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Invited review paper

Toxicity analysis of freshwater and marine sediments with meio- and macrobenthic organisms: a review

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Abstract

Benthic metazoans play a key role as test organisms in toxicity analyses of aquatic ecosystems. This report gives an overview of the species of benthic metazoans used for the assessment of toxicity in freshwater and marine sediments, as well as of the criteria relevant to the choice between test species and procedures. The main applications of these organisms are mono-species bioassays, test-batteries, analyses of benthic communities and bioaccumulation studies. Sediment toxicity assays, including acute and chronic exposures, have been developed for nematodes, insects, oligochaetes, polychaetes, crustaceans, molluscs and echinoderms. At least 30 species of freshwater and 71 species of marine and estuarine benthic metazoans have thus far been used in sediment toxicity bioassays. Although aquatic pollution is a world-wide problem, most sediment toxicity bioassays have been developed for organisms native to Europe and North America. The most common bioassay endpoints are mortality, development, growth and behavioural responses. The value of genetic, biochemical, physiological and pathological responses as toxicity endpoints is currently being investigated. The quest for additional test species and protocols is still a worthwhile endeavour in sediment ecotoxicology.

Table of contents

Introduction

Meio- and macrofauna inhabit freshwater and marine sediments, along with bacteria and protozoans. These

metazoans occupy a variety of trophic levels, acting as prey, predators, herbivores, omnivores, collectors, gatherers, shredders and filter feeders. In addition, many meiobenthic species influence through predation the abundance and biomass of bacteria in the sediment. Hence, these organisms control to a large extent the cycling dynamics of energy, nutrients and organic matter in the benthos and the water column (e.g. Carpenter, 1988; Minshall, 1988). Contamination is known to alter zoobenthic communities in freshwater (e.g. Malueg et al., 1984a, b) and marine environments (e.g. Becker et al., 1990), thus affecting significantly the integrity of ecosystems. The disappearance of certain benthic species, e.g. of hydrocarbon-degrading microorganisms, could reduce significantly the capacity of the water system to recover from subsequent, additional contamination (see Lee & Levy, 1989).

Benthic macroinvertebrates have been used as indicators of aquatic ecosystem health at least since the seminal work by Kolkwitz & Marsson (1909) early this century (see Cairns & Pratt, 1993). Analyses of composition and function of benthic communities, as well as toxicity bioassays with single species, have been used for tests of water quality, given the sensitive response of benthic organisms to toxic compounds (e.g. Bargos et al., 1990; Elder, 1990; Nelson, 1990). Benthic metazoans, in addition to other organisms, have long been used to assess the toxicity of a range of pollutants in single contaminant tests of aqueous solutions (reviews in Murphy, 1980; Hellawell, 1986; Coull & Chandler, 1992). The routine use of these organisms to assess the toxicity of effluents (e.g. Oshida et al., 1981; US EPA, 1988, 1989) and sediments, in which complex mixtures of chemicals interact, is a development of the previous decade which acknowledges the sensitivity of benthic meio- and macroinvertebrates as indicators of ecosystem pollution (Burton et al., 1992; Hill et al., 1993).

Most toxic pollutants of aquatic systems have a strong affinity for particulate material and eventually become associated with the sediment. As a result, sediments can accumulate concentrations of pollutants which exceed by several orders of magnitude those in the water column (contributions in Dickson et al., 1984). Benthic organisms play an integral role in the effects, fates and cycling of these contaminants in the ecosystem (Nalepa & Landrum, 1988). These metazoans are particularly vulnerable to toxic compounds, given their close contact with sediment particles and interstitial water for extended periods of their life cycle. Catallo (1993) reviews the ecological effects of pollution in wetland ecosystems, including specific sections on benthic organisms. Perturbations in the benthos due to the toxic effect of introduced agents can be manifested at various levels, including molecules and cells, organisms, populations and communities, and whole ecosystems, in decreasing order of response sensitivity (Burton, 1991). Most single-species tests of toxicity in sediments have focused on alterations within organisms (gross morphology, cells and molecules), their physiology, life history variables and behaviour. Although the majority of such tests involves exposure of live organisms to potential toxicants, there are exceptions such as the *in vitro* enzyme inhibition test for the cladoceran *Daphnia* and mayfly nymphs *Hexagenia* developed by Buikema et al. (1980b). Some animal welfare considerations relevant to bioassays with invertebrates are found in Olson et al. (1991).

The various approaches used in sediment quality assessments with biological assays are summarized briefly in Adams et al. (1992). Meanwhile, a vast number of sediment toxicity tests have been made with benthic animals including field studies, laboratory tests and analyses of micro- and mesocosms (see reviews in Buikema & Cairns, 1980; Nebeker et al., 1984; Chapman, 1986a, 1988; Ahlf & Munawar, 1988; IJC, 1988; Lamberson & Swartz, 1988; Giesy & Hoke, 1990; Giesy et al., 1990; Burton, 1991; Burton et al., 1992; Coull & Chandler, 1992; Hamer et al., 1992; Lamberson et al., 1992; Hill et al. 1993; Hooftman & Gimeno, 1993; Ahlf, 1994; US EPA 1994a, b; and contributions in Burton, 1992). Methods for the assessment of sediment toxicity in marine benthos are described in Chapman (1986a, 1988), Chapman & Becker (1986), Reish & Lemay (1988), Swartz (1987), Clark et al. (1989), US EPA/US ACE (1991), ASTM (1993a, 1995), Carr & Chapman (1992), Environment Canada (1992), Lamberson et al. (1992); Hill et al. (1993) and Luoma & Ho (1993). Only a small proportion of these tests, however, have become established in routine diagnoses of sediment toxicity. The aim of this report is to give an overview of test procedures with benthic metazoans for the assessment of sediment toxicity in freshwater and marine ecosystems, as well as of criteria relevant to the choice between such bioassays. Introductory overviews to the terminology, design and interpretation of aquatic toxicity tests are found in Buikema et al. (1982) and Elder (1990). Definitions of commonly used technical terms are given in Table 1.

Biological tests are widely recognized as an essential tool in toxicity assessments, given the limitations

Table 1. Definitions of some technical terms used in the text (adapted from Hill et al. 1993, with some additions).

bioassay: an experiment in which single test-species are exposed in the laboratory to samples of a field sediment (or extracts of this) potentially containing one or more contaminants, with the aim of measuring possible biological effects of those contaminants.
toxicity test: an experiment in which single test species are exposed in the laboratory to a clean natural or artificial sediment which has been dosed (*spiked*) in the laboratory with a known chemical or a mixture of chemicals, generally at a range of concentrations. The purpose of the experiment is to measure the degree of response associated with specific concentrations of the chemical(s).
whole sediment: the sediment and its interstitial water, also referred to as the *solid phase*.
interstitial water (pore-water): the water occupying the spaces between sediment particles, and that may be removed from the whole sediment by pressure/vacuum filtration, centrifugation or compression.
elutriate: an extract of whole sediment, obtained by "washing" with water (*aqueous elutriate*) or an organic solvent (*organic elutriate*).
overlying water: the water in the test chamber overlying the sediment, in a bioassay or toxicity test.
short (acute) and long-term (chronic) tests: This categorization refers to the duration of exposure to the test substance/s or of the monitoring of effects following exposure, relative to the life-span of the test organism. A long-term exposure should allow sufficient time for the contaminant to reach a steady state in the tissue of the test animals.
meiobenthos: organisms which pass a net with a mesh size of 0.5 or 1.0 mm and are retained at a mesh size of 0.04 mm.
macrobenthos: organisms which are retained in a net with a mesh size of 0.5 or 1.0 mm.
microcosm: enclosures of aquatic ecosystems smaller than 1 m^3.
mesocosm: enclosures of aquatic ecosystems larger than 1 m^3(Hobbie & Wakeham, 1988).

of chemical-analytical methods arising from lack of knowledge about the bioavailability of most pollutants, especially of heavy metals (Knezovich et al., 1987; Landrum & Robbins, 1990; Pavillon, 1990; Hill et al., 1993). Analytical quantification of the bioavailability of sediment associated pollutants is complex, given the great variation in concentrations and exposure modes within the sediment, as well as the diversity of habits of benthic organisms (Lee, 1991). In field sediments contaminated with complex mixtures of chemicals, biological-response-tests are currently the only way to assess potential toxicity (Hill et al., 1993). Moreover, since sediment toxicity results from the action of such mixtures, including synergistic and antagonistic effects, the bioassay with benthic organisms is a method of pollution assessment whose validity is not dependent on correlations with sediment chemistry (Oakden et al., 1984a; Thomas et al., 1986; Swartz et al., 1984, 1988). When identification of toxic compounds in sediments is desired, bioassays can be used to prioritize sites for chemical analysis (Giesy & Hoke, 1989). Several bioassay techniques have been used to rank freshwater sediments on the basis of toxicity to benthic organisms (e.g. Prater & Anderson, 1977b; Samoiloff et al., 1983a; Malueg et al., 1984b; Le Blanc & Surprenant, 1985).

Bioassays with benthic freshwater and marine species have had important applications, beyond the demonstration of sediment toxicity in areas of concern. For example, these bioassays have generated data required to set water and sediment quality criteria (e.g. Zarba, 1988; Elder, 1990; van der Gaag et al., 1991; but see Franklin, 1983), because toxicity tests with benthic organisms are direct indicators of impact, whereas

chemical concentrations *per se* are not. In addition, the bioassays have been instrumental for the planning of remedial action in (1) mapping sediment toxicity horizontally and vertically, (2) prioritizing sites for further analyses or potential remediation and (3) assessment of the effectiveness of remedial action (Giesy & Hoke, 1989; Baudo et al., 1990; see also Cairns, 1988a). Furthermore, the toxicity responses of benthic metazoans have provided insight into the history of contamination based on vertical sediment profiles (e.g. Warwick, 1980; Swartz et al., 1991). Bioassays might even be a useful tool in broadly identifying classes of toxic components of contaminated substrates (e.g. soils: Thomas et al., 1986).

The power of laboratory bioassays, however, should be viewed in the light of problems and limitations associated with their application to sediments. These fall into four categories (Lamberson et al., 1992): 1) alterations of the toxicological properties of the sediment during sampling and handling, 2) sensitivity of test organisms to natural sedimentary features and laboratory conditions, 3) toxicological uncertainties arising from the narrow range of contaminants as yet tested and from the difficulties in measuring exposure concentrations, and 4) poorly understood ecological interactions and relevance. Minimizing these problems, and the potential for over- and underestimation of toxicity (Luoma & Carter, 1993), has been and still remains a challenge in the development of standardized protocols for sediment bioassays.

Sediment toxicity assays with benthic metazoans

Bioassays with 101 species of infaunal and epibenthic metazoans for freshwater and marine sediments are listed in Tables 2 and 3, respectively. In the 'remarks' column of the tables, reference is made to how well established the bioassay has become. The three categories are 'standard method', 'method widely used' and no reference to this criterion, in decreasing order of general acceptance. For the most common tests this judgement is taken from Hill et al. (1993). In the remaining cases, we based the judgement on our own coverage of the literature. The degree of popularity and standardization, however, is not necessarily a measure of the adequacy of the bioassay, for recently developed test procedures of low present popularity, may nevertheless already fulfill most requirements for ideal bioassays. Table 4 lists a selection of toxicity bioassays with benthic metazoans, which have not been specifically applied to sediments, but which enrich the taxonomic range of potential applications in sediment toxicology.

Choice of bioassay

An ideal laboratory bioassay should be rapid, simple, replicable, inexpensive, standardized, sensitive, discriminatory, ecologically relevant, relatable to field effects and useful in developing regulatory criteria (Giesy & Hoke, 1989). These criteria are closely fulfilled by those bioassays which have been standardized and widely recommended (see Tables 2 and 3). In selecting an appropriate bioassay, however, the first decision relevant to this review, is whether a test with benthic metazoans should be included in the toxicological evaluation of a particular area of concern. Sediment toxicity assessments have been based on the biological responses of microbes, primarily bacteria and algae, benthic and nektonic invertebrates, amphibians and fish (overview in Giesy & Hoke, 1989). The specific aim of the toxicological study determines the choice of organism. For a rapid and sensitive screening operation, microbial assays (e.g. bacteria bioluminescens: Bulich, 1983, 1984) can yield the required information both in freshwater (Giesy et al., 1988) and marine environments (Becker et al., 1990). A more in depth toxicological analysis, however, requires the inclusion of bioassays with benthic invertebrates because of their higher discriminatory power and ecological relevance (Giesy et al., 1988; Becker et al., 1990).

The main applications of metazoan benthic species to assess the toxicity of sediments are in mono-species tests, test-batteries and analyses of benthic community structure and function. Other applications of bioassays with zoobenthic organisms include the monitoring of sediment toxicity with multi-species tests, bioaccumulation studies and analyses of morphological deformities. Giesy & Hoke (1989) give guidelines for the choice of test organism, test design and data analysis, with reference to advantages and disadvantages of sediment bioassays with some freshwater, benthic metazoans (see also Elder, 1990; ASTM, 1993b). Luoma & Ho (1993) describe the appropriate uses of marine and estuarine sediment bioassays, with recommendations for the choice of test organism and for the collection and handling of sediment samples (see also ASTM, 1991b, 1993b). Further guides to standardized methods and recommended procedures for the assessment of sediment toxicity are found in publications

Table 2. Freshwater, sediment toxicity bioassays with benthic metazoans (nat. = natural, sed. = sediment).

