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Methods used by Environment Canada in the Testing of Oil Spill Dispersants

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Environmental Impact Control Directorate
July, 1977

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METHODS USED BY ENVIRONMENT CANADA IN THE TESTING OF OIL SPILL DISPERSANTS

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A Report Submitted to:

Research and Development Division
Environmental Emergency Branch
Environmental Impact Control Directorate
Environmental Protection Service
Department of Fisheries and the Environment

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July, 1977

REVIEW NOTICE

This report has been reviewed by the Environmental Impact Control Directorate, Environmental Protection Service, and approved for publication. Approval does not necessarily signify that the contents reflect the views and policies of the Environmental Protection Service. Mention of trade names and commercial products does not constitute endorsement for use.

ABSTRACT

This report presents in detail the methods used in the testing and evaluation of the toxicity and effectiveness of oil spill dispersants by the Environmental Emergency Branch of the Department of Fisheries and the Environment.

RESUME

Le présent rapport indique en détails les méthodes utilisées par la Direction des interventions d'urgence, ministère des Pêches et de l'Environnement, pour évaluer la toxicité et l'efficacité des dispersants des nappes d'hydrocarbures.

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1. INTRODUCTION

Before an oil spill dispersant is used in Canada it must pass certain toxicity and effectiveness criteria. These criteria are outlined in the "Guidelines on the Use and Acceptability of Oil Spill Dispersants" (Anon. 1973).

The purpose of this manual is to explain, step by step, the procedures used to test oil spill dispersants for toxicity and effectiveness, thereby facilitating the transfer of this technology from the public to the private sector.

Further information pertaining to acceptability criteria or testing procedures can be obtained by contacting either:

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5151 George Street, 16th Floor,
Halifax, Nova Scotia, B3J 1M5

Trade names are given for some equipment to serve as a guide to specifications if equipment has to be purchased. Mention of trade names does not constitute endorsement for use by the Department of Fisheries and the Environment.

2. EFFECTIVENESS

2.1 Function of Effectiveness Testing

The effectiveness of an oil dispersant is its ability to disperse a surface slick of oil into the water column and to hold the emulsion there. Oil dispersant effectiveness is determined by the Simulated Environmental Tank (SET) test (Murphy and McCarthy 1969).

The SET test is a simple method by which oil spill dispersants can be evaluated for effectiveness under standardized conditions of temperature, water quality (freshwater or saltwater with a salinity of 30-35%) and oil type with varying dispersant-to-oil ratios. The standardized procedure allows for inter and intra-laboratory comparison of results for the testing of new dispersants.

2.2 General Requirements

2.2.1 Spacial Requirements

The first consideration is the location in which the SET's are to be conducted. A room with a concrete floor and open drains is most desirable. Although these tests can be performed in fairly cramped quarters, it is suggested that the room selected for use be one which measures at least 12' by 12' to allow for the washing of the SET barrel, and one which provides ample space for a table or counters for the necessary glassware. This room must be equipped with both a freshwater and saltwater supply (30-30%) and at least five electrical outlets, one of which must be 220 V. It is suggested that each electrical outlet be fitted with a bakelite switchbox so as to eliminate the plugging in and pulling out of cords, thus reducing the possibility of electric shock to the operator.

2.2.2 Equipment Required

The Guidelines require all SET tests to be conducted at a water temperature of $1 \pm 1^{0}C$. In order to reach and maintain this water temperature the DFE (Halifax) lab uses a Remcor Force Flow Liquid Circulator (230 volt, 60 cycle, single phase, Model CFF100A). When supplemented with ice, 6 to 8 SET's can be conducted in a $7\frac{1}{2}$-hour day. An even better method of cooling the water would be to have a walk-in cooler in which several thousand litres of water could be cooled and replenished as required.

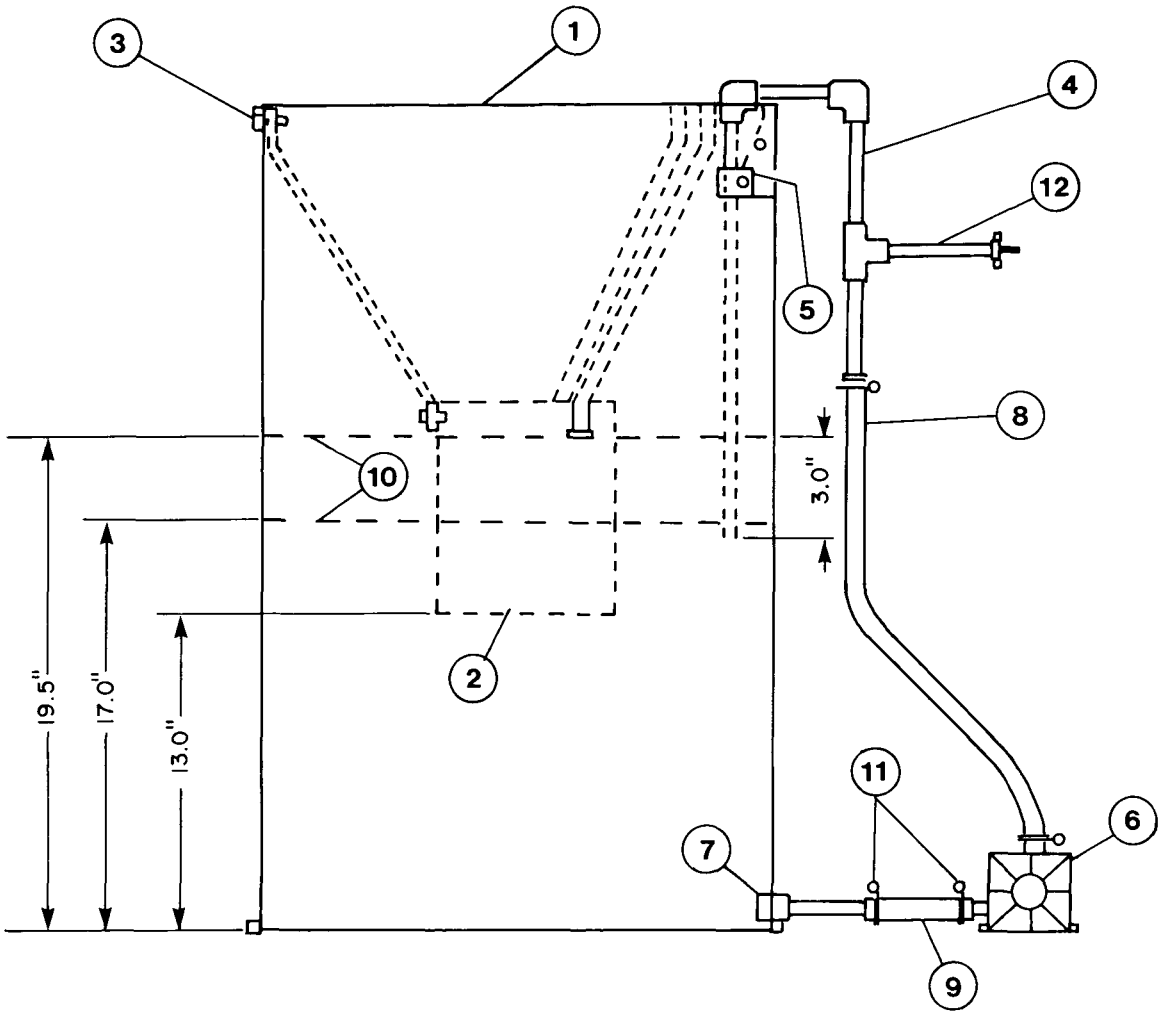
The Remcor unit, if used, requires the use of a submersible pump to recirculate the water through the cooling unit. A "Little Giant" P8496 series pump, available from Canadian Laboratory Supplies, Ltd. is used in the DFE (Halifax) laboratory.

The actual SET test requires the use of three additional pumps: (1) a recirculating centrifugal pump of approximately 20 l/min at 2-ft head (e.g. Cole-Parmer Model 7000, 1/35 HP), to recirculate the water-dispersant-oil emulsion in the SET barrel; (2) a pump capable of a water pressure of 35-45 psi to agitate the oil-chemical mixture, together with a 25' reinforced garden hose with a garden spray valve as an accessory; and (3) a large-volume pump to quickly fill the SET barrel and thus minimize warming of the cold water.

2.3 Detail of SET Test Equipment

2.3.1 SET Tank

The SET tank (see Fig. 1) is a 45-Imperial gallon steel drum painted with white epoxy paint on the inside to make it easier to clean and to see any oil which adheres to the side of the tank. On the inside of the barrel there are two marked levels 17" and 19.5" from the bottom. The reason for having these marked levels is explained in Section 2.5.



