used to thicken spilled surface oil slicks to enhance burning and skimming operations (Buist et al., 2017; Fritt-Rasmussen et al., 2021). While the herder application volumes in a response operation are expected to be very low compared to dispersant application volumes, it is still important to understand the potential lethal and sublethal effects of these compounds to marine organisms. The application doses of herders are typically a factor of 10–100 times less compared to the operative dosage need for dispersant use, i.e., relative volume dosage herder to oil (HOR) is 1: 1000 to 1:500 (Buist et al., 2017). Thus, considerably less chemicals are used for herder application than for dispersants, suggesting lower risk of herders themselves causing aquatic toxicity. The thickening of oil slicks achieved by using chemical herders extends the window-of-opportunity for oil ignitability and increases the effectiveness of in situ burning (ISB) as a response measure. This thickening process also reduces the contact area between oil and sea water which may, for a period after application, cause reduced dissolution of water-soluble and potentially toxic oil components from the oil to the water. Thus, this process may reduce the acute impact of an oil slick on marine organisms. As herders are used at very low HOR, the herders may not be expected to contribute significantly to toxicity compared to oil itself.

Operational use of herders has been tested in several large-scale field experiments, e.g., in the Barents Sea (Buist et al., 2010) and in the North Sea (Cooper et al., 2017). Fritt-Rasmussen et al. (2017, 2021) performed laboratory-scale herding and burning experiments to study the fate and effects of the herders ThickSlick-6535 (TS-6535) and Siltech OP-40 (OP-40) to improve the knowledge base used to evaluate the environmental risk by using herders, especially in connection with oil spill response in Arctic seas. Some of their key findings were that most of the herder around an oil slick remains on the water after the slick has been burned in situ, they detected very low concentrations (parts per billion) of herder in the water under the, and no detectable levels of herders were found in the smoke plume. There were challenges in measuring herder concentrations in water, but Fritt-Rasmussen et al. (2021) indicated that TS-6535 biodegraded within 7 days and did not bioaccumulate in the Arctic copepod *Calanus hyperboreus*, while OP-40 did not biodegrade initially and tended to bioaccumulate in *C. hyperboreus* and affected grazing activity. A review by Bullock et al. (2019) evaluated peer-reviewed and available grey literature on the use of ISB with a specific focus on the use of chemical herders to aid ISB in Arctic waters. One of their recommendations were that toxicity test should be performed using herders at concentrations realistic for response action with relevant Arctic species.

The main aim of the current work was to assess if using herders as an oil spill response tool could cause toxicity to cold-water marine organisms or cause changes in the toxicity of the resulting water-soluble fraction of spilled oil. Two hypotheses were identified: i) Environmentally relevant concentrations of herders (when applied to spilled oil) will not cause acute effects on marine organisms, and ii) Herder application do not contribute to toxicity of herder-treated surface oil spills. To test these hypotheses, acute toxicity testing on early and sensitive life stages of the cold-water copepod *Calanus finmarchicus* and North Atlantic cod (*Gadus morhua*) was used. First, both species were exposed to herders alone to provide toxicity thresholds. Second, LE-WAFs of crude oils with and without herders were prepared and toxicity tested. The two species tested are abundant in the Northern part of the Atlantic Ocean, representing key positions in the sub-Arctic food web (Melle et al., 2014; Sakshaug et al., 2009) and North Atlantic cod is the most important species for commercial fisheries in the Northeast Atlantic Ocean.

2. Materials and methods

2.1. Selected chemical herders and crude oils and their properties

The herders included in the tests were ThickSlick 6535 (TS-6535) and Siltech OP-40 (OP-40) (supplied from SL Ross, Canada). Both herders have been placed on the U.S. Environmental Protection Agency (EPA) National Oil and Hazardous Substances Pollution Contingency Plan (NCP) Product Schedule for consideration of use in US waters and have been commercially available since June 2012. TS-6535 is a blend of 65% of the surfactant sodium monolaurate (Span 20), which is the active ingredient, and 35% of the solvent 2-ethyl butanol. OP-40 is a patented polydimethylsiloxane copolymer which is the surfactant and active ingredient.

Two Alaskan North Slope (ANS) crude oils were used in this study: Alpine and North Star. To mimic crude oils that had been on the sea surface for approximately 1 h, the two oils were artificially weathered through evaporation at 150 °C (Daling et al., 1990; Stiver and Mackay, 1984). The 150+ °C residues had higher densities and higher viscosities than the fresh residues (see Supporting Information SI 1, Table S1 for details). The two 150 °C+ residues also displayed some different physico-chemical properties: The North Star 150 °C+ residue had a density of 0.838 g/mL, pour point of 9 °C, viscosity of 20 cP (at 10 °C, 10 s^{-1}) and flash point at 33 °C. The Alpine 150 °C+ residue had a density of 0.877 g/mL, pour point of 0 °C, viscosity of 43 cP (at 10 °C, 10 s^{-1}) and flash point at 40 °C (Sørheim, 2016). Differences in physical-chemical properties are important information for choice of oil spill response, including the use of herders, and for the toxicity to marine species. Representative gas chromatograms (GC) of the oils are shown in Supporting Information (SI1, Figs. S2 and S3).

2.2. Selected experimental organisms

Potential effects on primary consumers in the cold-water marine food chain were tested using early life stages (eggs to nauplii stage 3 (N3)) of the copepod *C. finmarchicus*. This copepod species is the most abundant zooplankton species in the Northern part of Atlantic Ocean, representing a key position in the sub-Arctic food web (Slagstad, 2009), and is an excellent model species for Barents Sea and Alaskan zooplankton species. Eggs for experiments were collected from mature individuals of the inhouse Sealab culture of *C. finmarchicus*. Details regarding copepod cultivation is described in Hansen et al. (2007).