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
Infaunal organisms						
INSECTA						
Chironomus sp.	mortality, behaviour (sed. selection)	24–48h, 48–60h	solid	nat.sed.		Gannon & Beeton (1969)
Chironomus sp.	mortality	48h, 10d	solid	spiked sed., copper		Cairns et al. (1984)
C. decorus	mortality	48h	aqueous, substrate bound (food)	copper		Kosalwat & Knight (1987b)
C. riparius	mortality, growth	13d (14d)	solid	nat. sed.		Ingersoll & Nelson (1990)
C. riparius	adult emergence	29d	solid	nat. sed.		Ingersoll & Nelson (1990)
C. riparius	mortality	24h	solid	nat. sed.	fourth instar larvae	Lydy et al. (1990)
C. riparius	mortality, growth, adult emergence	10d (up to 30d)	solid	any sed.	standard method	ASTM (1994a), BBA/IVA (1994)
C. riparius	mortality, adult emergence	28d	solid	any sed.	standard method	ASTM (1994a), BBA/IVA (1994)
C. tentans	larval mortality, growth	17–20d	solid	nat. sed. with heavy metals		Wentsel et al. (1977a)
C. tentans	adult emergence	17–20d	solid	nat. sed. with heavy metals		Wentsel et al. (1978b)
C. tentans	mortality, growth	14d	solid	Kepone		Adams et al. (1985)
C. tentans	larval mortality, growth, bioaccumulation	10d, 15d	solid	test sed.	part of a multi-species test	Nebeker et al. (1984)
C. tentans	adult emergence	25d	solid	test sed.	part of a multi-species test	Nebeker et al. (1984)
C. tentans	growth	10d	solid	nat. sed.	standard method	Giesy et al. (1988)
C. tentans	mortality, growth, adult emergence	10d (up to 25d)	solid	any sed.	standard method	ASTM (1994a)
Hexagenia sp.	mortality	4d	solid (and pore-water)	nat. sed.		Bahnick et al. (1980)
Hexagenia sp.	enzyme inhibition	1.5h	extract	test sed.		Buikema et al. (1980b)
Hexagenia sp.	mortality	24–48h, 4d	extract	test sed.	reference data for enzyme inhibition test	Buikema et al. (1980b)
Hexagenia sp.	mortality, growth	14d	solid	test sed.	method widely used	IJC (1988)
Hexagenia sp.	mortality, growth, behaviour (burrowing), molting frequency	4–10d, 21d	solid	test sed.	method widely used	Giesy & Hoke (1989), Bedard et al. (1992), ASTM (1994a)

Table 2. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
H. limbata	mortality	4d	solid	nat. sed.	part of multi-species test	Prater & Anderson (1977a, b)
H. limbata	mortality	10d	solid	copper in nat. sed.	combined with a 2 d *Daphnia* test	Malueg et al. (1983, 1984a)
H. limbata	mortality, bioaccumulation	10d, 28d	solid	test sed.	part of a multi-species test	Nebeker et al. (1984)
H. limbata	nymph production	–	*in situ*	nat. sed.		Edsall et al. (1991)
H. rigida (eggs)	embryonic development, egg hatching	35d	(not specified)			Friesen (1979)
Stenonema modestum	mortality, reproduction, growth	7–14d	elutriate	nat. sed.		Diamond et al. (1992)
Paratanytarsus parthenogenica	adult emergence, egg hatchability	21d	elutriate	dredged sed.		Le Blanc & Surprenant (1985)
OLIGOCHAETA						
Limnodrilus claparedeanus	mortality, growth, reproduction	500d	solid	nat. sed.		Wiederholm et al. (1987)
L. hoffmeisteri	mortality, growth, reproduction	500d	solid	nat.sed.,Cu spiked sed.		Wiederholm et al. (1987)
L. hoffmeisteri	mortality	4d	sed. slurry	endrin spiked sed		Keilty et al. (1988a)
L. hoffmeisteri	behaviour (reworking), mortality, weight, bioaccumulation	40–55d	sed. slurry	endrin spiked sed.		Keilty et al. (1988b)
L. udekemianus	mortality, growth, reproduction	500d	solid	nat. sed.		Wiederholm et al. (1987)
Lumbriculus variegatus	mortality, growth, reproduction, bioaccumulation	10–28d	solid	cadmium spiked sed., nat. sed.	method widely used	Carlson et al. (1991), Phipps et al. (1993)
L. variegatus	mortality, growth, reproduction, behaviour (burrowing)	1d, 2d, 14d	solid	nat. sed.		Dermott & Munawar (1992)
Potamothrix hammoniensis	mortality, growth, reproduction	500d	solid	nat. sed.		Wiederholm et al. (1987)
Pristina leidyi	mortality, reproduction	48h, 15–18d	aqueous and solid	reference sed.	short generation times	Smith et al. (1991)

Table 2. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
Stylodrilus heringianus	mortality	4d	sed. slurry, solid	endrin spiked sed.		Keilty et al. (1988a)
S. heringianus	behaviour (reworking), mortality, weight, bioaccumulation	40–55d	sed. slurry	endrin spiked sed.		Keilty et al. (1988b, c)
S. heringianus	behaviour (avoidance)	10min - 4d	solid	nat. sed.		White & Keilty (1988)
Tubifex tubifex	reproduction	200–300d	solid			Milbrink (1987)
T. tubifex	mortality, growth, reproduction	500d	solid	nat.sed., Cu spiked sed.		Wiederholm et al. (1987)
T. tubifex (mature animals)	mortality, reproduction	28d	solid w/o resident fauna	nat. sed. (sieved through 250mm)	method widely used	Reynoldson et al. (1991), ASTM (1994a)
- various species -	mortality	4d	solid	various toxicants in nat. sed.		Chapman et al. (1982a-c)
NEMATODA						
Panagrellus redivivus	mortality, growth, maturation	4 d	extract	nat. sed.	method widely used	Samoiloff et al. (1980; 1983a, b), Samoiloff (1987)
Caenorhabditis elegans	growth, reproduction	3d	solid	nat. sed.		Traunspurger et al. (submitted)
Epibenthic organisms						
ISOPODA						
Asellus aquaticus	mortality	42d	aqueous	Cadmium		Green et al. (1986)
A. communis	mortality	4d	solid	nat. sed.	part of a standard multispecies test	Prater & Anderson (1977a, b)
AMPHIPODA						
Diporeia (Pontoporeia) spp.	mortality	4d	solid (and pore water)	nat. sed.		Bahnick et al. (1980)
Diporeia (Pontoporeia) spp.	behaviour (avoidance), mortality, bioaccumulation	5d, 28d	solid	spiked and nat.sed.	standard method	Gossiaux et al. (1993), ASTM (1994a)
D. affinis	mortality, behaviour (choice)	24–48h	solid	nat.sed.		Gannon & Beeton (1971)

Table 2. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
D. hoyi	mortality	7d	solid	nat.sed.	protocol after R. Dermott	Munawar et al. (1989)
Gammarus sp.	mortality, behaviour (choice)	24–48h, 48–60h	solid	nat. sed.		Gannon & Beeton (1971)
Gammarus sp.	mortality, feeding rate	28–56h	solid	test sed.	method widely used	Pascoe et al. (1992)
Gammarus sp.	mortality	48h, 10d	solid	spiked sed., copper		Cairns et al. (1984)
G. fasciatus	mortality, growth, reproduction	56–70d	solid (and aqueous)	nat. sed.		Borgmann et al. (1989)
G. lacustris	mortality, bioaccumulation	10d, 28d	solid	test sed.	part of a multi-species test	Nebeker et al. (1984)
G. pulex	Scope for growth[1]	6d	water	zinc		Naylor et al. (1989)
G. pulex	behaviour	14h	water	phenolic compounds		Borlakoglu & Kickuth (1990)
G. pulex (only males)	scope for growth	6d (7d)	aqueous	artificial pond water	laboratory and *in situ* application	Maltby et al. (1990a,b)
G. pulex	Feeding rate	7–28d	sed. in an artificial stream	water dosed with toxicant	laboratory and *in situ* application	Maltby (1992)
G. pulex	mortality, feeding rate	30–60d exposure + 10d test	solid	nat. sed. with zinc	(based on Naylor et al., 1989)	Roddie et al. (1992)
Hyalella sp.	mortality	48h, 10d	solid	spiked sed., copper		Cairns et al (1984)
H. azteca	mortality, development, growth, reproduction	14d - several months	(not specified)			de March (1979)
H. azteca	mortality	10d	solid	test sed.	part of a multi-species test	Nebeker et al. (1984)
H. azteca	mortality of adult and young, bioaccumulation	28d	solid	test sed.	part of a multi-species test	Nebeker et al. (1984)
H. azteca	mortality, growth	28d(–56d)	solid (and aqueous)	nat. sed.		Borgmann et al. (1989), Borgmann & Munawar (1989)
H. azteca	mortality	10d	solid	nat. sed		Nebeker et al. (1989), Ingersoll & Nelson (1990)
H. azteca	growth	29d	solid	nat. sed.		Ingersoll & Nelson (1990)
H. azteca	mortality, growth, development, reproduction	10d - 30d	solid	any sed.	standard method	Kubitz (1992), ASTM (1994a)

[1] difference between energy intake through food and energy lost via respiration

Table 2. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
Pontoporeia spp.						(see *Diporeia spp.*)
DECAPODA						
Orconectes virilis	mortality, development	30d - 100d	(not specified)		*in situ* and laboratory application	Leonhard (1979)
Palaemonetes sp.	mortality	4d	elutriate	dredged sed.		Jones & Lee (1978), Lee et al. (1978)
GASTROPODA						
Helisoma sp.	mortality, bioaccumulation	10d	aqueous, solid	cadmium spiked sed.		Carlson et al. (1991)
H. trivolvis	mortality, growth, fecundity	70d - 210d	(not spe-cified)		multi-generation test	Flannagan & Cobb (1979)
BIVALVIA						
Sphaerium sp.	mortality	10d				Crane et al. (1993, not consulted)
Multi-species tests						
Hyalella, Chironomus, Gammarus (and *Daphnia*)	mortality	10d	solid	spiked sed. copper		Cairns et al. (1984)
Hyalella, Gammarus, Hexagenia, Chironomus	mortality	4d or longer	undisturbed	nat. sed., *in situ*	mixed species test in cage	Nebeker et al. (1984)
Hexagenia limbata, Asellus communis, (Daphnia magna and fish)	mortality	4d	solid	nat. sed.		Prater & Anderson (1977a, b)

Table 3. Marine and estuarine sediment toxicity bioassays with benthic metazoans (nat. = natural, sed. = sediment).

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
Infaunal organisms						
AMPHIPODA						
Ampelisca abdita	mortality, emergence, reburial	10d	solid	test and nat. sed.	standard method	Breteler et al. (1989), Scott & Redmond (1989), Di Toro et al. (1990), US EPA/US ACE (1991), ASTM (1993a)
A. abdita	mortality, behaviour (tube building)	4d, 10d	solid	diluted nat. sed.		Rogerson et al. (1985)
Amphiporeia virginica	mortality	10d	solid	test sed.	standard method	Environment Canada (1992)
Bathyporeia sarsi	mortality, behaviour (reburial)	10d	solid	nat. sed.	standard method	van den Hurk et al. (1992)
Corophium insidiosum	mortality	10d	solid	test sed.	standard method	Reish & Lemay (1988)
C. volutator	mortality, behaviour (reburial)	10d	solid	nat. sed.	standard method	Environment Canada (1992), van den Hurk et al. (1992), Roddie et al. (1994)
C. volutator	behaviour (burrowing)	1h	solid	sulphide spiked sed.		Meadows et al. (1991)
Eohaustorius estuarius	mortality, emergence, reburial	10d	solid, estuarine sed.	test sed.	standard method	PSEP (1991), US EPA/US ACE (1991), ASTM (1993a), Environment Canada (1992)
E. sencillus	mortality, behaviour (choice)	72h	solid	spiked nat. sed. with EDTA and trace metals		Oakden et al. (1984a)
Foxiphalus xiximeus	mortality	10d	solid	test sed.	standard method	Environment Canada (1992)
Grandidierella japonica - adults	mortality, emergence, reburial	10d	solid	nat. + test sed.	standard method	Nipper et al. (1989), ASTM (1993a), Environment Canada (1992)
G. japonica - juveniles	mortality, growth, behaviour (reburial)	28d	solid	nat. sed.		Nipper et al. (1989)
Hyalella azteca	mortality of adult and young	10d	solid	estuarine sed.	tolerant of salinity	Nebeker & Miller (1988)

