Inter-laboratory comparison of five marine bioassays for evaluating the toxicity of dredged material

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Four laboratories were compared to ascertain the reproducibility of test methods for five bioassays: the ten day whole sediment bioassay with the amphipod Corophium volutator*, the fourteen day whole sediment bioassay with adult sea urchins* Echinocardium cordatum*, the Microtox solid phase bioassay with the bacterium* Vibrio fischeri*, the oyster larvae sediment elutriate bioassay using* Crassostrea gigas *and the sediment pore water bioassay with the rotifer* Brachionus plicatilis*. The bioassays were all conducted according to the standard operating procedures of the National Institute for Coastal and Marine Management/RIKZ and carried out with one control sediment and three moderately contaminated dredged materials from the Netherlands. Reference toxicant tests were also performed for every bioassay, to assess the condition of the test species. Reproducibility and inter-laboratory variability were evaluated by calculating coefficients of variation for the sediment bioassays and considering the ability of each laboratory to achieve the test acceptability criteria and to identify a number of confounding factors.*

The bioassays involving the amphipods and sea urchins had an acceptable inter-laboratory variability, with average coefficients of variation of 20% or less. The Microtox solid phase bioassay showed high reproducibility and the least variability among laboratories, with average coefficients of variation of 12%. In contrast, the results for percent net response in the oyster larvae bioassay were very variable and poorly reproducible: in three of the four sediments the coefficients of variation exceeded 100%. Two laboratories did not meet the test acceptability criterion for oyster embryo development in the control sediment. Survival in the rotifer bioassay also varied greatly among laboratories with average coefficients of variation of 48%.

We conclude that the main sources of inter-laboratory variability were 1) individual differences in the skill and experience of laboratory technicians (for the amphipod, oyster larvae and rotifer bioassays); 2) the use of different batches of test organisms (sea urchin bioassay) and 3) the use of different dilution water (oyster larvae bioassay).

Keywords: Corophium volutator, *Echinocardium cordatum*, *Brachionus plicatilis*, *Crassostrea gigas*, *Vibrio fischeri*

Introduction

Sediment bioassays are commonly used to evaluate the suitability of dredged material for offshore disposal; to rank contaminated sites for clean up and to estimate the efficacy of remediation (Burton et al., 1996a). They provide useful information on the bioavailability and combined effects of the cocktail of toxicants in

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sediment. Since the mid-1970s, they have been widely used in North America for regulating the ocean or inland water disposal of dredged material (EPA/USACE, 1998). Standard operating procedures (SOP) for marine sediment bioassays (Environment Canada, 1992b; ASTM, 1995) describe the exposure of various species of marine invertebrates to sediment, sediment extract, or pore water under controlled laboratory conditions, evaluating different endpoints such as survival, growth or reproduction.

In Europe in recent years, the growing interest in measuring biological impact has generated demand for SOPs to be developed for various indigenous test species (Fleming et al., 1996). In The Netherlands, the environmental management of contaminated dredged material from marine harbours entails deciding whether to dispose of the material offshore or in confined disposal facilities. These decisions are currently based solely on the chemical composition of the bulk sediment. A national document on water management in the Netherlands for the period 1998–2006 (V&W, 1998) set a target of improving the assessment of the hazards of contaminated dredge materials. It has been suggested (Stronkhorst et al., 1996, 1997) that sediment toxicity bioassays could become a standard part of the procedure for licensing the offshore disposal of dredged material. This has led to SOPs being developed for a number of bioassays for evaluating dredged material in The Netherlands (RIKZ, 1999). These SOPs provide detailed information on every aspect of the bioassay, including: 1) collection and preparation of sediments; 2) preparation of test chambers and experimental set-up; and 3) Quality Assurance/Quality Control aspects (use of positive and negative controls, measurement of water quality conditions).

In addition to standardisation, two other important prerequisites to enable bioassays to be used as regulatory tools are good reproducibility and acceptable inter-laboratory variability. However, very little information is available on the ability of different laboratories to reach the same conclusion when evaluating given marine sediment, or on the sources of inter-laboratory variability. The various factors suggested as possible causes of bioassay variability include the source, handling and sensitivity of the test organisms, the quality of the overlying water, and the training and experience of laboratory personnel (Mearns, 1986; Williams et al., 1986; Schlekat et al., 1995; Burton et al., 1996b). To elucidate these factors, we conducted an inter-laboratory evaluation involving five bioassays and four laboratories active in marine sediment toxicity testing in The Netherlands and Belgium. The five bioassays and the reasons for selecting them are:

1. The 10-d whole sediment survival bioassay with the amphipod *Corophium volutator*: this bioassay has become a benchmark in Europe for contaminated marine sediment testing due to its availability and ecological relevance for intertidal areas (Chapman et al., 1992; Van den Hurk et al., 1992; Thain et al., 1996; Ciarelli et al., 1997, 1998; Stronkhorst et al., 1997);
2. The 14-d whole sediment survival bioassay with the sea urchin *Echinocardium cordatum*: this bioassay is ecologically relevant for the North Sea region, has a large dynamic range and great sensitivity (Stronkhorst et al., 1997) and is considered a reproducible bioassay (Bowmer, 1993);
3. The Microtox solid phase bioassay with the bacterium *Vibrio fischeri*: this 20-min sediment dilution test observing the reduction in bioluminescence has been recommended as a screening tool (Burton et al., 1996a; Svenson et al., 1996; Cheung et al., 1997);
4. The oyster embryo-larvae development bioassay using *Crassostrea gigas*: This 48-h sediment elutriate bioassay developed by Thain (1991) is considered to be a sensitive early-life-stage-test and has been proved to discriminate among different levels of sediment contamination in the North Sea (Butler et al., 1992; Thain et al., 1996; Van den Hurk et al., 1997) and
5. The sediment pore water survival bioassay with the rotifer *Brachionus plicatilis* (Rotoxkit^{TM}): This 24-h pore water bioassay is regarded as a cost effective acute toxicity bioassay (Snell and Persoone, 1989).

