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Biological effects of contaminants: *Corophium* sp. sediment bioassay and toxicity test

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**Biological effects of contaminants:
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J. Thain and B. Roddie

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The method described here is a whole-sediment reworker bioassay using burrowing amphipods. This method description covers the use of *Corophium* spp., as this is the genus most commonly used in Europe, but the procedure can be used with any infaunal amphipod. 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. The bioassay endpoint is mortality.

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Key words: whole-sediment bioassay, toxicity testing, infaunal amphipod, *Corophium* spp.

1 INTRODUCTION

Burrowing amphipods have been used for testing sediments for some time in North America and standard guidelines for such tests have been produced by ASTM (1990). Several European laboratories have evaluated the guidelines using European species such as *Corophium* spp. The guideline suggested below is based on procedures used since 1991, by B.D. Roddie, ERT Ltd., Leith, Edinburgh, UK, J.E. Thain, CEFAS Laboratory, Burnham-on-Crouch, UK, and P. van den Hurk, AquaSensa, Amsterdam, The Netherlands. It has been extensively used for the testing of chemical products that are liable to incorporation into seabed sediments, and for assessing the toxicity of potentially contaminated seabed sediments.

Adult *Corophium* are exposed to contaminated field sediments (bioassay) or chemically spiked sediments (toxicity test) for ten days. During this period, burrowing behaviour may be assessed by counting the number of amphipods on the sediment surface or actively swimming. At the end of the experiment, the amphipods are sieved from the sediment and the number of surviving animals is recorded. Mortality is assessed by comparing the survival rate with the initial number of animals, and the mortality data are analysed either by the use of an appropriate LC₅₀ method (toxicity test) or by the use of an appropriate analysis of variance technique to compare treatments with controls (bioassay).

The method as described may be used to:

- a) assess the relative toxicity of field-collected sediments;
- b) assess the toxicity of highly contaminated sediments by serial dilution with clean sediment;
- c) assess the toxicity of sediments to which chemicals have been added in controlled amounts;
- d) map the spatial or temporal distribution of toxicity in a study area.

The method does not purport to simulate natural exposure conditions, nor to provide data directly predictive of ecosystem responses. The method aims to provide information concerning the immediate effects of sediment contaminants during short-term exposure.

Experience to date suggests that *Corophium* can be used in sediments with a wide range of particle-size characteristics and organic content. Good survival has been observed in both fine and coarse clean sediments.

The method requires no assumptions concerning the nature or identity of sediment-sorbed contaminants, and is appropriate for the assessment of the effects of unknown mixtures. Because the method evaluates the effects of all chemicals present, however, it cannot readily be used for controlling individual pollutants at source.

At present, *Corophium* is the genus most commonly used in Europe, but it should be borne in mind that the procedure described below can be used with any infaunal amphipod and, indeed, in the future other species may prove to be of equal value.

An important conclusion of a ring test conducted for this method was the need for guidance to provide a basis for the choice of chemicals for which a sediment test will be required and the spiking method to be employed. These issues are addressed in Annexes 2 and 3.

2 MATERIALS

The following materials and equipment are required:

- 1) Reference sediment collected from an area known to be free from significant contamination. In many instances, this is likely to be the site from where the amphipods were collected. For site-specific studies, it may be necessary, where possible, to use a sediment with the natural characteristics (grain size distribution, organic matter content, etc.) similar to those of the contaminated sediments being studied.
- 2) Reference sea water: natural sea water is preferred, but artificial sea water is acceptable. It must be free from significant contamination.
- 3) Adult *Corophium*, greater than 5 mm in length (excluding rostrum), collected from an area known to be relatively free from contamination. Either *Corophium volutator* or *Corophium arenarium* is acceptable, but an attempt must be made to identify which species is used, and at least to ensure that a mixture of species is not used.

Although only the minimum size is stipulated, test operators should be aware that, at some times of the year, the natural mortality rate of large animals (e.g., > 8 mm body length) can be unacceptably high. This appears in northern latitudes to be confined primarily to late summer and early autumn, when the previous year's over-wintering generation reaches the end of its natural lifespan. To avoid problems of excessive background mortality, laboratories may wish to consider restricting the upper as well as the lower size limit.