Table 3. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
Leptocheirus plumulosus	mortality	10d	solid, estuarine sed.	test sed.	standard method	ASTM (1993a), Environment Canada (1992), Schlekat et al. (1992)
L. plumulosus	mortality	10d, 20d, 28d	solid, estuarine sed.	test sed.		Schlekat et al. (1992)
L. plumulosus	mortality, growth, reproduction, morphol. development	10d–40d	solid	nat. estuarine sed.		McGee et al. (1993)
Paraphoxus epistomus	mortality	10d	solid	nat. sed.	part of a multispecies test	Swartz et al. (1979)
Rhepoxynius abronius and *R. fatigans*	mortality, behaviour (choice)	72h	solid	spiked nat. sed. with sewage, EDTA and trace metals		Oakden et al. (1984a, b)
R. abronius	mortality, behaviour (avoidance, reburial, emergence)	10d	solid	nat. or spiked sed.	standard method	Swartz et al. (1985,1988, 1990), Lamberson & Swartz (1988), PSEP (1991), US EPA/US ACE (1991), van den Hurk et al. (1992), ASTM (1993a), Environment Canada (1992)
COPEPODA						
Amphiascus tenuiremis	reproduction, age structure	21d	solid	fenvalerate spiked sed.		Strawbridge et al. (1992)
A. tenuiremis	mortality	4d	aqueous, solid	cadmium spiked sediment	method widely used	Green et al. (1993)
Enhydrosoma propinquum	mortality	7d	solid	fenvalerate in nat. sed.		Chandler (1990)
Microarthridion littorale	mortality, reproduction	7d	solid	fenvalerate in nat. sed.		Chandler (1990)
Nannopus palustris	mortality, reproduction	7d	solid	endosulfan in nat. sed. (estuarine)		Chandler & Scott (1991)

Table 3. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
Paranychocamptus wilsoni	mortality, reproduction	7d	solid	fenvalerate in nat. sed.		Chandler (1990)
Pseudobradya pulchella	mortality, reproduction	7d	solid	endosulfan in nat. sed. (estuarine)		Chandler & Scott (1991)
MALACOSTRACA (Cumacea)						
Cyclaspis sp.	mortality	10d	solid	nat. sed.	part of a multispecies test	Swartz et al. (1979)
Diastylis alaskensis	mortality	10d	solid	nat. sed.	part of a multispecies test	Swartz et al. (1979)
Diastylopsis dawsoni	mortality	10d	solid	nat. sed.	part of a multispecies test	Swartz et al. (1979)
Lamprops quadriplicata	mortality	10d	solid	nat. sed.	part of a multispecies test	Swartz et al. (1979)
OLIGOCHAETA						
Monopylephorus cuticulatus	respiration rate	4–8h	elutriate	nat. sed.	sublethal toxicity test for nat. sed.	Chapman (1987)
- various species -	respiration rate	1–2h	extract	various toxicants in nat. sed.		Chapman et al. (1982c)
- various species -	mortality	4d	solid	various toxicants in nat. sed.		Chapman et al. (1982a-c)
- various species -	respiration rate	1–2h	extract	various toxicants in nat. sed.		review in Chapman & Brinkhurst (1984)
POLYCHAETA						
Arenicola marina	mortality, faecal production, bioaccumulation	10d			method widely used	Thain & Bifield (1993, not consulted)
Capitella capitata	life cycle (mortality, growth, reproduction)	35d, 50d	solid, elutriate	nat. sed.		Chapman & Fink (1984)

Table 3. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
C. capitata	mortality, juvenile growth	4d, 20d	solid	estuarine, nat. sed.	standard method	Reish & Lemay (1988)
Dinophilus gyrociliatus	life cycle, mortality, reproduction	7d	pore-water or elutriate only	nat. sed.	method widely used	Carr et al. (1986, 1989), Long et al. (1990)
Glycinde picta	mortality	10d	solid	nat. sed.	part of a multispecies test	Swartz et al. (1979)
Hediste (Nereis) sp.	mortality, juvenile growth	4d, 20d	solid		standard method	US EPA/US ACE (1991)
Neanthes sp.	growth (biomass)	20d	solid	nat. sed.	standard method	Johns et al. (1991)
Nephtys incisa	growth, scope for growth	10 d	solid	nat. sed.		Johns et al. (1985)
N. incisa	mortality, behaviour (burrowing)	4d, 10d	solid	diluted nat. sed.		Rogerson et al. (1985)
Nereis (Neanthes) arenaceodentata	mortality, juvenile growth	28d	solid and aqueous	diluted nat. sed. (also estuarine)	standard method	Dillon et al. (1993), Moore & Dillon (1993)
N. arenaceodentata	mortality, growth, fecundity	153 d	solid	diluted nat. sed.		Pesch et al. (1991)
N. arenaceodentata	mortality, juvenile growth	4d, 20d	solid		standard method	Reish & Lemay (1988), PSEP (1991), US EPA/US ACE (1991)
N. arenaceodentata	mortality, bioaccumulation	12d	solid		standard method	Dillon et al. (1993)
N. diversicolor	behaviour (burrowing)	20 h	solid	pesticides in nat. sed.		Møhlenberg & Kiørboe (1983)
N. virens	mortality	12d	solid	spiked sediment	(follows McLeese & Metcalfe, 1980)	McLeese et al. (1982)
Scoloplos armiger	behaviour (burrowing)	20 h	solid	pesticides in nat. sed.		Møhlenberg & Kiørboe (1983)
Streblospio benedicti	colonization, growth	7d	solid	endosulfan in nat. sed. (estuarine)		Chandler & Scott (1991)
NEMATODA						
Chromadorina germanica	mortality, reproduction	14d	solid	nat. sed. (also estuarine)		Tietjen & Lee (1984)
Diplolaimella punicea	mortality, reproduction	14d	solid	nat. sed. (also estuarine)		Tietjen & Lee (1984)

Table 3. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
GASTROPODA						
Littorina litorea	mortality, bioaccumulation	2d–4d, 10d	liquid	sewage sludge		Franklin (1983)
BIVALVIA						
Abra alba	behaviour (burrowing)	20 h	solid	pesticides in nat. sed.		Møhlenberg & Kiørboe (1983)
A. alba	faecal production, bioaccumulation	5d	solid	hydrocarbons spiked sed.	method widely used	Strømgren et al. (1993)
Cerastoderma edule	behaviour (burrowing)	20 h	solid	pesticides in nat. sed.		Møhlenberg & Kiørboe (1983)
C. edule	mortality, bioaccumulation	2d–4d, 10d	liquid	sewage sludge		Franklin (1983)
Macoma baltica	behaviour (burrowing)	20 h	solid	pesticides in nat. sed.		Møhlenberg & Kiørboe (1983)
M. inquinata	mortality	10d	solid	nat. sed.		Swartz et al. (1979)
M. nasuta	mortality, bioaccumulation	4d	solid		standard method	Reish & Lemay (1988)
Mercenaria mercenaria	behaviour (burrowing)	4d	solid	oil contaminated sed.		Olla & Bedja (1983)
Mulinia lateralis	mortality, growth	7d	solid	nat. sed.		Burgess & Morrison (1994)
Mytilus edulis	survival in air, bioaccumulation	15d exp.	—	test sed.		Eertman et al. (1993)
M. edulis	mortality, bioaccumulation	4d, 10/60d	liquid	sewage sludge		Franklin (1983)
Mya arenaria	behaviour (ET50 for burrowing)	< 4h,	solid	estuarine sed.	shipboard use possible	Phelps (1989, 1990)
Protothaca staminea	mortality, bioaccumlation	4d	solid		standard method	Reish & Lemay (1988)
P. staminea	mortality	10d	solid	nat. sed.		Swartz et al. (1979)
Yoldia limatula	behaviour (burrowing)	4d, 10d	solid	diluted nat. sed.		Rogerson et al. (1985)

Table 3. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
ECHINODERMATA						
Echinocardium cordatum	mortality	14d			method widely used	Bowmer (1993, not consulted)
Epibenthic organisms						
DECAPODA						
Carcinus maenas	behaviour (avoidance)	10 min	solid	pesticides in nat. sed.	- no response shown -	Møhlenberg & Kiørboe (1983)
Crangon crangon	behaviour (avoidance)	10 min	solid	pesticides in nat. sed.		Møhlenberg & Kiørboe (1983)
C. crangon	mortality, bioaccumulation	1–4d, 10/60d	liquid	sewage sludge		Franklin (1983)
C. septemspinosa	mortality	4d	solid	spiked sed.	standard method	McLeese & Metcalf (1980)
Mysidopsis bahia	mortality	4d, 10d	solid	sed. spiked with pyrethroids	standard method	Clark et al. (1989)
Palaemonetes pugio	mortality	4d	solid	sed. spiked with pyrethroids	method widely used	Clark et al. (1989), see also Buikema et al. (1980c)
Penaeus duoarum	mortality	4d, 10d	solid	sed. spiked with pyrethroids	method widely used	Clark et al. (1989)
Sicyonia ingentis	mortality, bioaccumlation	4d	solid		standard method	Reish & Lemay (1988)
COPEPODA						
Corophium spinicorne	mortality	10d	solid	fluoranthene in nat. sed.		Swartz et al. (1990)
Nitocra spinipes	mortality	4d	aqueous	estuarine water	method widely used (Baltic Sea)	Bengtsson (1978, 1981), Tarkpea et al. (1986), Dave et al. (1993)
Tisbe battagliai	mortality, development	4d	elutriate	nat. sed.	method widely used	Williams (1992)

Table 3. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
ECHINODERMATA						
Lytechinus pictus (adults)	mortality, growth, gonadal production, bioaccumulation	60d	solid	nat. sed.		Thompson et al. (1989)
L. pictus (adults)	mortality, behaviour, growth, gonad production, bioaccumulation	49d	solid	H_2S spiked sed.		Thompson et al. (1991)
Water column life stages[2]						
BIVALVIA (embryo/larvae)						
Corbicula fluminea	mortality	4d	solid	nat. sed.		Phelps (1993)
Crassostrea virginica	mortality, development	2d	aqueous		standard method	ASTM (1989), US EPA/US ACE (1991)
C. gigas	mortality, development	2d	solid	estuarine sed. possible	standard method	Chapman & Morgan (1983), ASTM (1989), Phelps & Warner (1990), PSEP (1991)
Mytilus edulis	mortality, development	2d	solid	nat. sed.	standard method	ASTM (1989), PSEP (1991), US EPA/US ACE (1991)
ECHINODERMATA (gametes)						
Arbacia punctulata	fertility	30min exp.	aqueous		standard method	Burgess et al. (1993), see also Nacci et al. (1991)
Dendraster excentricus	fertility	15- 60min	aqueous	reference toxicant		Dinnel et al. (1982, 1987)
Paracentrotus lindus	fertility	30min. exp.	aqueous	reference toxicant	standard method	Dinnel et al. (1987)
P. lividus	fertility	60 min.	solid and aqueous	nat. freshwater sed.		Pagano et al. (1993)
Sphaerechinus granularis	fertility	15 min.	solid and aqueous	nat. freshwater sed.		Pagano et al. (1993)
Strongylocentrotus droebachiensis	fertility	15–60min	aqueous	reference toxicant		Dinnel et al. (1982, 1987)
S. puspuratus	fertility	20min	elutriate	nat. sed.		Long et al. (1990) following Dinnel et al. (1987)

[2] these life stages have been shown to be predictive of sediment toxicity to benthic organisms (US EPA, 1991).

Table 3. Continued

Organism	Assay Endpoint	Duration	Sediment phase	Test substance	Remarks	Reference/s
(embryos)						
Arbacia punculata	mortality, development	2–4d	aqueous		standard method	PSEP (1991)
Dendraster excentricus	mortality, development	3d	aqueous	reference toxicant		Dinnel et al. (1982)
D. excentricus	mortality, development	2–4d	aqueous		standard method	PSEP (1991)
D. excentricus	mortality, growth, bioaccumulation	28d	solid	diluted nat. sed.		Casillas et al. (1992)
Lytechinus pictus	mortality, development	2–4d	aqueous		standard method	Reish & Lemay (1988)
Paracentrotus lividus	mortality, development	72h	solid and aqueous	nat. freshwater sed.		Pagano et al. (1993)
Sphaerechinus granularis	mortality, development	120h	solid and aqueous	nat. freshwater sed.		Pagano et al. (1993)
Strongylocentrotus droebachiensis	mortality, development	4d	aqueous (not specified)	reference toxicant		Dinnel et al. (1982)
S. droebachiensis	mortality, development	2–4d	aqueous		standard method	PSEP (1991)
S. franciscanus	mortality, development	2–4d	aqueous		standard method	PSEP (1991)
S. puspuratus	mortality, development	2–4d	aqueous		standard method	Reish & Lemay (1988), PSEP (1991)
S. puspuratus	echinocrome synthesis	2d	elutriate	nat. sed.		Long et al. (1990), following Bay et al. (1983)
S. puspuratus	cytology, cytogenetics	2d	elutriate	nat. sed.		Long et al. (1990), following Hose (1985)
S. puspuratus	development	2d	elutriate	nat. sed.	modification of Oshida et al. (1981)	Long et al. (1990)
Multi-species test						
Protothaca staminea, Macoma inquinata, Glycinde picta, Paraphoxus epistomus, and a cumacean	mortality	10d	solid	nat. sed.		Swartz et al. (1979)

Table 4. Some benthic metazoans used in toxicity analyses, but not thus far applied in sediment bioassays

