SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 22, No. 7, pp. 1535–1547, 2003

Printed in the USA
0730-7268/03 $12.00 + .00

USING MARINE BIOASSAYS TO CLASSIFY THE TOXICITY OF DUTCH HARBOR SEDIMENTS

JOOST STRONKHORST,*† COR SCHIPPER,† JOS BRILS,‡ MARCO DUBBELDAM,§ JAAP POSTMA,§ and NELLY VAN DE HOEVEN‖

†National Institute for Coastal and Marine Management/RIKZ, P.O. Box 20907, 2500 EX The Hague, The Netherlands
‡Netherlands Organization for Applied Scientific Research/TNO, P.O. Box 57, 1780 AB Den Helder, The Netherlands
§AquaSense Consultants, P.O. Box 95125, 1090 HC Amsterdam, The Netherlands
‖Ecostat, Vondellaan 23, 2332 AA Leiden, The Netherlands

(*Received* 6 *May* 2002; *Accepted* 20 *December* 2002)

Abstract—A procedure was developed to assess contaminated marine sediments from Dutch harbors for possible adverse biological effects using three laboratory bioassays: A 10-d survival test with the amphipod *Corophium volutator*, a 14-d survival test with the heart urchin *Echinocardium cordatum* (adults), and the bioluminescence inhibition test with the bacterium *Vibrio fischeri* (Microtox® solid phase test [SP]). Microtox results were mathematically corrected for the modifying influence of fine sediment particles. After a validation procedure on test performance and modifying factors, respectively, 81%, 99%, and 90% of the amphipod, heart urchin, and Microtox results were approved. Lower and upper threshold limits for biological effects were set at respectively 24 and 30% mortality for *C. volutator*, 27 and 35% mortality for *E. cordatum*, and 24 and 48 toxic units for the Microtox SP based on significant differences with control sediment and the performance of reference sediments. The bioassays clearly distinguished harbor sediments that give rise to acute effects and those that do not. Threshold limits for the amphipods, heart urchins, and bacteria were exceeded in, respectively, 9 to 17%, 33 to 40%, and 23 to 50% of the sediment samples. Highest effects were observed in sediments from the northerly harbors; there was significantly less response in sediments from the Delta Region and the port of Rotterdam (The Netherlands). The procedure outlined in this paper can be used for routine screening of contaminated dredged material that is proposed for open water disposal.

Keywords—Dredged material Adverse effects *Corophium volutator* *Echinocardium cordatum* *Vibrio fischeri*

INTRODUCTION

A substantial part of harbor sediments consists of silty material often contaminated with persistent compounds such as tributyltin, mineral oil, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and trace metals at levels with ecological risk ranges from low to high. Laboratory bioassays can improve the hazard assessment of sediments in this intermediate zone of contamination and make it easier to decide whether harbor sediments need remedial action or if dredged material may be disposed of in open waters [1]. This is because bioassays provide an overall measure of the combined effect and bioavailability of the sediment-associated contaminants and thus provide an insight into the possible biological implications of contamination. When interpreting bioassay results, however, serious difficulties may occur because of variability in laboratory handling, differences in sensitivity between batches of test organisms, or the influence of so-called confounding or modifying factors. The most relevant of modifying factors in silty marine sediments are grain size [2], un-ionized ammonia [3], and sulfide [4]. These constituents do not require any specific actions because they are natural factors that can be easily neutralized when brought into seawater.

This paper tries to develop a general procedure for discriminating between harbor sediments that give rise to adverse effects and those that do not. This includes the setting of assessment criteria for test performance, modifying factors, and classification of the biological responses in the bioassays applied. We present the results of an extensive survey on the possible adverse biological effects of silty marine harbor sediments from The Netherlands as observed in laboratory bioassays. Three marine bioassays were deployed and conducted according to recently established standard operating procedures [5]: A 10-d whole sediment survival test with the amphipod *Corophium volutator*, a 14-d whole sediment survival and reburial test with the heart urchin *Echinocardium cordatum*, and the bioluminescence inhibition test with the bacterium *Vibrio fischeri* (Microtox [AZUR Environmental, Carlsbad, CA, USA] solid phase [SP] test). *Corophium volutator* is a representative of the intertidal benthic fauna of estuaries along the North Sea and is widely used in sediment toxicity assessments in northwestern Europe [6–8]. *Echinocardium cordatum* was used because it is abundant in the North Sea and applied in sediment toxicity assessments there [9,10]. *Vibrio fischeri* bioassay is used worldwide as a screening tool for sediment toxicity assessments [11]. All three tests have shown acceptable interlaboratory variability [12] and, according to a preliminary survey, have sufficient discriminatory power for the present level of contamination of Dutch marine harbor sediments [13,14]. The relationship between the measures of toxicity presented here and the measures of chemical contamination in the investigated sediments will be addressed separately.

MATERIALS AND METHODS

The quality assurance and quality control procedures adopted in this study entailed the application of standard procedures

* To whom correspondence can be addressed (j.stronkhorst@rikz.rws.minvenw.nl).

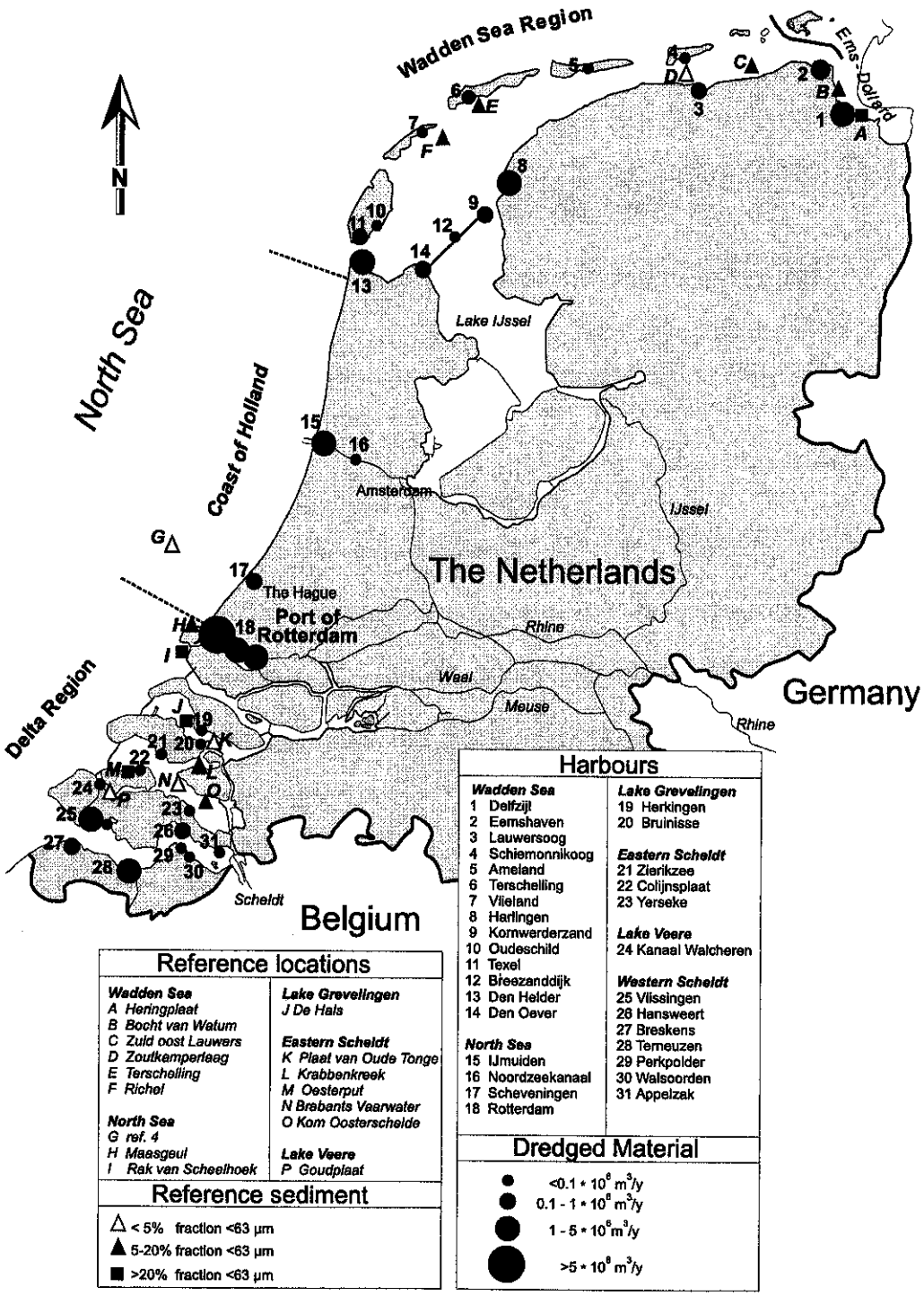

Fig. 1. Sediment sampling sites in harbors and at reference locations along the Dutch coast. Also indicated are the annual volume of dredged material and the sediment texture at the reference site.

for the bioassays and sediment sampling [5]; the confirmation of positive and negative controls for each batch of tests by applying test acceptability criteria during the monitoring program; and a verification on the influence of grain size, salinity, pH, ammonia, and sulfide in each of the tested sediments by applying modifying factor criteria (MFC).

