Continuous-flow Apparatus for use in Petroleum Bioassay

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INTRODUCTION

This study's objective was to build a system for long-term bioassay of dispersed petroleum derivative, with small amounts of insoluble material, in stable seawater concentration. Initially, the system was tested using No. 2 fuel oil for simplicity in handlin and because of its reported high toxicity content. A discussion of the problems encountered and possible application of the system to crude oils is included.

Current estimates place the annual input of anthropogenic hydro carbons to marine waters at 5 to 8 million metric tons (NATIONAL ACADEMY OF SCIENCES 1975). Although major spills of petroleum have the potential to produce catastrophic biological effects in localize areas, there is also growing concern about low concentration, chroni pollution by oil. Few laboratory studies have been designed to permit assessment of long-term effects from low concentrations of petroleum. This is due, in part, to the two following reasons.

First, until recently (VAUGHAN 1973; ANDERSON et al. 1974; TEMPLETON et al. 1974; RICE et al. 1975) the necessity to monitor waterborne oil during bioassay was not fully established. Many authors still fail to include analytical estimates of quality and concentration in petroleum bioassay (MORROW 1974; EISLER 1974; McAULIFFE et al. 1975). Without such measurements, quantitative assessment of effects, in terms of biological availability of contaminant or duration of exposure, is impossible. Since recognition of the need to measure quality and concentration in bioassay has just come about recently, the state-of-the-art as to the best method to use is unsettled. However, it is generally agreed that a variety of analytical tools will be needed for better bioassay techniques (NATIONAL ACADEMY OF SCIENCES 1975).

The second determent to chronic exposure studies has been the almost exclusive use of static or batch-treated bioassay systems. There is evidence that most toxic petroleum hydrocarbons pass out of such systems rapidly (ANDERSON et al. 1974; VANDERHORST et al. in press). At best, such systems are expensive to chemically character ize because an inordinate number of samples is required.

Soluble petroleum has often been stated to exhibit the most lethally toxic effects (SHELTON 1970; MOORE and DWYER 1974). However, there is evidence that water-insoluble petroleum may contribute to sublethal responses (ANDERSON 1972). Two major points stand

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out in terms of laboratory apparatus. Attempts to produce soluble phases of petroleum without some insoluble contamination have been unsuccessful, even in batch-prepared quantities. Also, oil-in-water dispersions containing large amounts of insoluble oil are highly unstable (ANDERSON 1974).

Oil consists of thousands of compounds which have widely different physical and chemical characteristics. Due to this complexity, the choice of apparatus will greatly influence the types and quantities of hydrocarbons available to biological test species. Thus, a system capable of producing stable concentrations of petroleum hydrocarbons in sea water is needed. This system will permit simultaneous development of long-term biological indicators of oil contamination and a standardized, sensitive set of analytical methods.

METHODS

A device was built to continuously extract dispersed No. 2 fuel oil derivative in sea water. Variability in bioassay exposure tank concentration of CCl_4-extractable organic material, using the undiluted and diluted dispersion, was evaluated by IR-spectrometry. IR analyses were performed using the method of SIMARD et al. (1951) on 1-ℓ samples collected through preplaced glass siphons at designated places in the exposure tanks. No. 2 fuel oil was used for testing (API reference oil III, 38% aromatic). Specific hydrocarbon content of an oil-in-water dispersion of oil, of the same type prepared by a batch shaking and settling method, is given by ANDERSON et al. (1974). Dilution water was metered by a constant head box and dripper-arm-equipped manifold as described by VAUGHAN (1973).

DISPERSION APPARATUS

An overall view showing arrangement for components of the apparatus is illustrated in Figure 1.

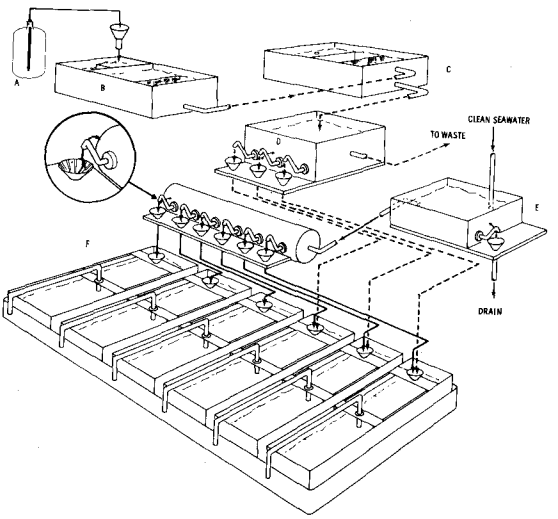

FIGURE 1. Fuel oil dispersion and bioassay apparatus

Components: A. Fuel oil metering; B. Fuel oil-sea water contacting; C. Separation; D. Dispersion metering; E. Diluent metering; F. Exposure

In operation, fuel oil and sea water are vigorously mixed in constant proportion. The mixture is allowed to partially separate based on specific gravity. Floating material is discarded and non-floating mixture (dispersion) is metered to bioassay tanks.