The objectives of this study were to determine for each bioassay: 1) the differences in mean responses among laboratories; 2) the ability of each laboratory to meet the test acceptability criteria (TAC) of the bioassays and to identify several confounding factors as described in the SOPs and 3) to discuss the possible causes of inter-laboratory variation, with an eye to improving overall performance in sediment toxicity testing.

Methods and materials

General design of the study

This study entailed testing four sediments with five bioassays. The four laboratories had previous

experience in applying at least two of the selected bioassays. To maintain anonymity, each laboratory was assigned a number (1 to 4). The National Institute for Coastal and Marine Management/RIKZ provided the SOPs (RIKZ, 1999) detailing the bioassay methods, and distributed aliquots of four sediments. To evaluate the variability in the responses of the bioassays among the sediment aliquots, one laboratory was selected to carry out a specific bioassay with three aliquots of each sediment.

Sediments

The control sediment and three moderately contaminated harbour sediments used were collected in October 1998. The control sediment was collected from an intertidal mudflat (Oesterput) in the Eastern Scheldt estuary in southwest Netherlands (51°36′N, 3°48′E). Two sediments were collected from the port of Rotterdam: Botlek section 66 (51°52′N, 4°18′E) and Botlek section 73 (51°53′N, 4°17′E) and the third were collected from Den Helder harbour, section 5 in northern Netherlands (52°57′N, 4°47′E). Dredged material from these locations is regularly dumped in the North Sea and Wadden Sea. In each section samples were collected at six locations with a Van Veen grab and pooled on board ship. This was repeated three times to obtain three separate aliquots. At the RIKZ laboratory, the sediments were homogenised for 30 min, divided into aliquots, sent to the participating laboratories in high-density polyethylene containers and stored at 4°C.

The grain size fractions (<2 μm, <16 μm, <63 μm) of the sediments were determined according to the Dutch standard NEN 5753. Total organic carbon content (TOC) was measured with a carbon analyser (conversion at 1200°C). Destruction methods for trace metal analysis were according to the Dutch standard NEN 5770 and total metal analysis was performed using atomic adsorption spectrometry and inductive coupled plasma (Cd, Cr, Zn, Cu, Ni, Pb, As) or cold vapour technique (Hg). Organochlorine pesticides (OCPs: α-, β- and γ-HCH, HCB, α-endosulfan, aldrin, dieldrin, endrin, isodrin, DDE, DDD and DDT) and polychlorinated biphenyls (PCBs: congeners 28, 52, 101, 118, 138, 153, 180) were quantified after extraction with pentane, cleanup over a SiO_2 column and determination on a GC with electron capture detector. Polycyclic aromatic hydrocarbons (PAHs: anthracene, naphthalene, phenanthrene, fluoranthene, benzo (a) anthracene, chrysene, benzo (a) pyrene, benzo (k) fluoranthene, benzo (ghi) perylene, and indeno (1,2,3-cd) pyrene) were quantified using HLPC-UV/Fluorescence. Mineral oil (the fraction C10-C40) was analysed with GC following the Dutch standard method NEN 5733. TBT was analysed according to the method described by Stäb et al. (1993).

Bioassays

The bioassays were conducted by all laboratories in November 1998 except for the sea urchin bioassays, which were performed in May 1999. The bioassays were conducted according to the SOPs in RIKZ (1999). Bioassay results were approved if responses to control sediment and reference toxicants satisfied TAC. For the bioassays with *Corophium volutator*, *B. plicatilis* and *V. fischeri* we adopted reference toxicants prescribed in previous protocols and for the bioassays with *E. cordatum* and *Crassostrea gigas* we used ammonium chloride.

Corophium volutator

All participating laboratories used amphipods of the same age collected at the same time and place as the control sediment (Oesterput, Eastern Scheldt, SW Netherlands). Test organisms were acclimatised for more than 1 week prior to testing. The amphipod bioassay consisted of a 10-d static exposure at 15 ± 2°C and involved 5 replicate test chambers (1-L beakers) per treatment, with each replicate containing 20 amphipods. All laboratories used seawater from the Eastern Scheldt for testing, except Laboratory 2, which used seawater from 'Ecloserie Marine' (France). Laboratory 1 tested variability among three aliquots from each of the four sediments. At the end of all bioassays, survivors were counted; the results are presented as mean survival percentages. A 72-h water-only reference toxicity test with cadmium chloride ($CdCl_2$) was performed to assess test organism sensitivity. This test was carried out in duplicate at 15 ± 2°C and a salinity of 32 ± 4 gl^{-1}. Each test beaker contained 20 amphipods.