Sediment sampling and storage

Sediment samples were collected in March to August 1999 and April to August 2000 in harbors along the Dutch coast. The harbors (Fig. 1) are located in four regions: Wadden Sea (locations 1–14), Coast of Holland (locations 15–17), Port of Rotterdam (location 18), and the Delta region (locations 19–31). Composite sediment samples were made following a stratified random sampling strategy. Based on historical data, the harbors were divided into segments of generally homogeneous areas of contamination. In each segment, six subsamples were collected with a van Veen grab (0.12 m²) from approximately the top 20 cm of the sediment and composed to a single test sample. Harbor sediments were collected at 195 stations; at 62 stations samples were collected in both 1999 and 2000 to investigate the agreement in toxicity classification between

years, making a total of 257 harbor sediment samples. In addition, reference sediments were collected at 16 sites along the Dutch coast (Fig. 1); at several reference sites samples were collected in both 1999 and 2000, making a total of 22 coastal reference sediment samples. The reference sediments represent the ambient conditions in Dutch coastal waters in terms of the background anthropogenic influence of present and historic contamination. After homogenization of the sediments, subsamples were made for the bioassays, sediment texture, and bulk sediment chemistry (not presented in this paper) and stored at 4°C for one to four weeks prior to testing and analysis. The control sediment was from the Eastern Scheldt, an estuary in southwest Netherlands regarded as the least contaminated area along the Dutch coast. Samples of these control sediments were used in the bioassays for quality control (see below) and for statistical comparison with the tested harbor sediments.

Physico-chemical characteristics of the sediments

The dry weight fraction of the sediments was determined at 105°C. Total organic carbon content was measured with a carbon analyzer (conversion at 1,300°C) in accordance with the Dutch standard method NEderlands Normalisatie-instituut (NEN) 5756. The fine sediment fraction <63 μm was determined using the gravimetric method according to NEN 5753.

Bioassays

In 1999 the selected bioassays were performed at the laboratories of TNO and in 2000 at the laboratories of AquaSense (Amsterdam, The Netherlands).

Corophium volutator. The amphipod *C. volutator* is abundant in silty intertidal sediments where it lives in *U*-shaped burrows and feeds on organic particles in the sediment. The amphipod survival bioassay was conducted according to the standard operating procedure SPECIE-01 [5]. Both laboratories used amphipods collected in the Eastern Scheldt, The Netherlands. The test consisted of a 10-d static exposure at 15 ± 2°C and involved five replicate test chambers (1 L beakers) per treatment, with each replicate containing 20 amphipods. Control responses were performed with silty sediment from the Eastern Scheldt containing approximately 40% <63 μm particles. A 72-h water-only reference toxicant test using ammonium chloride (NH_4Cl) was performed to assess the sensitivity of each batch of amphipods. On days 1 and 10 of the test, the pH and the concentrations of dissolved oxygen, ammonium, and salinity were measured in the overlying water to determine the influence of modifying factors.

Echinocardium cordatum. The burrowing heart urchin *E. cordatum* lives 5 to 10 cm deep in the sediment and feeds on detritus. It is one of the few species of macrobenthos that can be found throughout the North Sea. The test with *E. cordatum* was conducted according to the standard operating procedure SPECIE-03 [5]. Test organisms were collected from the coastal zone of Holland and had an individual wet weight of 10 to 40 g. The number of organisms used in the heart urchin bioassay was limited to 40 [15]. The bioassay involved a whole-sediment exposure at 15 ± 2°C in aquaria of 750 cm^2 containing 3 L of sediment and 4 L of overlying sea water. The system used is a continuous flow-through system (10 L/d) using uncontaminated, filtered seawater. Each sample was tested using four replicates and 10 organisms per replicate. Control responses were performed with sediment from the Eastern Scheldt. Survival was determined after 14-d exposure to the test sediment. The reburrowing rate of the survivors in clean control sediment was also recorded but provided no additional information, and therefore was not evaluated further. A 96-h water-only test using ammonium chloride (NH_4Cl) was performed to assess the sensitivity of each batch of heart urchins. On days 1 and 14 of the test, the pH and the concentrations of oxygen, ammonium, and salinity were measured in the overlying water to ascertain the possible influence of modifying factors.

Vibrio fischeri. The Microtox SP test with the marine bacterium *V. fischeri* was performed according to standard operating procedure SPECIE-02 [5]. The end point is inhibition of bioluminescence. Azur Environmental (Hook, UK) supplied the batches of bacteria. Sediments were tested as suspensions prepared with Microtox SP (MSP) reagent and diluted to 13 concentrations ranging from 0.005 to 19.7% sediment. Each concentration was tested in duplicate at 15 ± 1°C. Bioluminescence was measured after 20 min exposure. Control responses were analyzed using the same silty sediments as in the amphipod tests. Zinc sulfate ($ZnSO_4$) was used as reference toxicant. The pH and concentrations of oxygen, ammonium, sulfide, and salinity were determined in pore water that was collected by centrifugation (20 min at 3,780 *g*).

The major confounding factor in the Microtox SP test is the sediment texture [16]. We developed a statistical method to correct for the loss of bacteria due to adsorption to fine sediment particles (see Appendix). After this correction, the effect concentration E_t was estimated as the dry sediment fraction (percent, wt/wt) that causes a 50% light reduction. The results of the Microtox SP test are expressed as toxic unit (TU): TU = 1%/E_t.

Test acceptability, modifying factors, and threshold limits

For all three bioassays, test acceptability criteria for the reference toxicants (positive controls) and control sediments (negative controls) were compiled from previous and unpublished validation studies performed at our laboratories. Test acceptability criteria for reference toxicants were quantified as the mean plus or minus three times the standard deviations of earlier observations.

Modifying factor criteria defined for pH, salinity, sulfide, total ammonia, and the fine sediment fraction were compiled from previous investigations that have been partly reported by Postma et al. [17]. A sulfide criterion was set for the Microtox SP test but not for the two other bioassays because the oxygenated overlying water in these tests ensured that the sulfide was rapidly oxidized into nontoxic sulfate. However, the MFC for sulfide in the Microtox SP was used as a trigger for the two other bioassays. For ammonia, MFC were set for different pH values to account for the formation of the more toxic NH_3.

To classify the biological response in each bioassay, lower and upper threshold limits were set. One set of threshold limits was defined as the 95th percentile values for coastal reference sediments. Another set was defined for the amphipod *C. volutator* and heart urchin *E. cordatum* as the mortality that was always significantly higher than the mortality in control sediment. This later approach was not applicable for the Microtox SP bioassay. Instead, an arbitrary value twice the 95th percentile of the reference sediment performance was calculated.

Calculations

The statistical correction procedure for the Microtox SP is given in the Appendix. For the pairwise comparison between the control sediments and harbor or reference sediments, we

Table 1. Test acceptability criteria (TAC) for control sediments and reference toxicants and minimal, maximal, and average responses in the three selected bioassays during 1999 and 2000, respectively

	Amphipod *Corophium volutator*		Heart urchin *Echinocardium cordatum*		Bacterium *Vibrio fischeri*	
	Control sediment % mortality	Reference toxicant LC50 (mg NH_4^+/l)	Control sediment % mortality	Reference toxicant LC50 (mg NH_4^+/l)	Control sediment EC50,[a] % wet sediment	Reference toxicant EC50 (mg Zn^{2+}/l)
TAC	<10	36–220	<10	12–32	>0.6	2.1–8.5
Minimum	0 / 4	79 / 55	0 / 0	14 / 18	0.7 / 0.7	2.7 / 3.1
Maximum	13[b] / 9	146 / 181	8 / 6	24 / 31	3 / 3	6.9 / 4.7
Average	5 / 6	111 / 117	3 / 3	20 / 24	1 / 1	4.8 / 3.9
n[c]	17 / 15	7 / 13	11 / 17	6 / 10	9 / 10	6 / 11

[a] Specifically for reference sediment from site M (40% fines <63 μm) without corrections for dry weight and grain size.
[b] One batch exceeded the TAC.
[c] n = number of observations.

used Dunnett's test ($\alpha = 0.05$) with arcsine–square root transformed data on amphipod survival and heart urchin survival.

RESULTS

Acceptability of test results

The test acceptability criteria for the positive and negative controls are summarized in Table 1. Responses in the control sediments met the test acceptability criteria except for one batch of amphipods in August 1999. Due to an oversight, this latter series was not retested, and consequently the accompanying 17 test results were not approved and were excluded from further analysis. The amphipods, heart urchins, and bacteria that were used at the two laboratories during the monitoring exercise had corresponding sensitivity toward the reference toxicants. All observed concentrations that caused 50% mortality or 50% inhibition of bioluminescence were within the range of test acceptability criteria (Table 1). The test acceptability criteria for dissolved oxygen in the amphipod, heart urchin, and bacterium tests were set at >50%, >60%, and >30%, respectively, and were met in all cases.