- 4) 500 µm (approximately) sieve.
- 5) An orbital shaker or roller (for toxicity test preparation).
- 6) Shaker bottles of the appropriate type and capacity (non-leaching plastic bottles are generally the most practical, but if organic contaminants are being studied it may be preferable to use glass).
- 7) Test vessels: 1 litre tall-form glass beakers are most commonly used, but any vessel of a suitable material is acceptable which permits a minimum sediment depth of 15 mm and a sediment:overlying water volume ratio of approximately 0.2.
- 8) Aeration system: Individual air stones or plastic pipette bubblers can be used for each test container. However, they easily become blocked and require daily checking and maintenance. For routine testing, an example of a more practical option has been the use of the aeration system described in Annex 1.
- 9) Automatic 5 ml dispensing pipette or 10 ml pipettes.
- 10) Scoops or serving spoons for handling sediments.
- 11) pH meter (to 0.1 pH units).
- 12) Dissolved oxygen meter (mg l⁻¹ or as % saturation value).
- 13) Thermometer (to 0.1 °C).
- 14) Salinity or conductivity meter.
- 15) Aquaria for holding *Corophium* (approximate size 10 litres to 30 litres).

- 16) A balance that will weigh to two decimal places (i.e., 0.01 g), or to a precision greater than or equal to 1 % of the quantity being weighed.
- 17) Clean 100 ml beakers (glass or of a suitable inert material). One beaker is required for each test vessel (see item 7, above).

3 METHOD

3.1 Field Collection and Storage of Animals

Typical densities of *Corophium* sp. that occur on the shore are 10,000–50,000 m⁻², but population densities can fall dramatically in winter to below 1000 m⁻². A single test may require between 150–600 animals depending on the design and the degree of replication.

Procedure A: Collection in sediment

Collection of animals: *Corophium* sp. should be collected from a clean intertidal shore (mud or muddy sand). The animals should be collected by removing the top 5 cm of sediment with a spade and laying this carefully in plastic trays. Sea water may be poured over the sediment and the samples should then be returned to the laboratory as soon as possible.

Holding conditions: On returning to the laboratory, the overlying water should be aerated and maintained at around 15 °C, under flow-through or static conditions. Temperature, pH, salinity, and dissolved oxygen should be monitored according to the appropriate laboratory practices.

Procedure B: Separation from sediment at collection site

Collection of animals: *Corophium* sp. are sieved at the site of collection. This may be an advantage, particularly where natural densities are not great, in that a check can be made on the numbers collected. Animals collected in this way are transferred to the laboratory in natural sea water.

Holding conditions: On returning to the laboratory, the animals are held in aquaria (approximately 10 litres to 30 litres) in the presence of a small amount of detrital material under static or flow-through conditions, and should be used within 5–10 days. Temperature, pH, salinity, and dissolved oxygen should all be monitored according to the appropriate laboratory practices. Mortalities during holding should be acceptably low, if possible less than 10 %.

Whichever method of collecting the animals is used, it is essential to measure the salinity at the point of collection. If the salinity is low, the animals should be acclimated to full salinity sea water (at least 25) at a maximum rate of approximately 3 per day.

3.2 Preparation of *Corophium* for Use in Toxicity Tests and Bioassays

Corophium collected and held in the laboratory as described in Procedure A, above, should be prepared by sieving them out of their native sediment prior to initiating the test. Care should be taken to ensure that any animals damaged during sieving are not included in the test, either through the use of a quarantine period (e.g., two days) or by visual inspection, or both. The holding trays should be drained down and the sediment gently dug out with a scoop and transferred to a 500 µm sieve (e.g., stainless steel or nylon). The sediment should then be sieved and gently washed away with sea water. It is very important to take care at this point to avoid damaging the animals. Damage can be minimized by keeping the sieve mesh in water as much

as possible. The animals that remain on the sieve should be gently washed into a clean aquarium containing flowing or static sea water and gentle aeration must be provided.

Animals collected and held in the laboratory as described in Procedure B may be sieved or pipetted directly from the holding tank (see Section 3.6, below).

Salinity, dissolved oxygen, pH, and temperature should all be recorded at regular intervals, and they must stay within the range specified for the test (see Section 3.7, below).

3.3 Preparation of Test Sediments for Toxicity Tests

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

- 1) an organic content of between 0.5 % and 4 % (dry weight);
- 2) a silt/clay fraction ($< 63 \mu\text{m}$) of between 5 % and 20 %;
- 3) a median grain size of $90 \mu\text{m}$ to $125 \mu\text{m}$.