Fertilized Atlantic cod (*Gadus morhua*) eggs were supplied by NOFIMA (Tromsø, Norway). The eggs were obtained by strip-spawning one ovulating female from a broodstock at Havbruksstasjonen in Tromsø and fertilized with milt from one male cod. Eggs were incubated over night at 4 °C and fertilization success determined (>75%) the following morning. The eggs were then shipped in 10 L of oxygenated sea water double-bagged inside a Styrofoam box containing ice. Upon arrival, temperature (4 °C) and oxygen concentration (>8 mg/L) was measured. The eggs were then slowly acclimated to a temperature of 8.5 °C over the next 36 h before being used for toxicity testing (at 3 days post fertilization (dpf)).

2.3. Preparation of exposure solutions for toxicity testing of herders

Exposure solutions were generated by mixing different masses of herder (loading) into sterile filtered (0.22 μm) sea water. The herder loading rate (ratio of herder to water, expressed as mass per volume water) ranges were 18–847 mg/L and 0.5–19.5 mg/L for TS6535 and OP-40, respectively. The concentration ranges were chosen to cover the range of previously reported toxicity data on the two herders (Fritt-Rasmussen et al., 2017). Each concentration was prepared individually in 500 mL sterile filtered (0.2 μm) natural sea water (salinity: 33 ppt). Vortexed water-accommodated fractions (WAFs) (Singer et al., 2000) were mixed using energy sufficient to achieve a 20–25% vortex for 20 h, and then allowed to settle for 4 h in separation funnels to allow resurfacing of bulk particles. Thereafter, the water phases were collected. To accurately control the concentration of herder, small amounts of herder were weighed out on sheets of microscope cover

glass that were added to the WAF bottles. The actual weight of the herders added to each WAF bottle were recorded, and the toxicity data are based on these recorded and nominal data (g/L).

2.4. Preparation of low energy WAFs of oil in sea water

LE-WAFs were prepared following guidelines established by the Chemical Response to Oil Spills: Ecological Research Forum (CROSERF) (Aurand and Coelho, 2005). These guidelines were developed to standardize WAF preparation for laboratory exposures to aquatic organisms and analytical chemistry measurements (Faksness et al., 2015). LE-WAF were prepared in closed vessels with calm mixing (no vortex) of water below a surface layer of oil to avoid the formation of oil droplets in the WAFs. The dissolved oil components are expected to be the main driver for the toxicity to marine organisms (Carls et al., 2008; Olsvik et al., 2011), and having droplets in the media will alter the dissolution dynamics from the crude oil into the water phase (Nordtug and Hansen, 2021; Sandoval et al., 2017).

Different LE-WAFs were prepared using the pre-weathered crude oils (150+ °C residues) with and without the application of the two chemical herders. Briefly, 1.75 L of sterile filtered (0.22 μm) natural seawater collected from 80 m depth in Trondheimsfjorden was added to glass bottles (2 L) giving water to air headspace ratio of 4:1. If herders were used, they were first applied to the water surface (10 μL) followed by the oil. An oil-to-water loading of 1:340 (3 g oil/L seawater), giving a herder-to-oil ratio of 1 to 500, was used. 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. Preparation was carried out in darkness at approximately 10 °C using a standard mixing time of 72 h (Faksness et al., 2012). Additional LE-WAF systems were generated with North Star only to study if potential impact of herders on dissolution rates of oil compounds, and these systems were sampled after 24, 48 and 72 h. Samples for chemical analysis and toxicity testing were collected from the systems only once (one system was generated for each sampling time). The experimental matrix is shown in Table S2 in Supporting Information, SI 1.

2.5. Toxicity testing using early life stages of the marine copepod C. finmarchicus

To obtain *C. finmarchicus* eggs, reproducing females from the inhouse culture were transferred into 50 L barrels with clean seawater and provided with the unicellular algae *Rhodomonas baltica* (approximately 7500 cells/mL). Eggs were harvested after 24 h, resulting in an average age of the eggs of 12 h after fertilization at the start of the 96-h exposure test with copepod eggs. *C. finmarchicus* nauplii at stage 3 (N3) were hatched and reared from eggs collected from the same culture.

The solutions of the test substances were supplied to the toxicity test laboratory in borosilicate bottles (100 mL) and acclimatized to 10 °C prior to the exposure. Exposures to herders were performed in 4 mL borosilicate glass vials with Teflon lined caps (Chromacol), which were primed with exposure solution overnight prior to the exposure experiment. The exposure solutions were removed and replaced with fresh exposure solution on the day of the exposure initiation. The 100% exposure solutions were diluted in a series of seven concentrations with a spacing factor of 1.7 between dilutions with each quadruple replicate for each exposure concentration. Natural seawater was used as negative controls in 8 replicates. Positive controls with 3,5-dichlorophenol (0.5 mg/L) were used in same replication as for the exposure dilutions.

For the 72-h immobilization test using nauplii, the exposure vials were filled with minimal headspace to keep potential evaporative loss to a minimum during exposure and stocked with 25–30 nauplii at onset exposure. Mortality was monitored at end of exposure after 72 h under a low-power dissecting microscope. The test animals were not fed during exposure. The calculated values are 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.

Hatching success of *C. finmarchicus* eggs were monitored in 5 mL vials (n = 23–30 in each vial) filled with exposure solution or clean seawater as control. The number of hatched nauplii were recorded after 24 and 96 h. The temperature was 10 ± 2 °C and oxygen and pH was within the normal seawater range throughout the experiment.

2.6. Toxicity testing using early life stages of Atlantic cod Gadus morhua

The test procedure used in this study was adapted from OECD Test No. 236: Fish Embryo Acute Toxicity (FET) which uses zebrafish (*Danio rerio*) as test species (OECD, 2013), and the adapted method for North Atlantic cod is described in detail in Hansen et al. (2021a). Cod eggs are comparable to zebrafish eggs in size, but in contrast, cod eggs have a lower density (pelagic eggs) and develop at lower temperature and over a longer period from fertilization to hatch (80–120 degree days, d°). Compared to the OECD guideline, we used larger exposure beakers (100 mL), a higher number of individuals (approximately 100 eggs per beaker) and a lower temperature (8.5 ± 1 °C).