Echinocardium cordatum

The participating laboratories used adult heart urchins collected in May 1999 during four successive occasions at the same location along the Dutch North Sea coast and distributed to each laboratory separately. Though the individual weight of the sea urchins was more than that stipulated by the SOP (35 to 38 g versus 10 to 30 g), for logistical reasons the bioassays were nevertheless performed. Test organisms were acclimatised for more than 1 week prior to testing.

The bioassay consists of a 14-day whole sediment exposure in an aquarium of 750 cm^2 containing a ca. 6 cm thick layer of sediment overlain with water with a flow-through of 10 l per day. There were 3 replicates per treatment, each with 10 organisms. Laboratory 4 tested the variability of three aliquots of each sediment. All laboratories used seawater from the Eastern Scheldt for testing, except for laboratory 2, which used seawater from 'Ecloserie Marine' (France). At the end of the exposure, the number of dead organisms was recorded, as was the number of organisms that were unable to burrow into clean sand within 30 min. The results are expressed as mean survival percentage and mean percentage of organisms that were able to burrow into clean sand. A 96 h water-only reference toxicity test using ammonium chloride (NH_4Cl) was performed to assess test organism sensitivity. These bioassays were performed in 1L glass beakers, and three replicates, each with 5 organisms, were used. Bioassays were carried out at 15 ± 2°C and a salinity of 32 ± 4 g l^{-1}.

Vibrio fischeri

In the Microtox solid-phase bioassay the adverse effect of sediment-associated contaminants is measured as the inhibition of bioluminescence of the marine bacterium, *Vibrio fischeri* (Microbics, 1992). All laboratories obtained batches of bacteria through Azur Environmental (UK). Sediments were tested as suspensions prepared with Microtox solid-phase reagent and diluted into 13 concentrations ranging from 0.005 to 19.7% sediment. Laboratory 3 tested the variability among three aliquots. Bioluminescence was measured after a 20-min exposure period at 15 ± 1°C and a salinity >8 g l^{-1}. The effect was expressed as the sediment concentration that caused a 50% reduction in bioluminescence (EC_{50}; vol.% wet sediment). Zinc sulfate ($ZnSO_4$) was used as reference toxicant and dilution series with Microtox Solid Phase dilution was performed in the same way as was done for the sediment suspensions. The exposure period was 20 min.

Crassostrea gigas

The oysters used by all laboratories were supplied by Guernsey Sea Farm (Channel Islands, United Kingdom). Elutriates were prepared by pouring sediment and seawater into 1–l test chambers at a dilution of 1:50 (w/w). After homogenizing the sediment/water suspensions for 24 h on a roller bank at 0.3 m s^{-1} and the sediment had settled, embryos were added. Within 1 h after fertilization, approximately 40000 embryos were inoculated into each 1–l bottle, resulting in a concentration of about 40 embryos ml^{-1}. Exposures occurred in a temperature-controlled room for 48 h at 20 ± 2°C and a salinity of 32 ± 4 g l^{-1}. Two of the four laboratories used seawater from the Eastern Scheldt, but laboratories 2 and 4 used seawater from Guernsey Sea Farm and from Huisduinen (North Sea), respectively. The bioassay involved 5 replicates per treatment. Laboratory 1 tested the variability among three aliquots. After 48 h the overlying seawater was carefully poured into a clean container without disturbing the sediment. Sub samples of 10 ml were transferred into glass vials and preserved in 5% buffered formalin. Larvae were examined by microscopy and the number of normal D-shaped larvae were counted and related to the number of normal D-larvae found in the seawater controls. Results are expressed as Percentage Net Response (PNR), defined as:

$$\mathrm{PNR} = \frac{\mathrm{S} - \mathrm{C}}{100 - \mathrm{C}} * 100 \qquad (1)$$

where S = percentage of abnormal larvae in sediment elutriate and C = percentage of abnormal larvae in seawater control.

A PNR value of 0 or close to 0 indicates that the response was similar to that of the seawater control, and a PNR value of 100 indicates that all larvae showed abnormal development or died. A 48 h water-only reference toxicity test using ammonium chloride (NH_4Cl) was performed to assess oyster larvae sensitivity.

Brachionus plicatilis

Rotifer cysts were supplied by Florida Aqua Farms (Dade City, Florida, USA). Pore water was obtained by centrifuging 50 g of sediment for 20 min. at 2500 G. The pore water was then poured into 0.5–l opaque bottles and a dilution series using control artificial seawater in 10 ml volumes was prepared to obtain concentrations ranging from 0 to 100% pore water. Rotifer cysts were placed in a multi-well plate and incubated at 25 ± 2°C and a salinity of 32 ± 4 g l^{-1} for 28 h under a light source of 3000 to 4000 lux to induce hatching. The bioassay involved six replicates per concentration and five neonates per replicate were placed in each test container. After 24 h in the dark, the multi-well plate was placed under a microscope and the number of survivors recorded. Results are expressed as mean percentage survival after 24 h of exposure. A 24 h reference toxicant test with potassium bichromate ($K_2Cr_20_7$) was used to determine test organism sensitivity.