Physico-chemical characterization and modifying factors

The harbor sediments investigated varied strongly in their physico-chemical properties (Table 2). Most samples, however, were anoxic silty sludges with a low median dry weight fraction (40% dry wt), high median fine sediment fractions (51% <63 μm), and intermediate organic carbon content (median of 3.3% organic carbon). The mean pH of the pore water (7.6 ± 0.3) was lower than the pH of seawater used at the laboratories (8.0 ± 0.1; data not shown). The salinity of the pore water confirmed that sediments originated from marine locations and a few brackish sites. Most sediment contained substantial amounts of ammonia; high concentrations of total sulfide were found incidentally.

Table 3 presents the modifying factor criteria for grain size (percent <63 μm), pH, sulfide, salinity, and total ammonia. The MFC for ammonia in the bioassay with *C. volutator* shows a steep decrease with increasing pH. At pH = 8.2, for instance, the MFC for total ammonia equals a criterion for un-ionized ammonia of 1.3 mg/L.

All amphipod and heart urchin bioassays met the MFC for fine sediment because the sediments had no more than 74% fines (Table 2). The results of the Microtox SP test were corrected for grain size. In this bioassay, the physical interaction of the fine sediment fraction was the sole cause of light inhibition in 33% of the 257 harbor sediments and 60% of the 22 coastal reference sediments tested. These data were treated as observations below the level of detection (TU < 2). The pH was always within the limits, except at one site in the harbor of Delfzijl that had a pH of 9.2 because of a leak in a nearby underground industrial effluent pipeline. All three bioassays on sediment from this site were excluded. The MFC for sulfide was exceeded in two samples from Lake Grevelingen and one sample from the harbor of Lauwersoog (The Netherlands). For those three samples, the responses in the amphipod (8–65% mortality), heart urchin (10–80% mortality), and bacterium tests (<2–73 TU) were excluded.

The MFCs for salinity were only exceeded in the Microtox

Table 2. Physico-chemical characteristics of harbor sediments from The Netherlands (n = 257)

	Unit	Minimum	5th Percentile	Median	95th Percentile	Maximum
Sediment dry weight	% dry wt	11	24	40	69	87
Fine sediment fraction <63 m	% dry wt	<1.1	13	51	70	74
Fine sediment fraction <2 m	% dry wt	<0.5	5	23	33	38
Sediment organic carbon (OC)	% dry wt	<0.3	1.0	3.3	5.5	9.8
pH[a]	—	6.9	7.2	7.6	8.1	9.2
Salinity[a]	g/L	2	4	25	30	32
Total ammonia[a]	mg/L	<1	<1	52	113	200
Total sulfide[a]	mg/L	<0.1	<0.1	<0.1	0.7	104

[a] In pore water.

Table 3. Modifying factor criteria (MFC)[a] for the three bioassays in pore water (P) and overlying water (O)

	Amphipod *Corophium volutator*	Heart urchin *Echinocardium cordatum*	Bacterium *Vibrio fischeri*
Fine sediment fraction <63 m (%)	<90	<90	—
pH (−)	7.0–9.0 (O,P)	7.5–8.5 (O,P)	6.0–8.5 (P)
Sulfide (mg/L)	—[b]	—[b]	<16 (P)
Salinity (g/L)	4–40 (O)	>28 (O)	>8 (P)
Total ammonia (mg/L)[c]			
pH 7.0	192 (O)	—	—
pH 7.5	106 (O)	<25 (O)	—
pH 8.0	60 (O)	<15 (O)	—
pH 8.1	53 (O)	<15 (O)	—
pH 8.2	50 (O)	<15 (O)	—
pH 8.3	49 (O)	<15 (O)	<1,000 (P)
pH 8.4	39 (O)	<15 (O)	—
pH 8.5	31 (O)	<5 (O)	—
pH 8.6	25 (O)	—	—
pH 8.7	21 (O)	—	—
pH 8.8	16 (O)	—	—
pH 8.9	13 (O)	—	—
pH 9.0	10 (O)	—	—

[a] Partly based on Postma et al. [17].
[b] MFC for sulfide in the bioassay with *V. fischeri* was used as the trigger.
[c] Most critical at the end of the amphipod test and start of the heart urchin test.

SP test with brackish sediments from the central port of Rotterdam. Although these samples showed no significant light inhibition, they were excluded. The MFCs for ammonia reflect the higher sensitivity of the heart urchin *E. cordatum* than the amphipod *C. volutator*, whereas the bacterium *V. fischeri* appears to be insensitive to ammonia. Ammonia was the most important confounding factor in the test with *C. volutator*; it exceeded the MFC in 13% of the tests. These samples showed a concentration–response relation between ammonia content above MFC and amphipod mortality (Fig. 2). Overall, the proportions of test results approved were 81% for the amphipod test, 99% for the heart urchin test, and 90% for the bacterium test.

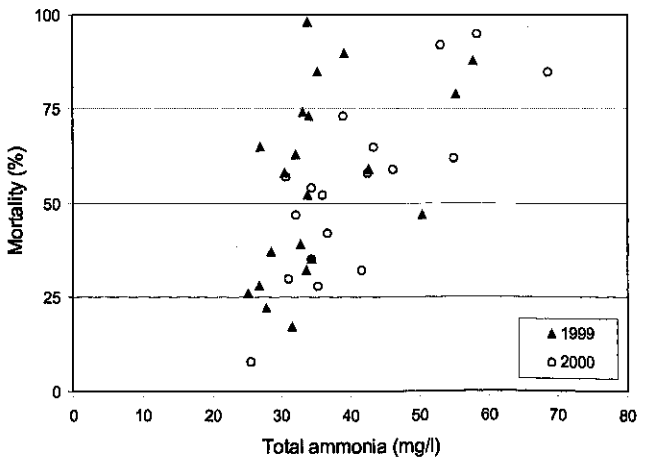

Fig. 2. Mortality of the amphipod *Corophium volutator* versus the concentration of total ammonia in overlying water of the tests that failed the modifying factor criterion for ammonia. Total ammonia concentration was measured at the end of the 10-d exposure period at pH of 8.4 to 8.6, salinity of 30 to 35 g/L, and temperature of 14 to 16°C.

Adverse biological response of coastal reference sediments

The data on toxicity of coastal reference sediments differed with sediment grain size classes (Table 4). The highest responses were found in the reference sediments with more than 20% fines. Median responses in the amphipod, heart urchin, and Microtox SP bioassays in these silty reference sediments were 20% mortality, 10% mortality, and 12 TU, respectively. The silty sediments from reference site I caused the most biological effects, i.e., 18 to 47% amphipod mortality and 10 to 27.5% heart urchin mortality. The more sandy-type reference sediments did not have a significantly adverse effect on the test organisms, except for sediments from site E, which had elevated amphipod and heart urchin mortalities of 28 and 82.5%, respectively.

Adverse biological responses of harbor sediments

A distinction has been made between samples that were not significantly different from the controls and those that were. Approximately two thirds of the harbor sediments responded significantly in the Microtox SP test, and half the harbor sediments showed statistically significant toxicity in the amphipod and heart urchin tests (Fig. 3). As expected, the subcellular endpoint in Microtox SP is more sensitive than the observations of survival of test organisms in the other two bioassays. In the amphipod bioassay, a significant difference from the control was observed at as low as 10% mortality in tested sediment. This occurred when the control mortality was low and the variation in both distributions small. Above 23% mortality in test sediments there was always a significant difference with the control sediments. The heart urchin bioassay was less powerful in detecting significant differences than the amphipod bioassay. In addition to differences in the number of test organisms (40 heart urchins instead of 100 amphipods per sample), the heart urchin bioassay showed a wider variation between replicates, which resulted in an overdispersion in the binomial distribution of the mortality rates. In this test, at 35%

Table 4. Basic statistics on the toxicity of reference sediments from Dutch coastal waters as determined by the three bioassays. The reference sediments are categorized by the fine sediment fraction (<63 μm)

Grain size class	Fine sediment fraction <63 μm (%)	Amphipod *Corophium volutator* (% mortality)	Heart urchin *Echinocardium cordatum* (% mortality)	Bacterium *Vibrio fisheri* (toxic unit)
<5% Fines (n = 7)[a]				
Minimum–maximum	1–3	4–18	0–5	1–1
Average	2	8	3	1
Median	3	7	3	1
5–20% Fines (n = 8)[b]				
Minimum–maximum	12–20	5–28	0–83	1–22
Average	17	13	14	8
Median	18	10	5	8
>20% Fines (n = 7)[c]				
Minimum–maximum	21–76	3–47	3–28	1–43
Average	39	22	12	15
Median	48	20	10	12
Overall (n = 22)				
Average	19	15	10	8
Median	18	11	5	1
95th Percentile[d]	—	30	27	24

[a] Locations D, G (2×), K, N, and P (2×). See Figure 1 for locations.
[b] Locations B, C, E, F, H, L, and O (2×).
[c] Locations A, I (3×), J (2×), and M.
[d] Used as threshold value.

mortality or more there was always a significant difference with the control sediments.