Three days post fertilization (3 dpf) approximately 100 cod eggs were transferred to individual borosilicate beakers containing filtered sea water (controls; N = 6 replicates) or exposure solutions (100 mL; N = 3 replicates per concentration). Five concentrations were used: 100% (undiluted), 30%, 9%, 3% and 1% diluted in filtered seawater. The eggs were exposed in open beakers for 96 h, and solutions were renewed after 48 h. After 96 h exposure, all eggs were transferred to new beakers containing clean filtered sea water for recovery. Upon hatching, larvae from replicate treatments were pooled into one larger beaker (containing 500 mL filtered sea water). Mortality (determined as eggs displaying coagulated embryos on the bottom of the beaker) and hatching were monitored daily until 14 dpf. A complete timeline for the experiment is given in the Supporting Information (SI 1, Table S3).

At approximately 3 days post hatch (dph), 12–37 larvae from each treatment were collected and individually immobilized in a glass petri dish filled with 3% methylcellulose and kept at 8 °C using a temperature-controlled microscope stage. Images were collected through a microscope (Eclipse 80i, Nikon Inc., Japan) with quipped with Nikon PlanApo objectives (2× for whole larvae images and 10× for close-up larvae images and videos), a 0.5× video adaptor and a CMOS camera (MC170HD, Leica Microsystems, Germany). Standardised images of larvae were used for biometric evaluation using automated image processing (standard length, body area, yolk-sac area, myotome height, eye area and eye-to-forehead distance) using a method described in Kvæstad et al. (2022) and blinded deformation ranking analysis adopted from methods used by Sørhus et al. (2015) and Hansen et al. (2018a). Briefly, larvae deformations were determined in severity degrees, where 0 was no deformation, 1 was moderate deformed and 2 were severely deformed. Representative images of larvae with highlighted traces of distances/areas are given in Fig. 3.

2.7. Chemical analyses

The water samples were spiked with the appropriate surrogate internal standards (SIS, *o*-terphenyl, naphthalene-d8, phenanthrene-d10, chrysene-d12, phenol-d6, 4-methylphenol-d8) for analyses of semi-volatile organic compounds (SVOC) and total petroleum hydrocarbons (TPH), and serially extracted with dichloromethane (DCM) following a modification of EPA method 3510C (USEPA, 1996). The extracts were concentrated to approximately 1 mL and the final extract was spiked with the appropriate recovery internal standards (RIS, 5α-androstane, fluorene-d10, and acenaphthene-d10) and analysed on GC/FID (gas chromatography/flame ionization detection) and GC/MS (gas chromatography/mass spectrometry). All internal standards have purity >98% and were purchased from Sigma-Aldrich (SigmaAldrich.com).

An aliquot of the crude oils was weighted directly into a graduated flask (10 mL) and dissolved in DCM. The extract (0.5 mL) was transferred to a GC vial, added internal standards, and analysed on GC/FID and GC/MS.

Samples were analysed for SVOC (decalins, PAHs and phenols) using GC/MS (modifications of EPA Method 8270D (US EPA, 2007) and for TPH using GC/FID (modification of EPA Method 8015C (US EPA, 2003). Volatile organic compounds (VOC, C5-C9), including BTEX (benzene, toluene, ethylbenzene, and xylenes) were analysed by use of P&T GC/MS (Purge and Trap Gas Chromatography Mass Spectrometry using a modification of EPA method 8260C (US EPA, 2006). A list of all target analytes is provided in Supporting information (SI2, Table S4). The total WAF concentration was calculated by adding up volatiles and TPH. The detailed chemical analyses of the WAFs were also used to calculate toxic units as described in Supporting Information SI 6.

2.8. Statistical analyses

Statistical analyses were conducted with GraphPad Prism V9.00 (GraphPad Software, Inc., CA, USA). Comparisons between treatments were performed with one-way ANOVA, followed by Tukey's multiple comparisons test or Kruskal-Wallis test, followed by Dunn's multiple comparison test for non-normal distributed data sets according to D'Agostino & Pearson omnibus normality test. Significance level was set to $p < 0.05$ unless otherwise stated. A nonlinear curve fit (third-order polynomial) was applied in figures displaying measured parameters plotted as a function of exposure concentrations.

3. Results and discussion

3.1. Toxicity of chemical herders

Although chemical herders are expected to be associated with spilled oil during application, exposure and potential impacts on pelagic organisms to the herders alone in the environment should not be overlooked. Ecotoxicity to primary consumers in the marine environment were assessed by testing the two herders on two early developmental stages of the marine copepod *C. finmarchicus* (eggs and N3). The tests showed that the effects on hatching was considerably less than those on the survival of the N3 stage (Table 1).