PARTS LIST

1. 45-gallon steel drum
2. Polyethylene cylinder
3. Nut, bolt and washer
4. Upper part of plumbing
5. Acrylic lock-unit for plumbing
6. Centrifugal recirculating pump
7. Drain
8. Tygon tubing 3/4" I.D. x 2.5'
9. Tygon tubing 3/4" I.D. x 6"
10. Marked levels at 19.5" & 17.0"
11. 1/2" screw clamps
12. Test sample outlet

Fig. 1 - Simulated Environmental Tank

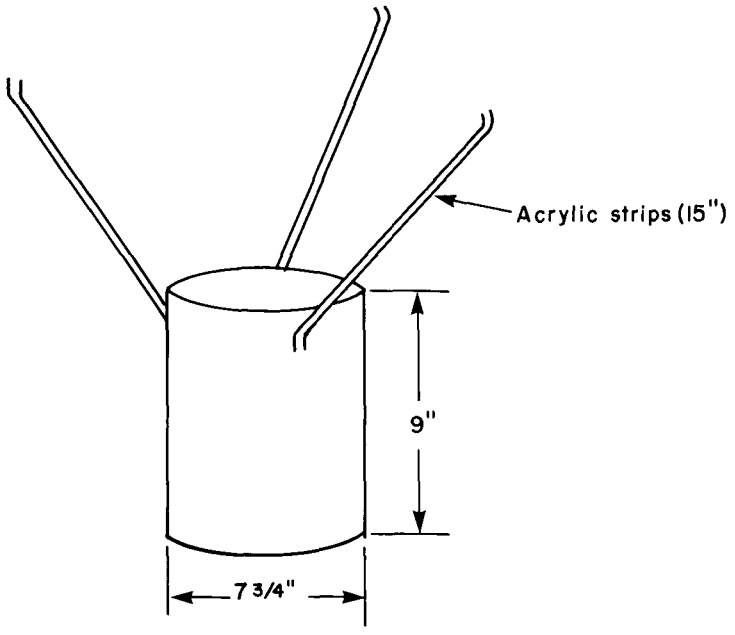


Fig. 2 - Polyethylene Cylinder

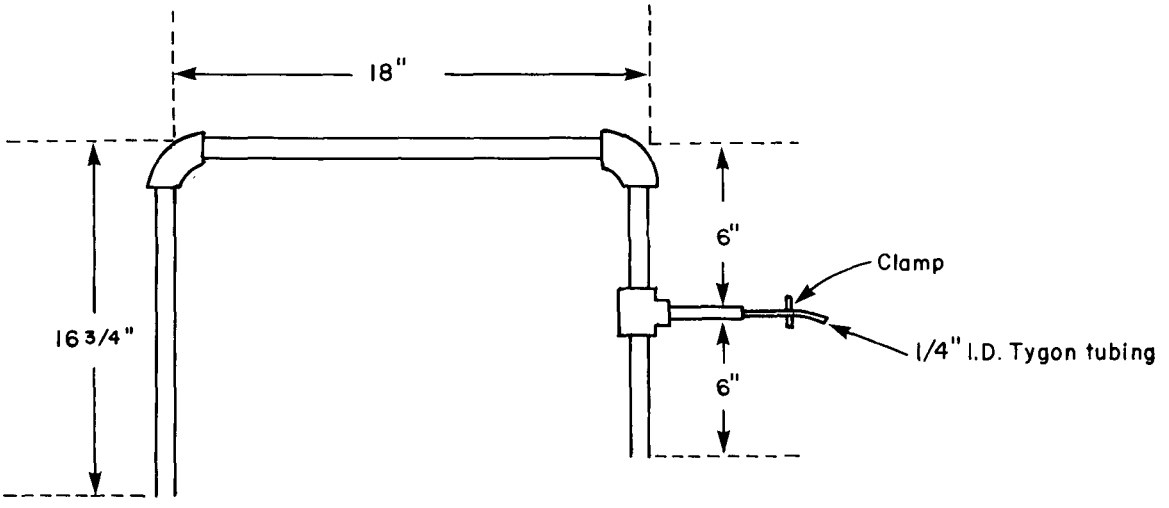


Fig. 3 - Upper Plumbing Apparatus

Attached to the SET drum are a permanently fixed cylinder on the inside and a removable plumbing unit for recirculating the contents of the SET barrel.

2.3.2 Plastic Cylinder

The cylinder fitted to the inside of the SET barrel is cut from a 2-gallon polyethylene bottle (Fig. 2). It is 7½" in diameter and 9" deep and keeps the oil and dispersant in as much contact as possible during the 10 minutes before agitation of the mixture. This cylinder is affixed permanently to the barrel by means of 15" long acrylic strips or other suitable material with non-corrodible screws.

2.3.3 Recirculation Apparatus

The upper plumbing apparatus, which is used to recirculate the emulsion and to draw off the aliquot, is constructed of standard ½" I.D. PVC piping, two elbows and one T fitting (Fig. 3).

The lower part of the plumbing fixture consists of the centrifugal recirculating pump to which is attached 3/4" I.D. tygon tubing connecting the pump with the upper plumbing apparatus and the drain at the bottom of the barrel, as shown in Fig. 1.

The drainage fixture is simply a ½" PVC threaded plug which has been inserted as near the bottom of the barrel as possible. The drain can be made waterproof by using rubber packing and oil-resistant gasket glue. A threaded piece of ½" PVC piping 2" long is attached to the threaded plug and this in turn is connected to the centrifugal recirculating pump by tygon tubing, thus completing the plumbing unit.

2.4 Laboratory Equipment Required

1. Spectrophotometer (Spectronic 20 Bausch and Lomb);
2. 12-100-ml. volumetric flasks;
3. 6-1000-ml. separatory funnels;
4. 1 separatory funnel rack;
5. 2-500-ml. graduated cylinders;
6. 6-200-ml. beakers;
7. 24-50 ml. beakers;
8. 48 small-mouthed, 8-oz amber storage bottles with screw tops;
9. aluminum foil;
10. 2 variable volume dispersers (125 ml.);
11. timer;
12. spatulas;
13. disposable pipettes;
14. 25-ml. graduated pipette;
15. 2 pro-pipette bulbs;

16. filter paper (Whatman qualitative 1 - 9.0 cm);
17. weight scale (Mettler Top-Loading Balance P1210);
18. 5 gal. A.C.S. grade chloroform;
19. 5 gal. lab-grade acetone;
20. 5 lbs. A.C.S. reagent grade anhydrous sodium sulfate;
21. 5 gal. crude oil - gravity (^{o}API):30-43; and residuum (700 F): 30 volumn percent minimum (e.g. Venezuelan Lago Medio crude);
22. 5 gal. medium bunker - viscosity (SSF at 122^{o}F): 200±20;
23. 5 gal. heavy bunker - viscosity (SSF at 122^{o}F): 300±20.

2.5 Effectiveness Test Procedure

The procedure is outlined in the Guidelines (Anon., 1973) and is represented here in more detail:

1. Cool a large supply of test water to 1±1^{o}C (5-gallon buckets of ice will help to quickly cool test water).
2. Assemble the plumbing apparatus to the SET barrel as illustrated in Fig. 1.
3. Fill the barrel to the 17" mark with 1±1^{o}C water.
4. With the recirculating pump off, carefully pour approximately 100 grams of the test oil into the centre of the "ring" (Fig.4). The exact amount of oil should be determined by weighing the oil container before and after the addition of the oil and this weight difference used in the effectiveness calculation (see Section 2.14).
5. Allow one minute for the oil slick to stabilize and come to equilibrium with the water temperature.
6. Add X grams of the dispersant (X=100, 30, 10, etc.) by spraying (using the polyethylene spray bottle) in a fine stream contacting as much oil as possible (Fig. 5). In the case of a concentrate product, use X=grams of the prediluted dispersant. The dispersant must be applied in less than 30 seconds. Weigh the bottle before and after the application of the dispersant to obtain the weight of the dispersant added.
7. Wait 10 minutes to allow the dispersant to contact the oil inside the ring.
8. Agitate the oil-chemical mixture using water at 35-45 psi from a rubber or plastic hose having a ½-inch inside diameter and fitted with a fully opened garden spray nozzle (Fig. 6). Agitation of the surface of the water in the tank should be continued until the volume in the tank has reached the 19.5" mark. In some case the high-foaming characteristics of a few dispersants make it difficult to see the 19.5" mark. This problem can be overcome by timing the interval required to reach the 19.5" mark prior to the test and duplicating this interval at this stage of the test procedure.