Data analyses

For each sediment, inter-laboratory differences in mean survival were determined using analyses of variance (ANOVA) when the data were normally distributed and the variances were homogeneous. If the assumptions of normality and homogeneity of variances were not met even after arcsine transformation, the non-parametric Kolmogorov-Smirnov test was used. Differences in toxicity between the control sediment and contaminated sediments were tested with the student-t test or, if an ANOVA had already been done, with Dunnett's test. The concentrations of the reference toxicants that caused 50% mortality (LC_{50}) or a 50% inhibition of bioluminescence (EC_{50}) were calculated using the trimmed Spearman-Karber method (Hamilton et al., 1977). Statistical differences among sub-samples were determined using ANOVA. Statistical significance was evaluated at $p < 0.05$.

Test reproducibility and inter-laboratory variability were quantified by calculating the Coefficient of Variance (CV, %) as follows:

$$CV = \frac{SD}{\bar{X}} * 100\% \quad (2)$$

where SD = standard deviation of grand mean, $\bar{X}$ = grand mean, that is, the overall mean of the laboratory means.

To check for the confounding influence of the water quality parameters on sediment toxicity, the data on sulphide, ammonia, salinity and pH in the different sediment matrices were compared with modifying factor criteria (MFC) published by Postma et al. (2002).

Results and discussion

Table 2 shows the physico-chemical and chemical characteristics of the sediments used in the inter-laboratory comparison. The three harbour sediments were silty sludge with a small dry weight fraction (ca. 30%) and a high content of fines (ca. 65%, <63 μm). The control sediment from the Oesterput intertidal flat in the Eastern Scheldt was more compact, with a 56% dry weight and 36% fines. The Botlek 66 and Botlek 73 sediments had similar concentrations of metals, PCB, PAHs, organochlorine, TBT and mineral oil, which were slightly higher than in the Den Helder sediment and substantially higher than the control sediment (3 to 9 times or, as in the case of TBT, ca. 100 times higher).

The laboratories that tested the sediments in triplicate found no significant sub sampling variation in any of the bioassays, and therefore these data were pooled. In all bioassays the concentration of dissolved oxygen was sufficiently high (data not shown).

Corophium volutator

Three of the four laboratories met the TAC for survival in the control sediment. These three laboratories obtained similar bioassay results with the harbour sediments (Figure 1). Laboratory 2, however, did not achieve the criterion of >90% survival (Table 1) and found significantly lower survival in the harbour sediments than the other laboratories. The two probable causes for these deviating results were this laboratory's different test conditions and less experience in handling the amphipods.

All laboratories met the TAC for the reference toxicant $CdCl_2$ (Table 2). The LC_{50} of Laboratory 4 was somewhat higher compared to the others, probably because the $CdCl_2$ stock solution was prepared in seawater instead of in de-ionized water as was done by the other laboratories.

All the laboratories reported that the Den Helder sediment was significantly toxic compared to the control sediment (Figure 1). The grand means in survival observed by all four laboratories ranged from 93% in the control sediment to 33% in Den Helder sediment (Table 3). The coefficients of variation (CV) ranged from 4% in the control sediment to 48% in Den Helder sediment (Table 3). On average, the CV was 21%, but it fell to 5% when the results of Laboratory 2 were excluded. These CVs are similar to or lower than those reported by Bowmer (1993) for a ring test involving 8 laboratories, which involved *Corophium volutator* being exposed to spiked sediments (CV of 39 to 52%). Our results were also in line with CVs reported for other amphipods, for instance, by Schlekat et al. (1995) for *Eohaustorius estuarius* (1.5–45%), *Leptocheirus plumulosus* (5–65%) and *Ampelisca abdita* (5–146%) and by Mearns et al. (1986) for *Rhepoxynius abronius* (6–81%).

In pore water of the Den Helder the average sulphide and ammonium contents of the sediment were rather high (15 and 133 mg l^{-1}, respectively) and may have been critical for *Corophium volutator*. However, concentrations of ammonium on overlaying water did not exceed the TAC (Table 4). This amphipod is one of the most suitable and recommended species for evaluating the toxicity of contaminated sediments

