ICES Techniques in Marine Environmental Sciences

No. 29

Biological effects of contaminants: Sediment bioassay using the polychaete *Arenicola marina*

J. THAIN and S. BIFIELD

Centre for Environment, Fisheries and Aquaculture Science
Burnham Laboratory
Remembrance Avenue
Burnham-on-Crouch
Essex, CMO 8HA
United Kingdom

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK-1261 Copenhagen K Denmark

December 2001

ISSN 0903-2606

TABLE OF CONTENTS

Section **Page**

ICES Techniques in Marine Environmental Sciences

Biological effects of contaminants: Sediment bioassay using the polychaete *Arenicola marina*

J. Thain and S. Bifield

Thain, J., and Bifield, S. 2001. Biological effects of contaminants: Sediment bioassay using the polychaete *Arenicola marina*. ICES Techniques in Marine Environmental Sciences, No. 29. 16 pp.

The method described here is a whole-sediment reworker bioassay using the polychaete *Arenicola marina*, a direct deposit feeder that is widely distributed in European coastal waters and on the east coast of North America. This method has been tested nationally in the UK as well as in ring tests under the Paris Commission. It is suitable for carrying out bioassays on field-collected sediments and also for toxicity testing. Bioassay endpoints include both mortality and a non-lethal indication of effect (inhibition of casting).

Key words: whole-sediment bioassay, *Arenicola marina,* toxicity testing, dredge spoil assessment

1 INTRODUCTION

The North Sea Task Force Monitoring Master Plan document of 1990 (NSTF, 1990) recommended the use of the oyster embryo bioassay on sediment elutriates for monitoring biological sediment quality. This recommendation was made in the absence of a suitable whole-sediment reworker bioassay. Subsequently, through initiatives in the UK National Monitoring Programme and the Oslo and Paris Commissions, a whole-sediment reworker bioassay using the polychaete *Arenicola marina* has been tried and tested. The technique was developed by the Ministry of Agriculture, Fisheries and Food (MAFF), Burnham-on-Crouch, UK and successfully ring-tested by the Paris Commission in 1993 (PARCOM, 1994), and used in the UK National Monitoring Programme in 1992 and 1993. The results confirm that *A. marina* is a suitable test species for use in a whole-sediment reworker bioassay. The technique is suitable for: 1) environmental quality monitoring; 2) dredge spoil assessment and monitoring; and 3) toxicity testing of substances expected to enter sediments.

Arenicola marina, commonly known as the lugworm, is a head-down, direct deposit feeder, ingesting bulk sediment from which it digests the detrital material. It lives in a "J"-shaped burrow of up to 50 cm in depth and is found predominantly in the intertidal zone, and occasionally in the sublittoral zone. It is widely distributed in Europe and on the east coast of North America.

For the bioassay lugworms, obtained from a bait supplier or collected from a wild population, are exposed to sediments over ten days, under static conditions. Healthy animals will bury themselves in the substrate almost immediately and cast regularly throughout the exposure period. Non-healthy animals will do one of two things: they will remain on the surface and die sometime during the exposure period, or they will bury themselves and cast little or not at all. In the latter case, dead worms may be found in the test sediments when they are sieved at the end of the experiment.

The objectives are:

1) primarily, to determine the initial concentration of a known contaminant which, in 10 days, kills 50 % of the exposed animals (10d LC_{50}), or to determine statistically significant mortality, as in the case of discrete sediment samples and dredge spoils.

2) additionally, to determine the initial concentration which, in 10 days, inhibits casting and assign a relative casting ranking to each of the exposure concentrations or field-collected sediments.

The method described below is suitable for carrying out bioassays on field-collected sediments and for toxicity testing. The latter is included on the grounds that it is a suitable method for testing the toxicity of many chemicals (used, for example, in the offshore oil industry) and in subsequent monitoring and survey work associated with the tested chemicals, for example, chemicals used in the offshore oil industry.

2 MATERIALS

The following materials and equipment are required to set up the bioassay.

1) The sediment to be tested and a reference sediment. The reference sediment is used in the control treatment or as the base sediment for spiking purposes. Reference sediment: A muddy sand from a marine site known to be free of significant contamination. Approximately 1 kg per test tank is required for each test vessel.

2) Lugworms *Arenicola marina*: Animals may be collected (dug) from a clean intertidal shore or obtained from a bait supplier. An ideal size of worm is 1 g.

3) Plastic tanks: Sandwich-box type container, approximate size 200 mm (length) × 90 mm (width) × 90 mm (depth); sufficient to provide a substrate surface area of 150–200 cm^2 to a depth of 3 cm. If larger test containers are used, then the sediment volume must be adjusted accordingly, such that the sediment depth is always ≥ 3 cm.

