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SEDIMENT REWORKER RING-TEST, 1993

Technical report.

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SUMMARY

This report summarises the test results obtained from the 1993 Paris Commission (PARCOM) sediment reworker ring test.

The main features of the test methods with the bivalve *Abra alba*, the polychaete *Arenicola marina*, the amphipod *Corophium volutator* and the sea urchin *Echinocardium cordatum*, as well as the biology and availability of the test animals are summarised briefly. The sediment spiking technique employed and the recommended parameters for choosing a suitable base sediment are presented. The test results are shown in tabular form, along with data on control mortality and a comparison with the 1991 ring-test. An overview of the effort required to carry out the individual tests prepared by TNO and WQI, Denmark is also given.

The ring test produced sufficient valid results for a thorough evaluation of each species on scientific and technical grounds.

It was observed during range finding tests by the expert laboratories that one of the test substance, Petrofree, was only toxic at far higher concentrations than usually demanded by most regulatory authorities and it was withdrawn from the *Arenicola marina*, *Corophium volutator* and *Echinocardium cordatum* tests.

Arenicola marina failed to achieve an LC50 result in 5 tests for the PAH fluoranthene and on the basis of this insensitivity is considered as a less suitable general test method than the other three.

The other three species were in general similarly sensitive to the three test products.

1. INTRODUCTION

This document was aimed at providing a basis for discussion at the final workshop held in The Hague on the 13th and 14th of December, 1993. It is intended as a factual record of the test methods, the test species used and the results obtained. In compiling it, every effort has been made to avoid contentious statements, so as to leave the evaluation of the tests themselves to the participants at the workshop.

Please note that the identities of the laboratories remain confidential. To this end, each laboratory has been coded from 1 to 16.

1.1 Participating laboratories

A total of 16 laboratories participated in the ring-test, a full list of which is given in section 11. Regrettably, half of the participants carried out only one of the test methods.

Table 1 *The number of test methods carried out by the individual participating laboratories.*

	No. of participating laboratories
Four test methods	2
Three test methods	1
Two test methods	5
One test method	8

The leading or expert laboratories for the four test methods are given below:

Abra alba	:	Bioconsult S.A., Norway
Arenicola marina	:	Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Burnham-on-Crouch (MAFF)
Corophium volutator	:	MAFF, UK
Echinocardium cordatum	:	The Netherlands Organization for Applied Scientific Research - TNO Institute of Environmental Sciences

A majority of Norwegian labs chose to participate in the *A. alba* test, while a majority of UK labs chose to participate in the *C. volutator* test. The other two test methods were subscribed to by a more international mix of laboratories.

1.2 Evaluation criteria

The data from the ring test was presented so that the following criteria could be discussed and evaluated at the workshop. This list is not exhaustive.

1. The relative sensitivity of the test methods, including the manipulation of this parameter through sedimentary conditions, end-point etc.
2. Control mortality during the tests
3. Sediment choice and availability.
4. Ecological relevance of the test species
5. Availability of the test animals internationally.
6. Cost effectiveness in terms of labour.

1.3 Ring test workshop

For an insight into the discussions at the ring-test workshop and the final outcome, the reader is referred to the relevant summary record presented to the Group on Oil Pollution of the Paris Commission at its meeting of February 1994.

2. SUMMARY OF THE TEST METHODS

A brief description of all four test methods is included below.

2.1 *Echniocardium cordatum*

Test type	:	Sub-chronic, whole sediment test,
Measured endpoints	:	14d LC50 under flow-through conditions
Results expressed as	:	$mg.kg^{-1}$ dry sediment weight
Environmental parameters	:	O_2 and pH on six occasions (aeration required)
No. of test animals per concentration	:	10
Test preparation time	:	1d
Exposure duration	:	14d
Volume/weight of sediment	:	3kg dry sediment per test concentration
Volume of water	:	100 to 140 litre per concentration.
Sediment type	:	Standardised muddy-fine sand

2.2 *Abra alba*

Test type	:	Acute, sediment suspension test
Measured endpoints	:	96-120h static EC50 (defecation rate of faecal pellets).
Results expressed as	:	$mg.l^{-1}$ suspension (8% sediment)
Environmental parameters	:	No measurements required, no aeration.
No. of test animals per concentration	:	30
Test preparation time	:	3-4d
Exposure duration	:	5d
Volume/weight of sediment	:	duplicate 200ml aliquots of 8% sediment suspension per concentration
Special materials	:	12 to 14 racks of 15 sieves (90µm mesh) per test
Sediment type	:	Organic rich, muddy sediment sieved at 45µm

2.3 *Corophium volutator*

Test type	:	Sub-chronic, whole sediment test
Measured endpoints	:	10d static LC50
Results expressed as	:	$mg.kg^{-1}$ dry sediment weight
Environmental parameters	:	O_2 and pH daily (aeration required)
No. of test animals per concentration	:	50-60 individuals
Test preparation time	:	5d
Exposure duration	:	10d
Volume/weight of sediment	:	1.5kg of dry sediment per concentration.
Sediment type	:	Standardised muddy-fine sand.

2.4 *Arenicola marina*

Test type	:	Sub-chronic, whole sediment test
Measured endpoints	:	10d static LC50
Results expressed as	:	$mg.kg^{-1}$ dry sediment weight
Environmental parameters	:	O_2 and pH daily (aeration required)
No. of test animals per concentration	:	15
Test preparation time	:	2d
Exposure duration	:	10d
Volume/weight of sediment	:	3kg of dry sediment per concentration.
Sediment type	:	Standardised muddy-fine sand

3. TEST CHEMICALS

The test chemicals were chosen so as to represent a broad spectrum of product categories commonly used in the offshore industry, i.e. a mud, a corrosion inhibitor and a biocide. One product (the bactericide Bioban P1487) had originally been tested in the previous Paris Commission ring-test in 1991 and was again included as a reference. Additionally, fluoranthene, a poorly soluble pure compound was included as a further reference compound.
They were deliberately chosen for their poor solubility or because they were known to have particolophilic properties.

Table 2 *Chemicals and methods tested.*

Leading laboratory	Test methods	No. of participating laboratories	Chemicals
TNO	*Echinocardium cordatum*	4	Bioban P1487, Servo CK337, Fluoranthene, Petrofree mud (TNO only)
MAFF	*Arenicola marina*	7	Bioban P1487, Servo CK337, Fluoranthene.
MAFF	*Corophium volutator*	14	Bioban P1487, Servo CK337, Fluoranthene.
Bioconsult	*Abra alba*	6	Bioban P1487, Servo CK337, Fluoranthene, Petrofree mud.

The test compounds can briefly be described as follows:

Fluoranthene is a four ring polycyclic aromatic hydrocarbon, which is only slightly soluble (0.1 ± 0.6 $mg.l^{-1}$ in seawater; Neff, 1979) and is known to have a moderate to high aquatic toxicity. It was not distributed to the ring test participants, all of whom secured their own supplies.