Fig. 4 - Adding Oil to SET Barrel

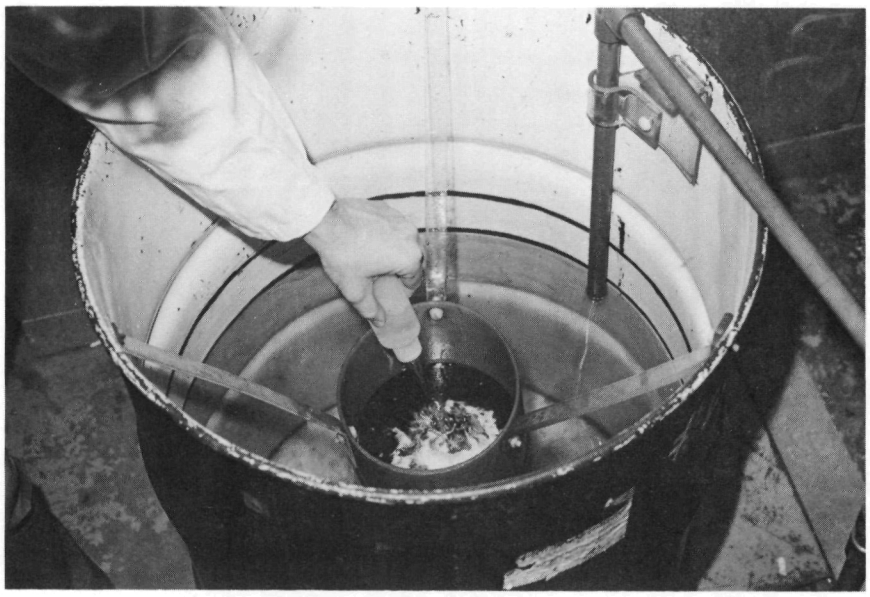


Fig. 5 - Adding Dispersant to SET Barrel

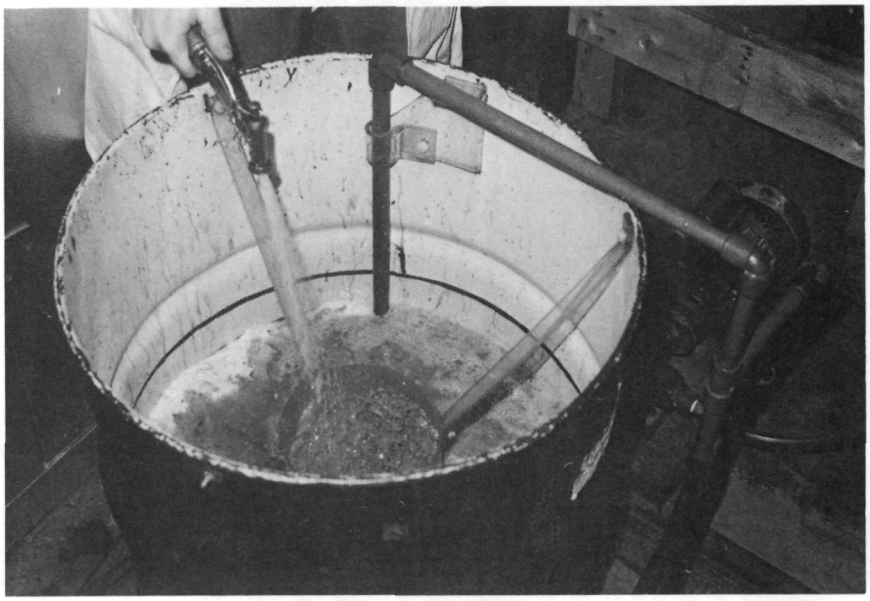


Fig. 6 - Agitating Dispersant-Oil Mixture

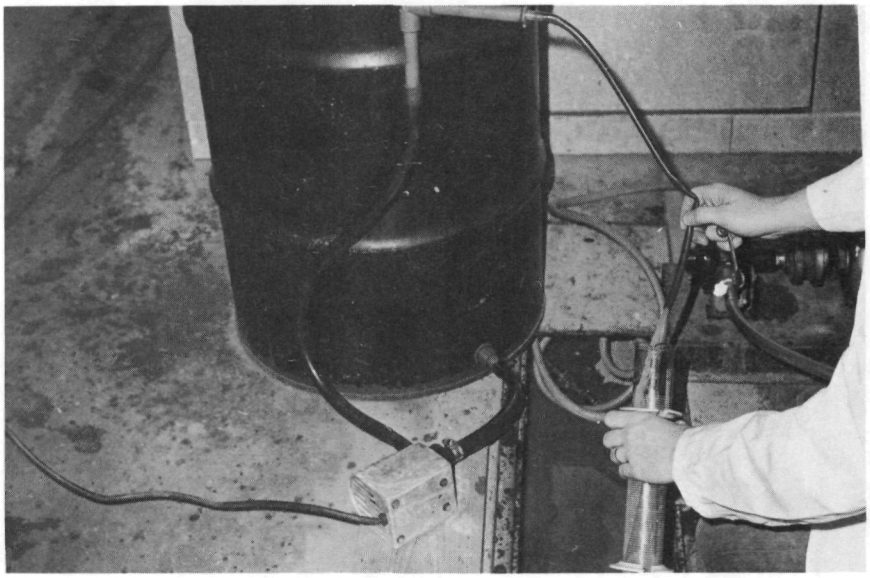


Fig. 7 - Withdrawing Sample

9. As soon as the hosing is completed, start the recirculation pump and continue its operation throughout the test at a rate of approximately 20 litres per minute.
10. Ten minutes after the termination of hosing, withdraw a 500-ml sample to determine the amount of initial dispersion (Fig. 7). Before taking the actual sample, the sampling line should be cleared first by collecting a few hundred mls in a beaker. After taking the sample return the collected oil-water mixture to the tank.
11. Immediately after collection transfer the 500-ml sample quantitatively to a separatory funnel and extract the dispersed oil with four 20-ml portions of chloroform and dilute to 100 ml with additional chloroform (Fig. 9). The first 20-ml portion of chloroform should be used to rinse the sample container prior to use during the first extraction. Pour extracted oil in chloroform solution over 2 grams of anhydrous sodium sulphate in an 8-oz amber glass storage bottle with an aluminum foil-lined cap. Samples should not be stored for more than three days before analysing.
12. Upon completion of each test the SET barrel must be throughly cleaned before another test is started (Fig. 9). BP1100X* as a cleaner and a fairly stiff brush to scrub the inside of the barrel are recommended. Care should also be taken to clean the plumbing apparatus by pouring some cleaner through it and rinsing both this and the barrel well so as to be sure there is no residue from either oil or cleanser which might influence the results of the next test.

2.6 Shortcuts

For a given oil and temperature, if the dispersant cannot disperse 65% of the oil or if the dispersant/oil ratio needed is greater than unity, the dispersant is considered to be ineffective under these conditions. If the tests are performed in a sequence moving from light to heavy oils, and high to low ratios, considerable time and money can be saved. It is suggested that the set of experiments be started with crude oil at a dispersant/oil ratio of 1:1 and at a temperature of 1°C. As stated previously, should the dispersant not disperse 65% of the oil, it is then considered ineffective under these conditions. It is also reasonable to assume that the dispersant is ineffective with the heavier oils and further tests are unnecessary. Similarly, if the dispersant is effective with crude oil at 1°C, but ineffective with the medium bunker oil at 1°C, then tests with heavy bunker oil at 1°C are not necessary. Because of the large variance associated with the SET test, it is necessary to replicate at least three times.

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* BP1100X is an oil spill dispersant available from BP Canada, Ltd., Montreal.