The LC_{50}s for the nauplii exposed to TS-6535 and OP-40 were 61.2 mg/L (90% CI = 54.1–69.3) and 1.00 mg/L (90% CI = 0.93–1.086), respectively. For *C. finmarchicus*, the survival endpoint was one order of magnitude more sensitive to both herders than hatching success (Table 1). This may suggest lower bioaccumulation of herders in eggs compared to the more active nauplii. For OP-40, the LC_{50} value for survival was comparable with literature data, while the EC_{50} values for hatching was the highest of all available toxicity thresholds reported for this herder (i.e., the least sensitive). The exact EC_{50} value of hatching success after OP-40 exposure could not be determined for *C. finmarchicus*, as it exceeded the maximum loading used in the test (19.2 mg/L). However, based on the extrapolated dose-response curve the 96 h-EC_{50} value for OP-40 is approximately 40 mg/L, significantly higher than available literature data. For the related Arctic copepod species *Calanus hyperboreus*, toxicity thresholds for herders were reported as values less than one of the exposure concentrations (NOEC) (Buist et al., 2017). After short-term exposure (24 h) to OP-40, the LC_{50} concentrations for *C. hyperboreus* (<12.5 mg/L) was higher than for *C. finmarchicus* (1.0 mg/L, 72 h), while for extended exposure (21 d), the LC_{50} of *C. hyperboreus* (<2.5 mg/L) was comparable to the 72 h LC_{50} for *C. finmarchicus* (1.0 mg/L). A similar trend was observed for TS-6535, the most comparable effect concentrations was 21 d-LC_{50} for *C. hyperboreus* and 72 h-LC_{50} for *C. finmarchicus*. Comparative acute toxicity studies with crude oil and marine diesel have shown that *C. finmarchicus* is also more sensitive than the Arctic copepod *Calanus glacialis*. Acute mortality occurs faster in *C. finmarchicus*, and this may partly be associated with its lower lipid content compared to *C. glacialis* (Hansen et al., 2013; Hansen et al., 2011). For exposure to surface-acting chemicals like chemical dispersants, sensitivity was more comparable between these two species, and in fact the Arctic *C. glacialis* were slightly more sensitive than *C. finmarchicus* to some dispersants (Hansen et al., 2014). Acute toxicity testing of the two herders reported by Fritt-Rasmussen et al. (2021) showed similar values as those indicated by Buist et al. (2017). They were also able to measure bioaccumulation of the active ingredients of the two herders, and much higher bioconcentration was observed for OP-40 than TS-6535. This was linked to the hydrophobicity (Log K_{ow}) of the ingredients, which was estimated to 3.15 (monolaurate) and 1.75 (2ethyl-1-butanol) in TS-6535, and 7.4 (3-(polyoxyethylene)) in OP-40. The same study showed that nominal concentrations of 0.3 mg/L of the two herders caused a very pronounced reduction in grazing activity by individuals exposed to OP-40, whereas no effect were seen in TS-6535-treated copepods. It is not straight forward to compare toxicity thresholds determined after different exposure times and experimental designs (Fritt-Rasmussen et al., 2021), but in general the effect concentrations decline with prolonged exposure time. The time to reach toxic effects may also be influenced by biological factors like body size and lipid content (Øverjordet et al., 2018). The *C. finmarchicus* nauplii used in this study had a very low biomass and lipid content compared to the large adult *C. hyperboreus* used in the experiments published by Buist et al. (2017), which may have contributed to the lower effect concentrations (i.e. higher sensitivity) found for *C. finmarchicus* nauplii survival.

Atlantic cod eggs were exposed for 96 h from day 3 post fertilization, and effect concentration (LC_{50}) was calculated based on accumulated mortality at the end of the exposure period. However, daily recordings showed that most of the mortality occurred during the first 48 h of the exposure (Supporting information SI 5, Fig. S7). After the exposure period, the eggs were transferred to clean water and delayed effects on hatching and survival until 3 days after hatching was recorded. For Atlantic cod, effect concentrations for 96-h survival and hatching success were similar for TS-6535, while for OP-40 the EC_{50} for hatching was slightly lower than the 96-h LC_{50} indicating a slight delayed effect from OP-40 exposure (Table 1). This is also in line with the differences in hydrophobicity of the active ingredients in the two herders tested. Compounds with high hydrophobicity (high LogK_{ow}) typically takes longer to partition to equilibrium between water and biomass (Øverjordet et al., 2018).

Both test species used in our studies were more sensitive to OP-40 than to TS-6535 for all endpoints, which is in line with available literature data for five other species (Supporting information SI 5, Tables S8 and S9). Atlantic cod was more sensitive to TS-6535 than *C. finmarchicus* for both end points. In contrast, *C. finmarchicus* was more sensitive to OP-40 than Atlantic cod embryos. The survival of Atlantic cod was monitored during the embryo stage (i.e., egg survival) while survival of *C. finmarchicus* was tested using hatched nauplii. This may explain the difference in the relative sensitivity of the two end points in the two species. Toxicity data were collected from literature to compare species sensitivity distributions for the two herders tested. Species sensitivity distribution curves (Fig. 1) were

Table 1
Effect thresholds (EC_{50} and LC_{50}) for cold-water marine copepod (*Calanus finmarchicus*, egg and nauplii) and fish (Atlantic cod *Gadus morhua*, eggs/larvae) exposed to chemical herders TS6535 and OP-40. Concentrations of herders are nominal (mg/L) based on variable loading in the experiments.

		Effect thresholds LC/EC_{50} (mg/L)	
Species and stage	Endpoint	TS-6535	OP-40
C. finmarchicus nauplii (N3)	Survival (72 h LC_{50})	61.2 (54.1–69.3)	1.00 (0.92–1.09)
C. finmarchicus (eggs – N1)	Hatching (24 h EC_{50})	559 (454–688)	>19.8
Atlantic cod eggs	Survival (96 h LC_{50})	42.8 (39.8–46.0)	8.21 (7.40–9.03)
Atlantic cod eggs	Hatching success (EC_{50})	39.6 (28.3–55.3)	4.77 (4.09–5.58)