Descriptively, the sediment should neither be a mud nor a coarse sand, but a muddy fine sand.

Sediment (approximately 40 kg wet weight or sufficient for the needs of the study) must be collected from an area known to be clean, and preferably from the same location from which the animals were collected. The aerobic layer of sediment (usually the top 5–10 cm) should be removed with a spade, and transferred to polythene bags or suitably cleaned vessels. The bags or containers should then be sealed, after excluding as much air as possible.

On return to the laboratory, the sediment should be sieved to $500 \mu\text{m}$ using reference sea water. Sieving in this way serves to adjust the interstitial salinity of the sediment, and excludes any benthic organisms which might interfere with the test or eat the test animals. It is very important that the sediment is washed in a closed system, e.g., a container with a limited volume of water, and that the slurry is left to settle for 24 hours before decanting the overlying water. The sediment should be carefully mixed before storage. The sieved sediment should then be placed into clean polythene bags and, after as much air as possible has been excluded, the bags should be sealed and stored in the dark at 4°C until they are required for use.

The sediment should be amended with the test substance as described in Annex 3.

3.4 Preparation of Test Sediments for Bioassays

All sediments used in tests must be collected and treated in a standardized manner. The provisions of ASTM E1391-90 (Standard Guide for Collection, Storage, Characterization and Manipulation of Sediments for Toxicological Testing), for instance, provide a suitable basis for a laboratory standard operating procedure.

Sediments should be collected by grab or core. Samples should be taken from the upper 2–4 cm, placed in polythene bags (excluding as much air as possible), and stored in the dark at 4°C . The information in the literature relating to the storage of sediments is conflicting (i.e., freezing, refrigeration with respect to time). As a general rule, sediments should be tested within two weeks of collection. If the nature of the sediment contamination is not known, or if heavy metals are the primary pollutants, then it may be advisable not to freeze the sediments, as there is evidence that this can alter toxicity. However, where the objective is to map toxicity, there is also evidence that, for hydrocarbon contamination, freezing for two to three months does not

distort the relationship between measured toxicity and contaminant concentrations and community indices determined for concurrently collected samples.

3.5 Test Preparation

It is recommended that at least 20 animals and two replicates be used per treatment in toxicity tests, where the objective is to establish an LC₅₀ value.

If it is intended to compare control with treatment mortality, then the degree of replication should reflect this requirement; for bioassays, it is recommended that at least three replicates with a minimum of 20 animals per replicate be used.

Approximately 150 ml of thoroughly homogenized sediment should be added to each test vessel. This should be sufficient to create a layer of 1.5–2 cm depth in a 1-litre vessel. The sediment should be smoothed and levelled with a polythene spatula. A disc of polythene cut to the internal diameter of the beaker should be lowered onto the sediment surface. This can conveniently be achieved by piercing the centre of the disc and threading a cable tie of appropriate length through the hole. Test sea water is then gently poured onto the polythene surface, and the vessel filled to the 850 ml mark. The polythene disc can then be gently removed.

After filling, each beaker is allocated a position within the test area using a random number sequence, and its position recorded. A separate record must be maintained of the relationship between sample identity and test vessel code number. When all test vessels have been prepared and positioned, the vessels are covered, and aeration pipettes introduced through apertures in the covers. Gentle aeration is applied, and the vessels are observed to ensure that no sediment resuspension occurs. If resuspension is noted, the aeration rate must be reduced until such resuspension is eliminated. Test vessels must then be left undisturbed for at least 16 hours to settle sediments before the addition of test animals.

The depth of sediment may vary in the test beakers. This is not critical except that the minimum depth must NOT be less than 15 mm, and the total sediment depth should not give rise to a sediment to water ratio of greater than approximately 0.2.

3.6 Addition of Experimental Animals to Test Vessels

Animals are prepared for each test as previously described (Section 3.2, above).

Corophium should be transferred to a tank or shallow tray in the reference sea water so that they can be selected more easily. This is accomplished by pouring the water from the holding tank through a 500 µm sieve, and gently washing the contents of the sieve into the tray. If animals have been held during acclimation in the absence of sediment, they may be sorted directly from the holding tanks.