Petrofree mud is a drilling mud based on an ester compound (62% active ingredient). It is a dark brown fluid with a fishy odour. It forms an invert emulsion in water and has little or no toxicity. No further details as to the identity of the ester were available from the manufacturers, Baroid Ltd., Aberdeen, U.K.

Bioban P1487, [4-(2-nitrobutyl)-morpholin (70%) and 4,4'-(e-ethyl-2-nitrotrimethylene)] produced by Angus Chemie GmbH, Germany, is a broad spectrum bactericide designed for application in oils and aqueous situations. It is described by the manufacturers as being "only moderately soluble" in water" (1100 $mg.l^{-1}$).

Servo CK337 is an inhibitor of CO_2 and H_2S corrosion, produced by Servo, Delden, The Netherlands. It is comprised of an amine neutralised phosphoric acid ester; that is described by the manufacturers as a "cationic surface active agent in an aromatic hydrocarbon solvent". About 40% active ingredient was reportedly present in the test product.

4. BASE SEDIMENT

4.1. Standardised base sediment guideline

The following is an extract from the guideline issued by the leading laboratories (MAFF & TNO) to all participants in the *A. marina, C. volutator* and *E. cordatum* tests (see McMinn et al,. 1993):

"It was agreed at the start meeting that some guidance should be given for selecting reference sediment for the *Arenicola marina*, *Corophium sp* and *Echinocardium cordatum* sediment reworker tests.

This guidance is aimed at:

a) evaluating toxicity of poorly soluble substances liable to incorporation into seabed sediments;
b) an attempt at ecological representation of sediments around North Sea oil and gas installations;
c) standardization of testing procedures and therefore better comparability of test results.

The reference/base sediment must have:

1) an organic content of between 0.5 and 4%;
2) a silt/clay fraction (< 63µm) of between 5 and 20%;
3) a median grain size of 90 to 125 µm.

It should be descriptively a muddy fine sand, not a mud and not a coarse sand. In practice not all laboratories may be able to characterise sediments in this way. This should **not preclude** any laboratory from taking part in the ring test. For the *Corophium* and *Arenicola* tests, the lead laboratory will carry out an independent characterisation of the reference sediments as stated in the protocol. If it is found that a reference sediment falls outside the above requirements than a test using such a sediment may not be accepted. We would strongly recommend that all laboratories attempt to characterise their reference sediment."

4.2 *Abra alba*: standard sediment suspensions

The following sediment preparation method is recommended in the *Abra alba* guideline (see Strømgren, 1993).

> "As a medium for *Abra* during toxicity testing is used a standard sediment suspension made of sieved sediment (< 45 µm). The sediment content in this standard suspension should be 8 ± 1% (dry weight). The sediment dry weight in the suspension is calculated by sub-sampling 20 ml of a well stirred suspension. This volume is transferred to a dried (65°C, 24 h) and pre-weighed filter. The filter with the sediment is dried (65°C, 24 h). The amount of seawater which has to be added to the sediment suspension to get a dry weight content of 8% is calculated according to the formula W = A (C-B-1) x 0.625, where A is the volume of the sediment suspension, B the tare of the filter, and C dry weight of the filter with sediment. The standard sediment suspension may be stored at 11°C with air bubbling. If air bubbling is continued for more than three days, the evaporation has to be compensated by addition of distilled water."

Furthermore, it is recommended that the sediment should contain sufficient organic matter to sustain an *Abra alba* population. This was not further defined.

5. SPIKING TECHNIQUE

5.1 Standardised procedures for spiking the sediment in the *A. marina, C. volutator* and *E. cordatum* tests

The following has been abstracted from the standardised method recommended by MAFF and TNO (see McMinn et al,. 1993 and Bowmer, 1993):

"This procedure is specific for the products used in this ring test. It should not convey to the reader any intention of acceptability for the future testing of such compounds.

Protocol requirements:
Echinocardium cordatum; at least 3 kg for one test vessel.
Arenicola marina; 3 kg, 1 kg for a test vessel; therefore enough for 3 replicates.
Corophium sp; 1.5 kg, 300 g for a test vessel; therefore enough for 5 replicates.

Material required for spiking 1.5 kg of sediment with a product. If larger mixing containers are used adjust the volumes accordingly. One 2000 ml mixing container (e.g. polycarbonate/polythene and wide mouthed); 1.5 kg wet weight reference sediment; 750 ml reference seawater; product to be tested; shaker or roller and a balance to weight to a precision greater than or equal to 1% of the quantity being weighed.

Note: Wet sediment does not mean a slurry nor does it mean slight damp. Typically, it should have the moisture content that it has on the foreshore as the tide goes out, i.e. ball up like a snowball in the hand.

1. All test material should be mixed thoroughly before being used. Liquid products should be placed on a platform shaker in their original container and allowed to mix for at least two hours before being used. Solid material should be left in their original container and shaken by hand or thoroughly mixed with a spatula.

2. The test materials must be prepared as follows. Each test concentration must be prepared individually; no serial dilution should take place at any stage in a-c below and there must be no serial dilution of a spiked sediment.

a) Fluoranthene:

Pre-dry to 98% dryness a small quantity of reference sediment e.g. 2 kg. Dissolve in 25 ml acetone a weighed amount of product to be tested (e.g. 0.33 g for 330 $mg.kg^{-1}$ final concentration in the sediment). Mix the 25 ml of acetone with 250 g of dried reference sediment; this can be carried out in a beaker using a spatula. Evaporate the contents of the beaker to dryness in a fume cupboard. Add the contents to the depression created in the sediment of the mixing container, see 5 below.

b) Servo CK 337:

Add a weighed amount of product to be tested (e.g. 0.33 g of 330 mg kg final concentration in the test sediment) to 50 ml reference seawater in a volumetric.

Shake vigorously by hand to dissolved. Add the contents of the volumetric immediately to the depression created in the sediment in the mixing container, see 5 below.

3. Tare a mixing container (2000 ml or other suitable size) on a balance.

4. Weight into it 800 g of the wet sediment (of known moisture content).

5. Create a depression or well in the centre of the sediment of sufficient size to take 250 g of sediment in 2.a above or 50 ml volume in the case of 2.b and 2.c above and add accordingly.

6. Weight out the required additional 700 g of wet sediment (450 g in the case of 2. above) in a separate container.

7. Add the 700 g of wet sediment to the mixing container taking care that the test material is not displaced from its well.

8. Carefully add 700 ml of reference seawater (750 in the case of 2.a) to the mixing container.

9. Seal the container and place it horizontally on a platform shaker set at 100 rev-1 or a roller and allow to mix for a minimum of 4h up to a maximum of 24 h. It is important to be consistent from one test material to the next. Therefore, please select a time and use it for all tests and report accordingly.