4) Sea water and deionized water: A volume of sea water sufficient to provide a layer about 3 cm above the sediment (this approximates to 500 ml per test tank). Sea water should be of natural origin from an unpolluted area, but artificial sea water is acceptable if control survival and behaviour during the 10-day exposure period are normal. Deionized water is required to dilute the sea water back to its initial salinity (30–35), as constant aeration during the experiment causes some evaporation. This is best achieved by marking the seawater level on the tank on day 0; daily topping up to this mark with distilled water may be required.

5) Aeration: Constant aeration is required to ensure that the dissolved oxygen content of the water in each vessel remains at a satisfactory level (approximately 90 %). Plastic disposable dispensing pipette tips are more suitable than air stones.

6) Sieve: A ~2 mm sieve of plastic, nylon or stainless steel is suitable, preferably of a diameter of > 150 mm.

7) Shaker or roller for use only in spiking experiments: Use a large orbital type that can be adapted to hold 2000 ml containers and to shake at 100 revolutions min^{-1}.

8) Constant temperature facility in the range 12 °C to 20 °C: Preferably hold at 15 °C although this is not essential, but water temperatures should not vary by more than ± 2 °C daily.

9) Eh probe and meter: Suitable for use in sediments at a depth of 3 cm.

10) Thermometer (to 0.1 °C).

11) Dissolved oxygen probe and meter (mg l^{-1} or as %).

12) pH probe and meter (to 0.1 pH unit).

13) Top pan balance (2 decimal places): To weigh sediments, for use only in spiking experiments.

14) Salinity meter (directly/conductivity or otherwise).

For Bioassays

In general, space limits the number of samples that can be bioassayed at one time. To carry out assays on 20–30 samples, the typical time in person-days for initial setting up: 3 days; daily maintenance: < 3 hrs; termination of experiment: 1 day; work-up of results: 1 day. In total: 9 person-days.

For Spiking Experiments (i.e., toxicity tests)

Typical time in person-days for initial setting-up: 3 days; daily maintenance: < 2 hrs; termination of experiment: 1 day; work-up of results: 1 day. In total: 8 days. In this time it should be possible to determine the toxicity of two or three compounds.

To carry out a test on one product using three replicates at each of five test concentrations and a control requires 18 kg of sediment and approximately 20 litres of sea water.

3 METHODS

1) Preparation of sediments:

   a) Collection of reference sediment: Lugworms can live in a range of sediments from mud to sand. For the test, a natural sediment should be collected, preferably a muddy sand from a foreshore known to be free of obvious contamination. This should be collected down to a depth of 150 mm. On return to the laboratory, the sediment should be sieved to 500 µm using reference sea water. Sieving in this way excludes any benthic organisms which might interfere with the test or eat the test animals. The sieved sediment should then be placed into clean polythene bags, kept damp at 4 °C, and used within two weeks of collection. This sediment is used as the control sediment in all bioassays and is used as the reference sediment in toxicity tests.

   b) Spiking sediment: Spiking methods are provided in detail in Annex 1.

      The control/reference sediment should have the following approximate characteristics:

      i) an organic content of between 0.5 % and 4 %;

      ii) a silt/clay fraction (< 63 µm) of between 5 % and 20 %;

      iii) a median grain size of 90 µm to 125 µm.

      Descriptively, it is neither a mud nor a coarse sand, but a muddy fine sand.

   c) Field-collected survey samples or dredge spoils: These samples should be collected as appropriate, e.g., using a Day grab or box corer, but due care and consideration should be given to the amount of sample collected, the homogeneity, depth, degree of anoxia, and replication. This will depend on the objectives of the sampling programme, but the test requires a minimum of approximately 2 kg of wet sediment.

2) Replication: At least two replicate tanks should be used for each test. In the case of spiked sediment, each exposure concentration should be prepared separately and no attempt should be made to serially dilute any sediment once contaminated with a test compound.

3) Adding sediment to tanks: Field-collected samples or spiked sediments should be tipped into a test tank and allowed to stand for 24 hours.

4) Addition of sea water and aeration: After 24 hours, clean sea water is added to each tank to a depth of about 3 cm above the sediment surface. Aeration is then supplied to each tank. The easiest way to do this is to fix a small plastic dispensing pipette tip (ideally 200 µl tip) to the end of the aeration tubing and attach it to the test vessel with a cable tie just above the sediment surface. Aeration should be adjusted to minimize disturbance to the sediment. The tanks should again be left to stand for 24 hours.

5) Experimental animals: The animals should already have been obtained from a supplier and, prior to the set-up of the test, they should be kept in a 20-litre holding tank at a stocking density of approximately 200 animals with aerated flowing sea water, 5–10 ml min^{-1}, and a 3-cm layer of reference sediment on the bottom of the tank. This is the preferred method of holding the animals. However, animals can be kept for up to seven days in static conditions.