The median amphipod mortality showed little variation among harbors (Table 5). Heart urchin mortality and bioluminescence inhibition, however, was higher in the northern harbors 1 to 15 than in the southern harbors (Tables 6 and 7). The spatial distribution of sediment toxicity is illustrated for the central part of the port of Rotterdam (Fig. 4). Gradients from inner harbor to outer harbor or shipping lane showed increasing toxicity in some but not all cases.

The median response in the heart urchin and Microtox SP bioassays was higher in harbor sediments (Tables 6 and 7) than in silty reference sediments (Table 4). In contrast and for reasons unknown, the median mortality of *C. volutator* in the silty reference sediments (20%) was higher than the median rates of 13% in most harbors investigated (Table 5).

Threshold limits

The threshold limits for *C. volutator*, *E. cordatum*, and *V. fischeri* based on the 95th percentile of the reference sediment performance were 30% mortality, 27% mortality, and 24 TU, respectively (Table 4). The threshold limits for the amphipod *C. volutator* and heart urchin *E. cordatum* based on the significance with control sediments were 24% and 35% mortality, respectively (Fig. 3). Test sediments above these latter threshold limits are always significantly more toxic than in control sediment, and therefore will have a type I error close to zero. Recognizing the indefinite ecological relevance of the Microtox SP test, we arbitrarily set a significant toxic level at 48 TU, which is two times the 95th percentile of the reference sediments. In summary, the lower and upper threshold limits were 24 and 30% mortality for *C. volutator*, 27 and 35% mortality for *E. cordatum*, and 24 and 48 TU for the Microtox SP.

Tables 5 to 7 present the frequencies these threshold limits exceeded in the harbors studied. The northern harbors showed the highest frequency in exceeding the threshold limits.

Correlation among the bioassays and with sediment texture

There was a very slight correlation between amphipod mortality and heart urchin mortality (Pearson $r^2 = 0.18$, $n = 232$) and between bioluminescence inhibition and heart urchin mortality ($r^2 = 0.17$, $n = 255$). Amphipod mortality did not correlate with the response in the Microtox SP test. Both the responses in the tests with *E. cordatum* and *V. fischeri* correlated slightly with the fine sediment fraction ($r^2 = 0.24$ and 0.17, respectively) and total organic carbon ($r^2 = 0.12$ and 0.20, respectively). Amphipod mortality was not correlated with either of these sediment characteristics. None of the threshold limits for the bioassays were exceeded when sediments had a fine fraction <63 μm below 10%.

Year-to-year variability

Weak correlations were found between the toxicity data of 1999 and 2000 from the same stations (Fig. 5). The data on amphipod and heart urchin mortality were not significantly different between the consecutive years, but the Microtox SP data were significantly higher in 2000 than in 1999 ($TU_{2000} = 0.7 \pm 0.1\ TU_{1999}$; $r^2 = 0.22$, $n = 55$). To determine the agreement in sediment toxicity classification, the lower threshold limits of each of the bioassays were used. For the amphipod, heart urchin, and bacterium bioassays, respectively, 90, 77, and 76% of the stations were classified similar in the consecutive years (p-value 0.049, 0.0001, and 0.0002, respectively, Fisher exact 2 × 2 test).

DISCUSSION AND CONCLUSIONS

Quality assurance/control and modifying factors

Figure 6 summarizes the procedures for test acceptability and the validation on modifying factors that were applied dur-

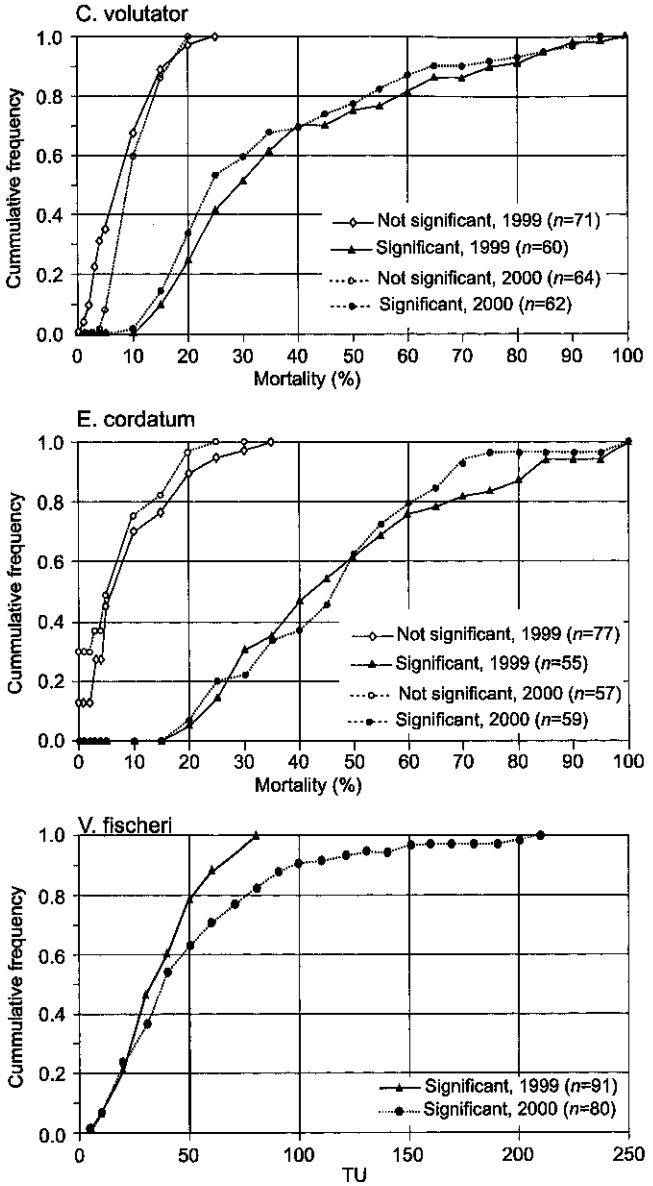

Fig. 3. Cumulative frequency of responses in the bioassays with the amphipod *Corophium volutator*, the heart urchin *Echinocardium cordatum*, and the bacterium *Vibrio fischeri* exposed to harbor sediments from The Netherlands, classified by their statistical difference from the control sediment. In 86 Microtox solid phase tests, no significant effects were measured.

ing this investigation. These procedures were complemented by audits conducted by the National Institute for Coastal and Marine Management at the contracting laboratories and by an interlaboratory study conducted previously [12]. The statistical method to correct for the interfering influence of sediment fines on the Microtox SP (see Appendix) marked 33% of the test results as giving a response due to these sediment fines only. One series of amphipod tests with high toxicity was rejected because the batch of amphipods exceeded the maximal expectable mortality in the control sediment. Moreover, 19% of the amphipod tests were rejected because of the confounding influence of pH, sulfide, and ammonia. Given that these procedures have improved the reliability of the bioassay results, it seems unlikely that the hazard assessment of Dutch marine harbor sediments in this survey was seriously biased by falsely predicted toxicity.

The main modifying factor in the amphipod bioassay was ammonia. The amount of ammonia over the tolerance limits is attributable to the static exposure in this bioassay, which caused both the ammonium concentration and the pH to increase steadily during the 10-d period—a phenomena also observed by others [18]. The outcome was an exponential rise in the levels of toxic un-ionized ammonia in the overlying water. In some cases, this might have hampered the identification of toxicity from persistent compounds. Such toxicity could not be established for several highly contaminated sites in the port of Rotterdam (Fig. 4) because of the coincidentally high ammonia levels. Under these circumstances, toxicity identification evaluation procedures may be used to identify the biological effects of toxicants other than the nonpersistent contaminants [19–21]. Alternatively, prior to testing, high ammonia levels can be reduced by various methods [22]. In our survey, time constraints precluded the application of adjustments for modifying factors, but such adjustments should be carried out when real-world decisions are to be made (Fig. 6). Finally, during the monitoring exercise all reference toxicant tests satisfied the test acceptability criteria, suggesting that conditions of organisms were within acceptable limits. However, it can be argued that water-only tests are inappropriate for identifying differences in stress in benthic species [23] and should be replaced by positive control sediments in future surveys.

Classification of harbor sediments

Testing sediment toxicity under controlled laboratory conditions is an essential element when acquiring evidence of the ecological risks of sediment-associated contamination. In addition to the quality assurance/quality control procedures mentioned above, the setting of assessment criteria is critical for both the scientific and legislative application of bioassays.