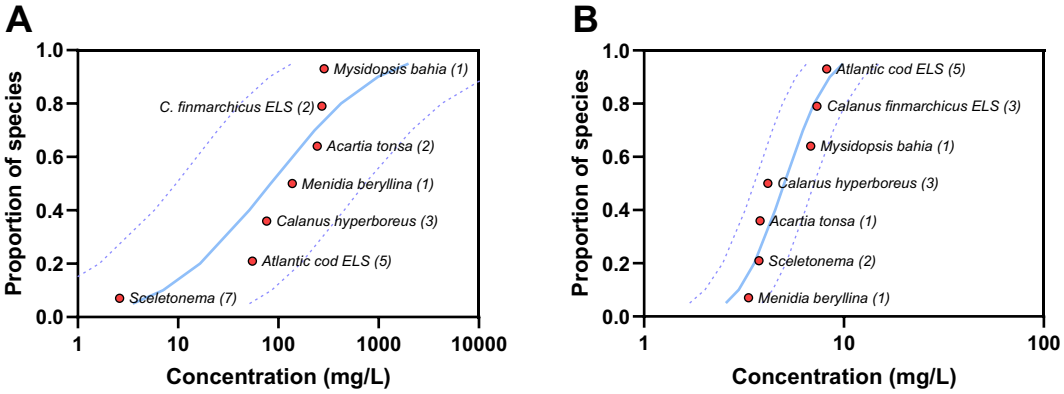

Fig. 1. Species sensitivity distribution curve for TS-6535 (A) and OP-40 (B). Based on mean LC/EC50 for each species where more than one value available (number of values in brackets). Solid line indicates the Central Tendency and broken lines indicate upper and lower Prediction interval (U.S. EPA, 2005, available at https://www.epa.gov/sites/production/files/2017-10/ssd_generator_v1.xlsm).

generated using literature data given in Supporting information SI 5. In general, the concentration range of effect concentrations for OP-40 is narrow, reflected in the effect concentrations ranging only 1.0 to >19 mg/L nominal loading, with an average of 7.7 mg/L. For TS-6535, the sensitivity distribution is wider with effect concentrations ranging 0.66 to 600 mg/L, with an average of 144 mg/L.

Of the seven species included in the fitted curve, the marine algae *Skeletonema costatum* appears most sensitive to TS-6535 (Fig. 1A). The second most sensitive species was the early life stages of Atlantic Cod, while *Calanus finmarchicus* appears as the second least sensitive species for TS-6535 exposure. In contrast, Atlantic cod and *C. finmarchicus* were the two least sensitive species to OP-40 (Fig. 1B). Despite survival of *C. finmarchicus* being the most sensitive endpoint after OP-40 exposure, there is no overall evidence that cold water species are more sensitive than the other tested species. The Arctic species *Calanus hyperboreus* appeared in the middle of both SSD curves, while the standard warm water test species were evenly distributed. Note that when the effect concentrations are given as above or below a given test concentration, this value was included in the fitted curve. This means that the species sensitivity may deviate from the exact concentration.

As herders are surface active chemicals, they may attach to surfaces of the exposure systems and that the use of nominal concentrations thereby may considerably overestimate the water concentrations. However, the toxicity values presented in the SSD-curves were all nominal and effect concentrations should therefore be comparable between species. It should also be noted that when used in oil spill response the ratio between oil and herder is at least two orders of magnitude in favor of the oil.

Very little information exists on environmentally relevant herder concentrations in water following applications to surface oil spills. In a study by Bullock et al. (2017) concentrations of herders were 16 mg/L in the water immediately after ISB and reduced to below detection limits (4 mg/L) 10 d after. Importantly, however, this experiment was conducted in a closed shallow pool (less than 1 m depth) and they used very high HOR (1:30 to 1:7). Thus, concentrations in a real spill scenario are expected to be significantly lower due to a higher potential for dilution. Also, as HOR is intended to be substantially lower (1:500 to 1:1000) than used in the

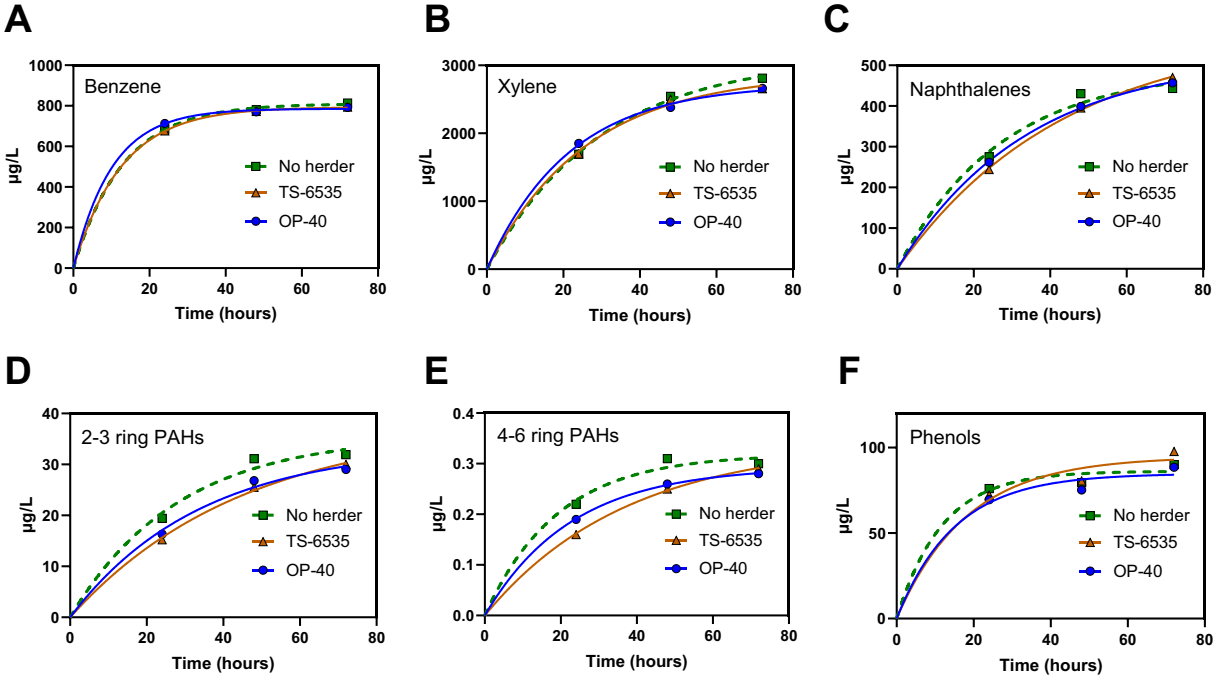

Fig. 2. Concentrations of benzene (A), xylene (B), naphthalene (C), 2.-3-ring polycyclic aromatic hydrocarbons (PAHs) (D), 4–6-ring PAHs (E) and phenols (F)of North Star 150 °C+ residue generated by CROSERF low energy stirring after 24, 48 and 72 h with and without herder (5.7 µL/L). Oil-to-water ratio was 1:340, herder-to-oil ratio 1:500.

study by Bullock et al. (2017), environmental herder concentrations are expected to be significantly lower than effect thresholds reported in the literature and in the present work.

