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Plastic Bags for Stable Storage of the Water-Soluble Fraction of Crude Petroleum Used in Aquatic Environment Toxicity and Tainting Studies

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Each year, an estimated 4,000,000 t of petroleum enter the marine environment through sea and land based discharges, atmospheric fall-out and other events (Clark and McLeod 1977). Aromatic petroleum hydrocarbons are tainting components of crude petroleum, and are potentially toxic because they are relatively soluble in water (Siron et al. 1987). Depending on concentration, they are also carcinogenic (Andelman and Snodgrass 1974; Rice et al. 1977a, b). Many laboratory studies have been conducted with marine organisms, both vertebrates and invertebrates, to assess the effects of the water-soluble fraction (WSF) of petroleum hydrocarbons with respect to physiology, toxicity, and tainting. When such experiments are conducted in a controlled water environment, the WSF is generally used, rather than specific hydrocarbons. This fraction has traditionally been prepared immediately before carrying out the experiment, or in several batches during the study, sometimes with results confused by erratic concentrations. Any procedure to store large amounts of WSF for long periods without changes in the composition would increase the feasibility and reproducibility of long-term studies. As part of an ongoing project which needs large volumes of WSF of constant composition to study the tainting effect of WSF on adult Atlantic salmon (Ackman and Heras 1992; Heras et al. 1993; Zhou and Ackman 1994; Zhou et al. 1994), we compared two different storage systems for the stability of contained WSF over time.

MATERIALS AND METHODS

Flotta North Sea crude petroleum, obtained from the Dartmouth, Nova Scotia refinery of Esso Petroleum Canada, was used to prepare the WSF. It was stirred with sand-filtered seawater in a 1000-L stainless steel mixing vessel equipped with a powerful mechanical stirrer, a bottom drain, and a jacket cooling-water system. Crude petroleum and seawater of salinity 31.6 $^{o}/_{oo}$, pumped from the Northwest

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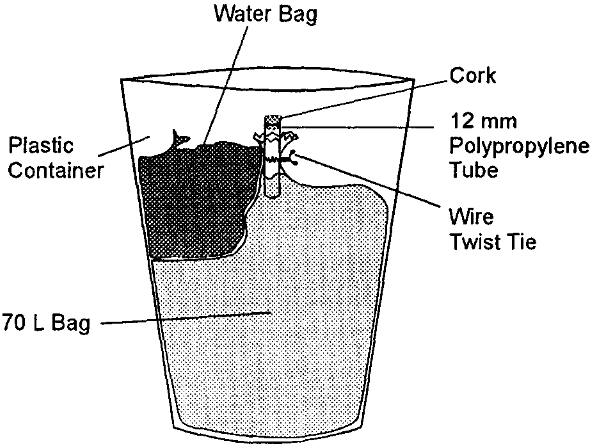

Figure 1. Schematic diagram of the bag sealing system designed to avoid a free air space.

Arm, an inlet near Halifax, and sand-filtered in the Dalhousie University Aquatron (Parrish et al. 1992), in a ratio of 1:99 (v/v), were stirred for 24 hr and allowed to settle for an additional 48 hr. The contents were kept cool by circulating water at 12°C through the outer jacket of the mixing vessel. WSF was removed through the bottom drain.

Five hundred liters of WSF were prepared immediately prior to the study and 65 L aliquots were stored in triplicate, using either 200-L plastic (polyvinyl chloride) containers with lids (40 cm diameter by 120 cm height; i.e., shipping barrels with loose fitting lids), or 70-L capacity bags (60 cm by 45 cm by 4 mm thick, made of polyethylene) dropped into a tapered domestic garbage container of convenient size. The bag was sealed with a wire twist tie wrapped around a piece of 12 mm id polypropylene tubing closed with a cork stopper (Fig 1). Care was taken to eliminate all air from the bag before sealing, and a 5-L bag filled with water was placed on the top so that every time a sample was taken, the storage bag would collapse, preventing formation of any free air volume inside the bag. The ambient storage temperature for the WSF containers was 12 °C. Clean seawater was stored in a similar way for provision of control blanks.

Analyses of the WSF concentrations was performed on individual samples from each of the three test containers taken at different time intervals over a 48-hr period. Samples were taken from the center of each container with a peristaltic pump (Cole Palmer Instruments Co., Niles, Illinois) using Viton® tubing. Samples were stored at 4 °C in amber bottles, previously rinsed with acetone and hexane, with air-tight Teflon-lined screw-cap lids, and were analyzed within 24 hours. The hydrocarbons of the WSF were extracted according to the Murray extraction procedure (Murray 1979; Murray et al. 1984) and analyzed immediately by gas-liquid chromatography (GLC).