The amphipod bioassay revealed that nearly half the harbor sediments we tested were significantly toxic in the batch-wise comparison to clean control sediment (Fig. 3), and that 17% of the samples exceeded the lower threshold limit (Table 5). This lower threshold limit is practically the same as several statistically based toxicity assessment criteria reported in the literature. For instance, Swartz et al. [24] used >24% mortality of amphipods to classify sediments as being toxic. Thursby et al. [25] and Phillips et al. [26] derived equivalent threshold values of 20% control-adjusted mortality for *Ampalisca abdita*, an amphipod with a similar life history to *C. volutator*. Given a control mortality of 5% (Table 1), it is equivalent to an absolute mortality rate of 25%. More interestingly, amphipod tests have been shown to be indicative of adverse biological effects in the field [27]. Long et al. [28] found that most toxic samples (amphipod mortality above 20% after adjustment to control) were associated with at least 50% reduction in benthic infauna diversity or abundance in coasts of the United States. For *C. volutator* such information is, to our knowledge, limited to a study in the vicinity of a North Sea oil platform where lower infaunal species diversity was associated with a *C. volutator* mortality rate above approximately 30% after 10 d of exposure to these sediments [8]. We conclude that the threshold value for *C. volutator* of 24% mortality is significant in terms of the statistics and the ecological implications for in situ sediments. Less stringent is the comparison with the upper threshold limit of 30% mortality, resulting in the rejection of one in 10 samples (Table 5). This upper threshold limit is close to the pass/fail criterion used in

Table 5. Basic statistics and classification of sediment toxicity in harbors along the Dutch coast according to the amphipod *Corophium volutator* bioassay

Harbor area[a]	Number of observations		% Mortality[b]			Percentage of samples exceeding threshold values for mortality	
	Total	Rejected	Median	Minimum	Maximum	≥24%	≥30%
1	14	1	16	1	80	8	0
2	6	1	23	15	26	40	0
3	5	4	13	13	13	0	0
8	18	6	16	3	55	42	25
4, 5, 6, 7, 10, 11	13	4	11	5	25	11	0
9, 12, 14	12	6	13	4	21	0	0
13	12	7	8	6	25	20	0
15	33	3	11	2	85	10	10
16	4	4	—	—	—	—	—
17	8	0	9	6	19	0	0
18 West	20	1	11	1	29	16	0
18 Southwest	22	0	9	3	49	14	5
18 Central	50	11	15	1	99	26	21
19, 20	4	0	18	13	19	0	0
21, 22, 23	6	0	10	6	19	0	0
25	10	1	21	7	34	11	11
27	3	0	10	9	24	33	0
28	8	0	13	6	24	13	0
26, 29, 30, 31	7	0	12	7	31	14	14
All	257	48	13	1	99	17	9

[a] See Figure 1.
[b] Based on approved data only.

the United States for 10-d amphipod mortality in dredged material proposed for ocean disposal, i.e., a maximal increase of 20% mortality over reference sediment [29]. Because the Dutch reference sediments had a median response of 11% mortality (Table 4), this would have resulted in a pass/fail criterion of 31%, which is only slightly higher than the proposed upper threshold limit for *C. volutator*.

According to the heart urchin bioassay, half the harbor sediments tested were significantly toxic in the batch-wise comparison with clean control sediment (Fig. 3) and also compared with the lower threshold limit for heart urchin mortality (Table 6). The upper threshold value of 35% mortality was exceeded in 36% of the samples. These are strong indications that harbor sediments along the coast of The Netherlands are

Table 6. Basic statistics and classification of sediment toxicity in harbors along the Dutch coast according to the heart urchin *Echinocardium cordatum* bioassay

Harbor site or area[a]	Number of observations		% Mortality[b]			Percentage of samples exceeding threshold values for mortality	
	Total	Rejected	Median	Minimum	Maximum	≥27%	≥35%
1	14	1	51	3	100	62	62
2	6	0	46	8	65	67	67
3	5	1	80	3	83	75	75
8	18	0	53	8	83	83	78
4, 5, 6, 7, 10, 11	13	0	43	0	75	54	54
9, 12, 14	12	0	33	13	68	67	42
13	12	0	49	0	78	83	83
15	33	0	20	0	100	36	33
16	4	0	3	0	5	—	—
17	8	0	8	3	18	0	0
18 West	20	0	9	3	33	10	0
18 Southwest	22	0	18	0	38	18	5
18 Central	50	0	23	0	100	42	24
19, 20	4	1	25	0	48	33	33
21, 22, 23	6	0	16	5	43	33	33
25	10	0	0	0	23	0	0
27	3	0	3	0	23	0	0
28	8	0	9	0	35	25	25
26, 29, 30, 31	7	0	5	0	45	14	14
All	257	3	20	0	100	40	33

[a] See Figure 1.
[b] Based on approved data only.

Table 7. Basic statistics and classification of bioluminescence inhibition of the bacterium *Vibrio fisheri* (Microtox solid phase test) exposed to sediments from harbors along the Dutch coast

Harbor site[a]	Number of observations		Toxic unit (TU) for light inhibition[b]			Percentage of samples exceeding threshold values for light inhibition	
	Total	Rejected	Median	Minimum	Maximum	≥24 TU	≥48 TU
1	14	1	32	1	171	69	31
2	6	0	41	1	119	83	33
3	4	0	73	28	85	100	80
8	18	0	50	28	150	100	50
4, 5, 6, 7, 10, 11	13	0	31	1	123	54	31
9, 12, 14	12	0	49	1	153	83	50
13	12	0	54	15	143	92	58
15	33	0	35	1	202	10	3
16	4	2	12	1	23	50	0
17	8	0	35	1	98	63	25
18 West	20	0	6	1	22	0	0
18 Southwest	22	5	22	1	55	47	6
18 Central	50	18	12	1	90	34	13
19, 20	4	0	8	1	21	0	0
21, 22, 23	6	0	29	9	78	50	17
25	10	0	15	1	27	20	0
27	3	0	1	1	14	0	0
28	8	0	1	1	23	0	0
26, 29, 30, 31	7	0	1	1	8	0	0
All	257	26	25	1	202	52	23

[a] See Figure 1.
[b] Based on approved data only.

still affected by pollution. Daan et al. [10] have conducted benthic community analysis and heart urchin toxicity tests using boxcosms with sediments from around an oil platform in the North Sea. Adverse biological effects from oil contamination were found at a site 50 m from the platform: faunal abundance and species diversity were reduced by approximately 60 and 30%, respectively. The mortality rate of heart urchin after 14 d of exposure to the same sediment in the laboratory was 30%. This percentage is close to the threshold limits we propose. Note also that heart urchin mortality in the oil-polluted sediment had risen to 85% after 110 d of exposure [10]. This indicates clearly that the standard heart urchin bioassay of 14 d does not show the full ecological risk of a given sediment. These results, however, could be biased by the fact that the oil contamination that occurred was patchy and no replicate testing was done [10].

Some authors claim that the Microtox test of sediment matrices is predictive for the effects of contaminants on benthic communities [30]. However, these studies as well as the arbitrary assessment criteria suggested by Ringwood et al. [16] cannot be applied to the light inhibition corrected for fine sediment fraction in this paper. The lowest detectable bioluminescence inhibition by sediment-bound contaminants in this survey resulted in a TU of 2. This is an order of magnitude below the lower threshold limit based on the 95th percentile of toxic units in Dutch coastal reference sediments. An upper threshold limit of 48 TU was defined to facilitate the classification of the Microtox SP data, but this value was set arbitrarily. Therefore, the use of the Microtox SP test to classify the ecological risks of harbor sediments needs further elucidation.

For the management of dredged material, bioassays are generally regarded as a useful screening instrument for ranking the hazard of dredgings prior to disposal. The proposed threshold limits can serve as pass/fail criteria for open water disposal, but such criteria will also depend on socio-economic constraints.

Spatial and temporal variations

The most striking spatial variation was the high average biological effects in the northern harbors and the relatively low average response in harbors of the Delta region and the port of Rotterdam. This occurred in the heart urchin and Microtox SP bioassays in both years (Tables 6 and 7). So far, no parameters have been identified that explain the high responses in the northern region. It is unlikely related to test performance or the influence of modifying factors. Statistical comparison between the bioassay results and data on bulk sediment chemistry, which will be carried out in the next stage of this study, might reveal cause and effect relationships.

In most harbors no clear spatial gradients were evident. This suggests that input of contaminants is diffuse and that mixing and removal of sediment is taking place. In the port of Rotterdam (Fig. 4), a few gradients were present, from high responses in the inner, poorly flushed harbors to the lower responses in the outer harbors or adjacent shipping lanes.