Organism	Assay Endpoint	Reference
FRESHWATER		
Brachycentrus americanus (Trichoptera)	mortality	Anderson & Shubat (1984)
Branchiura sowerbyi (Oligochaeta)	mortality, reproduction, development, degenerations	Naqui (1973), Casellato & Negrisolo (1989)
Chironomus attenuatus (Diptera)	mortality, lipid, nitrogen, caloric content	Thornton & Wilhm (1974)
Corbicula fluminea (Bivalvia)	oxygen consumption, condition index	Graney & Giesy (1988)
Dugesia japonica (Turbellaria)	head regeneration	Yoshioka et al. (1986)
Elimia clavaeformis (Gastropoda)	movements (*in situ* and lab.)	Burris et al. (1990)
Gammarus pseudolimnaeus	mortality	Burton et al. (1989)
Glyptodentipes pallens (Diptera)	behaviour (activity)	Heinis et al. (1990)
Ilyodrilus frantzi (Oligochaeta)	mortality	Chapman & Mitchell (1986)
Isonychia bicolor (Ephemeroptera)	molt frequency	Diamond et al. (1990)
Limnephilus lunatus, L. bipunctatus (Trichoptera)	mortality, adult emergence	Ließ (1993)
Monhystera disjuncta (Nematoda)	mortality, development	Vranken et al. (1984)
Nais communis (Oligochaeta)	mortality	Chapman & Mitchell (1986)
Polypedilum nubifer (Diptera)	reproduction	Hatakeyama (1987)
Pteronarcys dorsata (Plecoptera)	mortality	Anderson & Shubat (1984)
Quistadrilus multisetosus (Oligochaeta)	respiration rate	Brinkhurst et al. (1983)
Spirosperma nikolskyi (Oligochaeta)	respiration rate	Brinkhurst et al. (1983)
MARINE		
Ctenodiscus crispatus (Echinodermata)	physiology, behaviour	Shick (1976)
Crassostrea virginica larvae (Bivalvia)	mortality, development	Roberts (1980)
Ctenodrilus serratus (Polychaeta)	mortality, reproduction	Reish (1980)
Elasmopus bampo (Amphipoda)	mortality	Hong & Reish (1987)
Grandideriella lutosa, G. lignorum (Amphipoda)	reproduction, population changes	Connell & Airey (1982)
Rhyacodrilus montana (Oligochaeta)	respiration rate	Brinkhurst et al. (1983)
Variechaeta pacifica (Oligochaeta)	respiration rate	Brinkhurst et al. (1983)

by the American Society for Testing and Materials (ASTM, 1991a, b, 1993a, b, 1994a, 1995), the Society for Environmental Toxicology and Chemistry - Europe (Hill et al., 1993) and the U.S. Environmental Protection Agency (US EPA, 1994b). Publications by ASTM are updated regularly, thus the reader is encouraged to seek the latest issues for consultation. Overviews of strategies to combine the various test procedures, with recommendations relevant to the choice of strategy and test species in the palaearctic, are found in Hill et al. (1993) and Ahlf (1994, 1995).

Tabulated lists of some sediment toxicity bioassays, with rankings for sensitivity, ecological relevance, replicability, amenability etc., are found in Giesy & Hoke (1989) and Hill et al. (1993). The relative sensitivities of benthic organisms can vary considerably both in marine (Swartz et al., 1979; Williams L.G. et al., 1986; Chapman et al., 1985; Hong & Reish, 1987; Chapman, 1988; Becker et al., 1990; Long et al., 1990; Pastorok & Becker, 1990; van den Hurk et al., 1992) and freshwater sediment bioassays (Giesy et al., 1988; Burton, 1989; West et al., 1993). Intermediate or low sensitivity to contaminants must not be a drawback of certain test species, for bioassays with these organisms can be used to rank highly contaminated sites, where more sensitive animals would fail to detect differences.

In this report we describe the strengths and weaknesses of various bioassays with benthic metazoa and of combinations thereof. It is generally agreed that no single bioassay will usually fulfill the requirements of a comprehensive sediment toxicity assessment. Here, we avoid advocating a particular bioassay combination of apparent, general applicability. The choice of tests and their application strategy is ultimately dictated by the objectives of the study and the specific budgetary and biological circumstances at the site of concern. These variables affect the relative weighting

of the strengths and weaknesses described here, and thus lead to highly individual rationales concerning the choice of bioassays. The selection criteria for a bioassay include the critical test variables considered in the following sections.

Critical test variables

Consideration of several test variables is important in categorizing and applying sediment toxicity bioassays. These include overlying water quality, geochemical characteristics of the sediment, sediment test phase, exposure time, test species chosen, assay endpoints and controls, all of which affect in different ways the test sensitivity, its ecological relevance and the interpretation of results (Burton et al., 1992). The overlying water quality, which can affect organism responses, is influenced by the contact time between sediment and water (Burton et al., 1987). Contact times differ between modes of exposure used, such as static, recirculating, static-renewal, flow-through systems and *in situ* exposure, all of which have their strengths and weaknesses (see Buikema et al., 1982). Also, tests may need to include the range of environmental conditions expected in the overlying water of the sediment because water hardness, pH, and the sediment to water ratio in the assay are known to affect sediment toxicity (Anderson et al., 1984; Stemmer et al., 1990a, b). The matching of geochemical properties of the sediment sample to the tolerance limits of the test species is obviated in *in situ* tests, if native fauna are used as test organisms. Such natural properties of the sediment, however, could influence the bioavailability of toxicants and, therefore, produce contrasting test results between sites with similar effluent loads but different sediment properties. Warwick (1981) proposed the use of the nematode to copepod ratio as an indicator of differences in sediment granulometry between polluted sites. Studies of the influence of sediment characteristics, including "non- contaminant" factors, on the response of test animals are badly needed (Burton et al., 1992).

In addition, the potential seasonality of sediment toxicity and the patchiness of toxicity distribution in the area of concern dictate multiple sampling and coverage of seasonal variation (Burton, 1989; Munawar et al., 1989). Furthermore, the presence of indigenous fauna in the sediment sample can affect the results of the bioassay. This is particularly true for the high densities of oligochaetes typical of some contaminated sites and which can significantly affect the growth of test organisms such as chironomid midges, amphipods and mayfly nymphs during chronic exposures to the test sediment (Reynoldson et al., 1994). Removal of indigenous organisms from the sample is recommended prior to testing. This applies particularly to chironomid bioassays and to samples from sites with high densities of benthic invertebrates. Other, unwanted sources of variability between bioassay results are described by Schaeffer et al. (1987). The roles of sediment test phase, exposure time, assay endpoint and test species chosen, as critical variables of bioassay choices are discussed in separate sections below.

Exposure phases in sediment toxicity assessment

Exposure of benthic organisms to pollutants can take place either in the dissolved phase in the interstitial and overlying water or in the way of molecules adsorbed to sediment particles (Power & Chapman, 1992; Ahlf, 1995). Direct ingestion of particles and absorption through the body surface are the most important paths of contamination (Ahlf et al., 1992). The most commonly used modes of exposure in studies of sediment toxicity are aqueous or organic elutriates (extractable phase), interstitial water isolated from the sediment, whole sediment (solid phase) and *in situ* assays. Other modes of exposure are sediment slurries and sediment dilution series (e.g. Meador et al., 1990; Casillas et al., 1992; Nelson et al., 1993). Exposure of test organisms to sediment in suspension (e.g. Tsai et al., 1979; Schmidt-Dallmier et al., 1992) is relevant for example to the evaluation of dredged sediments for disposal at a dispersive aquatic site. Spiked sediments are commonly used in toxicity tests (Tables 2 and 3) to expose organisms to a range of toxicant concentrations. Since the sediment´s organic carbon content affects toxicity (e.g. Landrum & Faust, 1991; Lydy et al., 1992), there is still little agreement as to which substance should be used as reference sediment. Burgess et al. (1994) tested sand spiked with copper on marine macrobenthos. There is urgent need for the development of spiked reference sediments for freshwater invertebrates.

Most pollution effects on meiofauna have been tested *in vitro* with toxicants in the aqueous phase (Coull & Chandler, 1992). In principle, tests of aquatic toxicity with water column species (e.g. the cladoceran *Daphnia magna:* Buikema et al., 1980a; Baudo, 1987; see also Munawar et al., 1985) can be applied to interstitial water or elutriates isolated from whole sediment samples (Giesy & Hoke, 1989). Liquid-phase sediment assays were originally developed to test the toxicity of

polluted sediments or resuspensions thereof on water-column organisms (e.g. Schmidt-Dallmier et al., 1992). Such liquid sediment phases are amenable to dilution and are hence used to establish dose-response relationships of contaminated samples. Without an adequate field validation, however, the toxicity of these test phases to water-column dwellers does not allow inferences about the toxic effects of contaminanted sediments on benthic communities.

The results of toxicity tests on the same organism can vary, depending on the exposure phase used (e.g. Chapman & Fink, 1984; Fava et al., 1985; Lydy et al., 1990; McCauley et al., 1992; Green et al., 1993; West et al., 1993). Different strengths and weaknesses are associated with these procedures and some exposure modes may be appropriate in some cases but not in others (Burton, 1991; Ahlf, 1995). Studies comparing the toxicity of metals in different exposure phases show that ions in overlying and pore-water are the main path of contamination for most benthic organisms, while the toxicity of sediment bound metal is negligible (Cairns et al., 1984; Phelps et al., 1985; Nebeker et al., 1986; Green et al., 1993). Pore-water is predicted by equilibrium partitioning theory to be the controlling exposure medium in the toxicity of sediments to infaunal species (Di Toro, 1989; but see Lee, 1991). Thus, sediment quality tests exposing benthic organisms exclusively to pore-water can sometimes yield a useful approximation to the results of whole-sediment exposures (see also Ankley et al., 1991; Schubauer-Berigan & Ankley, 1991). The validity of pore-water tests as a model for whole-sediment analyses, however, depends on the chemical, physical and kinetic properties of the sediment (Landrum, 1989; Landrum & Robbins, 1990; Boese et al., 1990), as well as on the type of toxicant, the method used to obtain the pore-water sample and the test species chosen (Giesy et al., 1990; for marine sediments see Carr & Chapman, 1992). In reviewing the role of pore-water and whole sediment as sources of toxicity, Knezovich et al. (1987) concluded that their relative importance varies greatly, depending, among other things, on the type of contaminant and the species in question (see Green et al., 1993). The toxicity of water-borne copper to benthic *Neanthes arenaceodentata* (Polychaeta), for example, was higher in exposure chambers without sediment than in more biologically adequate chambers containing sediment (Pesch & Morgan, 1978). Interestingly, in tests with organisms like e.g. the mayfly *Hexagenia,* which ingest sediment and probably absorb toxicants through the intestine wall, the toxicity of pore-water exposure was still higher than that of whole sediment (Giesy et al., 1990). Elutriates, which have been extensively used to simulate the resuspension of sediments occurring during dredging projects, are of limited ecological relevance to toxicity assessments of undisturbed sediments.The associated oxidation of resuspended particles can change the toxicity properties of elutriate samples in comparison to those revealed by whole sediment analyses (see also Burgess et al., 1993). Moreover, some sediment toxicity effects are only associated with the whole sediment phase (Sasson-Brickson & Burton, 1991).

Overall, a test with benthic species involving direct exposure to a sediment sample is more adequate and ecologically relevant for an assessment of sediment toxicity than tests in isolated liquid phases (Lamberson & Swartz, 1988; see Ankley et al., 1990). Static bioassays, in which benthic organisms are exposed to whole sediment and overlying water in a beaker, have been used to test experimentally spiked sediments (Cairns et al., 1984) and field contaminated samples (Wentsel et al. 1977a, 1978b). Bioassays that test whole sediment with benthic organisms examine directly the effect of both bound and dissolved toxicants on the species which are most likely to be affected by sediment toxicity. Thus, the diagnosis of sediment quality should be centred around whole sediment analyses, with pore-water and elutriate tests only as complementary sources of information (Chapman & Fink, 1984; Lamberson & Swartz, 1988; Ahlf, 1994).

Burton (1991) and Burton et al. (1992) evaluated the strengths and weaknesses of the different sediment toxicity exposure phases. Advantages of whole sediment and *in situ* tests over tests in the aqueous or extractable phase include its holistic toxicity approach, high relative realism and ecological relevance, as well as a virtually unlimited sample quantity. Meanwhile, most sediment bioassays with benthic organisms are run on whole sediments (solid exposure phase, Tables 2 and 3). In contrast to liquid phase tests, however, whole sediment and *in situ* tests need to consider the possibility that indigenous biota in the sample affect the test results, for example through predation on the test species. Whole sediment tests are routinely used for rapid screening, initial surveys, studies of long toxicant exposure, assessment of sediment quality criteria and analyses of dose-responses (Burton et al., 1992). *In situ* tests are used principally to study resuspension effects, to determine sediment quality and for intensive system monitoring. They offer a highly realistic measure of toxicity, integrating all key components and eliminating extraneous influences associated with

handling and laboratory procedures. The latter, however, are inadequate to determine dose-responses and are less easy to reproduce, given, for example, the great variability of mesocosms and within-site- heterogeneity. Whole sediment bioassays are often more complex than *in situ* and liquid phase bioassays, because handling during sampling, storage and setup may change the physical, chemical and/or microbiological properties of the intact sediment through oxidation, mixing of layers, removal of interstitial water and disruption of sediment structure (Lamberson & Swartz, 1988; ASTM, 1991b, 1994a; Rubio & Ure, 1993).