6) Addition of experimental animals to test tanks: The animals are sieved from the holding tank and five individuals of a similar size are placed into each test container. During the first 6 hours, observations are made on the burying behaviour of the worms, although this may prove to be difficult as the animals may disturb the sediment whilst burying, giving poor visibility. After 24 hours, the tanks are observed for mortalities at the sediment surface, which should be recorded, and the worms removed and replaced with new individuals. If any further animals fail to burrow, they should not be removed. A daily record should be kept of the numbers of animals on the substrate surface because this may be a contaminant-related effect.

7) Casting: Healthy lugworms produce casts when active and feeding. To measure this activity, the number of casts in each tank should be noted and then all casts should be rubbed out (smoothed over gently with a spatula) and approximately 24 hours later the casts should be counted again. This process should be repeated for the duration of the test.

8) Experimental parameters to be measured:

   These parameters must be noted on appropriate data record sheets.

   a) Daily measurements: A permanent record should be kept of dead or moribund animals on the surface of the sediment and the number of casts. The salinity of the reference sea water should be monitored and recorded. Daily adjustments in the tank may be required (see Section 2(4), above). The salinity of the overlying test water should be maintained close to the day 0 value throughout the test by the addition of distilled water. Salinity can readily be monitored by marking the final level on the test container after the animals have been added on day 0; addition of distilled water can be made to this mark when required. A 1 mm drop in level is equivalent to an increase of approximately 1 in salinity, and a change of this magnitude may be used as a practical threshold for restorative action.

   b) Other measurements: Eh should be recorded immediately before the animals are placed in the tank and on the final day of the experiment. A reading at a depth of about 2 cm is taken at the front, middle, and rear of each tank.

   The following parameters should be measured at the start (before introduction of the test animals), after 24 hours, and at least twice further during the test, including day 10, and noted on appropriate record sheets: pH (normal range 7.5–8.5), dissolved oxygen (normally > 85 %), and temperature (15 °C ± 2 °C). Salinity must be measured at the start and finish of the test (normally a range of ± 4 during the experiment).

9) Termination of experiment: After the animals have been exposed for 10 days, the sediment containing the worms in each tank is gently sieved in a bath of sea water and the number of animals surviving is recorded.

10) Treatment of results: The endpoint of the test is mortality; this is defined as the initial addition of animals minus the number of surviving animals for each treatment. Dead animals may decompose during the test and for this reason "missing" animals are presumed and counted as dead.

In toxicity tests, the 10d LC_{50} should be calculated using an appropriate statistical method. The raw mortality data must be provided in the final report.

11) Validity criteria: The number of animals and replication used are small, therefore, control mortality should be 10 % or less.

12) An example of bioassay data: Table 1 shows sediment elutriate toxicity results using the oyster embryo bioassay and *A. marina* whole sediment bioassay results on the same sediments. Both techniques identified toxic sediments at the same sites. A more comprehensive data set for *A. marina* is given in Table 2 and demonstrates that, in addition to mortality, feeding behaviour is a useful endpoint; sediments from South Varne and Jarrow Slake on the River Tyne exhibited no mortality and the number of casts recorded was 1 and 3, respectively (cf. the control was 27).

13) The results of an interlaboratory comparison on two chemicals used in the offshore oil industry are given in Table 3; Bioban P1487 is a biocide and Servo CK337 is a corrosion inhibitor. The mean LC_{50}s in mg kg^{-1} dry weight for Bioban P1487 and Servo CK337 were 58.6 ± 7.2 s.d., n = 5 (C.V. 12.3 %) and 385 ± 99.7 s.d., n = 6 (C.V. 23.8 %), respectively. The use of an internal reference chemical may be desirable and this will be determined by the nature of the investigation. Cadmium is frequently used for water-phase testing, but a "sediment-bound" toxicant may be preferable.

4 REFERENCES

North Sea Task Force. 1990. North Sea Task Force Monitoring Master Plan. NSTF, London, North Sea Environment Report, 3: 1–37.

PARCOM. 1994. Summary record of the Eighth Meeting of the Working Group on Oil Pollution, The Hague. Paris Commission, London. pp. 8–11.

Table 1. Sediment elutriate (oyster embryo bioassay) and whole sediment bioassay results for samples collected in July 1992.