As for the coastal reference sediments, four out of the 22 sites investigated exceeded one or more of the lower threshold limits. Sediment from site O in the Eastern Scheldt caused 26% amphipod mortality coinciding with high dioxin-like responses in the DR-CALUX® bioassay [31]. Two of the three samples from site I, located at the mouth of the Haringvliet sluices that discharge freshwater from the Rhine and Meuse, caused an average 39% amphipod mortality, 25% heart urchin mortality, and 34 TU in the Microtox SP test. Field observation will have to conform if the benthic community structure at these sites is indeed as adversely affected as the laboratory bioassays suggest. The fourth reference sediment causing effects originated from the Wadden Sea (site E), which was

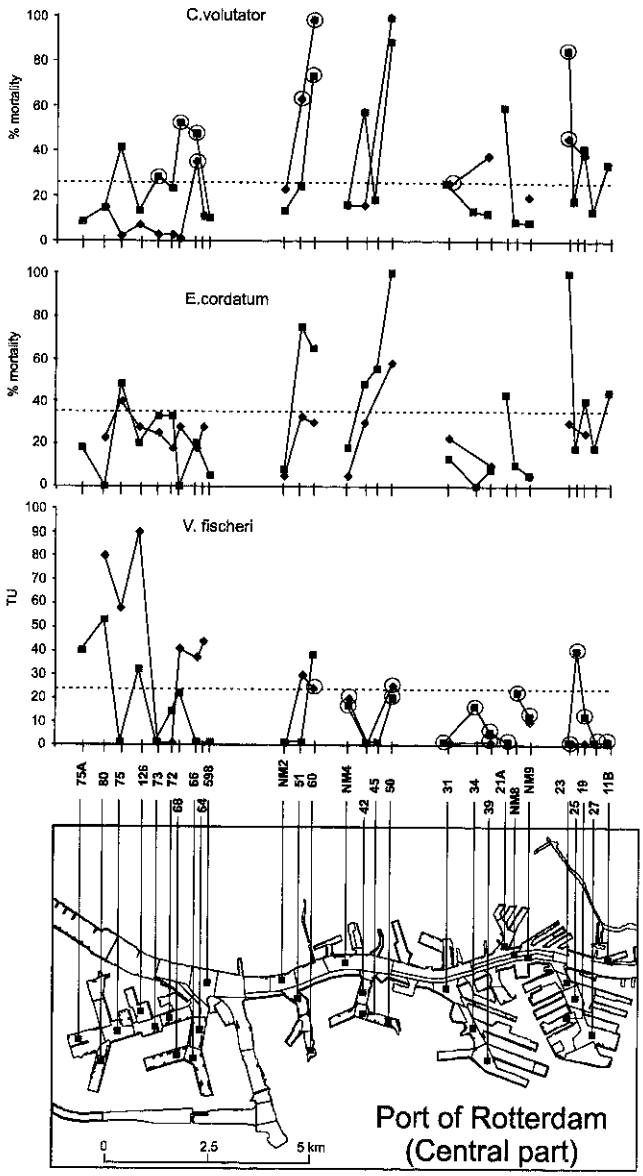

Fig. 4. Sediment toxicity in the central harbors of Rotterdam (The Netherlands) according to three marine bioassays in 1999 (filled diamonds) and 2000 (filled squares). Data that did not meet the test acceptability criteria or modifying factor criteria are encircled (open circles). The dotted lines indicate threshold values; TU = toxic units.

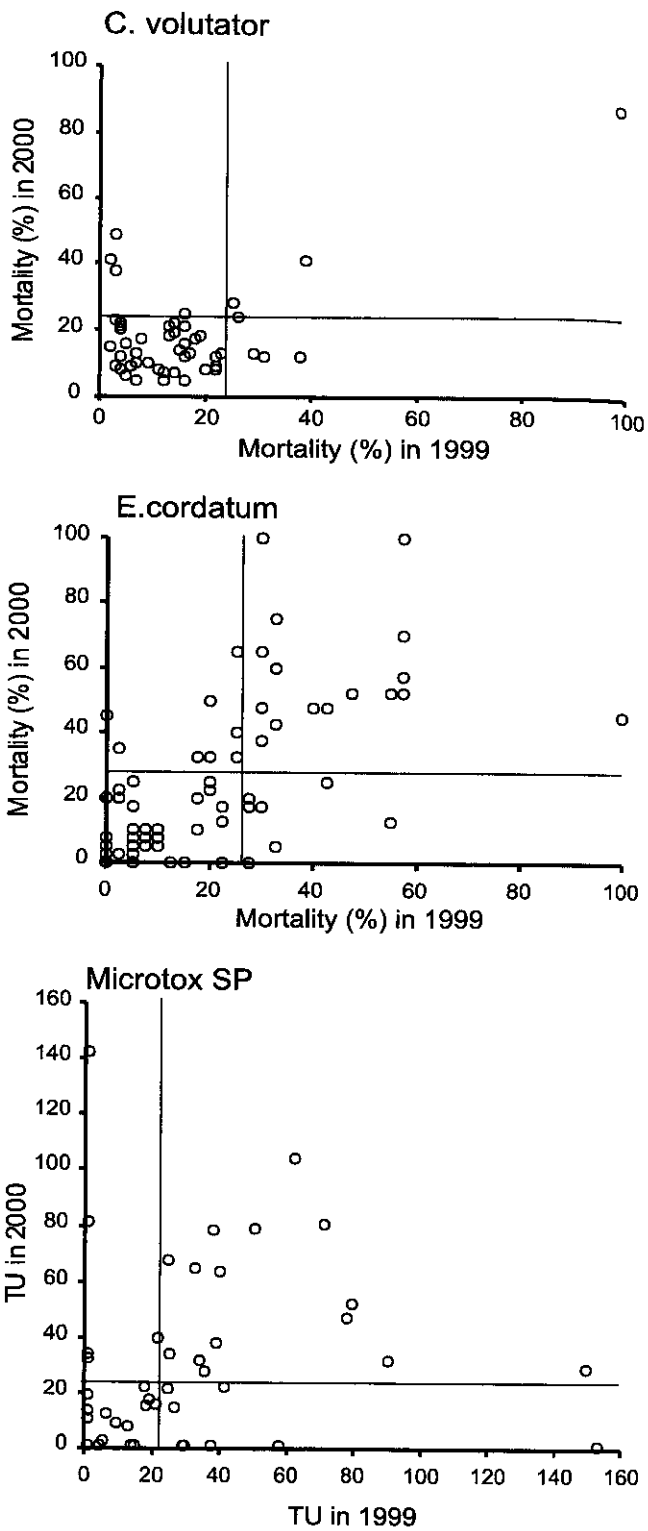

Fig. 5. Comparison between the sediment toxicity of the same sites investigated in 1999 and 2000 according to approved bioassay results with *Corophium volutator*, *Echinocardium cordatum*, and *Vibrio fischeri*; TU = toxic units.

unexpected since this location is not known to be contaminated.

In general, at least three quarters of the duplicate tests in the two consecutive years were uniformly classified. Nevertheless, the temporal variations in sediment toxicity were quite substantial (Fig. 5). This is probably related to conditions in the harbor section varying, for instance, because of dredging of (contaminated) material.

Evaluation of the bioassays

A selection of bioassays for sediment toxicity testing can be made on several theoretical and practical considerations [1,32,33]. The rationale for using the two selected whole mortality bioassays in this study was based on four arguments: namely (1) the organisms, tolerating a wide range of grain sizes, are realistically exposed to the whole sediment matrix; (2) the uptake of contaminants can take place through the water phase and by sediment ingestion; (3) the test organisms are ecologically relevant for the marine benthic ecosystems of The Netherlands; and (4) the endpoint mortality is directly linked to population dynamics. The decision to apply the Microtox SP bioassay was based on practical considerations, namely its reported correlation with invertebrate toxicity bioassays

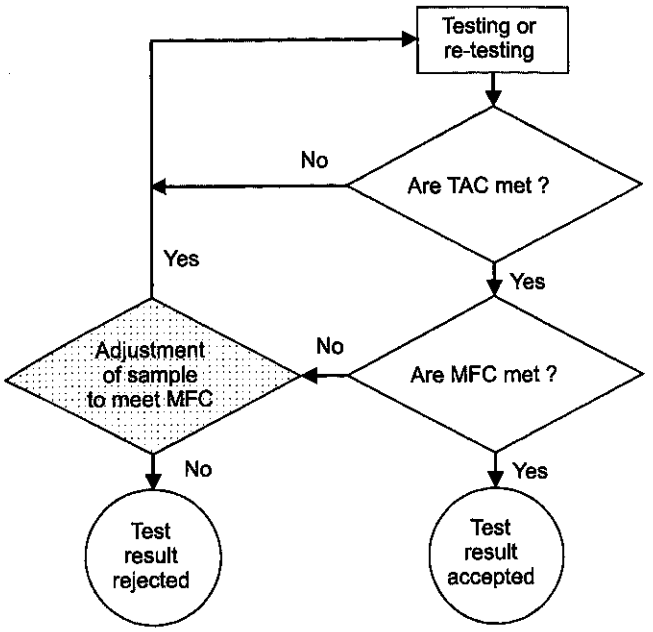

Fig. 6. Flow chart on the procedure for test acceptability and validation of modifying factors for sediment bioassays. TAC = test acceptability criterion; MFC = modifying factor criterion. In the present study samples have not been modified to meet MFC.