Individual animals are selected using an automatic or standard pipette. In each case, the opening of the pipette should be 8 mm in diameter, the ends of which should be heat-smoothed to avoid damaging the animals. Animals of a size of greater than approximately 5 mm are selected by gently sucking them up into the pipette and randomly assigning them to reference sea water in the 100 ml beakers (one 100 ml beaker is required for each test vessel). Randomly assigning animals ensures that there is no biasing for size or activity. When each beaker contains 10 (or 20, as appropriate) *Corophium*, the volume of the beakers is adjusted to an appropriate mark (e.g., 40 ml) using reference sea water. The animals are now ready to be added to the test vessels. At this point, the aeration in the test vessels should be temporarily stopped.

The test is initiated by randomly placing the groups of animals into the test container. This is best achieved by gently moving the beaker to a horizontal position where the rim is under the water surface and then gently pouring out the contents of the beaker.

NB: Always check that no animals have been left in the 100 ml beakers.

When all the animals have been transferred to the test vessels, the test has been initiated and aeration should be restored.

3.7 Routine Monitoring and Data Collection

The following parameters should be measured and recorded at the start (before introduction of the test animals), on one occasion during, and at the end of the test (immediately before termination): pH (normal range 7.5–8.5), dissolved oxygen (normally > 85 %), and temperature ($15^{\circ}\text{C} \pm 2^{\circ}\text{C}$). Salinity must be measured at the start and end of the test (normally, a range of ± 4 during the experiment).

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 beaker after the animals have been added on Day 0; the addition of distilled water can be made to this mark when required. A 5-mm drop in level is equivalent to an increase of approximately 2 in salinity and a change of this magnitude may be used as a practical threshold for restorative action.

Other observations: Records may be kept on the burrowing behaviour of the animals, although this may not be possible if the water in the test containers is turbid. Animals that are dead on the surface of the sediment are opaque in appearance. These mortalities should be recorded together with the number of animals that are not buried but may be seen swimming in the water column or browsing on the sediment surface. Where possible, dead animals should be removed.

3.8 Termination of the Test

The test is continued for 10 days, and on the tenth day all readings should be taken before the test is terminated. Termination is achieved by gently stirring up the sediment in the test beaker to form a slurry, and pouring the slurry into the 500 μm sieve. This is best achieved by keeping the sieve immersed in sea water. Any sediment left in the sieve should be gently washed away with reference sea water, and the number of animals alive and dead should be recorded. Death is defined as the absence of movement after gentle stimulation with forceps.

3.9 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 or be consumed during the test and for this reason “missing” animals are presumed, and counted as, dead.

For 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.

For bioassays, the mortality values for each replicate should be arcsin-transformed, and the means of the transformed values compared by analysis of variance with an appropriate test for significance of difference to the control (e.g., Tukey’s test, Dunnett’s test).