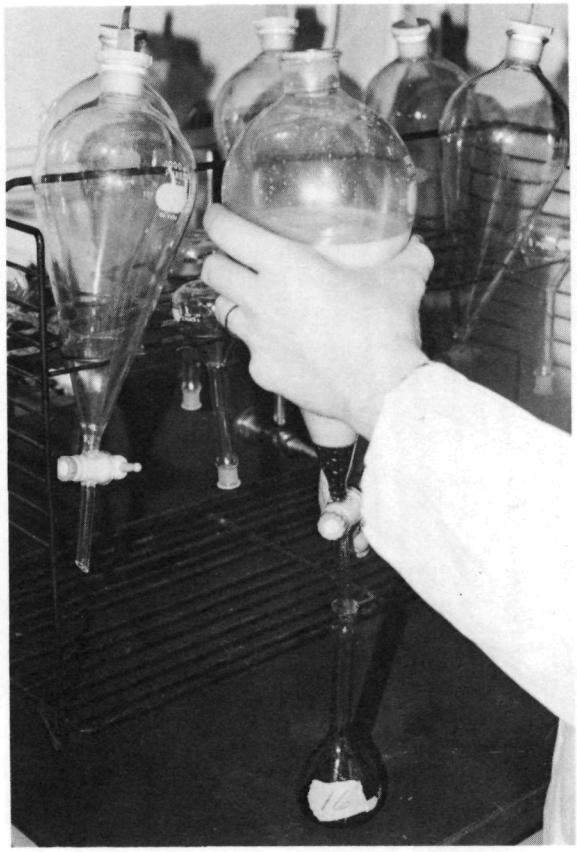

Fig. 8 - Extraction of Oil From SET Sample

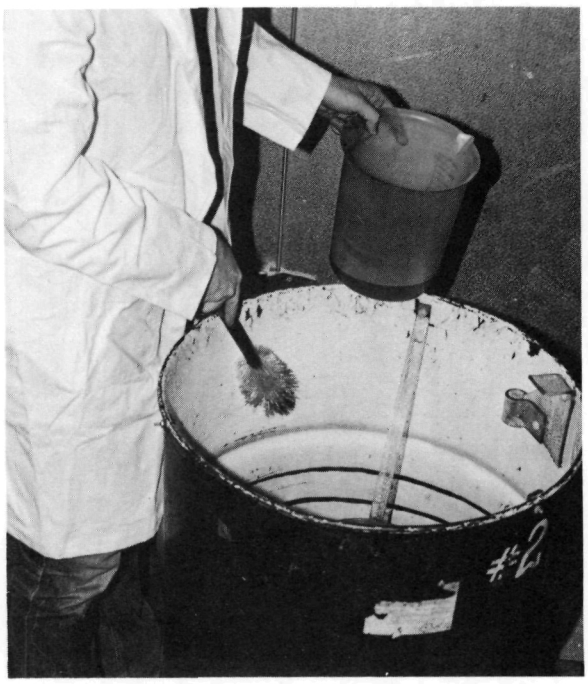

Fig. 9 - Cleaning SET Barrel

2.7 Number of Tests

Experiments are to be performed with the dispersant and three test oils: crude oil, medium bunker and heavy bunker fuel oils. Each dispersant-to-oil combination shall be tested at one temperature, 1°C, and at various dispersant-to-oil ratios in the series 1:1, 1:3, 1:10, etc. until a minimum dispersant-to-oil ratio needed to disperse 65% of the floating oil is reached. All tests should be conducted in triplicate and conducted in both freshwater and seawater.

2.8 Dispersion Stability Tests

To determine stability of the oil dispersion the "10 minute" tests described above are continued for a total of six hours, after which samples are withdrawn and measured for oil. These test will be conducted with crude oil at a dispersant-to-oil ratio of 1:3 and suitable temperature control to prevent the test water from warming above 1±1°C. The use of a cold room at this temperature would be the simplest method to achieve this goal.

2.9 Testing with Reference Dispersant

In order to compare experimental procedures and results with other laboratories, it is necessary to perform experiments with a reference dispersant. Mix 3 ml of a standard surfactant (IGEPAL CO 630, Chemical Developments of Canada, Ltd.) with 25 ml of 2-methyl-2, 4 pentanediol (hexane glycol). Then add this solution to 100 grams of test oil in a 250-ml separatory funnel and mix by agitating vigorously for 10 minutes. Then place on the surface of the water and hose with water immediately. A sample should be collected after 10 minutes and analysed as indicated below to obtain the dispersion value.

2.10 Dispersant Blanks

The SET procedure should be repeated using dispersants alone as a blank to determine the influence of test water and dispersants on the percent transmittance reading from the extracted sample. This reading can be subtracted from the SET test results to give the reading caused by dispersed oil alone.

2.11 Problem with High-Foaming Dispersants

In some cases the high-foaming characteristics of the dispersants cause much oily foam to collect at the surface of the water after 10 minutes. Since the oil in this foam may be dispersed but not measured, as it is not in the water column, the 10-minute test should be extended to a 1-hour test so that the foam will have some time to dissipate. If this is the case, the 1-hour test will have to be conducted either in a cold room or a water bath to keep the water temperature from rising above 2°C.

2.12 Alternate Extraction Solvent

As some dispersants are difficult to extract with $CHCl_3$, an alternate solvent such as hexane can be substituted. If hexane is used, two separatory funnels must be used as the oil-in-hexane solution rises to the top. A hexane stock solution will have to be prepared if this procedure is employed (see Section 2.13).

2.13 Stock Solutions

Before the SET aliquots can be analysed for effectiveness, a stock solution for each of the oils used must be prepared. This is done by adding exactly 5 grams of crude oil or 2.5 grams of medium or heavy bunker oil to a 500-ml volumetric flask and diluting this to the 500-ml mark with A.C.S. grade $CHCl_3$. For crude oil, 9 concentrations from 45%-5%, in gradients of 5%, are diluted to exactly 100 mls with A.C.S. grade $CHCl_3$. For the bunker oil stocks, concentrations of 40, 20, 10, 5, 2.5, 1 and .5% are prepared in the same manner. These stock solutions are stored in 8-oz amber bottles with tinfoil-lined screw caps for future use.

2.14 Analysis of Results

Each of the stock concentrations are analysed for %T on the Spectronic 20 at a wavelength of 530 nm for crude and 590 nm for the bunkers. Standard curves are constructed by plotting %T against weight of oil on 10" x 10" graph paper, for each of the stocks. Fig. 12 shows a normal crude oil standard curve.

Each of the SET samples are filtered through Whatman qualitative 1-9 cm filter paper into a 50-ml beaker (Fig. 10) and then analysed for %T on the Spectronic 20 (Fig. 11).

Using Fig. 12 as an example, it can be seen that if a particular sample gives a %T of 18, the actual weight of oil present in that sample is .265 grams. From this the percentage of oil dispersed can be calculated as follows:

$$\% \text{ dispersion} = \frac{\frac{A}{B} \times C \times 100}{D} \qquad (1)$$

where A = weight of oil in 500 ml sample (g) = .265
B = volume of sample (500 ml)
C = volume of water in SET (ml) = 136,000
D = weight of oil used in the test (g) = 100

Therefore, the percent of oil dispersed in the above sample was 72.

Fig. 10 - Filtering Extracted SET Samples

Fig. 11 - Analysis of Filtered SET Samples

In some cases there is so much oil in the sample that no light passes through, resulting in a 0%T reading. When this happens the sample must be diluted so as to get a %T reading of between 10 and 90. The calculated value must be multiplied by the dilution factor to get the final percent effectiveness.

2.15 Reporting

All tests should be replicated three times and the average calculated. The data are expressed as percent effectiveness of a dispersant against a particular oil for a variety of dispersant-to-oil ratios in either freshwater or saltwater. Effectiveness tables for both water types should be constructed as outlined in the Guidelines and made available to Environment Canada.

3. TOXICITY

3.1 Function of Toxicity Testing

The aim of the toxicity bioassay procedures is to establish whether the dispersant passes the toxicity criteria outlined in Section 3.3 of the Guidelines on the Use and Acceptability of Oil Spill Dispersants (Anon. 1973). The test does not necessarily attempt to imitate the exact situation which might prevail in nature, but involves standard procedures and conditions which are easily reproducible. Much of the following description of the bioassay test is quoted directly from the Guidelines (Anon. 1973).

The standard conditions involve the use of a standard species, a standard test water and temperature, and a specified test procedure. It is realized that it may be difficult or impossible in some instances to adhere to these conditions. Alternative, non-standard conditions may be used in such circumstances. However, in case of uncertainty or dispute about the validity of the results, information based on the standard test must be used.

There are three materials to be tested: (1) the dispersant alone, (2) dispersant-oil mixture, and (3) a reference toxicant. For the dispersant alone and the reference toxicant the median lethal concentration will be established after 4-day exposure. The dispersant LC50 will be triplicated. For the dispersant-oil mixture the average percent mortality at a concentration of 100 mg/l, replicated five times, must be established after 4-day exposure. This value is merely a convenient reference point for establishing pass/fail criteria for the test materials to an agreed standard fish. Obviously, the "safe" concentration causing no sub-lethal effects would be lower than those concentrations causing mortality after 4-day exposure and is often much lower.