Time of exposure to the test substance

The time of exposure to the test substance is a critical consideration in sediment tests, for extended exposure is associated with greater sensitivity (Birge et al., 1984; Malueg et al., 1984a, b; LeBlanc & Surprenant, 1985; Ingersoll & Nelson, 1990; Parsons & Surgeoner, 1991). Thus, the effect of small toxicant doses can sometimes be revealed in the form of sublethal effects after prolonged exposure. Short exposure tests, however, may be more appropriate, for example, in cases requiring a less sensitive and resource intensive approach which allows more testing. Although there are rigorous methodologies to extrapolate the effect of acute short-term exposures to effects of long-term exposures on the survival, growth and reproduction of aquatic organisms (Giesy & Graney, 1989), the great value of long-term exposures in bioassays is undoubted, despite possible complications arising from food provisioning to keep the test organisms alive (e.g. Wiederholm et al., 1987; Moore & Dillon, 1992). The development of chronic, sublethal sediment bioassays is important, firstly, because bioaccumulation is a slow process which can affect reproduction and, secondly, because benthic organisms generally experience such prolonged exposures to low contamination levels (Dillon, 1993). Most bioassays with commonly used test organisms include endpoints for chronic exposures (Tables 2 and 3).

The duration of tests with benthic metazoans varies greatly (Tables 2 and 3). Enzyme activity in *Hexagenia* and respiration rate of oligochaetes respond to very short exposures of 1–2 h, mortality is manifested in various organisms within 24 h and life cycle endpoints with oligochaetes can require exposures of up to 500 days. In general, short term exposure bioassays (< 4 d) produce fast results and allow efficient processing of large numbers of samples, but may be too brief to detect the more subtle, sublethal effects that would hinder reproduction or even produce mortality after a long exposure time (Lamberson & Swartz, 1988). If possible, therefore, sublethal responses should be concurrently recorded. Longer term (such as 10-day) mortality or growth tests may be more sensitive and the results may be less variable (Swartz et al., 1985). Consequently, in choosing among the available bioassays, one criterion should be that the test include endpoints for both short- and longterm exposures.

Assay endpoints

The strengths and weaknesses of various endpoints have been evaluated by Lamberson & Swartz (1988). They conclude that mortality is the most easily measured and most readily understood response criterion, allowing comparisons among species and among chemicals or other variables within a species. Mortality is the most common endpoint used in standardized bioassays for the assessment of sediment toxicity (Tables 2 and 3). Mortality is commonly expressed as values of LC50 (lethal concentration for 50% of test animals), EC50 (effect concentration, here: 50% reduction in young) or percent survival. Anderson & Shubat (1984) used another measure of mortality, which makes reference to exposure time rather than concentration (ExpT50). This measure complemented LC50 values in order to express lethality as a function of both, contaminant concentration and time of exposure. In that study, emphasis was made on the monitoring of post-exposure mortality, particularly for assays which resulted in zero mortality after conventional, short exposures. Although mortality is a very amenable toxicological response, Luoma & Ho (1993) recommend the use of endpoints more sensitive than mortality (behaviour, reproduction, larval settlement) for whole sediments, single-species assays. For a critical position on the use of LC50 values as thresholds in environmental toxicology see Cairns (1992).

Developmental criteria of benthic species such as growth, adult emergence and maturation rates (e.g. nematodes: Coomans & Vanderhaeghen, 1985) are important toxicity variables in that they cover various life stages of the test organims. The results can be highly variable, however, and the bioassay may take too long for large-scale screening or monitoring work. Such sublethal endpoints can nevertheless be useful for analyses of chronic exposure to toxicants. As with other sublethal effects, these endpoints require verifi-

cation of their negative impact on the reproduction of the organism in question.

Behavioural responses have been criticized on the grounds that they may be related to factors other than sediment contamination (Lamberson & Swartz, 1988) and that the variability of behaviour results in low resolution. The usefulness of behavioural endpoints, however, is specific to the test organism and bioassay used. Reburial and avoidance endpoints for the amphipods *Rhepoxynius abronius* and *Ampelisca abdita* for example, can be highly variable and insensitive when compared with other sediment bioassays (Long et al., 1990). The reburial endpoint of *Bathyporeia sarsi*, however, is sensitive and useful (van den Hurk et al., 1992). Some behavioural endpoints are highly sensitive, and given appropriate controls and calibrations could contribute greatly to a sediment toxicity assessment (see Møhlenberg & Kiørboe, 1983). *Gammarus*, for example, showed behavioural responses to phenolic toxicants at 1/20th of the acute LC50 dose (Borlakoglu & Kickuth, 1990). An automatic recording device for *Gammarus pulex* activity detected toxicity responses to the pyrethroid fenvalerate at concentrations as low as 0.1 ng/l, substantially below the minimum of 100 ng/l required for the detection of pyrethroids in water with analytical methods (Ließ, 1993). *Corophium volutator* responded behaviourally at two orders of magnitude less than the lethal dose for sulphides (Meadows et al., 1991). Induced disruption of precopulatory amplexus in *Gammarus pulex* after 24 h exposure to toxicants (Poulton & Pascoe, 1990) is a quick bioassay with great potential for *in situ* diagnostics of sediment quality. Choice experiments offering sediment samples with different test substance concentrations to benthic organisms have also been used in ecotoxicology (e.g. Møhlenberg & Kiørboe, 1983; Oakden et al., 1984a, b; Meadows et al., 1991).

Physiological endpoints, such as the respiration rate of oligochaetes (e.g. Chapman, 1987) or enzyme activity, can provide valuable means to assess sublethal sediment toxicity after a few hours of exposure to toxicants. Respiration rate tests respond to the presence of metals, as well as to all organic compounds that affect respiratory enzyme function (e.g. phenols, chlorophenols, pesticides, PCBs). Susceptibility to disease under toxic stress was proposed as a bioassay endpoint in crustaceans (Couch & Courtney, 1978), but it did not become established among standardized bioassays for sediment quality. Measurements of physiological variables often require sophisticated equipment thus making these response criteria less amenable for large scale testing of multiple samples than survival endpoints (also Reynoldson et al., 1991).

The etiological agents between toxicity and endpoint response are little understood. In a comparison of five marine bioassays, Long et al. (1990) found that whereas the endpoints within affinity groups have similar response patterns, some endpoints show negative correlations with endpoints in other affinity groups. In that study, for example, the *Mytilus edulis* percent normal development endpoint and the *Ampelisca abdita* avoidance endpoint were positively correlated and, therefore, contradicted each other. Two endpoints of the *Strongylocentrotus pupuratus* test, echinochrome content and percent normal development, also contradicted each other. Therefore, comprehensive assessments of sediment toxicity are best made with multiple endpoints, until the relationships between these endpoints and specific chemicals are quantified experimentally (Long et al., 1990).

Growth has been used as an endpoint in several bioassays for long-term exposure to sediment samples (Tables 2 and 3). Generally, a reduction in growth is considered as an adverse effect. The interpretation of growth results, however, must be accompanied by consideration of some potentially confounding factors, including intra-sediment nutritional differences, the potential for recovery, the subsidy effect (Odum et al., 1977) whereby low contamination levels*enhance* growth, and the fact that a reduction in growth need not necessarilly affect subsequent reproduction (see Moore & Dillon, 1993). Further research to elucidate the role of these factors is needed for a better interpretation of the growth endpoint in chronic bioassays.

Reproductive variables characterize the most ecologically relevant and sensitive life stage of the test organism (e.g. Reynoldson et al., 1991). Reproduction has become an established endpoint in the widely used *Tubifex tubifex* bioassay for a long term exposure to toxicants (28 d). Out of several reproductive variables tested, the total and per adult cocoon production emerged as the most robust endpoints in that bioassay (Reynoldson et al., 1991).

Choice of test species

The choice of test species is central in the design of a sediment toxicity test because organism morphology, ecological niche, feeding mechanism and physiology determine toxicant uptake, pathway and, thus, hazard (Knezovich et al., 1987). The choice of organism should include criteria such as (1) its importance in the

ecosystem dynamics, (2) its behaviour in the sediment and feeding habits, (3) its sensitivity to the test substance, (4) ease of the test method and (5) availability of a large reference base to interpret the results (see also Anderson et al., 1984; Giesy & Hoke, 1989; ASTM, 1993b). These criteria narrow down the options to those species which have been previously used in toxicity assessment and which have been well studied under field conditions. Generally, native species should be preferred over foreign species to analyse the toxicity of a given sediment, in order to ensure the ecological relevance of the results. In most *in situ* tests the choice is restricted to the animals naturally occurring at the study site, which are hence in the majority of cases ecologically relevant. The feeding habits are relevant for the choice in that, for example, species which ingest sediment particles (e.g. amphipods and oligochaetes) might be better suited to investigate toxicity of molecules adsorbed to the sediment, whereas smaller species with a higher body surface to volume ratio (e.g. nematodes) may be more sensitive to dissolved chemicals absorbed through the body walls (see Knezovich et al., 1987). The life style of the organism will further determine whether the source of contamination is primarily the overlying water, interstitial water or whole sediment, all of which can differ with respect to the dose of the test substance/s (Burton et al., 1992). When test organisms are collected from the field for laboratory bioassays, special care needs to be taken to avoid testing with individuals which may have developed a resistance to the toxicants in question (e.g. Bryan, 1979; Luoma & Carter, 1991). The isopod *Asellus meridianus*, the midge *Chironomus tentans*, and the polychaete *Nereis diversicolor*, for example, are more resistant to metals at contaminated sites than at sites free of such toxicants (Brown, 1976; Wentsel et al., 1978a; Bryan, 1979). Lastly, the cosmopolitans among the test species should be favoured in the tests, given the choice, because of their higher overall ecological relevance and better comparability between sites.

Advantages of using native species of benthic metazoa are that they have a high ecological relevance and that they can be easily collected from the field, thereby avoiding laboratory artifacts that may affect cultured organisms (e.g. Robinson et al., 1988). Test organisms sampled from the field, however, are subject to natural population variation, seasonality, weather and pollution, which may introduce unwanted noise into the analyses (Lamberson & Swartz, 1988). Also, these organisms may not be available at certain times of the year, thus hindering the coverage of seasonal variation in sediment toxicity. Cultured organisms are readily available and generally less variable than natural populations. Meanwhile, most established bioassays for freshwater and marine sediment analysis include well developed culture techniques for the test animals (references in Tables 2 and 3).

Organisms in sediment bioassays

Freshwater organisms

Giesy & Hoke (1989) list criteria for the selection of freshwater species for sediment toxicity tests. An overview of commonly used freshwater organisms in sediment toxicity bioassays is found in Burton et al. (1992) and ASTM (1993b). ASTM (1994a) describes the biology, handling and culturing methods, as well as test protocols for the mayfly *Hexagenia*, the amphipods *Hyalella* and *Diporeia*, the midges *Chironomus tentans* and *C. riparius*, and the oligochaete*Tubifex tubifex*. At least 30 species of benthic metazoans have thus far been used in freshwater sediment bioassays (Table 2).

Freshwater insecta

Nymphs of the burrowing mayfly *Hexagenia* (Ephemeroptera) are one of the few insects for which standardized test protocols have been developed in sediment toxicology (Table 2). The biology of this genus, field collection, culture and exposure methods are reviewed by Fremling & Mauck (1980). Henry et al. (1986) make further recommendations for the development of test protocols with this species. In a study of Detroit river sediments, lethality of *Hexagenia limbata* was the most sensitive bioassay, when compared with *Photobacterium phosphoreum* bioluminenscence inhibition, or lethality in *Daphnia magna* and *Chironomus tentans* (Giesy et al., 1990). Molt production in the mayfly *Stenonema modestum* and potentially in other ephemeropterans, is a sensitive, subacute endpoint recommended for seven day exposures to elutriates of contaminated sediments (Diamond et al., 1992).

Infaunal larvae of *Chironomus* midges (Diptera) are sensitive insects with high ecological relevance and amenability in toxicological studies. They are tolerant of various sediment types and, therefore, the genus is suitable for analyses with spiked reference sediments. The biological and procedural background which led to the development of modern bioassays with chirono-

mids is reviewed by Anderson (1980). The strengths of *Chironomus* in toxicity tests and the large data base on its responses to various toxicants led to a standardization of bioassay procedures with this genus (Table 2).

Chironomus tests should be started using first or second instar larvae, because *Chironomus* larvae become more tolerant to toxicants with increasing age (Nebeker et al., 1984; Williams et al., 1986). Field sediment samples should be treated with gamma irradiation to eliminate midge predators and other organisms which may bias the mortality and growth data in the *Chironomus* bioassay (Ingersoll & Nelson, 1990; Reynoldson et al., 1994). The culture method of *C. riparius* is described by McCahon & Pascoe (1988a; see also Maas-Diepeveen & van de Guchte, 1990; van de Guchte, 1992). Both, *C. riparius* and the amphipod *Hyalella azteca* are very robust with respect to the grain size of the sediment, but additional tests of the effect of other geochemical properties are needed, in order to assess the precision with which these organisms respond to contaminant toxicity (Ingersoll & Nelson, 1990; see Lydy et al., 1990, 1992). As for other single-species bioassays, the whole sediment *Chironomus* test should be complemented with other toxicity assays. A *Chironomus* pore-water test, for example, would reveal information on water soluble toxicants which may be missed by *Chironomus* in whole sediments contaminated with large amounts of insoluble petroleum hydrocarbons (Hoke et al., 1993).