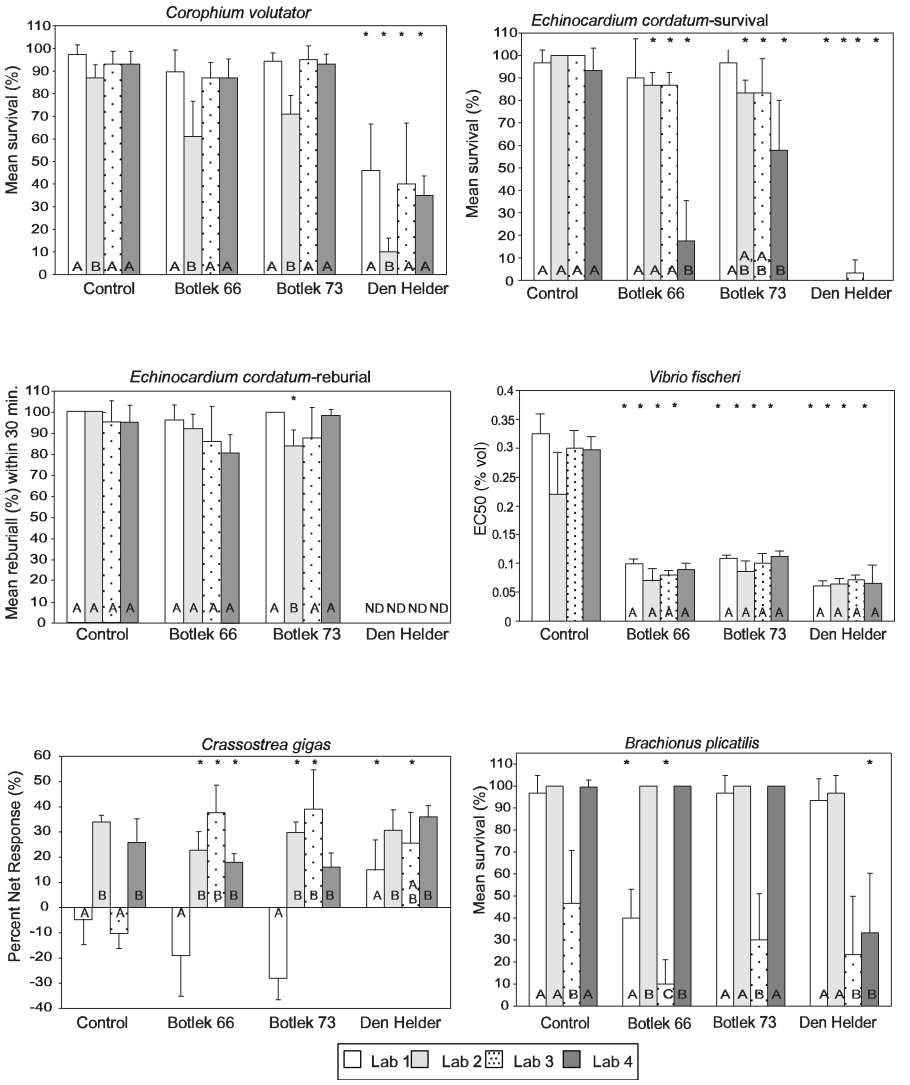

Figure 1. An inter-laboratory comparison of sediment toxicity tests with control sediment and three harbour sediments conducted by four laboratories using the *Corophium volutator* bioassay (mean survival ± SD), the *Echinocardium cordatum* bioassay (mean survival and re-burial ± SD), the *V. fischeri* bioassay (EC_{50} ± 95% CI), *C. gigas* embryo bioassays (PNR ± SD) and the *B. plicatilis* bioassay (mean survival in undiluted pore water ± SD). Laboratories that share the same letter are not significantly different from each other. Asterisks indicate significant differences of the harbour sediments from the control within each laboratory (ANOVA). The number of replicates (n) is: *Corophium volutator* n = 5 and n = 15 for Lab 1; *Echinocardium cordatum* n = 3 and n = 9 for Lab 4; *V. fischeri* n = 1 and n = 3 for Lab 3; *Crassostrea gigas* n = 5 and n = 15 for Lab 1; *B. plicatilis* n = 6 and n = 18 for Lab 2. ND = not determined.

in Europe because of its sensitivity, ecological relevance, abundance and availability (Van den Hurk et al., 1992; Chapman et al., 1992; Thain et al., 1996; Stronkhorst et al., 1997; Ciarelli et al., 1997, 1998). In the last decade, Laboratories 1, 3 and 4 have frequently used *Corophium volutator*, which probably accounts for the high reproducibility among these laboratories. Our findings support the use of this bioassay as a regular tool.

Echinocardium cordatum

All laboratories reported sufficiently high survival in the control sediment (Table 1). Exposure to Den Helder sediment, on the other hand, caused high mortality (Figure 1). In both cases, no significant differences among laboratories were found.

As already noted, to avoid possible differences in the sensitivity of the test organisms the SOP stipulates that

Table 1. Responses to control sediment (mean and standard deviation) and reference toxicants (mean and 95% confidence interval) for five marine sediment bioassays at the four participating laboratories. Data in bold exceed the corresponding test acceptability criteria (TAC) for control sediment and reference toxicant (RIKZ, 1999).

Test Species	Laboratory	Control Sediment	Reference Toxicant
Corophium volutator		Survival (%)	LC_{50} for Cd^{2+} (mg/l)
		TAC: >90%	TAC: 2–10.3
	Lab 1	97 (4.2)	3.6 (3.2–4.1)
	Lab 2	**87** (5.7)	2.7 (2.3–3.2)
	Lab 3	93 (5.7)	2.8 (2.3–3.4)
	Lab 4	93 (5.7)	7.8 (6.7–9.0)
Echinocardium cordatum		Survival (%)	LC_{50} for NH_4^+ (mg/l)
		TAC: >90%	TAC: 7–27
	Lab 1	97 (5.0)	>**60**
	Lab 2	100 (0.0)	>**60**
	Lab 3	100 (0.0)	>**60**
	Lab 4	93 (10)	28 (19–40)
Vibrio fischeri		EC_{50} (% vol.)	EC_{50} for Zn^{2+} (mg/l)
		TAC: >0.20	TAC: 2.1–8.5
	Lab 1	0.33 (0.30–0.36)	7.1 (6.6–7.4)
	Lab 2	0.22 (0.17–0.29)	7.4 (6.0–9.2)
	Lab 3	0.29 (0.27–0.33)	7.1 (6.9–7.3)
	Lab 4	0.30 (0.27–0.32)	3.9 (3.7–4.1)
Crassostrea gigas		PNR (%)	EC_{50} for NH_4^+ (mg/l)
		TAC: <10%	TAC: 4–10.6
	Lab 1	− 4.6 (10)	7.2 (7.0–7.3)
	Lab 2	**34.6** (2.6)	6.2 (5.4–7.0)
	Lab 3	− 10.2 (6.1)	6.4 (5.7 –7.2)
	Lab 4	**25.8** (9.4)	**3.5** (3.1–3.9)
Brachionus plicatilis		Survival (%)	LC_{50} for $K_2Cr_20_7$(mg/l)
		TAC: >80%	TAC: 210–460
	Lab 1	97 (8)	243 (203–274)
	Lab 2	100 (0.0)	371 (337–409)
	Lab 3	**47** (24)	328 (280–384)
	Lab 4	99 (3.3)	263 (225–308)