The hydrocarbon analyses were conducted with a Perkin Elmer (Norwalk, Connecticut) 8420 capillary gas-liquid chromatograph equipped with a flame ionization detector (FID) and a split injection system. The chromatography was conducted on a DB-1 methyl silicone fused silica capillary column (60 m, 0.25 mm id, 0.25 μm film thickness, J&W Sci.). The FID was set at 280 °C and the injector at 280 °C. The column temperature was held for 15 min at an initial temperature of 45 °C. It was then increased at a rate of 13 C° min^{-1} to a final temperature of 280 °C and held for 30 min. The carrier gas was helium at a pressure of 138 kPa. Hydrogen and air pressures were 90 and 159 kPa, respectively. A typical chromatogram is included in a prior publication (Heras et al. 1992). Concentration of hydrocarbons in the samples was calculated with respect to an internal standard (n-heneicosane, C_{21}), and corrected for GLC response and for the recovery efficiency of the Murray extraction method of WSF analyses. Blank readings were made daily and all solvents were glass distilled.

RESULTS AND DISCUSSION

The hydrocarbons of the WSF exhibited a GLC pattern similar to those previously reported for other crude petroleum WSF (Ernst et al. 1987; Heras et al. 1992), being dominated by monoaromatic compounds, primarily benzene, toluene and the xylenes. These low-boiling aromatics are the most soluble constituents of crude petroleum and represent more than 70% of the WSF. The alkanes present in the parent petroleum were greatly diminished in the WSF due to their low solubility in water. Among other aromatics of interest were complex patterns of substituted naphthalenes. The main polymethylbenzenes appeared to be 1,2,4- and 1,2,3-trimethylbenzenes. The WSF chromatograms did not show more than traces of polynuclear aromatic hydrocarbons (PNAH) of mass greater than dimethyl naphthalenes. Apart from aromatics, the WSF showed a high level of cyclohexane, almost equal to that of the benzene. No contamination from either the polyvinyl chloride container or the polyethylene bags were detected by GLC of the extracts from the control seawater.

After the WSF has been transferred into the seawater, several factors cause hydrocarbons to be lost, resulting in changes in both concentration and composition of the WSF. As shown in Fig. 2 the total concentration of the WSF stored in either container, was not significantly different for the first 6 hr, but soon the losses of hydrocarbons from the plastic barrels were greater than losses from the bags, and after 48 hr of storage 44% of the starting hydrocarbons were lost from the barrels compared with 35% from the bags (Table 1). This difference may be caused by the fact that the air volume in the bags was virtually nil while in the barrels the air volume was ≤ 10 L at the beginning and increased during sampling. Another reason was that the barrels were poorly sealed against leakage of volatile WSF in vapor form. In the course of an experiment, this

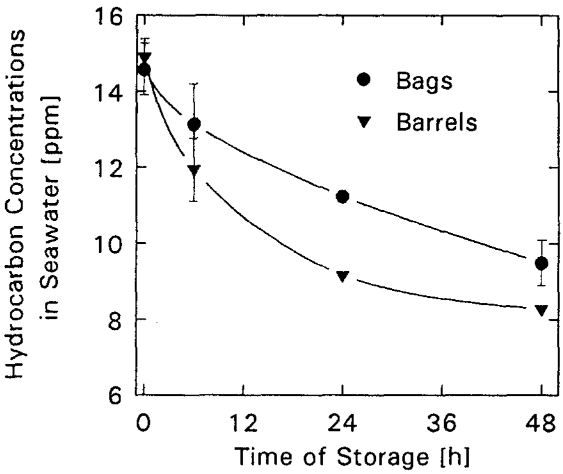

Figure 2. Hydrocarbon concentration [mg L^{-1}] of the WSF stored in bags and barrels.

Table 1. Changes in concentration from the major groups of hydrocarbons during storage in bags and barrels. Numbers are mean results for three storage containers ± SD.

	Barrels 0 hr	6 hr	24 hr	48 hr	% lost
Alkanes	2.51±0.01	2.07±0.32	1.87±0.04	1.91±0.04	23.9
Monoaromatics	11.06±0.42	8.98±0.38	6.40±0.19	5.75±0.03	48.0
Diaromatics	0.40±0.04	0.25±0.02	0.13±0.01	0.11±0.01	72.5
PAH*	0.48±0.02	0.32±0.05	0.38±0.04	0.25±0.01	47.9
Other	0.47±0.05	0.34±0.05	0.39±0.03	0.27±0.01	42.6
Total [ppm]	14.92±0.47	11.95±0.82	9.17±0.17	8.28±0.05	44.5

	Bags 0 hr	6 hr	24 hr	48 hr	% lost
Alkanes	2.51±0.03	2.09±0.15	1.62±0.18	2.00±0.07	20.3
Monoaromatics	11.32±0.25	9.96±0.95	8.42±0.37	6.89±0.48	39.1
Diaromatics	0.45±0.13	0.34±0.09	0.18±0.03	0.16±0.02	64.4
PAH	0.51±0.19	0.37±0.02	0.44±0.05	0.30±0.06	41.2
Other	0.55±0.17	0.38±0.02	0.57±0.05	0.14±0.14	74.5
Total [ppm]	14.60±0.68	13.13±1.08	11.24±0.11	9.50±0.60	34.9

* Polyaromatic hydrocarbons

difference between bags and barrels could be even more dramatic, because the solution of WSF would be continuously consumed during most experiments, thus increasing the air space in the barrels. This could augment the chances of hydrocarbon volatilization.

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