Chironomus tentans in a growth assay is recommended by Giesy & Hoke (1989) as the benthic macroinvertebrate of choice in test-batteries for sediment toxicity bioassessment. In a comparative test with river sediments, the *C. tentans* 10 d growth inhibition bioassay showed the highest discriminatory power compared with Microtox^R and *Daphnia* assays, and it was as highly sensitive as Microtox^R(Giesy et al., 1988). The culture method for *C. tentans* is described by Batac-Catalan & White (1982). A 30–40% inhibition of growth in the test sediment corresponds to field conditions of toxicity which do not support viable communities of benthic invertebrates. In metal exposures, larval growth seems to be the most sensitive endpoint in *C. riparius* and *C. decorus* (Powlesland & George, 1986; Kosalwat & Knight, 1987a, b). The growth of *Tanytarsus dissimilis,* however, is not affected at concentrations between LC50 during exposures to copper, lead and cadmium (Anderson et al., 1980).

Sexual dimorphism can be a source of bias in growth data for chironomids. *C. riparius* is sexually dimorphic with respect to weight with males smaller than females, particularly when reared individually (Day et al., 1994). Although the effect of dimorphism on data interpretation is thought to be minimal when animals are reared in groups, it is recommended that both larval weight and head capsule width be measured as endpoints in sediment toxicity tests to differentiate reduced growth from retardation of instar development (Day et al., 1994).

Head deformities of chironomid larvae can be used as biological indicators of toxic stress (Wiederholm, 1976, 1984; Warwick, 1985, 1988). This laborious method of toxicity assessment requires considerable training as well as standardized preparation and mounting techniques. Its advantages include (1) the immediacy of response, such that deformities reflect accurately the toxicity prevailing in the sediment at the time of sampling and (2) the fact that head capsules preserve well in the sediment, therefore permitting palaeoanalysis of contaminant pressure in a historical context (e.g. Warwick, 1980; see also Swartz et al., 1991). A quantitative measure of deformity incidence is the Index of Severity of Antennal Deformation.

Freshwater oligochaeta

The infaunal life style of aquatic oligochaetes, their considerable contribution to the benthic biomass at some sites, and the range of responses of different species to individual and combined stress make these organisms attractive for use in sediment toxicity bioassays and as field pollution indicators (review in Chapman & Brinkhurst, 1984). Infaunal oligochaetes are overall in closer contact with the sediment than, for example, epibenthic amphipods and, therefore, are more likely to reflect sediment toxicity independently of toxic effects of soluble pollutants in the overlying water. Most oligochaetes are amenable to laboratory culturing. Nevertheless, there are some drawbacks associated with oligochaetes, including (1) difficulties of species identification, (2) fragility during handling, (3) quick decomposition of juveniles after death, which can affect reproduction measures, and (4) adherence of sediment particles to the mucus layer and alimentary canal, which can reduce the precision of weight measurements (Wiederholm et al., 1987; Giesy & Hoke, 1989).

Tubifex tubifex was chosen by Wiederholm et al. (1987) and Reynoldson et al. (1991) as the preferable species for the development of a meanwhile widely used toxicity bioassay, because of its short genera-

tion time compared with *Limnodrilus* sp., *Potamothrix hammoniensis* and *Quistodrilus multisetosus*. In addition, the cosmopolitan *T. tubifex* is found over the widest range of habitats and, therefore, seems to be largely insensitive to natural variation in sedimentological variables. Its wide distribution makes it ecologically relevant to many freshwater areas of concern. The *T. tubifex* bioassay is quick to set up, simple, inexpensive and uses readily available materials. As such, it overcomes most of the difficulties pointed out by Giesy & Hoke (1989) for the use of oligochaetes in laboratory bioassays. The addition of food to the test culture is not recommended (Wiederholm et al., 1987). One weakness of this test, however, is the need for pretreatment (e.g. sieving) of the sediment to remove the resident fauna, a procedure which could alter the toxicity properties of the sample (Reynoldson et al., 1991). Gamma irradiation of the sediment sample, as recommended by Ingersoll & Nelson (1990), could be used to obviate this problem. The testing of sediments from oligotrophic water bodies, however, may require the choice of another species, or even organism group, because the above mentioned oligochaetes are nutrient dependent and will not typically occur in this trophic state. The cosmopolitan naidid *Pristina leidyi* has great potential for chronic toxicity analysis using reproduction as an endpoint, because it has a remarkably short generation time of 3 to 7 days, which represents a methodological advantage over tubificids (Smith et al., 1991). Respiration rates under toxic stress are known for various freshwater oligochaetes including *Limnodrilus hoffmeisteri, Quistadrilus multisetosus, Spirosperma nikolskyi, Stylodrilus heringianus* and *Tubifex tubifex* (Brinkhurst et al., 1983). Respiratory responses could be developed into sensitive endpoints for freshwater sediment bioassays (c.f. marine oligochaetes).

Oligochaetes are often thought to be an insensitive group because they become the predominant benthic macroinvertebrate upon eutrophication. This, however, is mainly due to their capability to tolerate prolonged periods of anoxia (Reynoldson, 1987). Their relative, long term sensitivity to toxic compounds is mostly unkown, for lack of controlled comparisons of sublethal effects with other benthic organisms (Reynoldson et al., 1991). A recent comparative chronic test with contaminated natural sediments indicates that *Lumbriculus variegatus* is as sensitive as the amphipod *Hyalella azteca* , but less sensitive than *Diporeia hoyi* (Dermott & Munawar, 1992). Reports regarding the sensitivity of oligochaetes to sediments contaminated with metals have been conflictive. Malueg et al. (1984b), Milbrink (1987) and Wiederholm et al. (1987) documented high sensitivity, whereas Chapman et al. (1980, 1982a, b) and Wentsel et al. (1977b) found oligochaetes to be highly tolerant of metals. Assessment of the sensitivity of oligochaetes to toxicants is further complicated by the fact that these organisms can develop site specific resistances (Wentsel et al., 1978a). A simple statement about the sensitivity of oligochaetes (or any other organism group) to contaminants is unlikely to be valid, for differences between species, as well as between their responses to different toxicants can be significant (Chapman et al., 1980, 1982 a, b; Chapman & Brinkhurst, 1984; Wiederholm et al., 1987).

Freshwater crustacea

The most frequently used benthic crustacean in freshwater sediment toxicology is the amphipod *Hyalella azteca.* In contrast to other amphipods, this species presents few culturing difficulties (e.g. de March, 1979, 1981). This species is highly sensitive, discriminatory and tolerant of natural variation in sediment grain size (e.g. Ingersoll & Nelson, 1990). The sensitivity of the freshwater amphipods *Hyalella* and *Gammarus* to toxicants is either similar to, or greater than, the sensitivity of the cladoceran *Daphnia* (Borgmann et al., 1989). Given its geographical distribution limited to America, however, the genus *Hyalella* has no ecological relevance for sediment evaluations in other regions of the world.

A review of procedures for the use of freshwater amphipods of the genus *Gammarus* in toxicity bioassays is found in Arthur (1980). Among North American freshwater gammarideans, *G. lacustris* was the species recommended by Arthur (1980) for toxicity bioassays, based on culturing success and availability of information on the requirements of the species (see McCahon & Pascoe, 1988a, b). This species belongs to the most sensitive among several taxonomic groups of invertebrates in the Great Lakes to a range of contaminants (Williams et al., 1984). The effect of various metals and organic contaminants on *G. lacustris* has been tested in several short-term exposure studies (e.g. Abel & Garner, 1986; references in Giesy & Hoke, 1989). Juveniles are the most sensitive life stage in this species (McCahon & Pascoe, 1988b). It should be noted, however, that *Gammarus* is partly associated with the water column, and therefore may not faithfully reflect the toxicity conditions of the sediment. For

this reason, McCahon & Pascoe (1988a) recommend instead the use of *Asellus aquaticus,* a species which is in continuous contact with the sediment (see Green et al., 1986).

The burrying poxocephalid amphipod *Diporeia spp.*, inhabiting the Great Lakes, is one of the six benthic invertebrates recommended by ASTM for standardized sediment ecotoxicology (ASTM, 1994a). *Diporeia* spp. was formerly named *Pontoporeia hoyi* and earlier named *Pontoporeia affinis*, thus figuring with three synonyms in the ecotoxicological literature (ASTM, 1994a). Surprisingly, no benthic ostracods have thus far become widely established as test species in sediment toxicology. Taub (1989) included the freshwater ostracods *Cypridopsis sp.* and *Cyprinotus sp.* in a microcosm design.

Freshwater nematoda

The current and potential applications of nematodes in ecotoxicological research are reviewed by Traunspurger et al. (1995). Nematodes are becoming increasingly important test organisms in sediment toxicology (Samoiloff, 1987; Bongers & van de Haar, 1990; Traunspurger et al., 1995). These infaunal animals are the most abundant and species richest group of metazoa in benthic ecosystems (e.g. Traunspurger, 1991). The *Panagrellus redivivus* bioassay developed by Samoiloff et al. (1980, 1983a, b) follows a quick and highly standardized procedure, which includes lethal, sublethal and gene level endpoints during a 4 d exposure to sediment extracts with standard dilutions. This test is often used in combination with the Microtox^R bioassay developed by Bulich (1983, 1984). Gregor & Munawar (1989) consider the sediment extraction/fractionation procedure of the *Panagrellus* bioassay an alteration of natural bioavailability of the test substances and, therefore, recommend that assay only as complementary to their advocated elutriate test with the Algal Fractionation Bioassay. Popham & Webster (1979), Coomans & Vanderhaegen (1985) and Van Kessel et al (1989) developed bioassays with *Caenorhabditis elegans* exposed to toxicants in agar. Traunspurger et al. (submitted) elaborated on these bioassays with *C. elegans* to test whole sediment samples. Some of the advantages of nematode bioassays are that the test is easy and quick to perform, the culture of some species is simple and nematodes are obtainable from all aquatic systems.

Freshwater mollusca

In evaluating the strengths and weaknesses of molluscs as bioassay organisms, Giesy & Hoke (1989) concluded that bivalves and gastropods are well suited for laboratory and *in situ* studies of toxicant bioaccumulation and for environmental monitoring (e.g. Green et al., 1989), but less so for use in toxicity bioassays for screening purposes. Some disadvantages of using clams as bioassay organisms are that lethality is often difficult to determine and that clams can close their shells to avoid irritants, thus hindering the determination of actual exposure doses in short-term laboratory tests (Giesy et al., 1983). While this conclusion might apply to the adult life stage, meanwhile bioassays with bivalve embryos play an important role in toxicity assessments of marine sediments (Table 3). The standardization of aequivalent assays with freshwater species is still pending. One consideration in the application of such assays is that the sensitivity of bivalves can vary considerably between toxicants (Little, 1978). Giesy & Hoke (1989) noted that the majority of ecotoxicological studies with marine and freshwater molluscs have focused on metals and hydrocarbons. Thus, little information is available on the sensitivity of any species to a broad range of other organic contaminants.

Marine organisms

Criteria for the selection of marine species for sediment toxicity tests are summarized in Lamberson et al. (1992). Not surprinsingly, these criteria differ little from those for the choice of freshwater organisms (e.g. Giesy & Hoke, 1989). At least 71 species of benthic metazoans have been thus far used in marine and estuarine sediment bioassays (Table 3). Peddicord (1980) describes bioassays with sediment elutriates using shrimps, crabs, mussels, tunicates and lobster not mentioned in Table 3. Pastorok & Becker (1990) compared several marine bioassays in exposures to contaminated natural sediments from Puget Sound. The ranks of statistical sensitivity in that study were in decreasing order as follows: *Photobacterium phosphoreum* (Microtox) = *Dendraster excentricus* embryo abnormality > *Rhepoxynius abronius* mortality = *Eohaustorius estuarius* mortality > *Neanthes arenaceodentata* biomass > *Neanthes arenaceodentata* mortality = *Dendraster excentricus* chromosomal abnormality > *Rhepoxynius abronius* nonreburial > *Eohaustorius estuarius* nonreburial = *Panope generosa* mortality. Other

relative sensitivities of marine bioassays are mentioned in sections below.

Marine crustacea

Amphipods in general are among the first species to disappear from benthic marine communities in contaminated areas (Belian-Santini, 1980; Swartz et al., 1982), and thus may be considered as sensitive indicators of sediment pollution. When toxicity is found, however, it is difficult to determine whether the observed effects are due to toxicants associated with sediment particles ingested or to chemicals dissolved in interstitial water (Lamberson & Swartz, 1988). Further tests are then necessary to pinpoint the source of contamination, should this be a desired goal. This is one reason why mono-species tests are presently just one component of sediment quality assessments.

