Technical Guideline No. 08– Processing and analysis of water, biota and sediment samples for the determination of hydrocarbon contamination using gas chromatography -mass spectrometry

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To be used in conjunction with:

GUIDELINES FOR THE ENVIRONMENTAL MONITORING AND IMPACT ASSESSMENT ASSOCIATED WITH SUBSEA OIL RELEASES AND DISPERSANT USE IN UK WATERS

1 Purpose and Scope

Water samples may be taken for a number of reasons during an oil spill response and monitoring programme, particularly for hydrocarbon and PAH analysis in order that the degree of contamination can be assessed.

Sediment and biota samples are taken for similar purposes; in the latter case possible contamination of the human food chain is an important consideration.

This document covers the extraction and analysis of water, biota and sediment samples primarily for PAH, but the method can be extended to other hydrocarbons (particularly *n*-alkanes, pristane and phytane) which allow the oil to be characterised and the degree of degradation following release investigated.

The method described here is one which has been in routine use for over 35 years and has proved robust and reliable. Other methods can be used for PAH and hydrocarbon analysis, but the main advantage of this method is the minimal losses of volatile low-molecular weight PAH, such as the naphthalenes, which are present at high concentrations in most crude oils. This due to the fact that it utilises wet biota and sediment samples. Drying samples can lead to losses of these compounds, and freeze-drying presents a risk of contamination due to backstreaming of oil from the rotary vacuum pumps in general use into the vacuum sample stage.

This document supports and should be used in conjunction with environmental monitoring guidelines for subsea oil releases (Law et al., 2014)

2 Health and Safety considerations

These processes involve the use of solvents and other chemicals and should be subject to CoSHH assessment prior to use. Such assessments will outline the necessary use of personal protective equipment, local exhaust ventilation and other precautions deemed necessary.

3 Procedure

3.1 Considerations

Sample contamination can occur during all stages of sample collection, preparation and analysis. Controlled conditions are therefore required for all procedures. On ships, there are multiple sources of PAHs, such as the oils used for fuel and lubrication and the exhaust from the ship's engines. It is important that likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination could occur. It is advisable to collect samples of the ship's fuel, bilge water, and oils and greases used on winches, etc., which can be used as fingerprinting samples at a later date, if there are suspicions of contamination in particular instances.

Plastic materials must not be used for sampling and storage because of possible adsorption of the PAHs onto the container material. Sediment samples should be transported in closed containers; a temperature of 25 °C should not be exceeded. Live biota should be transported in closed containers

at temperatures between 5 °C and 10 °C. For live animals, it is important that the transport time is short and controlled (e.g. maximum of 24 h).

If the samples are not analysed within 48 h after collection, they must be stored at below −20 °C.

Because PAHs are sensitive to photodegradation, exposure to direct sunlight or other strong light must be avoided during storage of the samples, as well as during all steps of sample preparation, including extraction and storage of the extracts (Law and Biscaya, 1994).

3.2 Sample extraction

In all cases, known amounts of a range of surrogate standards (deuterated PAH, deuterated alkanes and 2,2,4,4,6,8,8-heptamethyl nonane) are added at the start of the extraction/digestion process for quantification purposes.

3.2.1 Water

Water samples (usually 2.7 litres if collected using a Winchester sampler (see Kelly et al., 2000)), can be extracted using liquid-liquid extraction with *n*-pentane. Two 50 ml aliquots are used and combined, then dried over anhydrous sodium sulphate.

3.2.2 Sediment

Sediments can be extracted wet or dry, but due to losses and potential contamination in the drying process, wet extraction is recommended. Before taking a subsample for analysis, the samples should be thoroughly mixed using a metal spatula. The mass of sample extracted depends on the expected concentrations. For monitoring purposes, around 40-50 g would normally be extracted, but much less may be required if high levels of contamination are expected. As sediment concentrations are usually expressed on a dry weight basis (as the wet weight is an artefact of sampling and sample handling subsequently) then a separate aliquot of the sediment is taken and dried to constant weight at 105 °C – 16 hours (or overnight) is generally sufficient time for this to occur.

Wet sediments can be extracted by mixing with organic solvents, using a stepwise procedure. Extraction is enhanced by shaking, Ultra Turrax-mixing, ball-mill tumbling, or ultrasonic treatment. Water-miscible solvents, such as acetone, methanol, or acetonitrile, are used in the first step to remove the water from the matrix. Two subsequent extractions are required to ensure extraction efficiency.

Soxhlet extraction can also be used, and wet sediments should be extracted in two steps. First, a polar solvent, such as acetone, is used to extract the water from the sediment, the solvent flask is replaced, and the extraction continued with a less polar solvent or solvent mixture (e.g. acetone/hexane). Thereafter, the extracts must be combined.