[11,30]; the large knowledge base that is available through worldwide applications [11,34]; the good interlaboratory reproducibility [12,35]; and low costs. The confounding influence of sediment silts in this test has been recognized [16] and tackled by the mathematical correction procedure. Alternative Microtox test procedures using sediment extracts were not selected, as they also have their limitations [36].

The bioassays used in this study provide a relative measure of nonspecific toxicity and do not indicate specific toxicological syndromes. Moreover, all three bioassays used in this study have short-term exposures relative to the life cycle of the organisms. It could be argued that the acute tests suffer from a lack of sensitivity. For instance, Ciarelli et al. [37] performed chronic tests on the growth of *C. volutator* exposed to the same type of sediments as in our study and found effects in all tests. Other, potentially more sensitive, bioassays have been considered in the past for testing marine sediment in the Netherlands but have been rejected for various reasons. A 10-d amphipod test using *Bathyporeia sarsi* was excluded, for this organism dwells by nature in sediment with a much lower fine sediment fraction than the average harbor sediment [13]. A 4-d pore water test using the copepod *Acartia tonsa* was considered not appropriate because of its predominant sensitivity to ammonia, even after ammonia-reduction procedures [38]. The 2-d elutriate test with oyster *Crassostrea gigas* larval development bioassay was rejected because of the large variations between the test series and poor interlaboratory repeatability [12]. Despite the short-term exposure, the three bioassays in our study have been demonstrated to have sufficient discriminatory power to distinguish between those Dutch harbor sediments that give rise to adverse effects from those that do not.

During our survey it emerged that the availability of heart urchins in the wild is sometimes variable and that mortality between replicates cause statistical overdispersion. The reason for the variation between replicates is still unknown, although several aspects have already been considered. For instance, variation in exposure levels and sensitivity between the replicates seems unlikely, since sediments are well mixed and test organisms are randomly selected. We checked for negative influences of decaying biomass of dead heart urchins on remaining survivors in the same compartment, but found none (data not shown). We therefore recommend culturing the test organisms and improving the statistical performance of the heart urchin bioassay.

We conclude that the amphipod and bacterium bioassay discussed in this paper are suitable for regulatory use in granting permits for disposal of dredged material in Dutch coastal waters, in addition to the chemical screening that has been used until now.

Acknowledgement—The research presented here was part of a larger study on the development of a combined chemical–biological assessment framework for contaminated harbor sediments suggested for disposal in Dutch coastal waters and was financed by the Dutch Ministry of Transport, Public Works, and Water Management. We thank the field crews of the regional directorates of the Ministry for collecting the sediments. We are indebted to E. Leene, C.M. Keijzers, and S. Dalloesingh (AquaSense) for their testing efforts. Data management assistance by B. Haenen, K. van Essen, and J. Honkoop (RIKZ) was very much appreciated. We are grateful for the suggestions on the draft manuscript by A. Brouwer and B. van Hattum. J. Burrough advised on the English.

REFERENCES

1. Hill IR, Matthiessen P, Heimbach F. 1993. Guidance document on sediment toxicity tests and bioassays for freshwater and marine environments. Workshop Report. SETAC-Europe, Renesse, The Netherlands, November 10–12.
2. DeWitt TH, Ditsworth GR, Swartz RC. 1988. Effects of natural features on the phoxocephalid amphipod, *Rhepoxynius abronius*: Implications for sediment bioassays. *Mar Environ Res* 25:99–124.
3. Moore DW, Bridges TS, Gray BR, Duke BM. 1997. Risk of ammonia toxicity during sediment bioassays with the estuarine amphipod *Leptocheirus plumulosus*. *Environ Chem Toxicol* 16: 1020–1027.
4. Wang F, Chapman PM. 1999. Biological implications of sulfide in sediment—A review focusing on sediment toxicity. *Environ Toxicol Chem* 18:2526–2532.
5. Schipper CA, Stronkhorst J. 1999. Handbook for toxicity testing of dredged material. RIKZ-99.012. Technical Report. Rijksinstituut voor Kust en Zee/National Institute for Coastal and Marine Management, The Hague, The Netherlands.
6. Van den Hurk P, Chapman PM, Roddie B, Swartz RC. 1992. A comparison of North American and West European infaunal amphipod species in a toxicity test on North Sea sediments. *Mar Ecol Prog Ser* 91:237–243.
7. Grant A, Briggs AD. 2002. Toxicity of sediments from around a North Sea oil platform: Are metals and hydrocarbons responsible for ecological impacts? *Mar Environ Res* 53:95–116.
8. Roddie BD, Thain JE. 2001. Biological effects of contaminants: *Corophium* sp. sediment bioassay and toxicity test. ICES techniques in marine environmental sciences, 28. International Council for the Exploration of the Sea, Copenhagen, Denmark.
9. Bowmer CT. 1993. Method for the assessment of acute toxicity of contaminated sediment using the burrowing heart urchin *Echinocardium cordatum*. Test guideline for OSPAR sediment reworker ring test. Report IMW R 93/317. Organization of Applied Scientific Research TNO, Delft, The Netherlands.
10. Daan R, Lewis WE, Mulder M. 1990. Biological effects of discharged oil-contaminated drill cuttings in the North Sea. Report NIOZ 1990–5. Netherlands Institute for Sea Research, Texel, The Netherlands.
11. Doherty FG. 2001. A review of the Microtox toxicity test system for assessing the toxicity of sediments and soils. *Water Qual Res J Can* 36:475–518.
12. Stronkhorst J, Ciarelli S, Schipper CA, Postma JF, Dubbeldam MC, Vangheluwe M, Brils JM, Hooftman RN. 2003. Interlabor-