Position	Location	Oyster embryo bioassay	*Arenicola marina* bioassay	
		Sediment PNR‡	% mortality	Total No. casts
	Shoebury sand	na	0	27
53°33.33'N 0°6.05'E	River Humber: Bull anchorage	–7.1	0	23
53°36.0'N 0°3.6'W	River Humber: Pyewipe outfall	10.5	0	23
53°36.1'N 0°5.9'W	River Humber: Diffuser	3.3	0	18*
53°36.6'N 0°4.6'W	River Humber: No. 6B buoy	5.9	0	17*
53°37.4'N 0°8.7'W	River Humber: No. 10A buoy	–0.1	0	12*
53°38.6'N 0°11.0'W	River Humber: No. 11A buoy	–1.1	0	11*
54°37.4'N 1°9.34'W	River Tees: Redcar jetty	100 *	100	0*
54°35.3'N 1°11.4'W	River Tees: No. 25 buoy	85.2*	30	12*
54° 44.12'N 0°52.87'W	Off River Tees	5.5	0	27
54°55.05'N 1°21.43'W	River Wear: between South Piers	2.7	0	21
54°54.58'N 1°22.8'W	River Wear: Wearmouth Bridge	27 *	0	34
54°54.66'N 1°23.4'W	River Wear: Hetton Staiths	7.1	0	19*
54°54.99'N 1°23.55'W	River Wear: Deptford Quay	6.8	0	25
54°54.8'N 24°24.24'W	River Wear: Queen Alexandra Bridge	–0.1	0	21
55°00.47'N 1°23.40'W	River Tyne (mouth)	7.4	0	13*
55°0.47'N 1°25.84'W	River Tyne: Lloyd's Hailing Station	11.2	0	25
54°59.17'N 1°27.86'W	River Tyne: buoy off Jarrow Slake	13.4	0	0*
54°58.25'N 1°35.2'W	River Tyne: Ouse Burn	11.5	10	16*
54°57.45'N 1°38.1'W	River Tyne: Teams Confluence	15	0	25
55°0.53'N 1°7.80'W	Off River Tyne	–6.7	0	21
55°10.41'N 3°9.37'E	Dogger Bank	–3.2	0	27

* Statistically different from the control ($p < 0.05$).

‡ PNR = percent net response.

na = not applicable with respect to control.

Table 2. Results of *Arenicola marina* 10-day whole sediment bioassay for samples collected in the North Sea, English Channel, and Irish Sea in July 1992.

Position	Location	% mortality	Total casts in 10 days
	Shoebury sand	0	27
53° 33.33' N 0° 6.05' E	River Humber: Bull anchorage	0	23
53° 36.0' N 0° 3.6' W	River Humber: Pyewipe outfall	0	23
53° 36.1' N 0° 5.9' W	River Humber: Diffuser	0	18*
53° 36.6' N 0° 4.6' W	River Humber: No. 6B buoy	0	17*
53° 37.4' N 0° 8.7' W	River Humber: No. 10A buoy	0	12*
53° 38.6' N 0° 11.0' W	River Humber: No. 11A buoy	0	11*
54° 37.4' N 1° 9.34' W	River Tees: Redcar jetty	100	0*
54° 35.3' N 1° 11.4' W	River Tees: No. 25 buoy	30	12
54° 44.1?' N 0° 52.87' W	Off River Tees	0	27
54° 55.05' N 1° 21.43' W	River Wear: between South Piers	0	21
54° 54.58' N 1° 22.8' W	River Wear: Wearmouth Bridge	0	34
54° 54.66' N 1° 23.4' W	River Wear: Hetton Staiths	0	19*
54° 54.99 N 1° 23.55' W	River Wear: Deptford Quay	0	25
54° 54.8' N 24° 24.24' W	River Wear: Queen Alexandra Bridge	0	21
55° 00.47' N 1° 23.40' W	River Tyne	0	13*
55° 0.47' N 1° 25.84' W	River Tyne: Lloyd's Hailing Station	0	25
54° 59.17' N 1° 27.86' W	River Tyne: buoy off Jarrow Slake	0	0
54° 58.25' N 1° 35.2' W	River Tyne: Ouse Burn	10	16
54° 57.45' N 1° 38.1' W	River Tyne: Teams Confluence	0	25
55° 0.53' N 1° 7.80' W	Off River Tyne	0	21
55° 10.41' N 3° 9.37' E	Dogger Bank	0	27
55° 29.96' N 4° 10.0' E	Bremerhaven Workshop Transect: station 9	0	20
55° 25.04' N 8° 09.11' E	Off Esbjerg: No. 4 buoy	0	18*
54° 03.98' N 8° 07.51' E	Bremerhaven Workshop transect: station 1	0	17*
54° 01.98' N 8° 02.85' E	Bremerhaven Workshop transect: station 2	0	13*
54° 00.08' N 8° 00.06' E	Bremerhaven Workshop transect: station 3	0	17*
54° 00.86' N 7° 48.85' E	Bremerhaven Workshop transect: station 4	0	21
54° 06.53' N 7° 24.02' E	Bremerhaven Workshop transect: station 5	0	33
54° 25.01' N 6° 14.90' E	Bremerhaven Workshop transect: station 6	0	24
54° 50.11' N 5° 34.96' E	Bremerhaven Workshop transect: station 7	0	24
55° 06.02' N 5° 00.07' E	Bremerhaven Workshop transect: station 8	0	26
52° 50' N 2° 50' E	Smith's Knoll	0	30
52° 0' N 2° 20' E	Off Thames	0	23
51° 30.8' N 0° 58' E	Thames Warp	0	35
50° 56' N 1° 16.8' E	South Varne	0	3*
50° 40.03' N 3° 10.10' W	English Channel	0	23
50° 24.79' N 3° 30.22' W	English Channel	0	21
50° 25' N 3° 16' W	English Channel	0	21
50° 02.01' N 4° 22.03' W	English Channel	0	16*
54° 1.1' N 2° 56.3' W	River Mersey: No. 7 Buoy	0	17*
53° 28.65' N 3° 15.94' W	Inner Liverpool Bay	0	15*
53° 30.5' N 3° 5.6' W	River Mersey: C12 buoy	0	28

* Disruption in feeding behaviour: significantly different from the control ($p < 0.05$).