The sediment is then ready for testing.

Please note:
The addition of water to the sediment is important to ensure good mixing of the test material with the sediment. The action of rolling to shaking is different. If a roller is used it may be possible to reduce the water added to the mixing container in 8 above and achieve the same degree of mixing. Please report any such deviations as appropriate.

The procedure for mixing sediments is based on wet weight. It is imperative that the dry weight of a representative sample of the reference sediment be determined at the time of mixing . This necessary because sediments will vary in moisture content and all exposure concentrations *must* be standardised on a dry weight basis. Dry weight determinations are conducted by oven drying for 24 h and then exposure concentrations are back calculated."

5.2 Recommended method for spiking the sediment for the *Abra alba* test method

The following method has been abstracted in its entirety from the *A. alba* ring-test guideline (see Strømgren, 1993):

"The test medium is prepared by adding test substance to the standard sediment suspension in quantities to obtain the planned test concentration in mg per kg total medium. The test substances selected for the Ring test are not water soluble, and stock emulsion are prepared by micro-encapsulating the test substance in presence of standard sediment suspension. For micro encapsulation solutions of acacia gum and gelatine are required. Acacia gum and gelatine, 50 g of each, are supplied to the participating laboratories by the leading laboratory (enclosed).

The acacia gum solution is prepared by adding 12.5 g of acacia gum to 100 ml aqua dest at 30-50°C, and shaking vigorously by hand until all acacia gum is dissolved. Store at < 10°C. The gelatine solution is prepared by adding 12.5 g gelatine to 100 ml aqua dest, 30-50°C, and shaking vigorously by hand until the gelatine is dissolved. Store at < 10°C.

Micro-encapsulation

The test substance is micro encapsulated in a concentrated sediment suspension. The concentrated sediment suspension (≈ 100 ml) settling 500 ml standard sediment suspension in a measuring cylinder for 1 h and decanting abt. 400 ml of the water phase.

The gelatine and acacia gum solutions and the concentrated sediment suspension are heated in a water bath at abt. 50°C for 30 minutes. All components are shaken by hand after abt. 10 and 20 minutes. The test substance should be of room temperature (≈ 20°C), and if it is not homogeneous the container has to be shaken vigorously by hand for at least 2 minutes. The blender glass should be heated in the water bath for at least 5 minutes.

After heating in the water bath the components are added in the following order into the blender glass: 1) 10 ± 1 g gelatine solution, 2) 10 ± 1 g acacia gum solution, 3) abt. 100 ml concentrated sediment suspension and) 5 ± 0.05 g of the test substance. The mixture is blended for 2 minutes, and poured into a 500 ml measuring cylinder. About 50 g ice made from aqua dest is added and the emulsion gently shaken. The measuring glass is then filled with seawater to a final volume of 500 ± 5 ml. This stock emulsion contains 5 g of test substance in 500 ml.

Contamination of test medium

Test medium is contaminated by adding given volumes of stock emulsion to 500 g of standard suspension. A 500 ml glass bottle is tared and 500 ± 2 g of well stirred standard sediment suspension is added. The different volumes of the stock emulsion added to the bottles may be read from the dilution table enclosed. If the volume of the emulsion added is larger than 2.5 ml, a corresponding volume of standard sediment suspension is removed from the bottle before addition. After contamination, the test substance in the bottle is vigorously shaken by hand for 30 seconds and stored at the test temperature, 11°C. The test medium should be stored in the bottles for 12-14 h before starting the exposure of the test animals."

6. ECOLOGICAL PROFILE OF THE TEST SPECIES

6.1 *Echinocardium cordatum* (Pennant)

Taxonomy: Echinodermata, Echinoidea,
Common name: En, sea potato; Nl, zeeklit

Biology and ecology
E. cordatum has a bilaterally symmetrical test or shell, with five rows of tube feet across its surface. It has a covering of golden brown, mobile spines which are formed into a crest dorsally. The mouth is found on the underside of the test.

Near-shore populations grow rapidly to maturity in 2 to 3 years at a shell length of 2 to 3 cm and as young adults may occur at densities of several hundred per m^2. The species has separate sexes, and spawns in the autumn; development is planktotrophic. Populations containing several year classes exist which are dominated by animals of 0-5 to 1cm in length.

Distribution & demography
It can be found in northern temperate European coastal waters from the mean low water mark to several hundred meters depth offshore. According to Buchanan (1967), it tends to inhabit sediments where the silt-clay content is less than 20%. The species is both wide-spread in sandy sediments in the North Sea and in estuaries and bays. It is a common faunal element in the southern and northern areas of oil and gas exploration in the North Sea.

Trophic preferences & feeding method
It feeds by directly ingesting sediment and is nearly always found with its intestine full of sand. There is some suggestion that it may gather organic matter through a funnel shaped channel that it maintains with the surface.

Burrowing depth
It is typically found burrowing to a depth of 3 to 20cm in muddy fine sand.

Establishment of mortality
Mortality is nearly always preceded by surfacing from the sediment and often by dis-orientation, where the animal spirals or turns ventral side up. *E. cordatum* is considered to

be dead when the spines and/or the inflatable gill structures around the mouth no longer show any movement.

6.2 *Abra alba* (Wood)

Taxonomy: Mollusca, Lamellibranchiata, Tellinidae
Common name: none

Biology and ecology
Crowe (1985) provides an extensive work on the biology and ecology of this species. *Abra alba* has thin elliptical valves about 10-15 mm in length, which are usually white in colour. It has a long (up to 3 cm) inhalant siphon which it uses for ingesting particles from the surface of the sediment. As in many tellinid bivalves, e.g. *Macoma balthica*, the exhalant siphon is somewhat shorter.

Crowe (1985) reports that *A. alba* lives for one year in Kinsale harbour, Ireland, based on the lack of growth rings in the shell and a length frequency analysis of populations. At other locations, it may live for 2 to 3 years.

Distribution & demography
It is found predominantly in muddy sediments with a high silt-clay and organic matter content. It occurs at densities of up to several thousand individuals per m^2; extreme densities are known in the vicinity of sewage sludge dumping grounds.

Trophic preferences & feeding method
Abra alba feeds by sucking sedimentary particles in through its inhalant siphon. It presumably sorts the material on its gills and ejects hard particles as pseudofaeces. It produces oblong faecal pellets of >100 µm in length.

Burrowing depth
It burrows in the upper 2.5 cm layer of sediment, and is rarely found at depths below 5 cm.

Establishment of mortality
The valves of *Abra alba* shut tightly when the animal is disturbed. In moribund individuals, the valves gape open. If there is no adductor muscle response when the shell of a gaping individual is mechanically stimulated, then the animal is assumed to be dead.