Fuel Oil Metering Device: A 3.8-ℓ glass mariotte bottle was used to provide a constant supply of fuel oil to the contacting tank (contactor) (Figure 2).

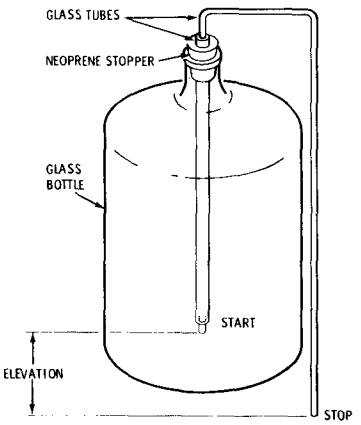

FIGURE 2. Mariotte bottle used for metering fuel oil

At the measured delivery rate (2.4 mℓ/min, S.D. = 0.09) a fresh bottle of No. 2 fuel was required for each day of operation. This material was obtained from a sealed drum of oil by glass siphon. Measurements of flow rate were for actual volumes discharged in 1 min intervals. Samples were taken immediately after placing a bottle into service and just prior to its removal. A necessary precaution for use of this system is to equilibrate source fuel oil and ambient laboratory temperature before metering to deal with viscosity and vapor pressure changes in the bottle. Rate of flow may be adjusted by selection of glassware size and changes in the difference in elevation, as shown in Figure 2.

Contactor: A fiberglass aquarium (34 x 34 x 74 cm) received the metered fuel oil and metered sea water (15,000 mℓ/min). To enhance initial mixing, the oil and sea water passed through a funnel (2 cm diameter at discharge) into the first of two identical polyvinyl chloride (PVC) boxes (21 x 10 x 12 cm) positioned at the inflow end of the contactor (Figure 3). The partition between the two boxes was 19 cm high as compared to 21 cm outside box height. Thus, the oil and sea water were vigorously dispersed by the force of incoming sea water in the first box. The dispersion received additional mixing as it flowed over the partition into the second box. The mixture flowed from the second box into the contacting tank proper via a hole (2 cm diameter) 3 cm below the top of the box.

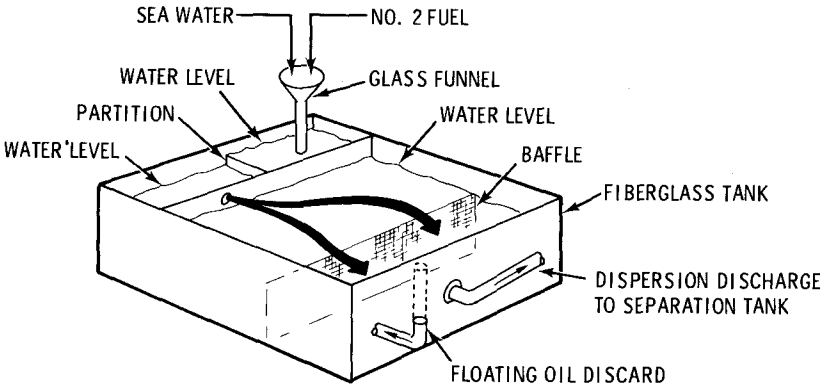

FIGURE 3. Contactor used for initial mixing and separation of fuel oil dispersion

Separation Devices: The remainder of the contactor, and the separation tank itself, were devoted to separation of floatable oil from the dispersion. The process was accomplished by providing surface outlets for discard of floating oil and discharge pipes near the bottom of the contactor and separation tanks for movement of dispersed oil to the next step in the process. The separation was enhanced by provision of both the contactor and separation tanks with baffles to move the dispersion toward the surface. The separation tank is shown in Figure 4. Flow directions (indicated by arrows) are given in both Figures 3 and 4.