3.2. PAH dissolution from oil during herder application

After application of oil and herder onto the water surface in the bottles when generating LE-WAFs, visual observations (Supporting information, SI 1, Fig. S1) confirmed that for the WAFs without herder, the oil slicks were uniformly spread on the water surface. The herder TS-6535 kept the oil slick contracted for at least 72 h, while the slick started to change already the first day when OP-40 was applied. These observations are in agreement with Rojas-Alva et al. (2020) who also observed that TS-6535 could hold its oil thickening capability for longer time (up to 350 h) than OP-40, the latter being most efficient (greater thickness) for shorter periods (6 h).

Water samples taken 24, 48 and 72 h after setting up the LE-WAFs with North Star were analysed using GC-FID and GC–MS to assess if herders caused differences in dissolution rates for oil components. Results from all chemical analyses, including GC chromatograms, are given in Supporting Information (Supporting information SI 3). According to the GC chromatograms there were no indications of the presence of oil droplets in any of the LE-WAFs (no alkanes observed), so solely the dissolved fractions were detected (Figs. S4 and S5). The sum of WAF concentrations of selected component groups from the oil over the 72-h stirring period is shown in Fig. 2. In general, the representations of the various groups show that the LE-WAFs are approaching equilibrium but are not entirely equilibrated after 72 h. There was little difference in the average dissolved concentrations of the lighter components (Fig. 2A–C) between LE-WAFs with and without herder applied. However, for the PAHs (Fig. 2D–E) dissolved concentrations after 24 and 48 h were clearly lower when herders were added compared to the untreated oil, indicating a decrease in dissolution rates caused by the herders. This is related to the octanol water partitioning coefficient of the components (K_{ow}) (Supporting information SI 3, Fig. S6). The individual WAF concentrations of volatile components (N = 24–27) were, however, on average significantly lower for oil added OP-40 and TS-6535 than that of the untreated oil after 48 and 72 h. The corresponding concentrations of semi-volatile components (N = 45–50) were always significantly lower in WAFs from the oils treated with herder compared to that of the untreated oil (Supporting information SI 3, Fig. S6 and Table S7). Variations in time to equilibrium of oil in water are related to in oil type and composition, oil-to-water loading, stirring time length, temperature and mixing energy (Faksness et al., 2008). As these parameters were all kept identical in our LE-WAF experiments, differences in dissolution rates are likely caused by a combined effect of the increased thickness of the surface oil (increased diffusion time through the oil) and a smaller contact area between oil and water caused by the herders (reduced contact surface area).

3.3. Acute toxicity of LE-WAFs with crude oil with and without herder

Two Alaskan crude oils were used to prepare LE-WAFs in toxicity studies: Alpine and North Star. Both oils were artificially pre-weathered to 150 °C+ (estimated time on sea less than one h). WAFs were generated with or without the herders TS-6535 and OP-40. Gas chromatograms and detailed chemical characterization are given in Supporting Information (SI 3). LE-WAFs prepared for ANS crude oils with and without herders were used to exposure early life stages of copepod (N3) and North Atlantic cod. For copepods, both the North Star and Alpine oils were tested, and for cod only Alpine was used for testing. Mortality measurements were used to provide toxicity thresholds, and these were based on chemical analyses of the exposure solutions at the onset of exposure (initial chemistry). Loss of SVOCs during exposure were measured in the exposure solutions used in the cod experiments (measured at the end of experiment). The total SVOC concentration declined with approximately 20%, and it was mainly naphthalene that contributed to the loss (approximately 50%) (Supporting Information SI 7, Table S13), most likely due to evaporation, as the exposure chambers were open.

No significant differences in effect concentrations were observed between the LE-WAFs of oil with and without the addition of herder (Table 2). The LC_{50} values are almost identical and confidence intervals are overlapping for the data on *Calanus finmarchicus*. The same was observed in the studies using Atlantic cod embryos, but for hatching success, LE-WAFs of oil alone have a 2-fold higher EC_{50} compared to the herder-treated oils. This suggests that adding herder increased the toxicity of the WAF, however, these LC_{50}-values are extrapolated, as toxicity (reduced hatching success) did not reach 50% for the highest exposure concentration. This also increases the confidence intervals for the LC_{50} thresholds, and they overlap. When comparing LC_{50}-values based on cod larvae survival until 9 days post exposure (when the experiment was terminated), the data are comparable. This is no surprise, as the mass of herder added to the oils (5.7 μL) is not able to cause a maximum herder concentration in the water anywhere close to the toxicity thresholds for the herders alone (Table 1). A Toxic Units approach, where the chemical compositions of the LE-WAFs are used to predict acute toxicity thresholds, confirmed the observed comparability between LE-WAFs with and without addition of herders (see Supporting Information SI 6 for details).