6.3 *Corophium volutator* (Pallas)

Taxonomy: Arthropoda, Crustacea, Amphipoda
Common name: NL, slijk garnaaltje

Biology and ecology
As in most amphipods, the body is laterally compressed. The animal is greyish-white to brownish in colour with a pair of large antennae which are some 70% or more of the length of the body. It may occur at densities of up 50,000 to 100,000 per m^2, although figures of several thousand are probably more usual. The sexes are separate and some 10 to 40 embryos are brooded in the pouch of the females, following fertilisation by the male in the burrow. Up to 5 d after hatching, the young leave the brood pouch at a length of 1 mm. *C. volutator* grows to a length 8 and 10mm (males and females respectively) and can live for up to one year. The young reach sexual maturity after 35 to 50d.

Distribution & demography
This species occurs in soft intertidal sediments, on mud flats, in salt marsh pools and brackish ditches around the coasts of Europe and North America. It can be found from the mid-tide zone to 0.5 m deep in the sublittoral and favours an organic content of ca. 7% (see Möller & Rosenberg, 1982 and Watkins, 1942).

Trophic preferences & feeding method
The animal reputedly lives on organic matter, detritus, bacteria and diatoms at the sediment surface and is classified as a selective deposit feeder. It scrapes material from the sediment surface outside its burrow with its antennae and sorts it on the sieve-like setae of the first and second gnathopods. It has been shown to ingest sedimentary particles of 4 to 63 μm and may also browse on larger particles by scraping them with its antennae (Nijkamp, 1992). It has been shown to be attracted to both microalgal mats and pieces of raw beef laid on the surface (pers. obs.), and emerges from the burrow to feed on the open sediment surface.

Burrowing depth
The animal burrows into the upper sediment layer, where it maintains a tube of 3 to 12 cm in depth.

Recognition of mortality
Mortality is recognised by total immobility (after mechanical stimulation in cases of uncertainty), combined with a slight colour change.

6.4 *Arenicola marina* (L.)

Taxonomy: Annelida, Polychaeta, Arenicolidae,
Common name: En, lugworm; NL, zeepier;

Biology and ecology

A. marina reaches an adult size of 10 to 15 cm in length with a wet weight of 3 to 8 g per individual. Larger specimens of up to 30 cm long and weighing 50 g have been found occasionally (pers. obs.). The animal is divided into a distinct thorax and abdominal area or tail. The colour varies from pink or brown in young animals to black in older specimens.

The sexes are separate, the oocytes and sperm being matured free in the body cavity (outside the gonads). Spawning is epidemic in October in The Netherlands; the eggs are fertilised in the burrow of the mother, where they undergo an abbreviated, demersal development prior to migration into the plankton when they have already developed several chaetigerous segments, followed by settlement further up the shore (Farke & Berghuis, 1979).

Distribution & demography

This species is found predominantly in the intertidal zone, but may occasionally occur in the sublittoral. It is widely distributed in tidal flat ecosystems in Europe and the east coast of North America and is replaced by a related arenicolid species on the Pacific coast of Canada.

Trophic preferences & feeding method

The animal is a head-down, direct deposit feeder, ingesting bulk sediment from which it digests the detrital material.

Burrowing depth

It maintains a 'J' shaped burrow of up to 50 cm depth (Ashworth, 1904).

Recognition of mortality

A. marina moves by internal hydrostatic pressure directed against powerful longitudinal and circular muscles. However, when dead, the body loses turgor and becomes flaccid; blood can be seen under the skin where vessels have degenerated. Mechanical stimulation elicits no response.

7. SUMMARY OF THE RING-TEST RESULTS

7.1 Methods of calculating the data

The leading laboratories each used their own standard method for calculating the EC50 and LC50 values of all data-sets received from the participating laboratories.

MAFF employed the Stephan binomial method (Stephan, 1977) for the *A. marina* and *C. volutator* data, while TNO used the Kooijman (1981) non-parametric method on the *E. cordatum* data. In this latter method, at a given time, the mortality probability of an individual is assumed to be logistically related to the logarithm of the concentration. Bioconsult used the algorithm: $Y = aX + b \log X + c$ for calculating the EC50 values in the *A. alba* test, where X is the concentration and Y is the response in percent of the control. The fit of the model was indicated by computation of the R^2 values.

An example of the difference in LC50 calculation produced by the MAFF and TNO methods on two data-sets is given below. It can be seen that the TNO method produced a marginally less sensitive LC50 and a generally narrower standard error. The *A. alba* calculation method could not be compared in a like manner as it is used for calculating EC and not LC50 data.

Table 3 *A comparison of the LC50 data calculation methods used by TNO and MAFF ($mg.kg^{-1}$ dry weight).*

Test method	**Corophium**		**Echinocardium**	
Data	TNO		MAFF	
Calculation method	TNO (Kooijman)	MAFF (Stephan)	TNO (Kooijman)	MAFF (Stephan)
Bioban P1487	71.5 (59.9-85.4)	71.8 (56-100)	121 (77.3- 191)	93.3 (73.2-119.3)
Servo CK337	251 (217- 292)	240 (180- 320)	168 (103- 273)	139.5 (83.4-223.5)
Fluoranthene	16 (13.9-18.4)	14.8 (10-18)	77 (17.5- 338)	70.4 (16.6-387)

7.2 Test results

An overview of the results obtained is presented in this section. When comparing the data, the following point should be borne in mind: in terms of reproducability, Canton & Adema (1970) and Brown (1968) (both in Anon, 1980) indicate that LC50 values "may easily differ from each other by a factor of 2 to 3" when a test method is repeated with the same test substance on different occasions. Only data for which a calculated LC/EC50 is available have been included. However, the complete dataset, including all 'less than' values, etc. can be found in Annex I.

Table 4 provides a summary of the numbers of successfully calculated test results. It can be seen for some tests, that there were a sizeable number of 'greater than' results, caused by various factors. Where *A. marina* was concerned, the majority of these were due to the insensitivity of the test animal to fluoranthene.

Table 4 *The numbers of successfully calculated results.*

	No. of test substances	Total No. of tests attempted	No. of calcu-lated results	No of '>' or '<' results	Data un-calcu-latable	No data
Abra alba	Bioban P1487, Servo CK337, Fluoranthene, Petrofree mud.	24	22	1	0	1
Arenicola marina	Bioban P1487, Servo CK337, Fluoranthene.	21	12	7	1	1
Corophium volutator	Bioban P1487, Servo CK337, Fluoranthene.	38	26	12	0	0
Echino-cardium cordatum	Bioban P1487, Servo CK337, Fluoranthene, Petrofree mud (TNO only).	13	11	1	1	0

With *Corophium*, the high number of > or < results is related to some laboratories which experienced problems with the test method and this type of data is spread evenly across the test substances.