Table 3. Results of *Arenicola marina* ring test using Bioban P1487 and Servo CK337.

Test Substance 95 %	Laboratory	LC_{50} mg kg^{-1} dry weight	Confidence Interval
Bioban P1487	1	54.4	50–100
	2	69.2	65–100
	3	62.7	56–100
	4	51.7	32–100
	5	55.0	33–100
Servo CK337	1	343.9	330–560
	2	239.3	100–320
	3	421.4	316–562
	4	369.2	320–560
	5	393.3	300–1000
	6	543.6	377–671

ANNEX 1

METHOD FOR CHEMICALLY SPIKING A SEDIMENT SAMPLE

SPIKING PROCEDURE FOR TOXICITY TESTS

The process of chemically spiking sediments described below has the primary objectives of ensuring that the test substance is evenly distributed throughout the test sediment, and that adequate contact between the substance and the sediment is promoted.

The method does not purport to reproduce the adsorption characteristics which may occur under equilibrium conditions or as a consequence of chronic exposure in the field.

IDENTIFICATION OF CHEMICAL PROPERTIES

In accordance with Appendix 1, it is desirable to identify initially the partitioning and adsorptive properties of the material to be tested.

Materials with a log P_{ow} of greater than approximately 4 should initially be dissolved in an organic solvent of acceptably low toxicity (e.g., methanol or acetone) before addition to a small quantity of dried sediment. In general, all substances or products of low solubility should be coated initially onto dried sediment before introduction to the main mass of wet sediment.

Materials which are known to be soluble or dispersible may be mixed with a small quantity of sea water before direct addition to the wet base sediment.

Powders should be dissolved or dispersed in an appropriate medium before addition to either dried sediment or wet sediment.

EXAMINATION OF CHEMICAL PROPERTIES

In cases where the properties of the chemical or product are not clearly identified by prior information, an examination of the behaviour of the material in sea water may provide a guide to the most appropriate preparation method.

The procedure described below is based on MAFF guidelines current in 1993.

The sample must be thoroughly homogenized before use. The original container should be placed on a roller or shaker table for one hour. If a shaker table is used, the speed or revolution should be set at approximately 150 rpm.

Add 1 g (± 0.01 g) of the homogenized sample to 1 litre (± 0.01 litre) of 0.2 µm-filtered sea water in a clean conical flask or separating funnel of approximately 1.5 litre volume.

Stopper the vessel, and shake vigorously by hand, inverting the vessel at least ten times. Approximately five minutes' treatment in a laboratory ultrasonic bath (power rating not constrained) is an acceptable alternative. In either case, the choice of method must be documented.

Allow the contents of the vessel to settle for approximately 1 hour, and then observe the contents, recording and classifying the visible characteristics as follows:

1	No floating or settled materials, liquid or solid	
	a) Clear solution mixture	A
	b) Homogeneous emulsion or fine/colloidal suspension	A
	c) Neutrally buoyant droplets, particles or floc	B
2	Floating, but no settled, liquids or solids	B
3	Settled, but no floating, liquids or solids	C
4	Floating and settled liquids or solids	D

SEDIMENT PHASE PREPARATION

Source of Sediment and Preparation

Sediment should ideally be obtained from the same location from which the test population is obtained. This sediment should be initially characterized in terms of particle-size and organic content, which should lie within the limits specified in Section 3(1) of the body of this document.

Sediment should be collected and processed for testing as described in Sections 3(1) to 3(3).

Chemical Amendment of Sediment

The quantity of sediment prepared per test substance concentration will depend on the size of the test vessels, and on the depth of sediment required by the test procedure.

For tests conducted in accordance with the PARCOM ring test of 1993 (10-day test with *Arenicola marina*), a minimum depth of 30 mm of sediment is required in each replicate test vessel.

Immediately before the addition of a chemical or chemical solution, the base sediment must be thoroughly homogenized, and a sample of approximately 20 g wet weight removed and placed in an air-tight container. This sample must be weighed ($\pm$ 0.01 g) in a tared container and then dried at approximately 60 °C for about 24 hours.

The dried sample must be cooled to room temperature in a desiccator, and re-weighed.

The ratio of the wet sample weight to the dry sample weight (net of container weight in both cases) should be calculated and entered on the study record. IT IS OF PRIMARY IMPORTANCE THAT CARE BE TAKEN TO AVOID ANY ALTERATION IN THE WATER CONTENT OF THE SEDIMENT BETWEEN THE TIME AT WHICH THIS DETERMINATION IS MADE AND THE TIME AT WHICH THE TEST SUBSTANCE IS ADDED TO THE SEDIMENT.