The use of morphological and developmental effects on fish larvae exposed to crude oils have previously been linked to delayed and long-term effects sometimes observed months after embryonic exposure (Bender et al., 2021; Hansen et al., 2021a; Laurel et al., 2019). Compared to hatching success as toxicity threshold variable, the delayed mortality observed in cod larvae up until 3 dph caused a significant drop in LC_{50} (i.e., higher toxicity) for all treatments (Table 2). Surviving larvae imaged 3 dph were assessed for morphometry and developmental malformations. Unfortunately, due to a sudden fungi growth in the Alpine + TS 30% WAF, data for this treatment was taken out and considered invalid. Only the two highest concentrations from the three treatments caused morphometric differences compared to controls (Table 3). Significant reductions ($p < 0.0001$) in standard length, eye diameter and body area, in addition to increased yolk fractions were observed for larvae exposed as embryos to 100% LE-WAF for the Alpine oil with and without herder application. Also, for the 30% LE-WAF without herder application, there were significant differences ($p < 0.01$) between controls and exposed larvae (except for body area). For LE-WAFs where herder was applied, only standard length ($p < 0.01$) and yolk area was significantly affected (OP-40,

Table 2
Effect concentrations for low-energy water accommodated fractions (LE-WAFs) of oil (North Star and Alpine) with and without addition of herder. Effect concentrations are given as mg/L total WAF concentration components, and they were adjusted to control mortality. WAFs were generated from oils artificially weathered by heating (150 °C) by CROSEF low energy stirring for 72 h (LEWAF). Oil-to-water ratio was 1:340, herder-to-oil ratio 1:500 (5.7 μL/L WAF).

			Effect concentrations LC_{50} (mg/L)		
	Species	Endpoint	150 °C+ residue	150 °C+ residue and TS-6535	150 °C+ residue and OP-40
North Star	*Calanus finmarchicus* nauplii	Survival (LC50, 72 h)	2.15 (1.89–2.44)	2.27 (1.96–2.62)	2.46 (2.14 to 2.82)
Alpine	*C. finmarchicus* nauplii	Survival (72 h)	0.710 (0.617–0.813)	0.660 (0.559–0.774)	0.701 (0.569–0.854)
	Atlantic cod eggs	LC_{50} hatching after 9 days recovery	8.34[a] (5.34–14.37)	4.89[a] (3.78–6.44)	4.10 (2.72–6.42)
	Atlantic cod eggs/larvae	Mortality 3 days post hatch LC_{50}	1.12 (0.55–2.23)	0.79 (0.44–1.43)	1.88 (1.54–2.42)

[a] Extrapolated above 100% WAF.

Table 3
Morphometric data on cod larvae (3 dph) exposed to low-energy water accommodated fractions (LE-WAF) of Alpine crude oil (150 + residue) with and without application of ThickSlick-6535 or Siltech OP40 for 4 days during embryogenesis. All data are given as mean ± standard deviations (SD), with number of fish larvae (N). Significant differences between controls and exposed groups are given **p < 0.01 and ****p < 0.0001.

			Standard length (mm)	Eye diameter (mm)	Body area (mm^2)	Yolk fraction
		N	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	Control	37	4.90 ± 0.18	0.29 ± 0.01	1.89 ± 0.08	0.26 ± 0.02
Alpine	0.8%	18	4.77 ± 0.35	0.29 ± 0.01	1.86 ± 0.10	0.27 ± 0.03
	2.7%	17	4.81 ± 0.25	0.28 ± 0.01	1.91 ± 0.07	0.28 ± 0.02
	9%	19	4.84 ± 0.16	0.29 ± 0.01	1.91 ± 0.08	0.28 ± 0.03
	30%	26	4.70 ± 0.16**	0.27 ± 0.01****	1.87 ± 0.09	0.29 ± 0.03**
	100%	24	3.41 ± 0.40****	0.22 ± 0.02****	1.56 ± 0.14****	0.39 ± 0.07****
Alpine + ThickSlick-6535	0.8%	19	4.90 ± 0.17	0.29 ± 0.01	1.89 ± 0.09	0.25 ± 0.02
	2.7%	16	4.94 ± 0.11	0.29 ± 0.01	1.88 ± 0.06	0.26 ± 0.02
	9%	27	4.88 ± 0.14	0.28 ± 0.01	1.90 ± 0.06	0.27 ± 0.03
	30%	0	NA	NA	NA	NA
	100%	30	3.53 ± 0.53****	0.21 ± 0.02****	1.51 ± 0.12****	0.36 ± 0.06****
Alpine + Siltech OP-40	0.8%	16	4.74 ± 0.46	0.28 ± 0.03	1.83 ± 0.10	0.26 ± 0.05
	2.7%	16	4.87 ± 0.16	0.28 ± 0.01	1.86 ± 0.08	0.27 ± 0.02
	9%	23	4.81 ± 0.12	0.28 ± 0.01	1.88 ± 0.05	0.27 ± 0.03
	30%	21	4.70 ± 0.13**	0.28 ± 0.01	1.87 ± 0.09	0.30 ± 0.02**
	100%	12	2.97 ± 0.30****	0.21 ± 0.01****	1.49 ± 0.12****	0.41 ± 0.05****

p < 0.01). Such morphological effects have previously been observed after embryonic exposure to fresh and weathered oils (Hansen et al., 2018a), fuel oils (Hansen et al., 2021b) and produced water (Hansen et al., 2019b).

The frequency of craniofacial, jaw and marginal finfold deformations increased with exposure concentration (heat plots displayed in Fig. 3). At the highest exposure concentration for all three treatments, 100% of the larvae displayed jaw and craniofacial deformations, and they also had abnormal marginal finfold. These effects are driven by petrogenic compounds like PAHs (Incardona et al., 2004; Incardona et al., 2006), and there is no clear evidence that can be extracted from these data suggesting that herders contribute to the toxicity of the petrogenic compounds of the LE-WAFs.

4. Conclusions

Two hypotheses were tested in the present study: i) Environmentally relevant concentrations of herders will not cause acute effects on marine organisms, and ii) Herder application do not contribute to toxicity of herder-treated surface spills. Both tested cold-water marine species, the copepod *Calanus finmarchicus* and North Atlantic cod, were more sensitive to exposure to OP-40 than to TS-6535, as all toxicity thresholds were higher for the latter. For *C. finmarchicus*, nauplii survival was one order of magnitude more sensitive parameter than egg hatching success for both herders. This may be due to lower uptake of the herders in eggs than in active nauplii.