3.10 Test Validity Criteria

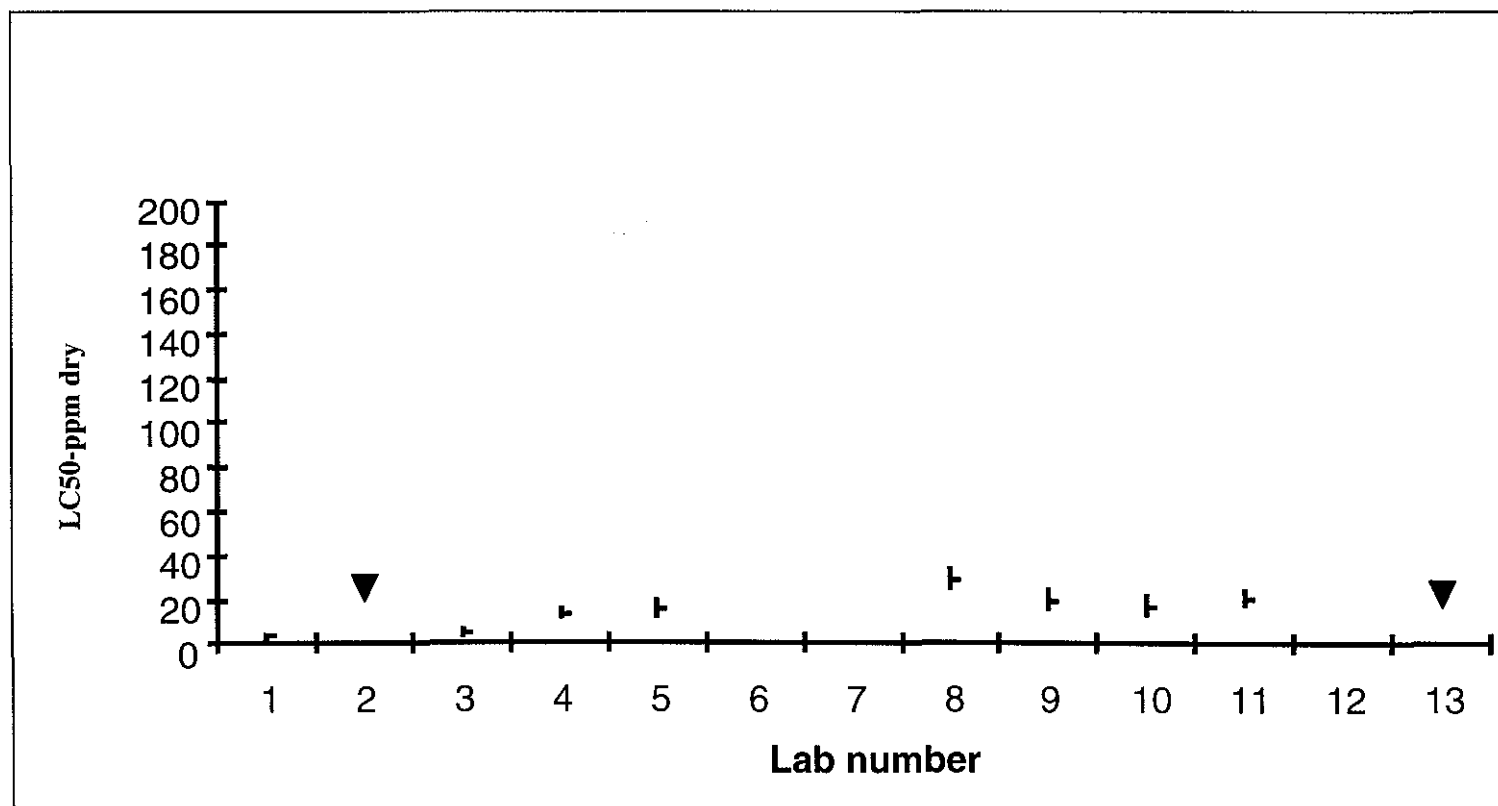
Control mortality should not exceed 15 % during the test, although practical experience has shown that, where higher mortalities are due to seasonal moribundity, a value of up to 20 % does not materially affect the relative response to different treatments. It is recommended that Abbott's correction be applied prior to the LC₅₀ calculation when mortality exceeds 15 %.

There is at present no established standard for reference toxicant effect. While cadmium has, for example, been used as a water-phase reference toxicant to assess test population sensitivity, it is preferable to use a sediment-bound toxicant to assess the sensitivity of the entire test system. Fluoranthene was used as a standard hydrophobic reference chemical during the 1993 PARCOM ring test of sediment reworker bioassays. Limited experience with this chemical suggests that a 10-day LC₅₀ of approximately 5–25 µg kg⁻¹ dry sediment is a reasonable initial compliance target.

4 EXAMPLE OF TOXICITY TEST DATA

Figure 1 illustrates the range of LC₅₀ values for the reference toxicant fluoranthene obtained by laboratories participating in the 1993 PARCOM sediment toxicity ring test. Vertical bars represent 95 % confidence intervals, and arrows represent "less than" values.