the weight of the sea urchins must be within a particular range (10 to 30 g). However, the sea urchins used in this inter-laboratory comparison were larger, which was probably why in three laboratories the tests with reference toxicant NH_4Cl resulted in LC_{50} above the maximum concentration of 60 mg l^{-1} (Table 1). The batch of sea urchins at Laboratory 4, however, had a LC_{50} of 28 mg l^{-1} and seemed more sensitive. Indeed, both Botlek sediments (Figure 1) showed higher mortality at Laboratory 4 than at the other laboratories. This single finding is in contrast with the limited ability of acute water-only reference toxicity tests to evaluate the condition of benthic species like the amphipod *Hyalella azteca* (McNulty et al., 1999). It is known that one of the factors that can affect inter-laboratory variability is the source of the test organism (Schlekat et al., 1995; Burton et al., 1996b). We believe that the transport and acclimatization of these test organisms prior to testing affected our results. The CVs for survival ranged from 3 to 50% (Table 3), which is comparable to the CVs of 18 to 61% reported by Bowmer (1993) in a previous inter-laboratory comparison with *Echinocardium cordatum* using spiked sediments. When the data from Laboratory 4 were excluded, the range in the CVs for sea urchin survival was only 2 to 9% (Table 3). The results after the heart urchins had been reburied in clean sand after 14 d exposure to the harbour sediments (Figure 1) did not differ significantly from the results of

Table 2. Physico-chemical and chemical characteristics of the sediments used in the inter-laboratory comparison. Values are means (SD) of 3 replicates.

Parameter	Unit	Sediment			
		Control	Botlek 66	Botlek 73	Den Helder
Physicochemical characteristics					
Dry weight	% dry wt.	56 (0.6)	30 (0.15)	32 (1.4)	23 (0.51)
Particle size <2 μm	% dry wt.	12 (0.0)	30 (1.5)	23 (3.2)	27 (4.0)
Particle size <16 μm	% dry wt.	16 (0.0)	52 (0.0)	40 (4.4)	52 (2.3)
Particle size <63 μm	% dry wt.	36 (1.5)	63 (6.1)	66 (4.0)	67 (4.0)
TOC	% dry wt.	1.5 (0.06)	3.6 (0.12)	3.5 (0.3)	3.2 (0.06)
Metals					
Arsenic	mg kg^{-1} dry wt	9.3 (0.2)	19 (1.5)	19 (1.0)	17 (1.1)
Cadmium	mg kg^{-1} dry wt	<0.5	1.8 (0.2)	2.1 (0.1)	0.63 (0.06)
Chromium	mg kg^{-1} dry wt	29 (0.0)	76 (7.0)	80 (2.1)	56 (4.0)
Copper	mg kg^{-1} dry wt	6.2 (0.4)	52 (5.3)	57 (3.6)	21 (1.5)
Mercury	mg kg^{-1} dry wt	0.17 (0.02)	0.94 (0.07)	0.86 (0.06)	0.39 (0.03)
Lead	mg kg^{-1} dry wt	18 (0.6)	68 (6.7)	74 (1.1)	41 (2.3)
Nickel	mg kg^{-1} dry wt	8.2 (0.1)	28 (2.5)	28 (0.6)	20 (1.1)
Zinc	mg kg^{-1} dry wt	54 (1.5)	283 (20.8)	313 (11.5)	123 (5.8)
Organic contaminants					
PAHs (sum of 10)	mg kg^{-1} dry wt	0.27 (0.2)	2.33 (0.6)	2.07 (0.2)	0.93 (0.4)
PCBs (sum of 7)	μg kg^{-1} dry wt	<7	31. 8 (5.2)	41.6 (11.9)	<7
Organochlorines	μg kg^{-1} dry wt	<1	<1	<1	<1
Mineral oil (C10–C40)	mg kg^{-1} dry wt	50 (0.0)	370 (132)	287 (127)	213 (45)
TBT	μg Sn kg^{-1} dry wt	1.3 (0.0)	105 (13)	203 (40)	69 (18)

Table 3. Inter-laboratory variability in survival of *Corophium volutator*, *Echinocardium cordatum* and *B. plicatilis* (%) and in inhibition of bioluminescence of *V. fischeri* (EC_{50}, vol. % sediment) for each of the sediments. Results are presented as grand mean, standard deviation (SD) and coefficients of variance (CV, %). N = number of laboratories.