In some cases the dispersant is to be diluted before application to an oil slick. For these "concentrate" dispersants the exposure concentrations to be tested would be made up in terms of the pre-diluted dispersant and not the concentrate. The pre-diluted dispersant would also be tested with No. 2 fuel oil.

The bioassays are conducted using the continuously stirred static test instead of the more sophisticated continuous flow tests, since they seem to be more realistic and probably more accurate for a material as difficult to handle as oil. This type of test incorporates the use of a multi-stirrer which is described in the following section.

3.2 Equipment Required

General equipment required is listed below:

1. thermometer (celsius);
2. pH meter;
3. DO meter;
4. salinity meter;
5. laboratory blender;
6. weight scale and glassware used for effectiveness testing will suffice for toxicity testing;
7. multi-stirrer (see Section 3.3) or laboratory stirrers;
8. 16 2' x 2' x 2' - molded polyethylene tanks;
9. temperature control system, either a water bath or an air cooling unit;
10. 16 rigid frame cages with covers.

3.3 Construction of Lab Multi-Stirrer

The multi-stirrer is a machine which simultaneously stirs 16 tanks to ensure circulation and maintain a homogeneous emulsion during the testing period.

The multi-stirrer used was built to stir 16 tanks (2' x 2' x 2') two abreast, and dimensions are therefore given accordingly.

The frame of the multi-stirrer is 17'6" x 2' and is constructed of steel dexion 225 (see Fig. 13, 14, 15). The stirring system is powered by two $\frac{1}{2}$-h.p. electric motors, which are positioned one on each end of the frame to stir one side of eight tanks. In order to reduce the r.p.m.'s down to $\simeq$ 450, a simple reduction system is employed for each motor. The motors are fitted with $2\frac{1}{2}$" x $\frac{1}{2}$" steel pulleys. A $\frac{1}{2}$" V-belt goes from this pulley to a 5" x $\frac{1}{2}$" pulley on a separate 8" x $\frac{1}{2}$" shaft, and then from a $2\frac{1}{2}$" x $\frac{1}{2}$" pulley on this shaft to another 5" pulley on the first stirring shaft (Fig. 15). All other pulleys are $2\frac{1}{2}$" x $\frac{1}{2}$".