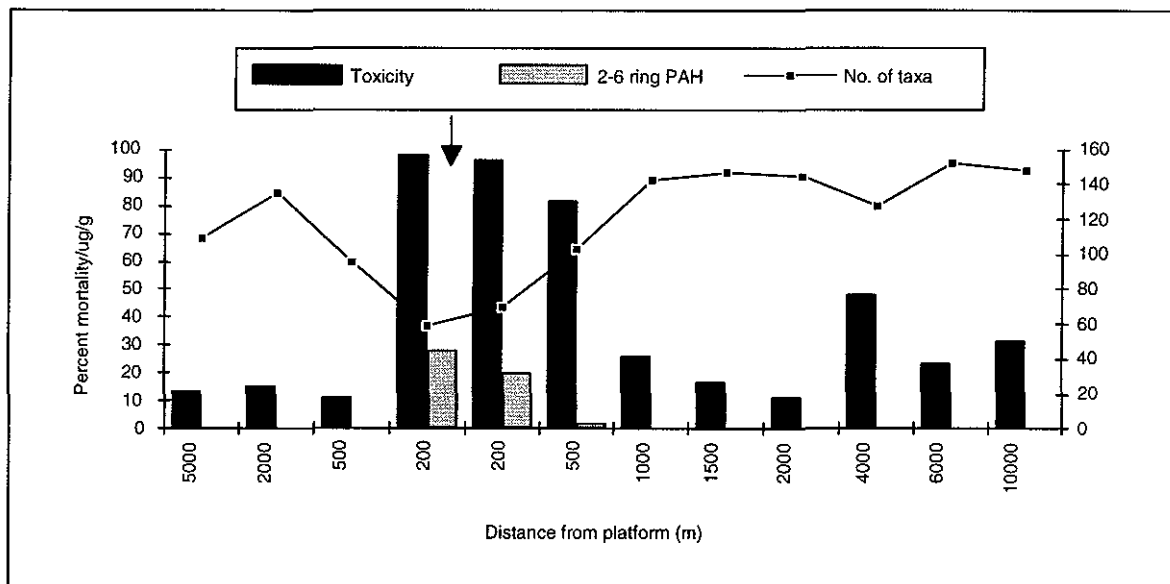
Figure 1. Results of the 1993 PARCOM ring test: fluoranthene LC₅₀ values in mg kg⁻¹ dry weight.



5 EXAMPLE OF BIOASSAY DATA

Figure 2 illustrates the effects observed in a survey of sediment toxicity in the vicinity of a North Sea oil platform. Samples were collected along a transect located on the axis of the residual current, in conjunction with samples taken for chemical and benthic community analyses.

Figure 2. Variation in sediment biology, chemistry, and toxicity in the vicinity of a North Sea oil platform. Toxicity is shown on the left-hand scale (as percent mortality), while the concentration of 2–6 ring PAHs in the sediment (in $\mu\text{g kg}^{-1}$ dry sediment) is shown on the right-hand scale.



6 REFERENCES

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ANNEX 1

AERATION SYSTEM FOR AMPHIPOD BIOASSAY

Components

To provide aeration for 10 test chambers, the following materials are required:

- One 63 mm external diameter ABS/PVC tubing 130 cm in length;
- One threaded tube connector;
- One piece of clear acrylic sheet 15 cm × 130 cm, 5 mm thick;
- Two No. 51 rubber/neoprene bungs;
- Ten 3 cm lengths of 8 mm external diameter vinyl/plastic tubing;
- Ten 1 ml pipette tips.