Test Species	N	Control		Botlek 66		Botlek 73		Den Helder	
		Mean (SD)	CV	Mean (SD)	CV	Mean (SD)	CV	Mean (SD)	CV
Corophium	4	93 (4)	4	81 (13)	17	88 (12)	13	33 (16)	48
volutator	3[a]	94 (2)	2	88 (1)	1	94 (1)	1	40 (6)	14
Echinocardium	4	98 (3)	3	70 (35)	50	80 (16)	20	1 (2)	–[d]
cordatum	3[b]	99 (2)	2	88 (2)	2	88 (8)	9	1 (2)	–[d]
Vibrio fischeri	4	0.28 (0.04)	16	0.09 (0.01)	13	0.11 (0.11)	12	0.07 (0.004)	7
Crassostrea gigas	4	11 (22)	195	15 (25)	162	14 (30)	209	27 (9)	34
Brachionus	4	86 (26)	30	62 (45)	72	82 (34)	42	68 (32)	47
plicatilis[e]	3[c]	99 (2)	2	80 (35)	43	99 (2)	2	74 (36)	48

[a] Excluding Lab 2 that did not achieve the TAC for control sediment.
[b] Excluding Lab 4 that use test organisms with a lower LC_{50} for the reference toxicant.
[c] Excluding Lab 3 that did not achieve the TAC for control sediment.
[d] Not calculated as survival was close to zero.
[e] Undiluted pore water.

Table 4. Range in salinity, pH and concentrations of ammonium and sulfide in different bioassay matrices, as observed at the four participating laboratories. None of the observations exceeded the modifying factor criteria (MFC[a]).

Parameter	Matrix	Test Species	Unit	MFC	Sediment			
					Control	Botlek 66	Botlek 73	Den Helder
Salinity	Overlying water[b]	*Corophium volutator*	g l^{-1}	4–40	31–36	29–32	29–33	30–35
	Overlying water[b]	*Echinocardium cordatum*	g l^{-1}	>28	29–33	29–33	29–33	28–33
	Pore water[c,d]	*V. fischeri*, *B. plicatilis*	g l^{-1}	>8, 1–32	30	17	16	30
	Elutriate	*Crassostrea gigas*	g l^{-1}	20–32	23–32	23–32	23–32	24–32
PH	Overlying water[b]	*Corophium volutator*	—	7–9	7.8–8.1	7.8–8.5	7.8–8.3	7.9–8.4
	Overlying water[b]	*Echinocardium cordatum*	—	7.5–8.5	7.7–8.2	7.8–8.1	7.8–8.1	7.8–8.4
	Pore water[c]	*V. fischeri, B. plicatilis*	—	6–8.5, 5–9	7.3–7.8	7.0–7.6	7.0–7.5	6.9–7.5
	Elutriate	*Crassostres gigas*	—	8–8.5	7.5–7.8	7.3–7.9	7.3–7.7	7.4–7.9
NH_4^+	Overlying water[b]	*Corophium volutator*	mg l^{-1}	<53[e]	<0.5–2	6–14	3–13	17–35
	Overlying water[b]	*Echinocardium cordatum*	mg l^{-1}	<15[e]	<0.5–4	0.1–4	0.1–8	2–9
	Pore water[c]	*V. fischeri, B. plicatilis*	mg l^{-1}	<1000, <1000	13–41	38–71	41–72	105–155
	Elutriate	*Crassostrea gigas*	mg l^{-1}	<3[e]	<0.5–3	0.4–0.8	0.6–1.3	0.2–2
Sulfide	Overlying water[b]	*Corophium volutator*	mg l^{-1}	<2	—	—	—	—
	Overlying water[b]	*Echinocardium cordatum*	mg l^{-1}	<5	—	—	—	—
	Pore water[c]	*V. fischeri*	mg l^{-1}	<16	0.1–0.3	0.1–0.3	0.1–0.3	1–28

[a] After Postma et al., 2002; RIKZ, 1999.
[b] At the end of the test.
[c] Undiluted and prior to testing.
[d] Single observation.
[e] At pH = 8.1.

reburying the urchins after exposure to the control sediment, except for Botlek 73 sediment at Laboratory 2.

The concentrations of ammonium in the overlying water (Table 4) met the TACs. In pore water of Den Helder sediment, however, the sulphide and ammonium contents may have been critical for *Echinocardium cordatum*.

We conclude that the sea urchin bioassay is an appropriate toxicity bioassay for marine dredged material because it shows limited inter-laboratory variability. The major bottleneck in this bioassay seems to be obtaining the proper life stage of the test organisms. This logistics problem could be solved by careful planning and by training the field staff.

Vibrio fischeri

All laboratories achieved the TAC for the control sediment and the reference toxicant $ZnSO_4$ (Table 1) and found that in all the contaminated sediments the bioluminescence was significantly less compared to the control (Figure 1). The EC_{50}s were very similar in all sediments; this shows that inter-laboratory variability was low. The CVs ranged from 6.6 to 16.4% (Table 3). This compares with the CVs ranging from 35 and 80% that Ross et al. (1999) found in study comparing 18 laboratories.

In pore water from Den Helder, a sulphide concentration above TAC was recorded by two laboratories (26 ± 1 mg l^{-1}). The other laboratories measured only 4 ± 2 mg l^{-1}, probably because sulphide is easily neutralized when brought into oxygenated conditions. In all other cases, pore water concentrations of sulphide and ammonium (Table 4) were below the MFCs and probably did not contribute to the observed toxicity.