The preparation of spiked sediments for whole-sediment toxicity tests is carried out in a manner dependent on the properties of the test substance.

Substances which fall into category A, above, should be added to sediments as a solution or emulsion prepared in a small volume of sea water.

Substances which are powders, are described as insoluble, or which fall into categories B, C, or D should be added intially to a small quantity of dried sediment and mixed thoroughly before being mixed with a larger volume of wet sediment.

Insoluble or poorly soluble substances should be dissolved in a suitable organic solvent, such as methanol or acetone, before addition to dried sediment.

Where a solvent is used, additional control sediments must be prepared at least at the highest concentration of solvent used in the substance treatments.

Test chemical concentrations may be prepared either as:

a) nominal concentrations per unit wet weight of the base sediment, and later corrected using the measured wet weight/dry weight ratio to units of mg kg^{-1} dry weight.

b) nominal concentrations per unit dry weight, by calculating the appropriate addition rate per unit wet weight on the basis of the measured wet weight/dry weight ratio.

Calculation of the required quantity of test substance must take into account the weight of any dry sediment used in preliminary preparation.

Where the test substance is prepared as an aqueous suspension or emulsion, the volume of water used should be kept to a minimum.

Mixing of test substance with sediment

The test substance and carrier medium should be added to the appropriate weight of wet sediment in a suitable container (e.g., a polythene or polypropylene bottle) of a suitable volume (e.g., 2 litres for an *Arenicola* test as referred to above); in general, a vessel volume of approximately twice the volume of sediment is acceptable. A clean spatula (e.g., stainless steel or polythene) should be used to initially disperse the test substance and carrier through the sediment.

Sufficient clean sea water is then added to create a freely flowing slurry. Care must be taken to minimize the volume of water added, but the quantity must be sufficient to allow the mixture to flow freely when the container is inverted or shaken.

The container should be labelled with the study number, the test substance number and the nominal concentration, and placed horizontally on an orbital shaker (with a displacement of at least 30 mm) at approximately 150 rpm for around three hours. The purpose of this procedure is to ensure that the test substance is evenly distributed throughout the sediment matrix; it is not intended to ensure any specified degree of partitioning of the test substance. A roller may also be used for mixing.

Introduction of spiked sediment to the test system

When the sediment preparations have been shaken for the specified period, the containers should be removed from the shaking apparatus.

Each container should be finally shaken thoroughly by hand, with at least five inversions, and the test operator should establish that the preparation moves freely and that no unmixed residues remain adhered to the walls of the container. This procedure must be carried out for each container immediately before the contents are dispensed to the appropriate replicate vessels.

The test medium should be dispensed in small aliquots to each replicate test vessel in turn, to minimize bias in the chemical content or particle-size distribution of the medium between vessels. Any supernatant water present must be included in the test system, and must be equally distributed between vessels before the solid material is dispensed.

If difficulty is encountered in dispensing the solid phase, a clean spatula may be used to manipulate the material; in this case, it is essential that a new spatula be used for each test substance, and that each test substance be dispensed in order from lowest to highest nominal concentration.

Sediment preparation containers must be used only once and should be disposed of as soon as practicable after dispensing the solid phase to the test vessels.

Preparation of the test system will then proceed in accordance with the guidelines.

APPENDIX 1

A GUIDELINE TO DEFINE PHYSICAL AND CHEMICAL PROPERTIES OF SUBSTANCES TO BE TESTED WITH A SEDIMENT TEST

Introduction

This guideline was prepared by Dr D. M. M. Adema (previously of TNO, the Netherlands) to provide a basis for the choice of chemicals for which a sediment test will be required. One condition essential in a scheme for selecting chemicals (for sediment-reworker testing) is that it should be done on the basis of physico-chemical properties which are already required in existing regulatory schemes.

As a matter of principle, a test with a sediment-reworker species should be carried out with chemicals which will, at least partly, end up at the sea bottom.

Chemicals for which a sediment test is required

The chemicals for which a sediment test is required include the following:

1) Chemicals which directly sink, i.e., chemicals with a density greater than that of sea water and with a low water solubility. The impact of these parameters on the actual behaviour of the chemicals will depend on environmental factors such as weather conditions. For a first discussion, it is recommended that the "Standard European Behaviour Classification" (SEBC) (see Figure 1) be followed and that all chemicals with the indication "S" (sinker) be included.

2) Chemicals which will end up at the sea bottom after transportation via biotic or abiotic particles. These are chemicals which adsorb onto particles, i.e., chemicals with a high partition coefficient between water and organic matter or other particles. Such partition coefficients are not widely known, but they are related to the well-known log P_{ow}, which has to be reported in any case as an indication for bioaccumulation potential.