atory comparison of five marine bioassays for the toxicity evaluation of dredged material in the Netherlands. *J Aquat Ecosyst Health Manag* (in press).
13. Stronkhorst J, van den Hurk P, Bowmer CT, Gerritsen AMM, Klamer HJC. 1996. Toxicity assessment of slightly contaminated dredged material from the port of Rotterdam. In Munawar M, Dave G, eds, *Development and Progress in Sediment Quality Assessment: Rationale, Challenges, Techniques and Strategies.* Ecovision World Monograph Series. Academic, Amsterdam, The Netherlands, pp 115–126.
14. Stronkhorst J, Maas JL, van de Guchte C, Hartnack J, Lourens JM, Vonck APMA. 1997. Dredged material, more or less harmful. Report FWVO 97.04. National Institute for Coastal and Marine Management, The Hague, The Netherlands (in Dutch).
15. Van der Hoeven N, Kater BJ, Pieters JF. 2002. Statistical tests and power analysis for three in-vivo bioassays to determine the quality of marine sediments. *Environmetrics* 13:281–293.
16. Ringwood AH, DeLorenzo ME, Ross PE, Holland AF. 1997. Interpretation of Microtox solid-phase toxicity tests: The effects of sediment composition. *Environ Toxicol Chem* 16:1135–1140.
17. Postma JF, de Valk S, Dubbeldam M, Maas JL, Tonkes M, Schipper CA, Kater BJ. 2002. Confounding factors in bioassays with freshwater and marine organisms. *Ecotoxicol Environ Saf* 53: 226–237.
18. Phillips BM, Anderson BS, Hunt JW. 1997. Measurement and distribution of interstitial and overlying water ammonia and hydrogen sulfide in sediment toxicity tests. *Mar Environ Res* 44: 117–126.
19. Burgess RM. 2000. Characterizing and identifying toxicants in marine waters: A review of marine toxicity identification evaluations (TIEs). *Int J Environ Pollut* 13:2–33.
20. Ho KT, Burgess RM, Pelletier C, Serbst JR, Ryba SA, Cantwell MG, Kuhn A, Raczelowski P. 2003. An overview of toxicant identification in sediments and dredged materials. *Mar Pollut Bull* 44:286–293.
21. Stronkhorst J, Schot ME, Dubbeldam MC, Ho K. 2003. A toxicity identification evaluation (TIE) of silty marine harbor sediments to characterize persistent and non-persistent constituents. *Mar Pollut Bull* 46:56–64.
22. Ferretti JA, Calesso DF, Hermon TR. 2000. Evaluation of methods to remove ammonia interference in marine sediment toxicity tests. *Environ Toxicol Chem* 19:1935–1941.
23. McNulty EW, Dwyer FJ, Ellersieck MR, Greer EI, Ingersoll CG, Rabeni CF. 1999. Evaluation of ability of reference toxicity tests to identify stress in laboratory populations of the amphipod *Hyalella azteca*. *Environ Toxicol Chem* 18:544–548.
24. Swartz RC, Schults DW, Ozretich RJ, Lamberson JO, Cole FA, DeWitt TH, Redmond MS, Ferraro SP. 1995. ΣPAH: A model to predict the toxicity of polynuclear aromatic hydrocarbon mixtures in field-collected sediments. *Environ Toxicol Chem* 14:1977–1987.
25. Thursby GB, Heltshe J, Scott KJ. 1997. Revised approach to toxicity test acceptability criteria using a statistical performance assessment. *Environ Toxicol Chem* 16:1322–1329.
26. Phillips BM, Hunt JW, Anderson BS, Puckett HM, Fairey R, Wilson CJ, Tjeerdema R. 2001. Statistical significance of sediment toxicity test results: Threshold values derived by the detectable significance approach. *Environ Toxicol Chem* 20:371–373.
27. McGee BL, Fisher DJ, Yonkos TL, Ziegler GP, Turley S. 1999. Assessment of sediment contamination, acute toxicity, and population viability of the estuarine amphipod *Leptocheirus plumulosus* in Baltimore Harbor, Maryland, USA. *Environ Toxicol Chem* 18:2151–2160.
28. Long ER, Hong CB, Severn CG. 2001. Relationships between acute sediment toxicity in laboratory tests and abundance and diversity of benthic infauna in marine sediments: A review. *Environ Toxicol Chem* 20:46–60.
29. U.S. Environmental Protection Agency. 1998. Evaluation of dredged material proposed for discharge in waters of the U.S. EPA 823/B-98/004. Washington DC.
30. Day KE, Dutka BJ, Kwan KK, Batsita N, Reynoldson TB, Metcalfe-Smith JL. 1995. Correlations between solid-phase microbial screening assays, whole-sediment toxicity tests with macroinvertebrates and in situ community structure. *J GT Lakes Res* 21: 192–206.
31. Stronkhorst J, Leonards P, Murk T. 2002. Using the dioxin receptor-CALUX in vitro bioassay to screen marine harbor sediments for compounds with a dioxin-like mode of action. *Environ Toxicol Chem* 21:2552–2561.
32. Long ER, Buchman MF, Bay SM, Breteler RJ, Carr RS, Chapman PM, Hose JE, Lissner AL, Scott J, Wolfe DA. 1990. A comparative evaluation of five toxicity tests with sediments from San Francisco Bay and Tomales Bay, California. *Environ Toxicol Chem* 9:1193–1214.
33. Dillon TM. 1994. A paradigm for developing sediment toxicity bioassays for the regulatory evaluation of dredged material. Paper D-94-4. U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.
34. Kaiser KLE, Devillers J. 1994. *Ecotoxicity of Chemicals to Photobacterium Phosphoreum.* Gordon and Breach Science, Reading, UK, p 879.
35. Ross P, Burton GA, Greene M, Ho K, Meier P, Sweet L. 1999. Interlaboratory precision study of a whole sediment toxicity test with the bioluminescent bacterium Vibrio fischeri. *Environ Toxicol Water Qual* 14:339–345.
36. Guzzella L. 1998. Comparison of test procedures for sediment toxicity evaluation with *Vibrio fischeri* bacteria. *Chemosphere* 37:2895–2909.
37. Ciarelli S, Vonck WAPMA, van Straalen NM, Stronkhorst J. 1998. Ecotoxicity assessment of contaminated dredged material with the marine amphipod *Corophium volutator*. *Arch Environ Contam Toxicol* 34:350–356.
38. Gerritsen AAM, Bowmer CT, Roza P, Voskamp T. 1994. The application of ammonium reduction techniques prior to toxicity testing of light to moderately contaminated harbour dredged sediments using the marine copepod *Acartia tonsa*. Report MW R 94/167. Organization of Applied Scientific Research TNO, Delft, The Netherlands.
39. Microbics. 1995. Microtox® acute toxicity solid-phase test. Carlsbad, CA, USA.

APPENDIX

The following is the correction procedure for the light inhibition caused by small sediment particles in the Microtox solid phase (SP) test.

It is assumed that the light emission by *Vibrio fischeri* is affected by the fine sediment particles (particles effect) and toxicants (toxic effect) in the sediment, and that these effects are multiplicative; therefore,

$$I(c) = I(0) \cdot F_{\text{toxic}} \cdot F_{\text{particles}} \tag{A1}$$

where $I(c)$ = bioluminescence at a sediment concentration c, $I(0)$ = bioluminescence in a blank sample, F_{toxic} is the effect of the toxicants in the sediment, and $F_{\text{particles}}$ is the effect of the small particles.

The toxic effect is, as in the standard Microtox data evaluation [39], described by the logistic relation (Eqn. A2), and the particle effect is described by a logistic relation using a slope parameter β of 1 (Eqn. A3):

$$F_{\text{toxic}} = \frac{1}{1 + (c/E_t)^{\beta}} \tag{A2}$$

$$F_{\text{particles}} = \frac{1}{1 + (c/\tilde{E})} \tag{A3}$$

where E_t is the effective concentration (EC50) due to the toxic components of the sediment, and $\tilde{E}$ is the EC50 due to the particles effect. Both E_t and EC50 are expressed in volume

percentage of dry sediment in the test medium. Combining Equations A1, A2, and A3 results in

$$I(c) = \frac{I(0)}{[1 + (c/E_t)^{\beta}] \cdot [1 + (c/\tilde{E})]} \quad \text{(A4)}$$

It is impossible to estimate all four parameters, $I(0)$, E_t, $\tilde{E}$, and β, from the data of a Microtox SP test because the toxic effect masks the particles effect or vice versa. Therefore, independent information about the particles effect was used to estimate $\tilde{E}$. If (1) bacteria are bound to the fine sediment fraction S and (2) the probability of binding is independent of S, the relation between log (S) and log (E) will be linear with slope -1. This relation was estimated using data from uncontaminated sediments from South Carolina, USA [16] and the Eastern Scheldt estuary, The Netherlands [14] (see Fig. A1). The slope of the linear regression between log (S) and log ($\tilde{E}$) is -0.94 and does not deviate significantly from -1. Using a slope of -1, the estimate of a in the relation $^{10}\log[\tilde{E}(S)] = a - {}^{10}\log(S)$ is 1.11 (SD = 0.75). It follows that

$$\tilde{E}(S) \approx 10^{1.11} \cdot S^{-1} \approx 21.8 \cdot S^{-1} \quad \text{(A5)}$$

This estimate of $\tilde{E}$ was applied in Equation A4, and then $I(0)$, E_t, and β were fitted with the least squares method. Before doing so, we checked if the effect observed in each Microtox SP test was more than could be explained by the fine sediment fraction alone. We estimated the EC50 of the sediment, assuming that the effect was caused by only one factor. That is, we estimated E in the equation

$$I(c) = \frac{I(0)}{1 + (c/E)^{\beta}} \quad \text{(A6)}$$

If $\hat{E}$, which is the estimate of E, was larger than or within the error range of $\tilde{E}(S)$, it was concluded that no distinction between the toxic effect and particles effect could be made; that is, E_t could not be estimated. Only if $\hat{E} < \epsilon \cdot \tilde{E}(S)$, Equation A4 was used to estimate E_t. For ϵ a value of 0.22 was used, which is equal to the one-sided 99% confidence limit for estimates of $\tilde{E}(S)$ (Fig. A1).

Software for this correction procedure is available from the authors.

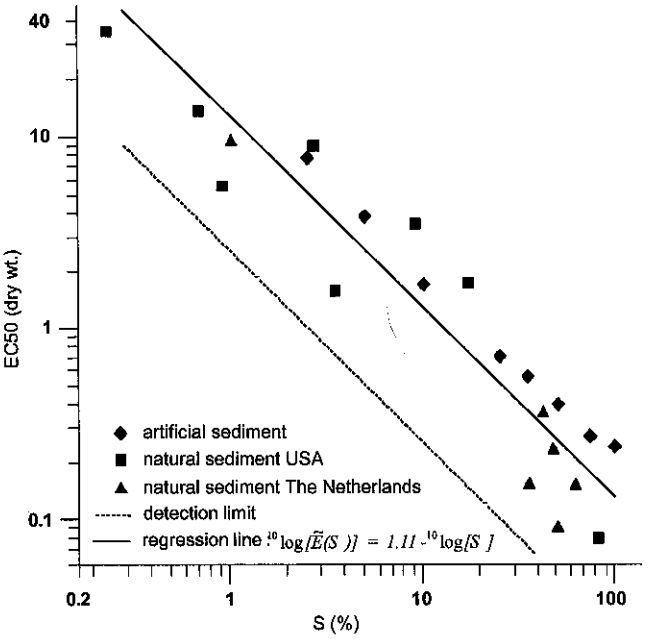

Fig. A1. The median effective concentration (EC50; percentage dry sediment) for bioluminescence inhibition for *Vibrio fischeri* as a function of the fine sediment fraction <63 μm (S) in artificial and natural clean sediments from the USA [16] and natural clean sediments from The Netherlands [14]. The detection limit for the toxic effect was set at 0.2 times the estimate of $\tilde{E}(S)$.