The Microtox solid phase is recommended as a screening tool to test large numbers of sediments and has shown correlations with the responses in other bioassays (Burton et al., 1996a; Svenson et al., 1996; Cheung et al., 1997). Our study demonstrated that this bioassay is easy and rapid to perform and that good analytical results can be obtained even if the laboratory has little experience with it, as was the case for some of the participating laboratories. However, it is difficult to interpret the Microtox solid phase results when sediments differing in particle size are used. Benton et al. (1995) found that as mean particle size decreases (i.e., percent silt or clay increases), apparent toxicity increases. In our case the contaminated harbour sediments contained more clay and silt than the control sediment and so the differences in toxicity is partly attributable to grain-size effects. The modifying influence of fine sediment particles needs to be resolved before this highly reproducible bioassay can be used for regulatory purposes.

Crassostrea gigas

Laboratories 2 and 4 did not meet the TAC for the PNR in elutriates of the control sediment. The two other laboratories observed more abnormal larvae in the seawater control than in the sediment elutriates, which resulted in negative PNR values (Table 1). Consequently, the PNR values for control sediment established at Laboratories 2 and 4 were significantly different from those of Laboratories 1 and 3 (Figure 1). The ammonium concentrations in the sediment elutriates (Table 4) was below the MFC and had no influence on the bioassay results. The TAC for the reference toxicant NH_4Cl was not met by Laboratory 4 (Table 1). Because of the poor agreement on these quality parameters, it was not worth subjecting the bioassay results (Figure 1) to further analysis.

There was very great inter-laboratory variability in all sediments: CV values exceeded 100%, except for Den Helder sediment, which had the lowest CV: 34% (Table 3). The greatest variability in mean responses was between Laboratory 1 and the other laboratories. It is probably attributable to Laboratory 1 having the most experience in carrying out this bioassay. It is a complex bioassay requiring a great deal of laboratory experience to obtain acceptable results. The critical steps seem to be the settling of sediment after homogenisation and the sub-sampling from the overlying water in the test vessels containing the larvae in suspension.

The oyster larvae bioassay for sediment has previously been used in several environmental monitoring programmes (Butler et al., 1992; Van den Hurk et al., 1997; Thain et al., 1996) and was reported to be a sensitive bioassay able to discriminate between different degrees of sediment contamination. No previous data were available on bioassay reproducibility with sediment elutriates. We conclude that at present the oyster larvae bioassay is not an appropriate routine bioassay for dredged material.

Brachionus plicatilis

In most cases the 6.25, 12.5 and 25 and 50% dilution series were similar to control values so we were unable to calculate LC_{50}s. Below we present the findings for survival in undiluted pore water.

Except for Laboratory 3, the laboratories met the TAC for undiluted pore water of control sediment (Table 1). Laboratory 3 also found significantly lower survival in the Botlek sediments compared to the other laboratories (Figure 1). The mean CV was high: 48% (Table 3). When Laboratory 3 was excluded from the analysis the mean CV fell to 24%.

At all laboratories, the LC_{50} values for the reference toxicant $K_2Cr_20_7$ were within the TAC limits. Data on the ammonium and sulphide concentrations in the pore waters was already available from the Microtox bioassay. Based on these results, none of the MFCs was exceeded for *B. plicatilis*.

This cyst-based rotifer bioassay is known to be rapid, highly repeatable, easy and cost effective for evaluating the toxicity of single compounds (Snell and Persoone, 1989). In 1989, the Rotoxkit bioassay was subjected to a major inter-calibration exercise involving 120 laboratories from Europe, Canada and the U.S.A. (Persoone et al., 1993). In 80% of the cases, the bioassay results were successful: the overall CV was 49%. Unsuccessful testing was attributable to excessive mortality in the control (9%), hatching problems (4%) and other problems (7%). In our study, we found great variability between Laboratory 3 and the other laboratories, which is probably attributable to Laboratory 3's limited experience in this bioassay. The practical reasons responsible for Laboratory 3's poor performance may be the small size of the rotifers and consequently the difficulty in handling the plastic micropipette when transferring them, and the turbid water due to debris in the hatching compartment of the multi-well plate, which hampered the microscopic detection and count of the organisms. Laboratory 2, which had the most experience in performing the rotifer bioassay, showed a high repeatability of bioassay results among sub-samples.

The results of this bioassay demonstrated that to perform the tests successfully, an essential prerequisite is sufficient experience in pore water handling and testing.

Conclusions

The study identified clear differences between bioassays in terms of reproducibility of tests and the ability of different laboratories to meet the TACs. On the basis of the results of this inter-laboratory comparison, the five toxicity bioassays can be categorised as displaying either 1) little variability and great agreement with TACs or 2) large variability and little agreement with TACs. The amphipod and the Microtox solid phase bioassays fall into the first category while the elutriate bioassay with *Crassostrea gigas* embryos and the pore water bioassay with *B. plicatilis* fall into the latter category. The heart urchin bioassay could probably be assigned to the first category if the test organisms are in the weight class stipulated in the SOP. In general, the sources of variability include factors such as the use of test organisms from different batches and different sensitivity, varying water quality conditions or differences in the degree of experience and proficiency in performing the toxicity bioassays. In two cases, TACs for control sediment and reference toxicants properly identified poor quality tests. Work is continuing on elucidating the sources of variability observed in this study and on introducing changes to reduce such variability in future toxicity testing.

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