7.2.1 *Abra alba*

Table 5 *The test results for the tellinid bivalve* Abra alba *(Leading lab. Bioconsult). 96-120h EC50, faecal pellet production in mg.kg-[1] dry weight.*

Test substance	Laboratory Code	EC50 $mg.kg^{-1}$ dry weight	Standard error
Bioban P1487			
	Lab 1	350	275-425
	Lab 2	525	487.5-562.5
	Lab 3	300	200-400
	Lab 4	212.5	175-250
	Lab 5	425	237.5-662.5
	Lab 6	125*	–
Servo CK337			
	Lab 1	212.5	187.5-237.5
	Lab 2	375	337.5-412.5
	Lab 3	200	137.5-262.5
	Lab 4	87.5	25-150
	Lab 5	462.5	350-575
	Lab 6	100	25-175
Fluoranthene			
	Lab 1	100	75-125
	Lab 3	187.5	162.5-212.5
	Lab 4	16.3	8.8-23.8
	Lab 5	137.5	100-175
Petrofree			
	Lab 1	6100	5663-6538
	Lab 2	5062	4288-5838
	Lab 3	6213	5163-7263
	Lab 4	8163	7225-9100
	Lab 6	4350#	3425-5275

* The LC50 was calculated by the Kooijman method as 327 $mg.kg^{-1}$ dry weight.

\# The LC50 was calculated by the Kooijman method as 8260 (7080-9630) $mg.kg^{-1}$ dry weight, although oxygen stress was a factor in this test (pers. obs.).

7.2.2 *Arenicola marina*

Table 6 *The test results for the polychaete Arenicola marina (Leading lab.: MAFF), 10d LC50, mg.kg^{-1} dry weight.*

Test substance	Laboratory code	LC50 mg.kg^{-1} dry weight	Confidence interval (95%)
Bioban P1487			
	Lab 5	54.4	50-100
	Lab 6	69.2	56-100
	Lab 7	62.7	56-100
	Lab 8	51.7	32-100
	Lab 9	55.0	33-100
Servo CK337			
	Lab 3	343.9	330-560
	Lab 6	239.3	100-320
	Lab 7	421.4	316-562
	Lab 8	369.2	320-560
	Lab 9	393.3	330-1000
	Lab 11	543.6	377-671
Fluoranthene			
	Lab 5	155.8	–
	Lab 6	>3300*	–
	Lab 7	>1000*	–
	Lab 8	>1000*	–
	Lab 9	>1000*	–
	Lab 11	>1000*	–

* Data included to show the trend with *A. marina* and phenanthrene.

Table 7 *The inhibition of cast production (faecal mounds) of the polychaete Arenicola marina (Leading lab.: MAFF), 10d EC50, mg.kg^{-1} dry weight.*

Test substance	Laboratory code	EC50 mg.kg^{-1} dry weight	Standard error (95%)
Bioban P1487			
	Lab 5	26.7	20-50
	Lab 6	26.2	18-32
	Lab 7	36.7	32-56
	Lab 8	38.6	32-100
	Lab 9	32.3	33-56
Servo CK337			
	Lab 3	279.2	320-560
	Lab 6	131.3	100-320
	Lab 7	126.5	100-180
	Lab 8	100	100-320
	Lab 9	223.8	100-320
	Lab 11	425.4	377-671
Fluoranthene			
	Lab 5	35.5	30-100
	Lab 6	100.0	100-320
	Lab 8	232.4	100-320
	Lab 9	13.9	18-33
	Lab 11	105.3	57-318

Int is interesting to note that while five of the six tests with fluoranthene and this species produced an LC50 result of > 1000, the EC50 (cast production) was obtained in all cases.

7.2.3 *Corophium volutator*

Table 8 *The test results for the Amphipod Corophium volutator (Leading lab.: MAFF), 10d LC50 mg.kg-[1] dry weight.*

Test substance	Laboratory code	LC50 mg.kg^{-1} dry weight	Confidence interval (95%)
Bioban P1487			
	Lab 4	114.5	105.4-143
	Lab 6	71.8	56-100
	Lab 8	54.3	32-100
	Lab 9	66.9	41-77
	Lab 10	117.2	–
	Lab 13	42.0	34-88
	Lab 15	64.0	41.1-128.3
	Lab 16	52.5	32-100
Servo CK337			
	Lab 4	331.5	164.7-429.9
	Lab 6	240.0	180-320
	Lab 8	107.7	32-100
	Lab 9	514.2	128-720
	Lab 10	302.2	130.8-439.1
	Lab 12	123.4	83.4-148.9
	Lab 13	473.3	220-1400
	Lab 14	424.1	189.4-757.6
	Lab 15	307.9	41.1-718.6
Fluoranthene			
	Lab 4	33.1	16.9-65.9
	Lab 5	33.1	10-60
	Lab 6	14.8	10-18
	Lab 8	5.8	3.2-10
	Lab 9	19.6	13-41
	Lab 10	22.1	12.4-42.6
	Lab 12	23.8	14.9-47.7
	Lab 14	22.3	9.2-82.2
	Lab 15	4.1	1.3-12.8

7.2.4 *Echinocardium cordatum*

Table 9 *The test results for the sea urchin* Echinocardium cordatum *(Leading lab.: TNO)., 14d LC50 in mg.kg^{-1} dry weight*

Test substance	Laboratory code	LC50 mg.kg^{-1} dry weight	Confidence interval (95%)
Bioban P1487			
	Lab 5	270	212-344
	Lab 6	179	141-227
	Lab 9	121	77.3-191
Servo CK337			
	Lab 5	151	96.4-238
	Lab 6	115	76.2-167
	Lab 9	168	103-273
	Lab 10	120	84-173
Petrofree			
	TNO	>32000*	
Fluoranthene			
	Lab 5	33	17.2-63.1
	Lab 6	34	18.9-62.5
	Lab 9	77	17.5-338
	Lab 10	116	47.2-286

* Data included to give an indication of the apparently low toxicity of Petrofree to *E. cordatum*.

7.2.5 General

From Table 10 below, it can be seen that for Bioban P1487, that *A. marina* and *C. volutator* were on average ca. 3 to 5 times more sensitive than *E. cordatum* and *A. alba*. For Servo CK337, *E. cordatum* was 2 to 3 times more sensitive than the other three species, while for fluoranthene, *C volutator* was on average ca. 3 to 5 times more sensitive than *E. cordatum* and *A. alba*.