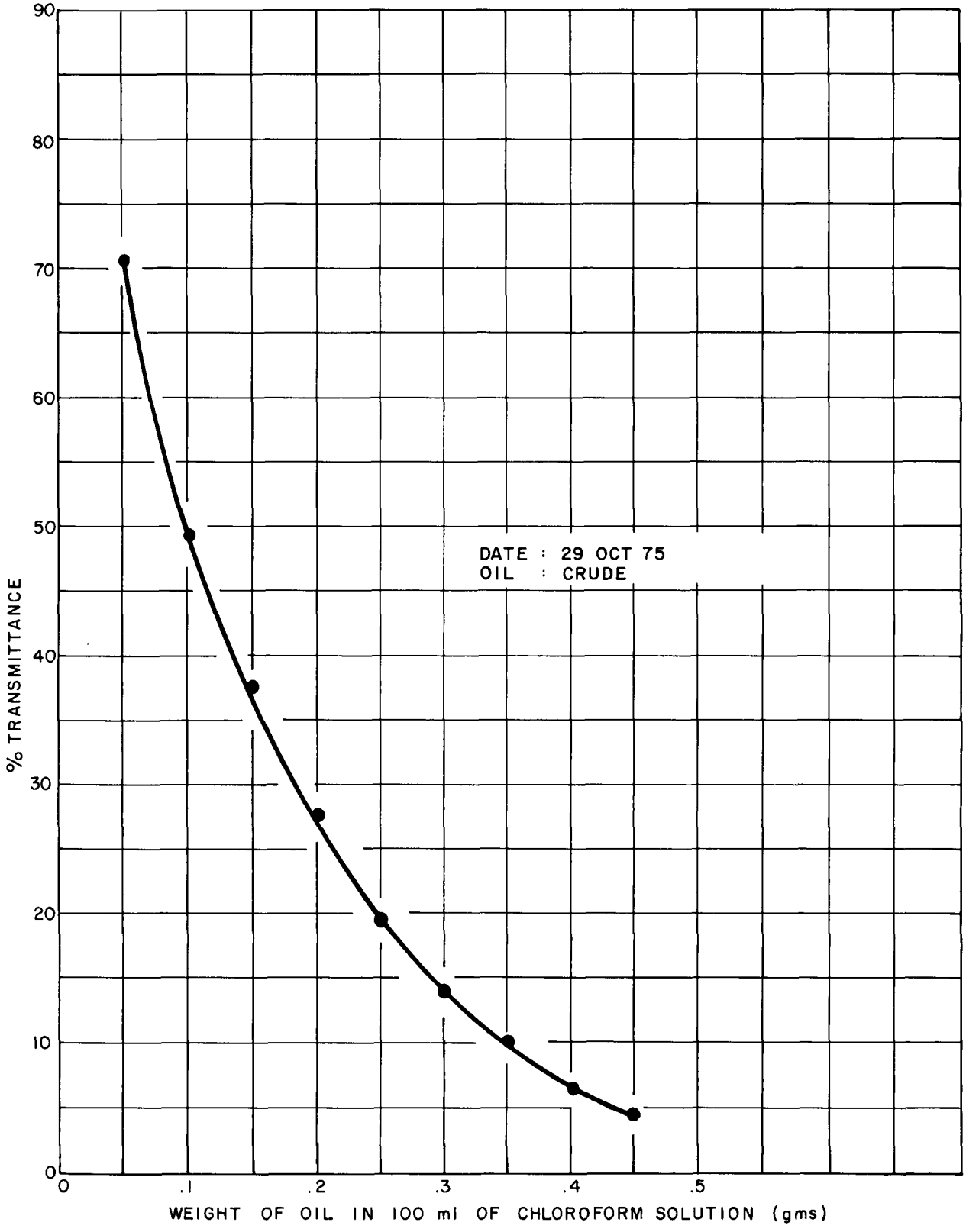

Fig. 12 - Standard Curve - %T against Oil Concentration

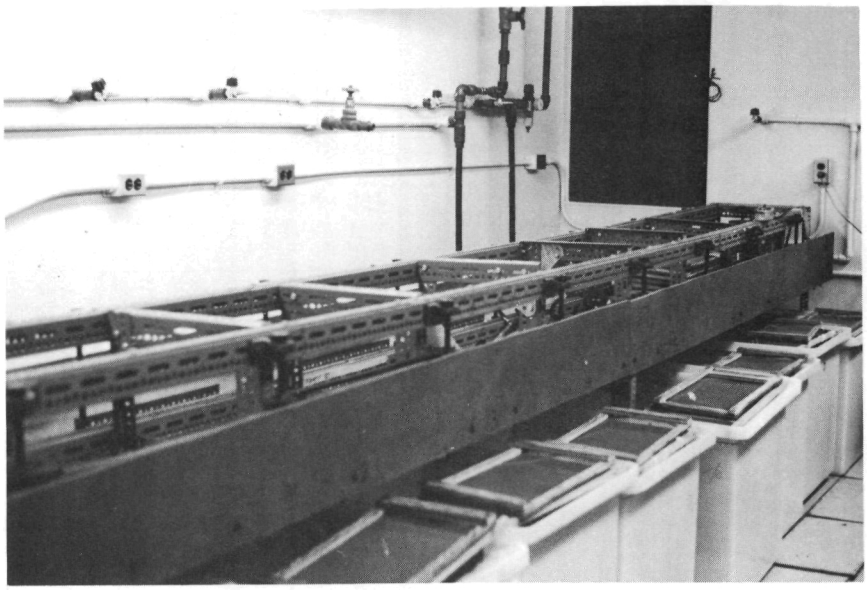

Fig. 13 - Multi-Stirrer With Tanks

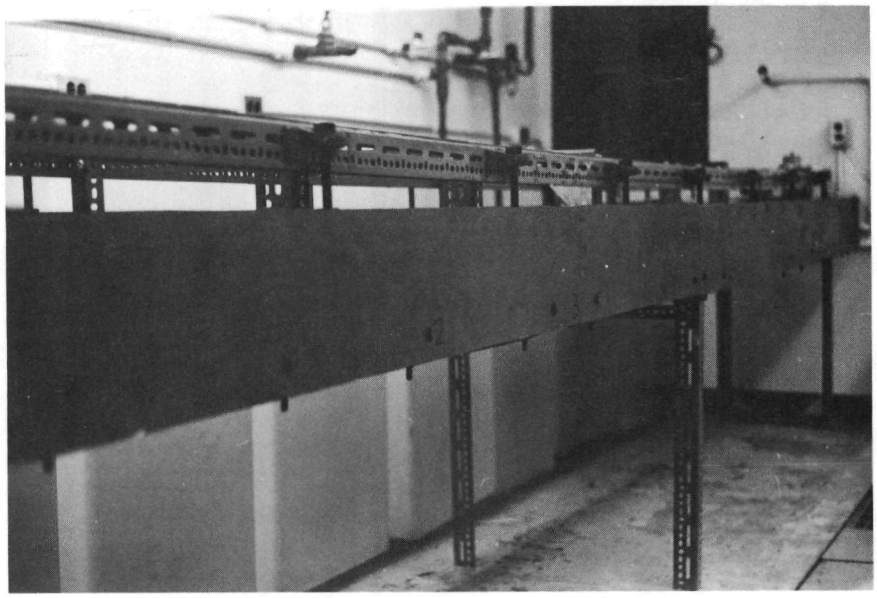

Fig. 14 - Multi-Stirrer

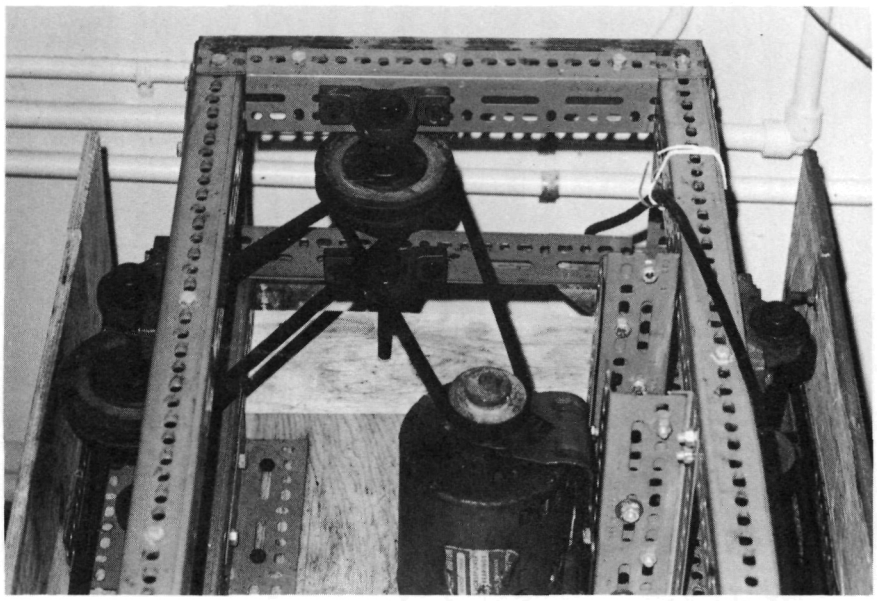

Fig. 15 - R.P.M. Reduction System

The stirring assembly consists of ½" steel shafts (cold rolled) 13" long, two 2½" x ½" steel pulleys and two ½" self-aligning, sealed pillow blocks (Fig. 16). At the end of each shaft a ½" coupling connects it to a ½" acrylic (cast) rod. The acrylic rod is fitted with two 5" diameter, 4-pitch propeller blades. These rods with affixed propeller blades should reach to within two inches of the bottom of the test tank. There will be eight stirring shafts on each side, excluding the reduction ones. These are positioned with 25" centres (shaft to shaft) and joined by 58" x ½" V-belts.

The bottom and sides of the multi-stirrer are enclosed by a plywood guard, with appropriate holes for the shafts, to keep any metal filings or wear from the V-belts from contaminating the test tanks.

A list of the required materials for the construction of 16-tank multi-stirrer is as follows:

1. 36 self-aligning, sealed pillow blocks;
2. 36-2½" x ½" steel pulleys;
3. 4-5" x ½" steel pulleys (plus bushings);
4. 2-½" h.p. electric motors;
5. 16-½" couplings;
6. 16-13" x ½" steel shafts (cold rolled);
7. 2-8" x ½" steel shafts (cold rolled);
8. ½" cast acrylic shaft (easily cut to required length in lab);
9. 14-58" x ½" V-belts;
10. 4-32" x ½" V-belts;
11. steel dexion, nuts, bolts and washers;
12. 2 sheets of 3/8" plywood.

If the toxicity tests are to be conducted with this apparatus, it is essential that the lab be equipped with an air cooling system in order to keep the test temperature at the required 15°C.

The total cost of this machine, excluding the test tanks and ½-h.p. motors, was $616.31 at October, 1974 prices. Modifications and improvements can be made in order to cut costs or to stir smaller or fewer test tanks.

There are alternative ways to stir the test tanks, such as multi-speed lab stirrers, but the cost of such equipment is somewhat prohibitive if 16 tanks are to be stirred simultaneously.

3.4 Freshwater Bioassays

3.4.1 Oil and Dispersants

The oil to be used in the bioassay tests is No. 2 furnace fuel oil (with a minimum aromatic content of 15% by volume and as specified

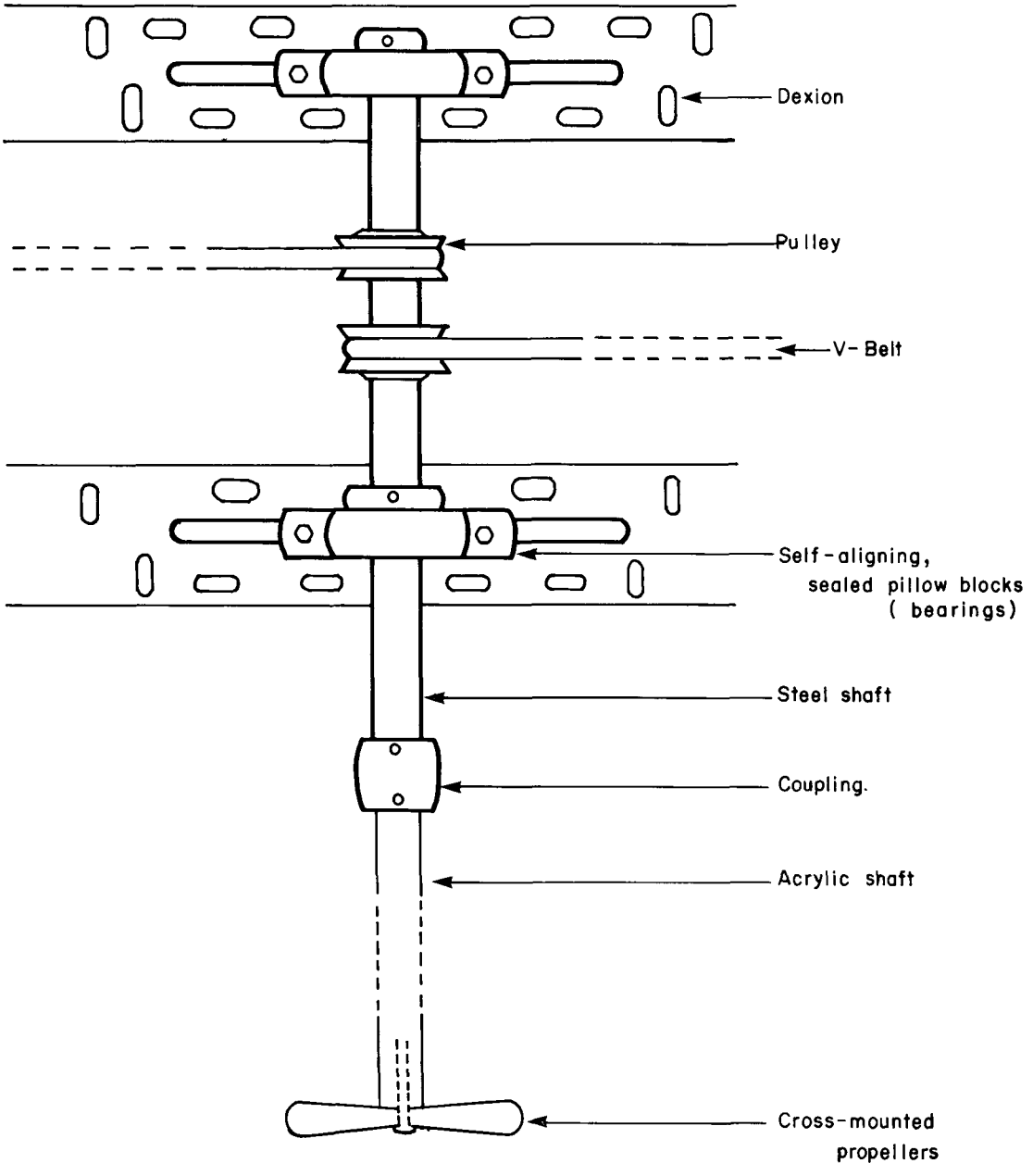

Fig. 16 - Multi-Stirrer Shaft Assembly

by ASTM D-396). The origin of this fuel oil should be specified (i.e. origin of crude oil and also refinery). The oil should be stored and transported in small, sealed containers (about 100 ml to 200 ml) to prevent loss of volatiles. Storage temperature should be approximately 4°C. A fresh container should be used for each new set of tests. Dispersant containers should be kept sealed when in storage and the appropriate batch number should be carefully noted on each.