Assembly Instructions

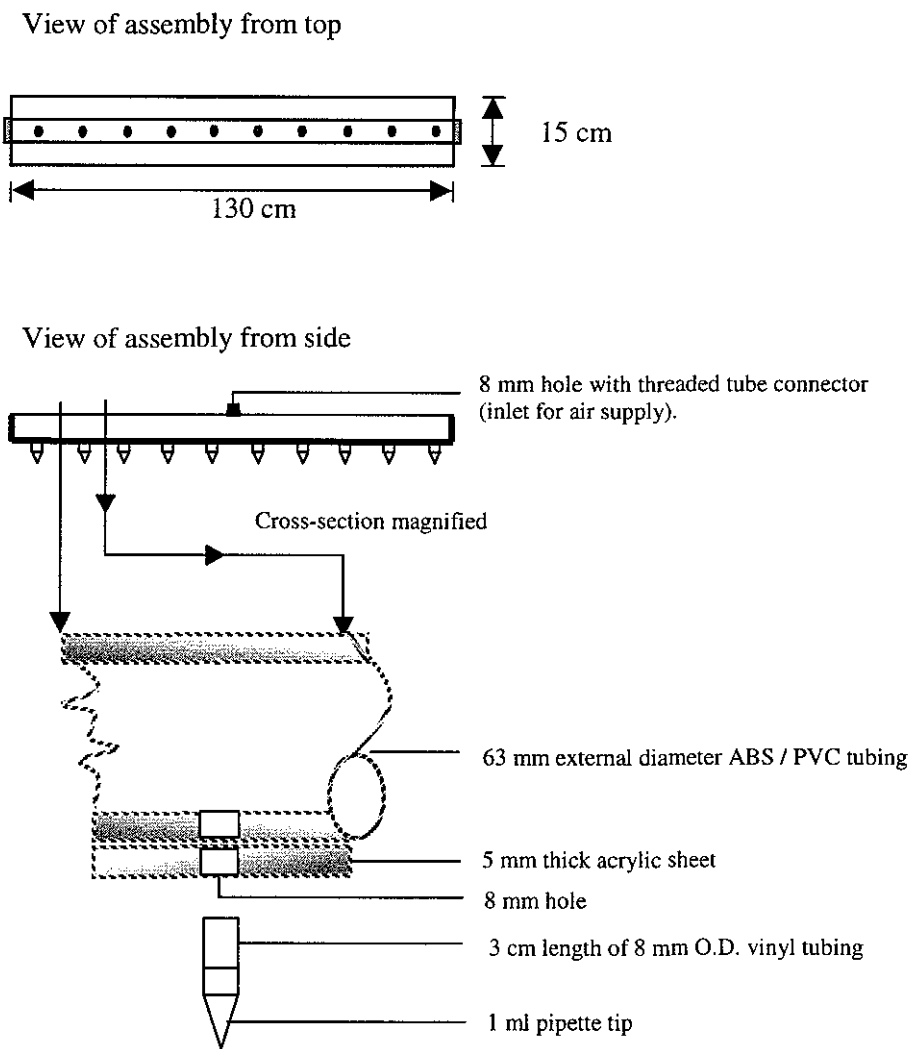
1. A centre line should be drawn longitudinally on the acrylic sheet and the centre marked.
2. Marks should then be made from the centre of the sheet. The initial marks should be 6.5 cm from the centre mark; thereafter, the marks should be made 13 cm from the preceding one.
3. These marks then provide the centres for the holes that have to be drilled in the acrylic sheet. They should be drilled out with an 8 mm drill.
4. It is essential that the first sheet be marked and drilled accurately, as it can then be used as a template for further systems.
5. The ABS pipe should then be firmly clamped to the template sheet, and drilled with the same 8 mm drill that has been previously used.
6. Another 8 mm hole should be drilled into the centre of the pipe, on the opposite side, and tapped to receive a threaded tube connector, which will provide the inlet for the air supply.

Assembly

1. Ten 2–3 cm lengths of the 8 mm O.D. tubing should be cut.
2. One end of the cut length of each piece of tubing should be pushed into the end of each of the 1 ml pipette tips. This process should be continued until ten of these units are assembled (see Figure A1.1).
3. The acrylic sheet and the pre-drilled pipe should be arranged so that the holes match, and the pipette tip/tubing assemblies should then be pushed into the holes. The fit should be sufficiently tight to ensure that the unit stays together and will remain air-tight. However, this can be achieved by using a silicon sealant, suitable for aquarium use.
4. The final stage is to insert the rubber bungs into the end of the ABS pipe. They should be pushed in as far as possible and should give an air-tight fit.

5. The end product should look something like Figure A1.1.

Figure A1.1. Diagram of an aeration system for an amphipod bioassay.



ANNEX 2

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 whole sediment 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 A2.1) 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 A2.1.

- 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).