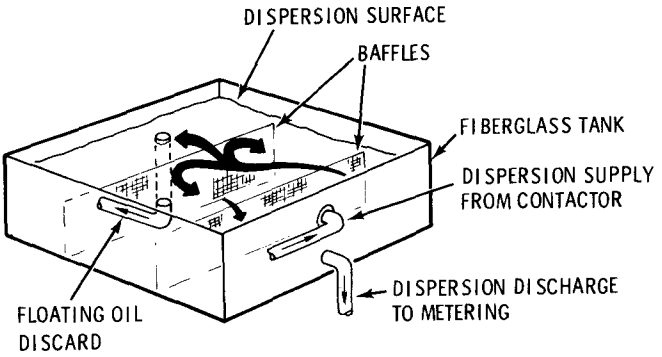

FIGURE 4. Tank for separation of floating oil from dispersion

Dispersion Metering Tank: A fiberglass tank, identical in dimension to the contactor and separation tanks, is illustrated in Figure 5. This tank provided a final floating oil discard and allowed refinement in head adjustment for delivery to bioassay tanks.

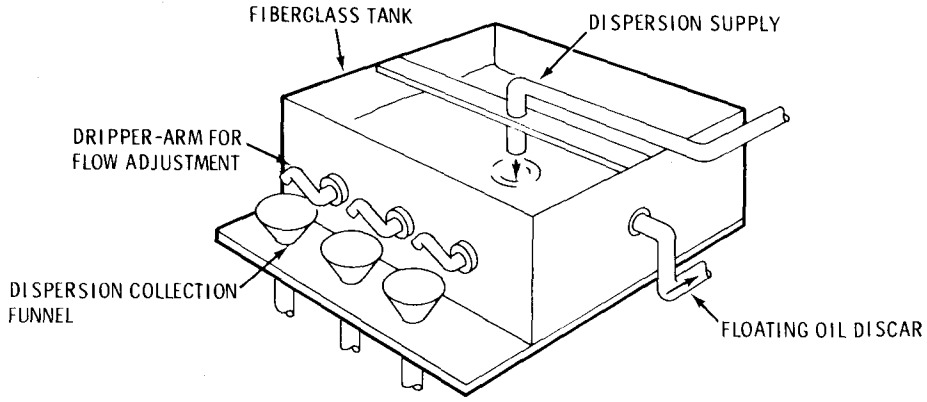

FIGURE 5. Dispersion metering tank

Flow rates in the apparatus were controlled by size of hardware and gravity. Table 1 lists flow rates at various stages of the process. Inputs were sea water and oil into the contactor with fates as indicated.

TABLE 1.
Flow rates (mℓ/min) measured in dispersion apparatus

POINT OF MEASUREMENT	FLOW RATE mℓ/min
Inputs:	
Fuel oil to contactor	2.4
Sea water to contactor	15,000
Approximate total	15,000
Fates:	
Floatable oil and sea water to discard from contactor	5,700
First stage dispersion from contactor to separation tank	9,300
Floatable oil and sea water to discard from separation tank	4,700
Second stage dispersion from separation tank to dispersion metering	4,600
Floatable oil and sea water to discard from dispersion metering	1,600
Final stage dispersion to exposure apparatus	3,000
Total	15,000
Discard for waste treatment	12,000
Usable dispersion for testing	3,000

Detailed specifications of the dispersion apparatus may be requested from the author.

Testing of Apparatus: Performance of the dispersion system was evaluated by measuring CCl_4-extractable organic material in time-series samples from bioassay exposure tanks receiving dilutions of the dispersion. Three seawater dilutions were tested and the intermediate dilution was sampled to determine spatial distribution of the dispersion in bioassay tanks.

In each experimental replicate, time-series samples were taken by pre-placed glass siphon from the mid-point of the bioassay tanks at 0.5-h intervals for 6 h, at 1.0-h intervals for a further 6 h, and in triplicate daily for the remainder of 96 h. To determine spatial distribution of the dispersion in bioassay tanks, triplicate samples were taken by pre-placed glass siphon at two depths and six locations within the bioassay tank receiving intermediate dilution.

The dilutions tested were: control, 2,000 mℓ/min sea water, no dispersion; high dilution, 1,600 mℓ/min sea water, 400 mℓ/min dispersed oil; intermediate dilution, 1,000 mℓ/min sea water, 1,000 mℓ/min dispersed oil; and low dilution, 400 mℓ/min sea water, 1,600 mℓ/min dispersed oil.

RESULTS

Distinguishable concentrations were obtained and maintained over the 96-h test periods for each of the dispersed oil dilutions. Mean concentrations were 0.4, 1.0, and 1.7 mg/ℓ total oil (CCl_4-extractable organics, IR). Ninety-five percent confidence intervals for adjacent dilutions overlapped on some days for the experiments taken in aggregate, but in no case did interval estimates overlap for the lowest and highest dilutions (Table 2).