3.4.2 Reference Toxicant

As described by LaRoche et al. (1970), "the use of a reference toxicant is desirable as a link between the findings of different investigators, as an internal standard to compare the relative toxicities of substances, and as a measure of condition of test organisms...". Laboratory grade dodecyl sodium sulphate (Fisher Scientific Company Limited), also known as sodium lauryl sulphate or DSS, is rapid, non-selective and consistent in its toxicity to aquatic animals.

3.4.3 Test Species

Rainbow trout (Salmo gairdneri Richardson) is specified here as the standard species for testing dispersants and oil in freshwater. This species should always be used unless circumstances make it impossible or undesirable to do so. If a non-standard species is selected, it should preferably be a sensitive sport or commercial fish of local importance. The species should be adaptable to healthy life in the laboratory for some weeks and readily available from a common source having a similar past history. The young of any Canadian species of the family Salmonidae (salmon, trout and char) would be best. There could also be special reasons for using an invertebrate species in the tests and this could be done with the same limitations as described above for non-standard fish. The rationale behind these requirements is chiefly that use of a standard species allows meaningful comparison of results from different laboratories and for different dispersants. Rainbow trout is a desirable species to use since it seems fairly typical of most sensitive cool-water fish in its resistance to pollutants, and is widespread in Canada. Indeed it is now common throughout much of the world and is becoming widely used as a standard species in bioassays (Sprague 1973). Another non-standard species is the fathead minnow (Pimephales promelas Rafinesque) which also has a wide distribution in Canada, even extending up into the Northwest Territories (Scott and Crossman 1973).

3.4.4 Size of Fish

Fish should be small - preferably weighing less than 4 grams. The length of the largest fish in a set of tests must not be more than 1.5 times that of the smallest. For a given species individuals should be of the same age and size range and should be obtained from the same source for any single series of tests.

3.4.5 Holding of Test Fish

The fish should be held and acclimated in tanks and accessories which are made of non-toxic material. Often used today are cylindrical fibreglass tanks with large bottom areas, and a central drain and standpipe taking water and debris from the bottom. Tanks should be exposed to an ordinary sequence of darkness and light, either from natural daylight or room lighting. Flowing water is desirable to carry away metabolic wastes, preferably at least one litre per minute for every kilogram of fish being held. Vigorous aeration with oil-free compressed air should be provided. The tank should contain one litre of water or more for every 10 grams of fish, and in addition, the volume of water in the tank should ideally equal the total flow during two or three hours, which would mean 90% replacement of water in 4.5 or 7 hours (Sprague 1973). Fish can be kept with less water than described above, but with increased risk of disease and stress problems. At least two weeks must be allowed for acclimation of fish to the dilution water and test temperature. Abrupt changes in quality of holding water must be avoided, especially extreme temperature changes. Fish should not be handled unnecessarily.

They should be transferred carefully, quickly, and as gently as possible to avoid placing the fish under unnecessary stress. Feeding during holding is important. The most desirable food is usually a specially designed and balanced fish food, in the form of moist frozen pellets for example. Other kinds may be available from feed suppliers. A recipe for preparing food is given by LaRoche et al. (1970) and beef liver might be substituted for the clams which they specify. Daily or twice-daily feeding to immediate repletion during the 5-day work week is usually satisfactory, but very small fish may require several feedings of small quantities of food every day. Fish must not be fed for 24 hours prior to or during bioassays. Disease should be virtually absent and mortality should be negligible if a stock of fish is to be considered suitable for bioassays. Weak fish will, of course, prejudice the assay so that the test material will appear to be more dangerous. Only those fish which feed actively and appear to be healthy should be used in the bioassays and any individual injured or dropped while handling must be discarded. Do not use fish which show a group mortality greater than 3% during the 2-week period preceding the bioassays.

3.4.6 Dilution Water

Any natural supply of clean, non-toxic water may be used for holding the fish. The best supply is usually water from a well, which is then aerated, possibly filtered, but not chlorinated. Surface water which is known to be clean may also be used. "Tap water" is often poisonous to fish, and in particular, small traces of chlorine, such as that which remains after carbon filtration, may cause mortality after long exposure. Copper or galvanized plumbing, brass fittings or the plasticizers from molded fittings may also cause toxicity and must be completely avoided.

The standard test water is specified here to have a total hardness of 50 mg/l as $CaCO_3$ and a normal balance of dissolved salts. This may be obtained by diluting well water with distilled water. This standard water is prescribed since there may be effects of hardness on the toxicity of some dispersants. However, if circumstances make it impossible to make up such water, any natural water may be used as described above. Another alternative is to prepare synthetic dilution water. Suitable formulae for both hard and soft freshwaters have been published (Abbott 1972, Cairns 1969).

3.4.7 Physicochemical Conditions

The temperature should be $15^{o}C$ ($59^{o}F$) for holding and testing. Fluctuation of $2^{o}C$ above or below this average is not critical during holding, and a maximum fluctuation of $\pm 1^{o}C$ from this average is acceptable during testing. The holding tanks should be aerated vigorously and the dissolved oxygen should not drop as far as 1 mg/l below saturation. Test tanks must not be aerated with respect to freshwater, it is desirable that the dissolved oxygen in the test water should not drop below 8 mg/l, and it need not do so if a satisfactory volume of test water is used. If the oxygen drops below 6.5 mg/l (a "moderate" level of protection according to Doudoroff and Shumway 1970), the test should be rejected and repeated with a greater volume of test water per gram of fish. The pH of the dilution water should normally be the stable one which prevails when the water is well aerated. The pH in freshwater tests should not vary more than 0.2 units, except as caused by the test material. If it does cause undue change in pH, this must not be adjusted, but accepted as part of its toxicity. Total hardness must be measured in the dilution water at the start of each series of tests. In each tank the temperature must be measured daily, and the pH and dissolved oxygen must be measured at least at the beginning and end of the test, and preferably daily.

3.4.8 General Conditions

The usual principles of good scientific investigation apply. For example, a control test containing only dilution water must be run for each series. All the test tanks, including the control, must have identical conditions, except for the concentration of the test material. The tanks must be freshly lined for each test with polyethylene or an equivalent liner. The selected concentrations should be formally randomized among the available test tanks and the fish should be randomized among the tanks as well. If more than one fish dies in the control test, this suggests unhealthy fish that are likely to be overly sensitive to wastes, residual toxicity from the container or lining or toxicity from the dilution water itself. Any mortality in the control tank should be regarded as unsatisfactory and anything greater than 20% mortality means that the series of tests must be rejected. The cause of the problem should be ascertained and eliminated before a new series is started.

There must be at least 2 litres, and preferably 3 litres, of test water per gram of fish per day. For a 4-day test this means at least 8 litres per gram of fish, and preferably 12 litres. This should ensure that, without aeration, the oxygen concentration would not be lowered more than 2 mg/l. Very small fish would require several times as much test water since their respiration rate would be higher per unit of biomass. The recommended volume of test water should also prevent serious accumulations of metabolic wastes and depletion of the toxicant by the fish.

3.4.9 Preparation of Test Mixtures

For the reference toxicant, dodecyl sodium sulphate (DSS), the required weight of material may simply be dissolved in a beaker full of dilution water taken from the tank selected for use. This water would then be poured into the tank, mixed well, and the fish added. For the dispersant and the dispersant/oil mixture, the following procedure is required. One-half litre of dilution water is taken from the appropriate tank. The required amount of oil (if any) and the required amount of dispersant should be weighed and added to the water. This should be homogenized in a high-speed laboratory blender. The mixture should then be added to the water in the test tank and dispersed by hand stirring. The laboratory stirrer in the test tank should then be adjusted as described in the following sections. It is essential that the oil/dispersant mixture stay in emulsion during the bioassay. It will be necessary to determine, by trial and error, the required blending speed and time, and the stirring speed in the test tank to accomplish this. After it is assured that negligible surfacing of oil is taking place (a 30-minute waiting period should be observed) the fish may be added to the tank.