Table 10 *Summary of the test data by test substance and test method; means, sd's and coefficient of variation. (all data in $mg.kg^{-1}$ dry weight)*

Test method and test substance	Mean LC/EC50 in $mg.kg^{-1}$ dry weight	± sd	Coefficient of variation (%)
Bioban P1487			
Echinocardium cordatum	190.0	±75	39.5
Abra alba	322.9	±144.1	44.6
Arenicola marina	58.6	±7.2	12.3
Corophium volutator	72.9	±28.1	38.5
Servo CK337			
Echinocardium cordatum	138.5	±25.3	18.3
Abra alba	239.6	±150.3	62.7
Arenicola marina	385.1	±99.7	23.8
Corophium volutator	313.8	±142.5	45.4
Petrofree mud			
Echinocardium cordatum	No data	-	-
Abra alba	5977	±1443	24.1
Arenicola marina	No data	-	-
Corophium volutator	No data	-	-
Fluoranthene			
Echinocardium cordatum	65.1	±39.6	60.8
Abra alba	110.3	±72.2	65.5
Arenicola marina	*	-	-
Corophium volutator	19.9	±10.3	51.9

* Mostly '>' data

7.3 Sedimentology results

Sedimentology of the A. marina, C. volutator and E. cordatum tests

The physical characteristics of the standardised sediment for the *A. marina, C. volutator* and the *E. cordatum* tests were analysed by courtesy of MAFF UK. From Table 11, it can be seen that many of the sediments failed to meet the guideline criteria on the basis of their median grain-size. However, all but one had the recommended organic matter content.
A multiple regression analysis (not reported here further) of the Bioban, Servo and Fluoranthene data for *A. marina, C. volutator* and *E. cordatum* against median grain size, the fraction <63 µm and the organic matter content failed to reveal any trend. There was no suggestion that the finer, more organic sediments reduced the toxicity of the three test substances in any way, nor was there any suggestion that the coarser or less organic sediments acted to increase toxicity.

These results confirm how difficult it can be to 'match' sediments to a recommended physical formula or guideline.

Table 11 *The granulometry of 10 sediments used in the* Arenicola marina, Corophium volutator *and* Echinocardium cordatum *tests (data courtesy of MAFF).*

	Median grain size (µm)	Fraction <63 µm (%)	Organic carbon content (%)	Estimated organic matter content (%)
Laboratory code				
Recommended range in guideline	**90 - 125**	**5 - 20**	**-**	**0.5 - 4**
Lab 4	77	38.4	0.64	1.2-1.6
Lab 6	97	5.4	0.12	0.2-0.3 *
Lab 8	73	9.3	0.19	0.3-0.5
Lab 9	143	6.9	0.43	0.8-1.0
Lab 12	156	16.0	1.22	1.2-3.5
Lab 13	37	81.9	0.76	1.4-1.9
Lab 14	176	7.2	0.73	1.3-1.8
Lab 15	74	17.5	0.21	0.4-0.5
Lab 16	163	9.3	0.37	0.7-0.9

* total organic matter was measured at 0.76% (weight loss on ignition)

Sedimentology of the Abra test sediment

No sedimentological data was made available with which to compare the different sediments used in the *A. alba* tests, or to allow any comparison with the the standardised sediment used in the other tests.

7.4 Control mortality

The control mortality data from all tests is presented below. The majority of the values in the tests were low, although not always within the 10% control mortality accepted by many OECD ecotoxicity test guidelines.

Table 12 *Mortality among control animals during all tests (% of the total control population per test, unless otherwise stated).*

	Abra alba	***Arenicola marina***	***Corophium volutator***	***Echinocardium cordatum***
Bioban P1487	32/462 individuals or 6.9±7.7 (All data)	0,7,0,0,40,-,0	18,16,40,5,3,6,4, 7,7,14,15,6,7	0, 10, 0, 0
Servo CK337	32/462 individuals or 6.9±7.7 (All data)	0,7,0,0,53,0,0	16,*,*,5,3,20,4, 20,27,14,*,6,7	0, 6, 0, 0
Petrofree mud	32/462 individuals or 6.9±7.7 (All data)	Not carried out	Not carried out	0
Fluoranthene	32/462 individuals or 6.9±7.7 (All data)	0,7,40,0,13,-,0	12,*,*,0,0,13,10, #,7,14,*,10,10	0, 0, 0, 0
Mean	6.6 ± 7.7	8.8 ± 16.4	11.0 ± 7.7	1.5 ± 3.1

* indicates shared control with Bioban test
\- indicates no data available
\# indicates test not carried out

Some of the observed control mortality with *C. volutator* may have resulted from the fact that animals of greater than 5mm were allowable in the test. Older (larger) animals from the previous year may have been included in the tested material, and by coincidence may have been dying off during the approximate period when the ring-test was carried out (Pers. comm. B. Roddie E.R.T.).

7.5 Comparison with the 1991 ring-test data (Bioban)

The 1991 Parcom ring-test also included sediment reworking species. However, due largely to poor participation, the results were deemed to be inconclusive. The test compound, Bioban P1487 was chosen again in the 1993 ring-test to allow some comparison with the 1991 data. The results are presented in Table 13.

Table 13 *A comparison of the results of the 1991 and 1993 sediment reworker ring-tests, with the compound Bioban P1487. (mg.kg-1 dry weight unless otherwise nidicated)*

Species	End point	Test conditions	Ring-test	
			1991	1993
Echinocardium cordatum	21d LC50	Industrial sand	22 (16-31) ≈180	
Echinocardium cordatum	14d LC50	Natural sediment		121 (73.3-191) 460
Abra alba	96-120h EC50	Data based on wet weight	74 (50-98) 87	10 42 (39-45)
Nereis virens	10d LC50		218 (100-1000) 1965 (1000-3300)	
Arenicola marina	10d LC50			51.7 (32-100) 69.2 (56-100)

All data are given as a range of lowest to highest values with the standard error in brackets

As can be seen from Table 13, the test conditions and methods have changed considerably between 1991 and 1993. The data for *A. abra* is similar, while the very large difference in sediment type and the shorter exposure time may explain why *E. cordatum* was five times less sensitive in 1993. The two polychaete worms give very different results, *N. virens* being very insensitive and highly variable, while *A. marina* is much more sensitive and has a low variability.

7.6 General remarks

7.6.1 Salinity

During the test programme, it became evident, that in static systems, some laboratories allowed the salinity to rise well above normal coastal values (31 to 35‰), as a result of evalopation. It was generally agreed that salinity should always be maintained at below 35‰ in all tests. A suitable lower level for testing all animals is 28‰.

7.6.2 Taxonomy

It is always recommended that field collected animals be correctly identified as being single species populations before use in testing. Quality assurance procedures should demand that a taxonomic inspection of a representative sample of each batch of animals is carried out and the results recorded. The following related species may sometimes be found at similar sampling locations.

- *Abra alba* is sometimes found with a visually similar species called *Abra nitida*. No data was forthcoming during the ring-test as to the presence or absence of *A. nitida* in the populations tested, which came from three locations in total.

- *Arenicola marina* is the only species in this genus on European coastlines and is difficult to confuse with any other species.

- *Corophium volutator* is sometimes found along with *C. arenaria*, which at some locations may replace it. Taxonomic data supplied by MAFF on eleven of the *Corophium* populations used during the ring-test indicated that two were comprised of *C. arenaria* and the other nine of *C. volutator*. For this reason, the guideline refers only to *Corophium* sp. (McMinn *et al.*, 1993).