TABLE 2.
Mean confidence intervals (C.I.) (95%) for IR measured concentration of dispersed No. 2 fuel*

	Mean and 95% C.I. of Concentration (mg/ℓ)								
	High Dilution			Intermediate			Low Dilution		
Day	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper
0	0.2	0.4	0.6	0.6	1.1	1.6	1.0	1.8	2.6
1	0.3	0.4	0.5	0.8	1.0	1.2	1.2	1.7	2.2
2	0.3	0.4	0.5	0.8	1.0	1.2	1.0	1.6	2.2
3	0.2	0.3	0.4	0.3	0.9	1.5	0.8	1.6	2.4
4	0.2	0.4	0.6	0.5	1.0	1.5	1.2	1.6	2.0
SUM	0.3	0.4	0.5	0.6	1.0	1.4	1.1	1.7	2.3

* Day-dilution intervals based on n = 9 samples per estimate;
Sum-dilution intervals based on n = 45 samples per estimate

Control tanks not receiving the fuel oil dispersion were monitored in each experiment, and did not reveal measurable oil in any instance.

A comparison of concentrations for a given dilution does not reveal significant differences in concentration with respect to the day of dispersion sampling. Measured concentrations were significantly less (P = 0.05) for samples taken at 1/2 h after initiation of oil flow at all dilutions and at 1 h after initiation for the

lowest dilution (highest concentration) when compared to subsequent samples.

Coefficients of variability for concentration, computed for data exclusive of the first 4 h in each experiment, ranged from 22.6% at the highest concentration to 25.7% at the lowest concentration (based on 54 samples at each concentration).

In the experiment designed to determine the spatial distribution of the dispersion in bioassay tanks, there were no significant ($P = 0.05$) differences in concentration with respect to depth of sampling, and the length or breadth of the exposure tank. Mean concentration in this experiment was 1.0 mg/ℓ, or equivalent to the intermediate dilution. Coefficient of variability for concentration for the distributed samples was 18%.

DISCUSSION

The data obtained in this study demonstrate the feasibility of continuously producing "stable" concentrations of No. 2 fuel oil dispersion in sea water bioassay tanks. Variability coefficients were comparable for time-series samples taken over the entire experimental period and for spatially distributed samples collected at very short intervals. A criticism commonly levied against the analytical tool used in this study (IR-spectrometry of CCl_4-extracts) is that it does not discriminate petroleum-derived hydrocarbons from other compounds having C-H bonds. The failure to detect measurable concentrations of CCl_4-extractable organics in controls, and the generally linear relationship between dilution and measured concentration, would seem to discount the validity of that criticism in the present evaluation.

The total amount of variability in the system (about 20%) is not surprising given the present state-of-the-art. This variability encompasses that associated with the delivery apparatus, the complex system of waterborne oil, and the method of measurement. For comparison, batch-treated experiments using identical exposure tanks and analysis method exhibited 100% variability in just 24 h (VANDERHORST et al. in press).

We do not yet have data on the composition of the material in the dispersion produced by this apparatus. ANDERSON et al. (1974) found oil-in-water dispersions of No. 2 fuel oil, Kuwait crude and South Louisiana crude oils to more nearly resemble parent oils than water soluble fractions of the same oils. Dispersion was not complete in our studies since the input of energy required for maintenance of complete dispersion would be difficult under laboratory conditions and extremely rare in nature.

The apparatus described here represents the culmination of lengthy experimentation. Vestiges from the development period remain in the system and might best be removed from reconstructions designed solely for the production of stable concentrations of dispersed oil. Most notable, perhaps, is the division of the system into units for contact separation and metering. That approach was used (and will remain in our systems) to allow versatility in the application of "weathering" factors at different points in the system. In a system designed solely for the production of an oil-in-water dispersion, these functions might well be performed in a single vessel with appropriate baffles and surface discharges.

Similarly, the PVC boxes at the inflow end of the contactor were initially used with stirring motors. The motors were found to be neither necessary nor desirable for the system with No. 2 fuel oil. Mean concentrations of CCl_4-extractable organics in the dispersion were essentially the same with or without the stirrers, and the motors proved to be a high source for system failure. As constructed, the system relies entirely on incoming seawater supply and gravity for operation. This is a highly attractive feature for systems intended for long-term use.

Because of increased viscosity, constant crude oil metering is not possible using the fuel oil metering device (mariotte bottle) described here. We have obtained satisfactory delivery rates (3.5 mℓ/ min) using a Lapp Pulsafeeder, Model LS-30 pump (Lapp Insulator Co., Inc., Leroy, N.Y.). Time-series data on the dispersions produced using crude oil have not been evaluated.

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