Amphipods are available in large numbers in marine and estuarine waters worldwide. Ease of collection, amenability in the laboratory, and the availability of culture procedures for some species make this group suitable for the development of toxicity bioassays in countries where species included in standardized test protocols are absent. Several species of marine amphipods, noticeably *Rhepoxynius abronius,* have become established as sensitive test organisms in sediment bioassays (Table 3). ASTM (1993a) describes the biology, handling and culturing methods, as well as bioassay protocols for *Rhepoxynius abronius, Eohaustorius estuarius, Ampelisca abdita, Grandidierella japonica* and *Leptocheirus plumulosus* (see also Environment Canada, 1992).*Grandidierella japonica* stands out as particularly tolerant of a wide range of sediment grain sizes (0.004 mm - 1 mm; Nipper et al., 1989). It is noteworthy that the prolonged holding of field collected amphipods can alter their toxicological sensitivity (Robinson et al., 1988). Lamberson et al. (1992) give an overview of the use of amphipods in marine sediment toxicology. The sensitivity and costs of these bioassays are discussed by Word et al. (1989). Marine research on toxicity and bioaccumulation of pollutants in gammaridean amphipods is reviewed by Reish (1993). Although the most common endpoint in single-compound toxicity tests with gammarideans is mortality in 4 d exposures (LC50), 10 d exposures became the standard in sediment toxicology (Table 3). Reburial capability is a sublethal endpoint which can be applied to most amphipod species. Acute 10-d exposures of *Leptocheirus plumulosus* to estuarine sediments exhibited sensitivity comparable to, or higher than, tests with juvenile *Hyalella azteca,* an amphipod used widely in freshwater sediment toxicology (McGee et al., 1993).

Hyalella azteca is suitable as a test organism for estuarine sediments in which the salinity may be too low for *Rhepoxynius* to survive (Nebeker & Miller, 1988). Although bioassays with the estuarine amphipods *Eohaustorius estuarius, Corophium volutator* and *Leptocheirus plumulosus,* the copepod *Nitocra spinipes,*as well as the bivalves *Mya arenaria* and *Crossastrea gigas,* are meanwhile established for analyses of estuarine sediments (Table 3; see also Crane et al., 1993), *H. azteca* offers the additional advantages of having a long tradition as a test organism, which generated a large data base of its responses to various toxic compounds, as well as the possibility of assessing the toxicity of river systems from upstream sites down the river and through the estuary with the same test species (Nebeker & Miller, 1988). *H. azteca* is the amphipod most sensitive to cadmium in 4 d water-only esposures, followed by *Leptocheirus plumulosus, Ampelisca abdita, Rhepoxynius hudsoni, R. abronius, Lepidactylus dytiscus* and *Eohaustorius estuarius* in decreasing order of sensitivity (Schlekat et al., 1992). In this comparison there is a 40-fold difference in the LC50 value between the most and the least sensitive species.

Decapod crustaceans are represented in marine bioassays principally by *Palaemonetes pugio,* the grass shrimp. A review of toxicity testing with this species, its biology and test methodology is found in Buikema et al. (1980c). Among other species, the ridgeback prawn *Sicyonia ingentis,* is recommended by the US EPA/US ACE (1991) for whole sediment testing because of its size, which makes it suitable for bioaccumulation analyses. Shuba et al. (1978) included copepods, shrimps, amphipods and isopods in a toxicological analysis of dredged material. Larvae are generally the life stage of preference for they have been found to be up to 500 times more sensitive than adults (Franklin, 1983).

Long et al. (1990) evaluated in a comparative test five marine bioassays (amphipods *Rhepoxynius abronius, Ampelisca abdita,* bivalves *Mytilus edulis,* echinoderms*Strongylocentrotus purpuratus* and polychaetes *Dinophilus gyrociliatus*) including whole sediment, elutriate and pore-water exposures. The *Rhepoxynius* survival bioassay was very sensitive and, overall, had a high concordance with the *Mytilus* test. In the *Rhepoxynius* test, however, survival was largely dependent on sedimentological variables. These variables had lit-

tle influence on the survival endpoint of *Ampelisca abdita,* which emerged as having less sensitivity but higher analytical precision for toxicants than the *Rhepoxynius* test. In another comparative study, *Corophium volutator* emerged as the most sensitive organism over *Bathyporeia sarsi* and *R. abronius* (van den Hurk et al., 1992). Other comparisons between various bioassays can be made from data in Chapman et al. (1987; see also Hong & Reish, 1987).

Marine oligochaeta

Enchytraeid oligochaetes have been recommended as indicators of marine pollution (Coates & Ellis, 1980). Most bioassays with marine oligochaetes use respiration rate as the endpoint of the test (Table 3). These assays require a sediment extract as test phase and should be complemented with a mortality assay in whole sediment (Chapman et al., 1982a, b, c). No bioassay with marine oligochaetes to test chronic exposures has become thus far established. In contrast, toxicity bioassays with freshwater oligochaetes are by and large based on mortality, growth, reproduction and behavioural responses, which can be quantified in whole sediment samples after both acute and chronic exposures.

Marine polychaeta

Polychaetes constitute over 40% of both number of species and specimens in the subtidal soft-bottom benthos, regardless of depth or latitude. Their ease of handling, short life history and amenable size makes them suitable organisms for marine bioassays. The materials and methods required for short- and long-term toxicity tests with *Nereis arenaceodentata, Capitella capitata* and *Ctenodrilus serratus* are described by Reish (1980), along with a brief review of the role of polychaetes in toxicity studies (see also Lamberson et al., 1992). These annelids form part of the standardized sediment bioassays used in the assessment of estuarine toxicity (Table 3). A pore-water/elutriate bioassay with *Dinophilus gyrociliatus* (e.g. Carr et al., 1989) complements the range of whole-sediment tests with polychaetes. The small size of *D. gyrociliatus,* however, makes it difficult to recover from the sediment sample at the end of the experiments. The intermediate performance of this survival and egg production bioassay among assays with other organisms is described by Long et al. (1990).

Nereis (Neanthes) arenaceodentata is widely distributed in shallow marine and estuarine benthos of Europe, North America and the Pacific (references in Dillon et al., 1993). The bioassays with this species include a sublethal, chronic exposure test of 28 d, in which growth of juveniles is recorded (see also US EPA, 1990; Pesch et al., 1991). The validity of this chronic test was confirmed by a positive correlation between juvenile (somatic) growth and subsequent reproduction (Moore & Dillon, 1993). In order to eliminate the confounding effect of gametic growth in this bioassay, Moore & Dillon (1993) recommend to limit the measurements of growth to the first 6 weeks post-emergence of the juveniles. *Nereis* sp. and *Hediste (Nereis)* sp. are the polychaetes recommended for whole-sediment toxicity testing by the US EPA/US ACE (1991). The relative sensitivity of polychaetes to pollution is little known. *Nereis virens* is considerably more resistant to organochlorine compounds than the decapod *Crangon septemspinosa* (McLeese et al., 1982). Bioassays with other polychaete species are discussed by Reish & Lemay (1988).

Marine nematoda

Nematodes are the most abundant and species richest organism group among the metazoans of marine sediments (Heip et al., 1985). Two marine nematode bioassays have been thus far developed (Table 3). These can be used to analyse both marine and estuarine sediments (Tietjen & Lee, 1984).

Marine mollusca

The role of molluscs as test organisms in marine toxicology was reviewed by Calabrese (1984). The review describes standard techniques employed in such work and presents examples of the results of toxicity tests. Although many species have been used for toxicity analyses, the most intensive testing has focused on only a few. These are the American oyster (*Crassostrea virginica*), the Pacific oyster (*Crassostrea gigas*), the blue mussel (*Mytilus edulis*), and the hard clam (*Mercenaria mercenaria*). Methodologies for embryo bioassays with marine bivalves are described in ASTM (1989). Bivalves have been used to investigate the toxicity of sediment particles in suspension (e.g. Tsai et al., 1979).

Bivalves are in general highly sensitive organisms to aquatic pollution. In a comparison of five marine sediment bioassays the embryo development test with *Mytilus edulis* was the most sensitive and had the highest discriminatory power and precision of those compared (Long et al., 1990). The *Mytilus* test may be a sensitive indicator of the toxicity of chemicals not rou-

tinely quantified in chemical analyses, but it may also respond sensitively to 'nuisance variables' (Long et al., 1990; see also Williams L.G. et al., 1986, and Becker et al., 1990). Although the whole-sediment acute test with juveniles of *Mulinia lateralis* is similar in sensitivity to equivalent bioassays with amphipods (*Ampelisca abdita* and *Eohaustorius estuarius*), utilization of the *M. lateralis* sublethal growth endpoint greatly increases test sensitivity (Burgess & Morrison, 1994).

Ventilation rate and faecal production of the deposit feeding clam *Macoma nasuta* (Specht & Lee, 1989) could be potentially developed into bioassay endpoints to assess sediment toxicity (c.f. *Abra alba*, Strømgren et al., 1993). The feeding mode of this infaunal species makes it more suitable for analyses of sediment toxicity than bivalves which feed on phytoplankton from the overlying water, and which are generally best suited for water quality monitoring (e.g. Nelson, 1990).

Echinodermata

Echinoderms have been used to evaluate toxicity of sediments in solid and aqueous phases (Table 3; overview in Dinnel et al., 1988). The latter phase has been tested with embryos and gametes, representing planktonic life-stages. Meador et al. (1990) indicated that the *Dendraster* embryo elutriate assay is appropriate for assessment of organic chemicals but not of metal contaminants associated with sediment. The relative sensitivity of echinoderm bioassays was evaluated by Nacci et al. (1986), Dinnel et al. (1987) and Dinnel & Stober (1987). The echinoderm tests show similar sensitivity to a variety of toxicants when compared to fish, crab zooea and Microtox^R assays of contaminated water. The fertility bioassays with *Strongylocentrotus droebachiensis* and *Dendraster excentricus* are similarly sensitive to two reference toxicants, suggesting that between species variation in sperm sensitivity is low within the echinoderms (Dinnel et al., 1982). The sperm fertility bioassay is of roughly equal sensitivity as the embryo development test with the same species (Dinnel et al., 1982). The short duration of the echinoderm fertility bioassay makes it particularly suitable for toxicity tests of chemicals which change form or degrade rapidly after introduction into the seawater or in the test containers. When evaluated against sediment bioassays with other macroinvertebrates, most endpoints of the *Strongylocentrotus purpuratus* echinoderm tests appear to be intermediate in sensitivity, precision and discriminatory power (Long et al., 1990). The results of this pore-water assay correlate highly with polynuclear aromatic hydrocarbon (PAH) concentrations in whole sediment (Long et al., 1990). The *Dendraster excentricus* embryo abnormality test emerged as the most sensitive bioassay, when compared with crustacean and polychaete bioassays exposed to contaminated natural sediments (Pastorok & Becker, 1990). The chronic *D. exenctricus* growth bioassay (Casillas et al., 1992) stands out among echinoderm tests for it has been applied to whole sediments. Meanwhile, echinoderm bioassays have been even applied to freshwater sediments (Pagano et al., 1993).

Benthic community analyses

Benthic community is a popular level of analysis of environmental impact of pollution. It represents the integrated toxicity conditions over a period of time, in contrast to lower levels of analyses (e.g. single species assays) which reflect the condition of organisms just at the time of sampling. In addition, the community level is more amenable to rapid scrutiny than the higher ecosystem level. Also, it is natural communities the reason of concern in most contaminated areas. The impact of sediment toxicity on these communities cannot be extrapolated with certainty from toxicological studies of single-species (e.g. Kimball & Levin, 1985), and, therefore, direct analysis of function and structure of benthic assemblages is argueably the more scientifically and ecologically relevant approach (Warwick, 1993). Schindler (1987) reviews the role of benthic community analyses in sediment toxicology. Specific reference to benthic communities in freshwater is made in reviews by Davis & Lathrop (1992) and La Point & Fairchild (1992), and to marine benthic assemblages in Diaz (1992). Information on types of (marine) mesocosms and their applications is found in Grice & Reeve (1982). Cairns et al. (1992) review studies with benthic microbial communities. Community analyses are often used to monitor environmental health.

Macrobenthic invertebrates are, with few exceptions, probably the best community for field studies in areas of concern, when compared with fish or periphyton assemblages (La Point & Fairchild, 1992). Advantages of macroinvertebrates include their ease of collection, by-and-large sedentary habits and lifespans (up to a year or more for many species) long enough to determine time-weighted chronic effects, but short enough to observe community structure changes within a reasonable period of seasons or years (White, 1988).

The method often uses intact sites as reference communities, but absolute measures of community degradation can be equally useful in the absence of such references (e.g. Warwick, 1993). Multiple samples must be collected throughout the seasons in order to adequately characterize community structure (Winner et al., 1980). ARGE (1991) uses the superficial benthic community, i.e. the abundance and biomass of species combined in a 'zoobenthic index', to monitor the water quality of the river Elbe.

The development of estuarine benthic communities has been used in the laboratory to assess the impact of substances dissolved in a flow-through system with seawater (Hansen & Tagatz, 1980). This system could be adapted to test the impact of contaminated sediments on colonization patterns. Additional, promising endpoints in this approach are species richness and standing crop (Pratt & Bowers, 1992), as well as measures of community similarity (Smith et al., 1990). Some community level endpoints have been used in ecotoxicology within the species assemblage of nematodes in areas of concern: Bongers (1990) used the ratio of r- to k-strategists in freshwater and marine benthos to estimate the impact of pollution (see also Bongers & van de Haar, 1990). Cantelmo & Rao (1979) focused on the numerical relationship between nematode species belonging to different feeding types in sediment samples spiked with pentachlorphenol. Other examples of community level assays are the *Gammarus* to *Asellus* ratio (Whitehurst, 1991) in whole sediments under laboratory conditions and the ratio of nematodes to copepods *in situ* (Rafaelli & Mason, 1981; Warwick, 1981; but see Coull et al., 1981).