   In the Netherlands, a log P_{ow} of 5 is taken as a threshold for setting criteria for sediment rather than for the aqueous phase. According to the EC or OECD guidelines, a log P_{ow} of 3 is the borderline for tests on bioconcentration. (Apparently it is generally accepted that from a log P_{ow} of 3, the partitioning between water and biota might lead to appreciable amounts of chemicals in biota. Such plant or animal material might end up on the sea bottom.) Not to exaggerate on either side, it is suggested that a test with a sediment-reworker species be required for chemicals with a log P_{ow} of 4 and higher. To provide an indication of log P_{ow} values, some of these values are listed for various chemicals and groups of chemicals in Table A.

3) Surface-active and particulophile (e.g., Vantocil™) substances, unless full and reliable information indicates the opposite of assumed adsorbability.

Preparations

It is generally preferable to test substances (as also concluded at the Aberdeen Workshop on Offshore Chemicals, 20–24 September 1993).

If, however, preparations should be tested, then a sediment test would be required if any of the intentionally added substances (at a concentration of > 1 %) in the preparation falls within one of the categories above.

Summary

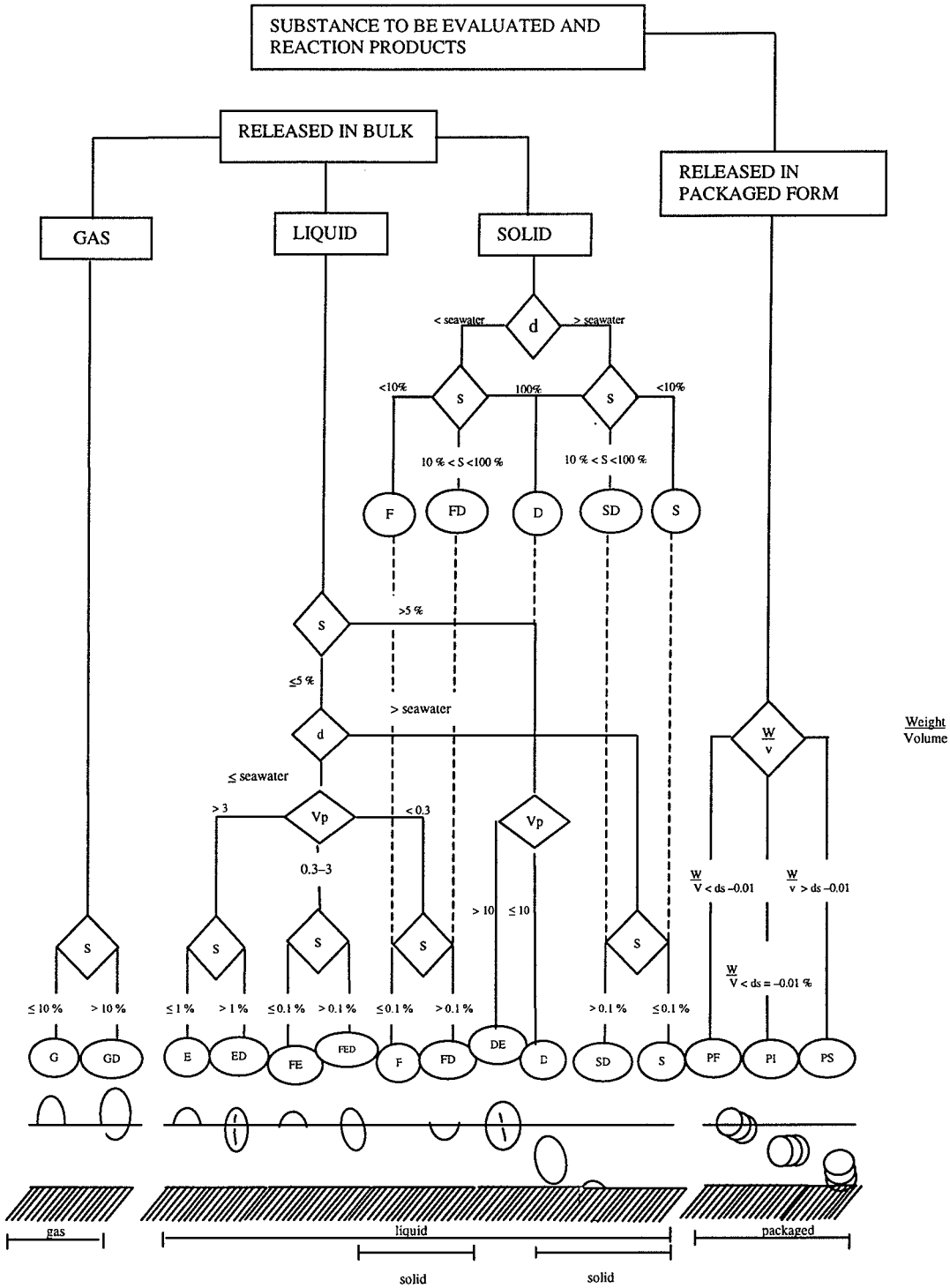
In summary, chemicals for which a sediment-reworker test is required are those which:

1) are partly or completely classified as sinkers according to the SEBC (Figure 1);

2) have a log P_{ow} of 4 or higher;

3) are surface-active chemicals (unless full and reliable information indicates the opposite of assumed adsorbability);

4) are known to adsorb to particles (are particulophile, e.g., Vantocil™) (unless full and reliable information indicates the opposite of assumed adsorbability).