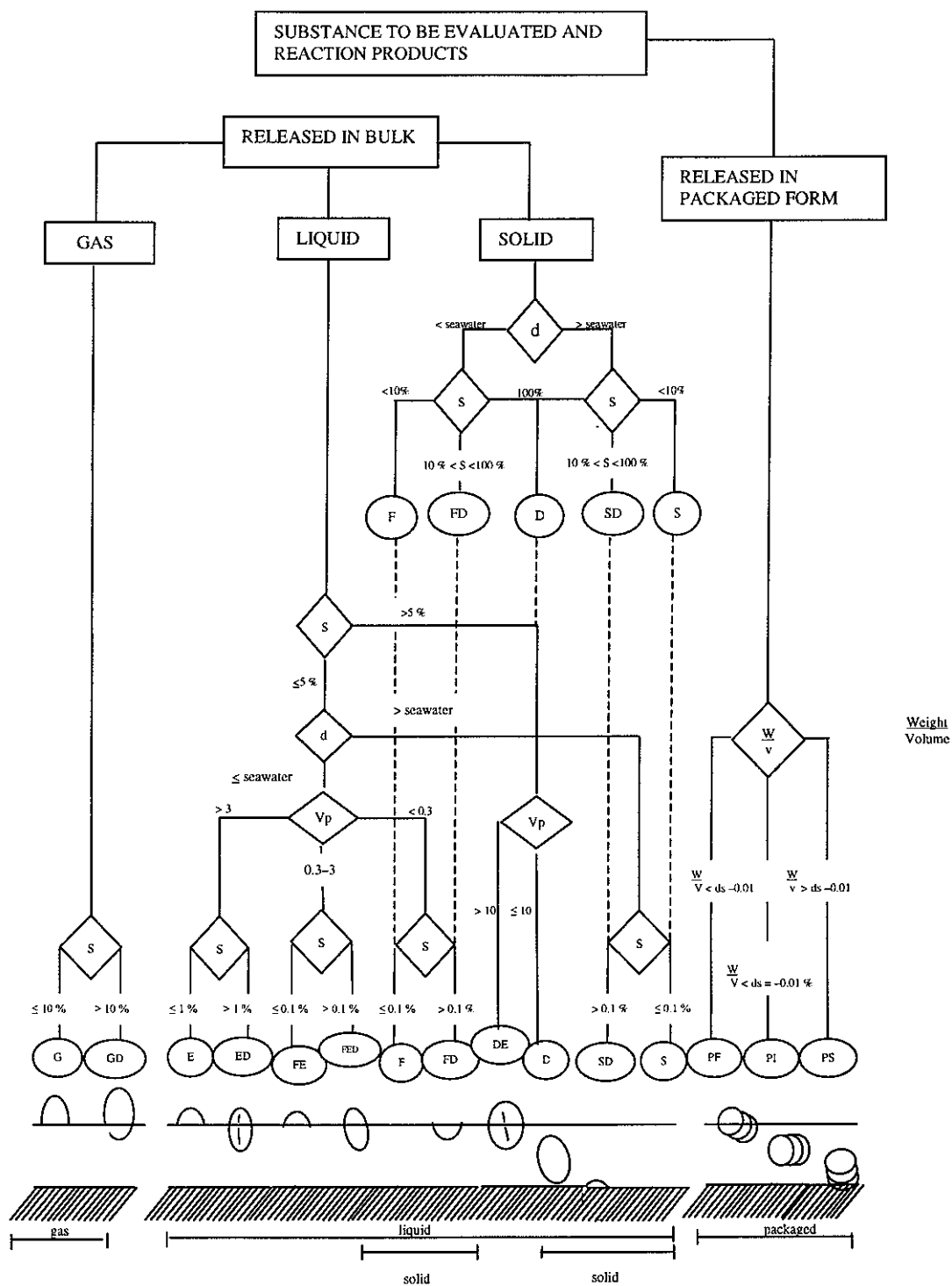
If, however, preparations should be tested, then a sediment test would be required if any of the intentionally added substances (at a concentration of > 1 %) in the preparation falls within one of the categories above.

Summary

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

- 1) are partly or completely classified as sinkers according to the SEBC (Figure A2.1);
- 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).

Figure A2.1 European Classification System – Flow Diagram (SEBC).



Air and water temperature: 10 or 20° C
Atmospheric 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

Table A2.1. Some log P_{ow} values.

Chemical	Log P _{ow}
benzene	2.13
toluene	2.59
<i>m</i> -xylene	3.09
ethylbenzene	3.13
isopropylbenzene	3.66
<i>n</i> -butylbenzene	4.28
diisopropylbenzene	5.01
<i>n</i> -dodecylbenzene	8.40
<i>n</i> -hexane	3.51
cyclohexane	3.18
<i>n</i> -nonane	5.09
<i>n</i> -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[<i>a</i>]pyrene	6.0

ANNEX 3

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 Annex 2, 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 3.3 of the body of this document.

Sediment should be collected and processed for testing as described in Section 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 Guideline (10-day test with *Corophium volutator*), a minimum depth of 15 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 (± 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 initially 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., 1 or 2 litres for a *Corophium* 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 about 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.

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