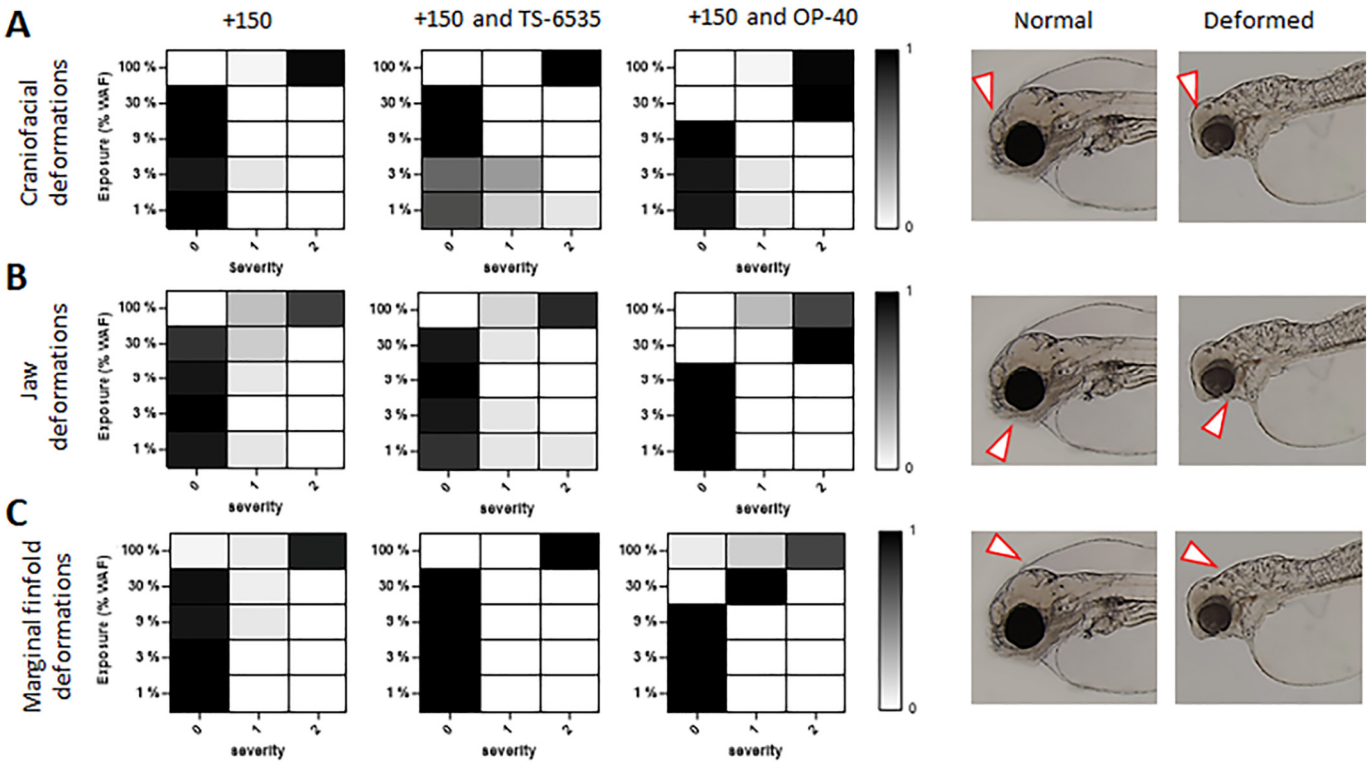

Fig. 3. Heat plots showing frequencies of specific deformations in cod larvae exposed to dilution series of WAFs of Alpine 150 °C+ residue with and without addition of herders. The oil:water ratio (OWR) was 1:340 and the oil:herder ratio was 1:500. Cod eggs were exposed for 96 h from 3 to 7 days post fertilization. After hatching (14 days post fertilization) surviving larvae were classified as normal (severity = 0), moderately deformed (=1) and severely deformed (=2) based on appearance as shown in the images to the right. The scale of the heat plot indicates the fraction of larvae within each combination of exposure and severity from none (white) to all (black).

For OP-40, the LC_{50} value for nauplii survival was in line with literature data, while the EC_{50} values for hatching was the highest of all available effect concentrations for this herder (i.e. the least sensitive). The exact EC_{50} value of hatching success after OP-40 exposure could not be determined for *C. finmarchicus*, as it exceeded the maximum loading used in the test (19.2 mg/L). However, based on the fitted dose response curve the 96 h EC_{50} value for OP-40 is approximately 40 mg/L, significantly higher than available literature data. Cod embryonic exposure revealed high sensitivity of this species compared to literature values for TS-3565, but low sensitivity to OP-40. Although dissolution of oil components from surface oil is somewhat delayed following herder application, no evidence of reduced ecotoxicity was provided following LE-WAF testing. Even though we observed differences in effect thresholds between the herders alone, no significant differences were observed between the toxicity of WAFs generated with oil alone and oil treated with the two herders. Application of chemical herders to oil slicks is not expected to add significant effects to cold-water marine species exposed to herder-treated oil slicks. The herders tested in the present work display acute toxicity at concentrations well above what would be expected following in situ application. The herder-to-oil ratio used is very low, and application would in any case only be justified if in situ burning follows immediately after herder application.

CRediT authorship contribution statement

Bjørn Henrik Hansen: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Writing - original draft; Writing - review & editing. **Trond Nordtug**: Data curation; Validation; Writing - original draft; Writing - review & editing. **Ida Beathe Øverjordet**: Methodology; Data curation; Writing - review & editing. **Dag Altin**: Investigation; Methodology; Data curation; Writing - review & editing. **Julia Farkas**: Investigation; Methodology; Writing - review & editing. **Per S. Daling**: Conceptualization; Writing - review & editing. **Kristin Rist Sørheim**: Resources; Project administration; Supervision; Writing - review & editing. **Liv-Guri Faksness**: Conceptualization; Data curation; Formal analysis; Supervision; Validation; Writing - original draft; Writing - review & editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153779.

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