- *Echinocardium cordatum* is occasionally found with another heart urchin *Bryssopsis lyrifera* in muddier sediments. *B. lyrifera* has grey-brown instead of golden spines, is squarer in shape and has a clearly outlined wide cross on the upper side of the test. The ring-test populations, which came from four separate sampling trips were comprised entirely of *E. cordatum*.

7.6.3 O_2 and pH

There was no indication that either O_2 or pH were disturbing or limiting factors in any of the tests reported here except for the *A. alba* test from Lab 6. However, it should be noted that the author only received this information verbally from the leading labs.

8. AVAILABILITY OF THE TEST ANIMALS

The availability of test animals is crucial to the effective harmonisation of test methods, as one or more species must become more or less universally available on a commercial basis. The data below includes the known distribution of the test species, all of which can be described as common within their specific biotopes. In principle, all four species are resident in 'sampleable' densities in most of the North Sea countries.

The actual availability is described in terms of the sources used in the present ring-test, although commercial sources may in fact be far more widespread.

Table 14 *Availability of the test animals.*

Species	Distribution	Supplier (distributor)	Alternatives
Echinocardium cordatum	Widely distributed *subtidally* in muddy-fine sand throughout the North Sea and coastal North Eastern Atlantic	RWS-North Sea Directorate (TNO); commercial supplier in the UK.	Secure new sampling locations in Denmark and Norway and commercial suppliers in The Netherlands.
Arenicola marina	Widespread in *tidal flat ecosystems* in muddy-fine sand	Various commercial worm suppliers and local collecting sites in The Netherlands, the UK and Denmark	
Corophium volutator	*Intertidal*, widely but locally distributed on the mid-shore in sometimes brackish muddy inlets and estuaries	Local field collections; many accessible sites in The Netherlands, the UK and Denmark	Develop laboratory culture techniques, secure sampling locations in Norway & Sweden.
Abra alba	Distributed patchily (locally dense) in *subtidal* mud in bays, estuaries and fjords throughout the coastal North Eastern Atlantic.	Ring test participants were supplied by Environor S.A., or secured their own independent supply.	Secure new sampling locations in the UK, and The Netherlands.

9. TIME AND MANPOWER ANALYSIS

9.1 Test method

9.1.1 TNO data

An analysis of all four tests was carried out by TNO, this is based on a number of constant factors as follows:

1. The tests were carried out under GLP and inspected by the Institutes Quality Assurance staff.
2. The sediment was collected as one lot of ca. 500kg for all test methods except the *A. alba* test and the figures are based on an average per test.
3. Preparation for the tests, cleaning up, reporting and other domestic activities have been standardised across the four tests.

It should be noted that the expert laboratories and those routinely familiar with the tests informally reported much shorter times in all cases.

Abra alba

Preparation of materials + dossier	2h
Sieving sediment:	8h
Selection of animals	4h
Sieve fecal pellets before preincubation	4h
Pre-incubation start and finish	2h
Spiking	2h
Start test	4h
analysis after 96h exposure	4h
analysis after 120h completion	4h
dry weight of faeces	3h
Calculation	0.5h
Quality assurance inspections	4h
Cleaning up	1h
Reporting	3h
Word processing	1.5h
TOTAL	**47h**

Corophium volutator

Preparation of materials + dossier	3h
Sediment collection + sieving: (0.5mm)	3.5h
WW/DW	0.5h
Test substance plus spiking	3h
Filling test vessels	1h
Water introduction	1.5h
Randomising test animals	4h
Environmental parameters x4	4h
Check on test animals x10	8h
Sieving and counting	4h
Cleaning up	1h
Calculation	0.5h
Quality assurance inspections	4h
Reporting	3h
Word processing	1.5h
TOTAL	**42.5h**

Arenicola marina

Preparation of materials + dossier	3h
Sediment collection + sieving: (0.5mm)	3.5h
WW/DW	0.5h
Test substance plus spiking	3h
Filling test vessels	1h
Water introduction	1.5h
Randomising test animals	0.5h
Environmental parameters x4	4h
Check on test animals x10	4h
Sieving and counting	4h
Cleaning up	1h
Calculation	0.5h
Quality assurance inspections	4h
Reporting	3h
Word processing	1.5h
TOTAL	**35h**

Echinocardium cordatum

Preparation of materials + dossier	3h
Sediment collection + sieving: (0.5mm)	3.5h
WW/DW	0.5h
Test substance plus spiking	3h
Filling test vessels	1h
Water introduction	2h
Randomising test animals	0.5h
Environmental parameters x4	4h
Check on test animals x14	4h
Sieving and counting	1h
cleaning up	1h
Calculation	0.5h
Quality assurance inspections	4h
Reporting	3h
Word processing	1.5h
TOTAL	**32.5h**

9.1.2 Water Quality Institute data

Abra alba	49h
Arenicola marina	44h
Corophium volutator	54h
Echinocardium cordatum	49h

9.2 The cost of test animals

The cost of test animals depends strongly upon whether a local supply is available, whether ship time is required and whether air or road freight is necessary. The following are global prices based on the experiences of the participating laboratories:

Abra alba	Hfl 600-1150
Arenicola marina	Hfl 60-400
Corophium volutator	Hfl 75-400
Echinocardium cordatum	Hfl 600-1000

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10. REFERENCES

Anon (1980). Biodegradability, Ecotoxicity and Bioaccumulation. Government Publishing Office, The Hague, The Netherlands.

Ashworth, J.H. (1904). Arenicola (the lug-worm). LMBC memoirs on typical British marine plants and animals. W.A. Herdman (ed.) Williams & Norgate Publ. London, 118pp plus plates.

Bowmer, C.T. (1993, unpublished). Parcom sediment reworker ring-test, 1993: Method for the assessment of acute toxicity of contaminated sediment using the burrowing sea urchin *Echinocardium cordatum*, 11 pp.

Buchanan, J.B. (1967). Dispersion and demography of some infaunal echinoderm populations. Symp. zool. Soc. Lond. 20: 1-11.

Crowe, W.A. (1985, unpublished Ph.D. Thesis). Aspects of the biology and ecology of the bivalvia in Kinsale Harbour (South Coast of Ireland) with particular reference to *Abra alba* (Wood) and *Mysella bidentata* (Montagu). National University of Ireland, 2 Vols., 156 plus 191pp.

Farke, H. & E.M. Berghuis (1979). Spawning, larval development and migration of *Arenicola marina* in the laboratory. Netherlands Journal of Sea Research, 13: 512-528.

Kooiman, S.A.L.M. (1981). Parametric analyses of mortality rates in bioassays. Water Res. 15 pp. 107-119.

McMinn, W., J. Thain, P. van den Hurk & B. Roddie (1993, unpublished). Paris Commission Ring-test Protocol: a sediment bioassay using an Amphipod *Corphium* sp., 14p.