Air and water temperature: 10 or 20° C
Atmosperic pressure: 100 kPa
s: solubility (%)
d: density
Vp: Vapour pressure (kPa)
Ds: density of sea water

G: gas
E: evaporator
F: floater
D: dissolver
S: sinker
P: package

Figure 1. European Classification System—Flow diagram (SEBC).

Table A. Some log P_{ow} values.

Chemical	log P_{ow}
benzene	2.13
toluene	2.59
m-xylene	3.09
ethylbenzene	3.13
isopropylbenzene	3.66
n-butylbenzene	4.28
diisopropylbenzene	5.01
n-dodecylbenzene	8.40
n-hexane	3.51
cyclohexane	3.18
n-nonane	5.09
n-decane	5.62
1-octanol	3.03
1-nonanol	3.53
1-decanol	4.03
1-undecanol	4.53
1-dodecanol	5.00
1-tridecanol	5.51
nonanone	2.88
decanone	3.40
undecanone	3.93
dodecanone	4.46
tridecanone	4.98
hexachlorobutadiene	4.63
monochlorobenzene	2.81
1,2-dichlorobenzene	3.53
1,2,4-trichlorobenzene	4.20
1,2,3,4-tetrachlorobenzene	4.94
pentachlorobenzene	5.69
hexachlorobenzene	6.44
lindane	3.53
trichlorobiphenyls	5.9
tetrachlorobiphenyls	6.6
naphthalene	3.5
phenanthrene	4.5
chrysene	5.6
benzo[*a*]pyrene	6.0

ICES Techniques in Marine Environmental Sciences

No. 1 Cadmium and lead: Determination in organic matrices with electrothermal furnace atomic absorption spectrophotometry
No. 2 Trace metals in sea water: Sampling and storage methods
No. 3 Cadmium in marine sediments: Determination by graphite furnace atomic absorption spectroscopy
No. 4 Lipophilic organic material: An apparatus for extracting solids used for their concentration from sea water
No. 5 Primary production: Guidelines for measurement by ^{14}C incorporation
No. 6 Control procedures: Good laboratory practice and quality assurance
No. 7 Suspended particulate matter: Collection methods for gravimetric and trace metal analysis
No. 8 Soft bottom macrofauna: Collection and treatment of samples
No. 9 Sediments and suspended particulate matter: Total and partial methods of digestion (*videotape available*)
No. 10 Organic halogens: Determination in marine media of adsorbable, volatile, or extractable compound totals
No. 11 Biological effects of contaminants: Oyster (*Crassostrea gigas*) embryo bioassay
No. 12 Hydrocarbons: Review of methods for analysis in sea water, biota, and sediments
No. 13 Biological effects of contaminants: Microplate method for measurement of ethoxyresorufin-O-deethylase (EROD) in fish
No. 14 Temporal trend monitoring: Introduction to the study of contaminant levels in marine biota
No. 15 Temporal trend monitoring: Contaminant levels in tissues of Atlantic cod
No. 16 Benthic communities: Use in monitoring point-source discharges
No. 17 Nutrients: Practical notes on their determination in sea water
No. 18 Contaminants in marine organisms: Pooling strategies for monitoring mean concentrations
No. 19 Common diseases and parasites of fish in the North Atlantic: Training guide for identification
No. 20 Temporal trend monitoring: Robust method for analysing contaminant trend monitoring data
No. 21 Chlorobiphenyls in marine sediments: Guidelines for determination
No. 22 Biological effects of contaminants: Cholinesterase inhibition by organophosphate and carbamate compounds
No. 23 Biological effects of contaminants: Determination of CYP1A-dependent mono-oxygenase activity in dab by fluorimetric measurement of EROD activity
No. 24 Biological effects of contaminants: Use of imposex in the dogwhelk (*Nucella lapillus*) as a bioindicator of tributyltin pollution
No. 25 Biological effects of contaminants: Measurement of DNA adducts in fish by ^{32}P-postlabelling
No. 26 Biological effects of contaminants: Quantification of metallothionein (MT) in fish liver tissue
No. 27 Soft bottom macrofauna: Collection, treatment, and quality assurance of samples
No. 28 Biological effects of contaminants: *Corophium* sp. sediment bioassay and toxicity test
No. 29 Biological effects of contaminants: Sediment bioassay using the polychaete *Arenicola marina*
No. 30 Chlorophyll *a*: Determination by spectroscopic methods