Extraction of wet sediments by pressurized liquid extraction (PLE) can also be carried out, requiring less solvent and time. Wet sediment is dried by mixing with sufficient anhydrous sodium sulfate to form a free-flowing mixture that is then packed into stainless steel tubes for extraction. Extractions are done at elevated temperatures and pressures.

A single step method can be sued where sediment samples are extracted using alkaline saponification using methanolic potassium hydroxide under reflux (Kelly et al., 2000). The digests are filtered through solvent-rinsed filter papers into separating funnels and extracted twice with n-pentane. These extracts are combined and dried over anhydrous sodium sulphate.

3.2.3 Biota

Normally, in a marine monitoring programme, mussels would be used to assess PAH levels in biota since they do not metabolise PAHs in the same way that teleost fish do. In a spill situation, however, sampling may be more opportunistic, and also commercial fish species will need be analysed in order to understand the risk to the human food chain.

Extraction can be carried out by either Soxhlet extraction, PLE or by alkaline digestion, followed by liquid – liquid extraction with an organic solvent. In the case of Soxhlet extraction, the wet tissue must be dried by mixing with a chemical drying agent (e.g. anhydrous sodium sulfate), in which case, a period of several hours is required between mixing and extraction, to allow complete binding of the water in the sample. Samples are spiked with recovery standard and should be left overnight to equilibrate. Apolar solvents alone will not effectively extract all the PAHs from tissues when using Soxhlet extraction. Mixtures such as hexane/dichloromethane are more effective

Alkaline digestion is conducted on wet tissue samples, making the drying procedure unnecessary.It has been extensively used in the determination of PAHs and hydrocarbons, and its use is well documented. It is usually conducted in alcohol (methanol or ethanol), which should contain at least 10 % water, and combines disruption of the cellular matrix, lipid extraction, and saponification in a single procedure, thereby reducing sample handling and treatment. Solvents used for liquid–liquid extraction of the homogenate are usually apolar, such as pentane or hexane, and they will effectively extract all PAHs.

For conducting PLE, the wet biota sample is dried by mixing with sufficient anhydrous sodium sulfate to form a free-flowing mixture. The mixture is packed into stainless steel extraction cells containing a glassfibre filter and sodium sulfate or glass powder to fill the cell. To ensure a better recovery, samples may be extracted twice and extractions may have to be done at elevated temperatures and pressure.

3.3 Screening

Screening analysis can be conducted using ultraviolet fluorescence spectrometry. A suitable spectrofluorimeter is one which is capable of scanning both excitation and emission monochromators simultaneously. All measurements are made within the linear range of the spectrofluorimeter so as to preclude self-absorption effects and consequent distortion of the spectra obtained. As there is no absolute measure of fluorescence emission, the instrument is calibrated over the range0 - 10 µg/ml of the oil of interest, and concentrations expressed as equivalents of that oil. The optimum excitation and emission wavelengths can be established using the source oil, but EXλ 310 nm EMλ 360 nm usually suffices for crude oils. Synchronous fluorescence spectra are run for each sample with an offset between monochromators of 25 nm. These data can be used to prioritise samples for GC-MS analysis by broadly indicating the relative contamination levels of each sample.

3.4 Clean-up

Extracts from all three sample types are reduced in volume to ca. 1 ml using a rotary film evaporator, then can be cleaned up.

Commonly this is done using an alumina column chromatography (1g of 5% deactivated alumina in a Pasteur pipette) and elution with 50% *n*-pentane/dichloromethane (two aliquots which are combined).

This clean up can also be achieved using automated methods such as gel permeation chromatography (GPC) and high-performance liquid chromatography (HPLC)

The extracts are reduced in volume, to between 100 µl and 1 ml, and transferred to crimp-top autosampler vials for analysis.

3.5 GC-MS analysis

Aliquots of the extracts are analysed by GC-MS (electron ionisation) in either full scan mode or using multiple ion monitoring, depending on the instrument configuration. A typical capillary column 25 - 50 m in length and the stationary phase is 5% phenyl methyl silicone fluid. The MS is set to record the molecular ions of the PAH to be determined and their deuterated analogues used as standards. For alkanes, the abundance of molecular ions is typically low, and a common alkyl fragment (e.g. 71, corresponding to C_5H_{11}) is used along with the retention time to identify individual compounds. Compounds and compound groups (such as C_1-naphthalenes) are quantified from mass fragmentograms and by comparison with that of the relevant surrogate standard. Analytical Quality Assurance requires that a Certified or Laboratory Reference Material is analysed within each batch and control charts are prepared in order that the day-to-day performance of the method can be monitored. A procedural blank is also included within each batch.

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