Möller, P. and R. Rosenberg (1982). Production and abundance of the amphipod *Corophium valutator* on the west coast of Sweden. Neth. J. Sea Research, 16:127-140.

Neff, J.M. (1979). Polycyclic aromatic hydrocarbons in the aquatic environment: sources, fates and biological effects. Applied Science Publishers, London, 262pp.

Nijkamp, H. (1992). Some representative species of the Wadden Sea and the dependence on the physical environment. A.I.D.Environment Sea Division. Amsterdam, 235 pp.

Stephan, C.E. (1977). Methods for calculating LC50. In: Aquatic Toxicology and Hazard Evaluation, F.L. Meyer & J.L. Hamelink (Eds.). ASTM, Philadelphia. ASTM STP, pp 65-89.

Strømgren, T. (1993, unpublished). Test protocol for Abro-test: PARCOM Ring-test, 11p + figs.

Thain, J. & S. Bifield (1993, unpublished). Paris Commission Ring-test Protocol: a sediment bioassay using the polychaete *Arenicola marina*, 8p plus Appendices: 1. Reference/Base sediment guideline; 2. Spiking procedure.

Watkin, E.E. (1941). The yearly life cycle of the amphipod *C. volutator*. J. Anim. Ecol. 10: 77-93.

APPENDIX I

Table I.1 *The complete test results for the tellinid bivalve* Abra alba *(Leading lab. Bioconsult). 96-120h EC50, faecal pellet production. in mg.kg-1 dry weight.*

Test substance	Laboratory Code	EC50 mg.kg^{-1} dry weight	Standard error (95%)
Bioban P1487			
	Lab 1	350	275-425
	Lab 2	525	487.5-562.5
	Lab 3	300	200-400
	Lab 4	212.5	175-250
	Lab 5	425	237.5-662.5
	Lab 6	125*	
Servo CK337			
	Lab 1	212.5	187.5-237.5
	Lab 2	375	337.5-412.5
	Lab 3	200	137.5-262.5
	Lab 4	87.5	25-150
	Lab 5	462.5	350-575
	Lab6	100	25-75
Fluoranthene			
	Lab 1	100	75-125
	Lab 2	Test failed	
	Lab 3	187.5	162.5-212.5
	Lab 4	16.3	8.8-23.8
	Lab 5	137.5	100-175
	Lab 6	*** >625	
Petrofree			
	Lab 1	6100	5663-6538
	Lab 2	5062	4288-5838
	Lab 3	6213	5163-7263
	Lab 4	8163	7225-9100
	Lab 5	No Result	
	Lab 6	4350#	3425-5275

* The LC50 was calculated by the Kooijman method as 327 mg.kg^{-1} dry weight.
\# The LC50 was calculated by the Kooijman method as 8260 (7080-9630) mg.kg^{-1} dry weight.
*** Indicates an incalculatable result.

Table I.2 *The test results for the polychaete Arenicola marina (Leading lab.: MAFF)., 10d LC50, mg.kg^{-1} dry weight.*

Test substance	Laboratory code	LC50 mg.kg^{-1} dry weight	Standard error (95%)	
Bioban P1487				
	Lab 3	>100		
	Lab 5	54.4	50	100
	Lab 6	69,2	56	100
	Lab 7	62.7	56	100
	Lab 8	51.7	32	100
	Lab 9	55	33	100
	Lab 11	>100		
Servo CK337				
	Lab 3	343.9	330	560
	Lab 5	***		
	Lab 6	239.3	100	320
	Lab 7	421.4	316	562
	Lab 8	369.2	320	560
	Lab 9	393.3	330	1000
	Lab 11	543.6	377	671
Fluoranthene				
	Lab 3	No data		
	Lab 5	155.8		
	Lab 6	>3300		
	Lab 7	>1000		
	Lab 8	>1000		
	Lab 9	>1000		
	Lab 11	>1000		

Note: a *** indicates an incalcalculatable result. However, a reliable '>' value may, or may not be available.

Table I.3 *The test results for the Amphipod Corophium volutator (Leading lab.: MAFF), 10d LC50 mg.kg-¹ dry weight.*

Test substance	Laboratory code	LC50 mg.kg^{-1} dry weight		Standard error (95%)	
Bioban P1487					
	Lab 4	114.5		105.4	143
	Lab 5	***	> 100		
	Lab 6	71.8		56	100
	Lab 7	***	< 56		
	Lab 8	54.3		32	100
	Lab 9	66.9		41	77
	Lab 10	117.2			
	Lab 11	***	> 70.9		
	Lab 12	***	> 148.9		
	Lab 13	42		34	88
	Lab 14	***	> 135.1		
	Lab 15	64		41.1	128.3
	Lab 16	52.5		32	100
Servo CK337					
	Lab 4	331.5		164.7	429.9
	Lab 5	***	> 600		
	Lab 6	240		180	320
	Lab 7	***	< 100		
	Lab 8	107.7		32	100
	Lab 9	514.2		128	720
	Lab 10	302.2		130.8	439.1
	Lab 11	***	> 239.2		
	Lab 12	123.4		83.4	148.9
	Lab 13	473.3		220	1400
	Lab 14	424.1		189.4	757.6
	Lab 15	307.9		41.1	718.6
	Lab 16	***	< 100		
Fluoranthene					
	Lab 4	33.1		16.9	65.9
	Lab 5	33.1		10	60
	Lab 6	14.8		10	18
	Lab 7	***	< 33		
	Lab 8	5.8		3.2	10
	Lab 9	19.6		13	41
	Lab 10	22.1		12.4	42.6
	Lab 11	***	< 26.2		
	Lab 12	23.8		14.9	47.7
	Lab 13	Not carried out			
	Lab 14	22.3		9.2	82.2
	Lab 15	4.1		1.3	12.8
	Lab 16	***	< 33		

Note: a *** indicates an incalcalculatable result,. However a reliable '>' value may, or may not be available.

Table I.4 *The test results for the sea urchin* Echinocardium cordatum *(Leading lab.: TNO)., 14d LC50 in mg.kg^{-1} dry weight.*

Test substance	Laboratory code	LC50 mg.kg^{-1} dry weight	Standard error (95%)	
Bioban P1487				
	Lab 5	270	212	344
	Lab 6	179	141	227
	Lab 9	121	77.3	191
	Lab 10	>341 <593		
Servo CK337				
	Lab 5	151	96.4	238
	Lab 6	115	76.2	167
	Lab 9	168	103	273
	Lab 10	120*	84	173
Petrofree				
	TNO	>32000		
Fluoranthene				
	Lab 5	33	17.2	63.1
	Lab 6	34	18.9	62.5
	Lab 9	77	17.5	338
	Lab 10	116	47.2	286

* Result calculated as the 10d LC50.