3.4.10 Fish Cages

The fish must be contained in a rigid frame cage of coarse netting, such as Vexar, or other non-toxic, non-absorbent material to protect them from the propeller blades. This also allows for the fish to be brought near the surface for observation of mortality without undue disturbance. The dimensions of the cage should be 19" x 10" (inside measurement) and 16" deep. A cover should be made for the cages (Fig. 17).

3.4.11 Number of Fish

It is desirable to have about 10 fish at each concentration. If the fish are large, it may be necessary to divide their numbers between two or more tanks at the same concentration in order to apply the recommended volumes of water per gram of fish. Should this entail difficulty it may be satisfactory to reduce the number of fish in each concentration to as low as five, which is the absolute minimum.

3.4.12 Dispersant/Oil Ratio

The ratio of dispersant to No. 2 fuel oil should be unity. This high ratio is used to ensure that the oil is entirely dispersed in the test tank and is not floating on the water surface during the bioassay. The concentration of the dispersant/oil mixture must be expressed in total mg of oil plus dispersant per litre of test water.

3.4.13 Concentrations of the Reference Toxicant to be Tested

Several concentrations of DSS should be tested*, with the objective of obtaining three partial responses between 30% and 70% mortality, if at all possible, and should be chosen from a geometric or logarithmic series. The series in "Standard Methods" (American Public Health Association et al. 1971) is suitable. For the first full test the following series of concentrations is suggested: 1.0, 1.8, 3.2, 5.6 and 10.0 mg.l. Sometimes it might be desirable to get a finer gradation by following up with tests at intermediate concentrations in the same series: 1.0, 1.35, 1.8, 2.4, 3.2, 4.2, 5.6, etc.

3.4.14 Observations for Mortality

The observation times should approximate a logarithmic series and a convenient set might be as follows: 1, 2, 4, 8±1, 14±2, 24, 33±3, 48, 72 and 96 hours. If a test were started in mid-morning, most of these observations could be made during normal daytime working hours, the single exception being an observation late in the first evening.

Fish should be considered dead when there is no respiratory or other overt movement and no response to gentle prodding. Dead fish should be removed as soon as observed at which time the length and wet weight should be recorded. The test should be terminated at 96 hours and the LC50 must be calculated for the reference toxicant DSS.

3.5 Seawater Bioassay

Since many oil spills occur in seawater, toxicity testing of oil dispersants in seawater must be incorporated into the Guidelines. Methods for seawater testing should approximate, as closely as possible, those for freshwater testing. Until otherwise specified by Environment Canada, the test organism should preferably be a sensitive and readily available marine species. Test water should have a salinity of 30-35‰.

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* The objective of this set of bioassays is to determine the lethal concentration for 50% of the individuals during 4-day exposure, signified by the term 96-hour LC50. The abbreviation, used in many fields of biological testing, is numerically equal to the notations TL50 and TLm, sometimes used in work with fish.

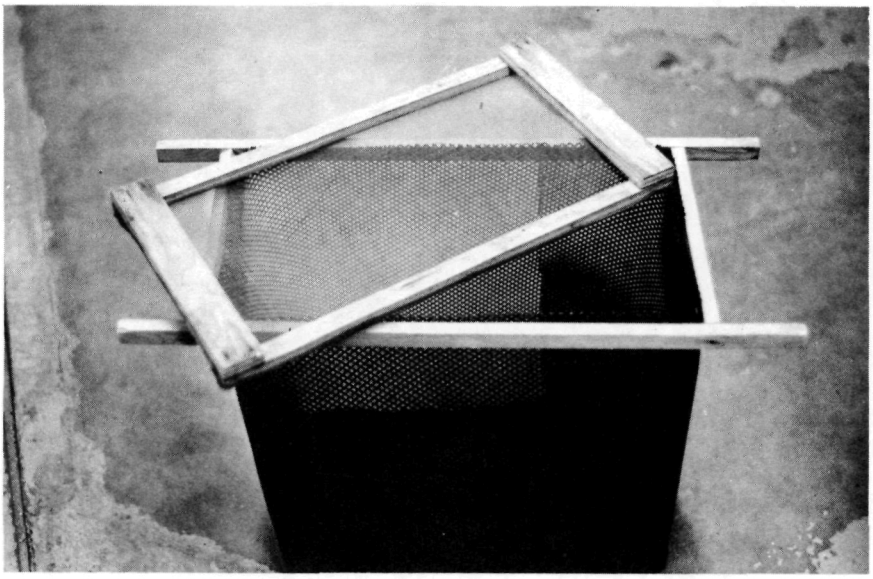

Fig. 17 - Fish Cage With Cover

Work is presently underway at EPS (Atlantic Region) to determine a suitable, standard marine species for toxicity testing of oil dispersants.

Results and all relevant information pertaining to holding conditions, feeding size and condition of a marine species should be reported when the toxicity of an oil dispersant has been determined in seawater. Source, salinity and conductivity of the holding and dilution water should be stated.

3.6 Analysis of Results

3.6.1 Dispersant/Oil Mixture

For this mixture the percentage mortality which occurs in each of the five replicate tanks at the desired concentration after 96 hours is noted. The mean ± S.D. must be reported. If this mean is less than 50% for the dispersant/oil mixture, the dispersant passes the toxicity criteria outlined in Section 3.3 of the Guidelines (Anon. 1973).

3.6.2 Dispersant and Reference Toxicant DSS

The mortality observed in each test tank at 96 hours is plotted against the concentration in the same tank. Concentration is on a logarithmic scale and percent mortality is on a probability or "probit" scale. Graph paper depicting such scales can be purchased from standard sources. Since the probit scale never reaches 0% or 100% mortality, any such points must be plotted with an arrow indicating their true position. A line is next fitted to the points by eye. Most consideration should be given to points between 30% and 70% mortality, and an effort made to minimize total vertical deviations of the line from the points. Only one successive 0% and one successive 100% should be utilized, these to be the ones nearest the centre of the range of concentrations used. If there is doubt about the placement of the line, it should be rotated to a flatter slope since this acknowledges more variability in the data. The concentration causing 50% mortality is read from the fitted line, and this is the provisional estimate of the 96-hour LC50.

The confidence limits must be estimated and reported. The common methods of fitting lines and making such estimates are not valid - special techniques are needed. Use of the simple nomographic techniques of Litchfield and Wilcoxon (1949) represents one such method, which requires about 10 to 15 minutes of work after some experience. Confidence limits of the LC50 may be estimated by the Litchfield-Wilcoxon method and these should be reported along with the LC50 and the "slope function" or "S".

Computer methods may also be used for probit analysis. Any suitable program based on the method of Finney (1971) may be used. An example of such a program is BMD03S in the BMD package (Dixon 1970).

Another example is the program called PROBITANAL which is available from the Institute of Computer Science at the University of Guelph. These programs compute the LC50, its confidence limits and the slope of the probit line, which should all be reported. In addition, a graph must always be drawn and inspected for reasonableness of the computed LC50. In cases where unusual probit lines exist, the investigator's judgement will carry more weight than the automatically computed LC50. Alternate methods of estimating the LC50 and its confidence limits are available and have been reviewed (Sprague 1969).

3.7 Reporting

1. Fish: species and source;
   age and approximate acclimation history;
   date obtained and dates of tests;
   acclimation history in brief since fish were obtained;
   feeding;
   average wet weights and lengths of all fish used in tests, and variability (range and/or standard deviation).

2. Water: source;
   special treatment before use;
   total hardness and pH;
   salinity;
   formula used if synthetic water;
   average and variability of daily or other scheduled measurements in test tanks and control tanks for temperature, pH, and dissolved oxygen. (Separate any tanks with unusual readings).

3. Equipment: blender - describe type, blending speed, blending time;
   test tank - describe arrangement including stirrer dimensions and stirring speed used.

4. Test Results: report the average percent mortality in the five replicate tanks for the dispersant plus fuel oil mixture at a concentration of 100 mg/l (± S.D.). The percent mortality in the individual tanks should also be reported.

Report the 96-hour LC50, its 95% confidence limit and the slope of the probit line for the reference toxicant, DSS, and the dispersant alone.

Report any mortality which occurred in the control tank.

3.8 Criteria

The dispersant with rainbow trout in freshwater and a suitable species in seawater should have a 4-day LC50 >1000 mg/l. The 1:1 mixture of dispersant plus No. 2 fuel oil at a concentration of 100 mg/l shall not cause more than an average of 50% mortality after 96 hours of exposure.

ACKNOWLEDGEMENTS

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