Benthic community responses to industrial discharges in rivers have been found to agree well with the results of an aquatic toxicity bioassay with the nektonic *Ceriodaphnia dubia* (Eagleson et al., 1990). Similarly, a reduction in benthic macroinvertebrate abundance, diversity, biomass and number of taxa at copper contaminated sites demonstrated the ecological relevance of toxicity bioassays with *Daphnia* and *Hexagenia* conducted at these sites (Malueg et al., 1984a, b). The recent development of large artificial streams offers opportunities to manipulate benthic communities, a useful step towards the validation of the results of single-species, micro- and mesocosm toxicity tests (Swift et al., 1993).

The evaluation of riffle/run macroinvertebrate communities in lotic freshwater systems has been used to assess the health of benthic ecosystems following a standardized methodology (Ohio EPA, 1987; see also Winner et al., 1980; Barbour et al., 1992; Ließ, 1993). An advantage of lotic systems, is that reference communities are usually available above the discharge site. Conversely, in lentic systems a potential gradient may not be as easily identified, thus making the comparison with control sites more problematic (La Point & Fairchild, 1992). Other applications of the community analysis approach are described by Johnson & Wiederholm (1989), Reynoldson & Zarull (1989) and Becker et al. (1990).

Analyses of benthic community responses to contaminants have generated lists of indicator macroinvertebrates and pollution indeces based on species composition (reviews in Pearson & Rosenberg, 1978, and in Hellawell, 1986 p. 423 ff. and appendices therein). These can be used for preliminary diagnoses or to monitor sediment toxicity (see Gray & Pearson, 1982). In freshwater, for example, certain chironomid species are commonly found to prevail in the vicinity of toxic dischargers, whereas ephemeropterans, plecopterans and trichopterans are typically absent (Winner et al., 1980; Sheehan, 1980; Malueg et al., 1984a, b; Schloesser, 1988; Plafkin et al., 1989; Eagleson et al., 1990; see also Hellawell, 1986). The assemblage characteristic of contaminated regions of a marine inlet of the North American Pacific coast is an overabundance of the polychaete *Tharyx multifilis* and the mollusc *Axinopsida serricata* along with a notorious scarcity of amphipods (Becker et al., 1990; see also Swartz et al., 1986). Luoma & Carter (1991) reviewed the toxicity of trace metals to aquatic benthos. They concluded that the effect of trace metals on higher levels of organization, i.e. population and communities, is little understood and is unlikely to be elucidated by a simplistic approach which, as yet, ignores important complexities of the system (also Luoma & Carter, 1993).

Experiments have shown that aquatic community variables, such as e.g. abundance, can be more sensitive to toxicity than mono-species tests (Lampert et al., 1989). Standardized multi-species aquatic toxicity tests have been meanwhile developed which exploit this sensitivity (Taub, 1989; Taub et al., 1989). An overview of multi-species tests for aquatic toxicity, but which involve sediments, is found in Ahlf (1994, Table 3.12 therein). This approach has a great ecological relevance in that it allows extrapolations about the effect of pollution to the ecosystem. Despite of standardization of microcosm protocols (Taub, 1989) and of various applications of micro- and mesocosms to the assessment of terrestrial (e.g. Mothes-Wagner et al.,

1992) and aquatic toxicity (e.g. Giesy, 1980; Cairns, 1985; Livingston, 1988; Maund et al., 1992; Merlin et al., 1992), no multi-species communities have thus far been used in a standardized bioassay for sediment quality assessments (but see Alden & Butt, 1988).

The use of benthic communities in sediment toxicology has been criticized on several grounds. (1) The results of toxicity analyses using community variables *in situ* are often insufficient, for they can fail to discriminate between changes in community composition due to chemical toxicants, water quality fluctuations (e.g. dissolved oxygen, temperature, pH, salinity), changes in organic content, differences in physical variables (e.g. sediment features, water depth) and in biotic interactions, such as competition and predation (e.g. Schlekat et al., 1992). (2) The results are usually site and season specific. (3) Determination of an endpoint for community responses to toxicity is problematic (Luoma & Carter, 1993). (4) The complexity of these systems and the costly data evaluation greatly limits the number of replicates. Despite of these drawbacks, recent developments in the field of microbial microcosms suggest that this methodology can potentially become an integral part of sediment test batteries (e.g. Cairns & Pratt, 1987; Henebry & Ross, 1989; Cairns & McCormick, 1991; Cairns et al., 1992). Similarly, analyses of absolute measures of benthic community stress *in situ* have been recently simplified to a degree that makes them amenable for incorporation into tiered strategies for a rapid and cost-efficient evaluation of sediment toxicity (Warwick, 1993). In general, therefore, toxicological analyses of benthic communities are presently useful only in combination with other test methodologies, such as single-species bioassays and chemical analyses of the sediment (La Point & Fairchild, 1992).

Combined bioassays, test-batteries and the step-wise approach

The various strengths and weaknesses of the presently available bioassays, as well as their selective sensitivity to certain toxicity variables, dictate a combination of several tests for an adequate assessment of sediment quality (Cairns, 1983a). While some of the proposed test-batteries are limited to microorganisms (e.g. Ross & Henebry, 1989; Ahlf et al., 1991), in others the meio- and macrobenthic species play a central role (e.g. Reynoldson & Day, 1993) or complement other mono- species bioassays with microbes, nektonic invertebrates and vertebrates (e.g. LeBlanc & Surprenant, 1985; Dutka & Kwan, 1988; Giesy & Hoke, 1989, 1990; Krantzberg & Pope, 1989; Munawar et al., 1989; Baudo et al., 1990 p.330; Elder, 1990; Kwan et al., 1990; Dutka et al., 1990, 1991; Hoke et al., 1993; Ahlf 1994). Often, the rationale in the design of test batteries or multispecies assemblages for sediment analysis is to use a hierarchical approach covering the cellular, species, population and community level with a wide discriminatory and sensitivity range. Benthic metazoans in such tests are typically *Chironomus* sp., *Hexagenia limbata, Hyalella azteca* and/or *Tubifex tubifex.*

The cladoceran *Daphnia* is a highly sensitive organism used for sediment tests, especially to metals. *Hyalella* and *Chironomus*, which are very amenable in the laboratory, are highly sensitive to organic toxicants in whole sediment and would, therefore, complement adequately the *Daphnia* results for soluble compounds. Nebeker et al. (1984) describe combined bioassays with *Daphnia* and benthic metazoans to test short and long term exposure to toxicants. A *Daphnia*, 48 h whole sediment or elutriate test is recommended as a fast and inexpensive initial screening procedure for acute toxicity of sediments, before resorting to further testing with benthic species. Short term exposures can also be tested in mixed species beakers with whole sediment and overlying water using the amphipods *Hyalella,* or *Gammarus*,the burrowing mayfly nymph *Hexagenia* and the midge *Chironomus* , as well as in a combined test with *Daphnia* and *Hexagenia.* Long exposures use *Hyalella* life cycle data and *Chironomus* larval growth, survival and adult emergence as endpoints. The toxic response shown by an organism in a multi-species exposure is likely to differ from its equivalent response in a mono-species bioassay because the presence of other organisms can affect the bioavailability of toxicants in complex ways (Malueg et al., 1983; Keilty et al., 1988b). Giesy & Hoke (1989) recommend a battery of screening tests, which includes Microtox^R, *Chironomus tentans* growth, *Daphnia magna* mortality and an algal assay (e.g. Munawar & Munawar, 1987).

One of the most commonly used multispecies bioassays was originally developed for freshwater sediments by Prater & Anderson (1977a, b) and later modified by other investigators (e.g. LeBlanc & Surprenant, 1985). It consists of burrowing or epibenthic organisms placed in the test sediment, while water column dwellers (*Daphnia magna* or the fish *Pimephales promelas*) are simultaneously exposed to the circulating overlying water. *Daphnia* are kept in

suspended cages, avoiding direct contact with the sediment. Benthic species typically used in this test are the insect larvae of *Hexagenia* or *Chironomus*, the amphipods *Hyalella* or *Gammarus*, or the isopod *Asellus*. The effect of the burrowing activity of *Hexagenia* on *Daphnia* survival in this apparatus was investigated by Malueg et al. (1983). The combination of both species was found to be a more sensitive test than the assay with *Daphnia* alone. Swartz et al. (1979) proposed a multispecies bioassay for marine sediments consisting of pelecypods, polychaetes, amphipods and cumaceans. Some investigators use combinations of benthic microbes for sediment analyses (e.g. Cairns et al., 1985).

Simultaneous testing with mono-species bioassays and tests at higher levels of organization, i.e. multi-species, multi-trophic, community and ecosystem level, have been also advocated as a way to overcome the interpretative limitations of single-species assessments of toxicity (e.g. Maciorowski & Clarke, 1980; Cairns, 1983b, 1988b; Kimball & Levin, 1985). Test batteries for aquatic systems should combine representative species from various trophic levels, e.g. microbes, algae and invertebrates, to ensure a broad coverage of toxicity (Burton, 1989).

The contributory role of benthic metazoans in assessments of sediment toxicity is best illustrated by test procedures which integrate various techniques. The sediment-quality-triad approach, which incorporates chemical and ecological measures into biological test-batteries, can, for example, combine (1) chemical analyses, (2) toxicity tests, e.g. effect on *Daphnia* reproduction, and (3) an assessement of biological effects *in situ* (benthic community health), e.g. incidence of malformations in *Chironomus* and its population density (Chapman, 1990, 1992; Chapman et al., 1992; van de Guchte, 1993). These three components were originally combined by Malueg (1984a, b) to investigate the toxicity of freshwater sediments contaminated with copper. The sediment- quality-triad was further developed and tested in marine ecosystems (Long & Chapman, 1985; Chapman, 1986b; Chapman et al., 1987). This approach thus combines the potential cause (chemistry) with the effect (biology) of degradation resulting from sediment pollution. A more comprehensive, integrated assessment of sediment toxicity has been recently done in the Great Lakes (Ankley et al., 1992). It included (1) analysis of benthic community structure, (2) determination of bulk sediment and pore water toxicity to microbial, invertebrate and vertebrate species, (3) identification of specific sediment toxicants, (4) evaluation of the presence of carcinogenic and/or mutagenic compounds in the sediments via microbial and fish assays, (5) determination of possible toxic and/or teratogenic effects of sediment contamination on avian populations, and (6) risk assessement for several contaminants of specific concern in the sediments and associated biota.

In a step-wise approach developed to assess sediment quality various test procedures are applied in sequence, depending on the results of the previous step (Reynoldson & Zarull, 1989; Chapman et al., 1992; US EPA, 1992; Ahlf, 1994). In contrast to test-batteries in which all tests are run simultaneously, this approach emphasises flexibility in test design and uses a sensible ranking of diagnostic methods, thereby optimizing the economic aspect of sediment assessments. The effects of toxicants on benthic metazoans, in addition to physical and chemical variables, are an essential component of stepwise-assessments of sediment quality.

A useful strategy for the use and choice of bioassays with benthic metazoa is well illustrated in the following studies. Williams et al. (1986) compared three bioassays commonly used in marine sediment toxicology. These are the bioluminiscence of the bacteria *Photobacterium fisherii* (Microtox$^{R)}$ in sediment extracts, oyster embryo abnormalities (*Crassostrea gigas*) in sediment slurries and mortality of the amphipod *Rhepoxynius abronius* in whole sediments. These three bioassays, along with a characterisation of *in situ* community structure, compose a test-battery used to examine sediment toxicity in Puget Sound, USA (Pacific). There was a high overall level of agreement between these tests, based on rank-order comparisons. At some sites, however, there were clear discrepancies in the responses of the three bioassays, probably due to differences in sensitivity to the kinds of contaminants in the various samples and/or to the duration and medium of exposure. In that comparison, the oyster embryo test was the least and Microtox^{R}the most sensitive of the three. The same three bioassays were compared with respect to their ability to predict alterations in benthic assemblages due to chemical toxicity (Becker et al., 1990). This comparison was made to examine the ecological relevance and hence validity of sediment bioassays performed in the laboratory. Although all three bioassays were reasonably successful in predicting the presence or absence of moderately to severely altered assemblages, the tests differed markedly in their ability to identify only the altered assemblages. The Microtox^{R}bioassay was the most sensitive: it successfully identified the highest percentage of altered assem-

blages, but it also reacted positively to nearly half of the sites considered as unaltered. The oyster embryo test was intermediate in sensitivity, whereas the amphipod bioassay, the least sensitive of the three, identified only half as many altered assemblages as Microtox^R. Effectiveness to detect only altered assemblages was highest for the oyster embryo test and considerably lower for both Microtox^R and *Rhepoxynius*. Most false predictions of the amphipod bioassay were seemingly due to the occurrence of much fine-grained material in the sediment at those sites, a condition which is known to increase the mortality of *Rhepoxynius* in the absence of toxicants (DeWitt et al., 1988). When this factor was taken into account, the *Rhepoxynius* bioassay was as effective as the oyster embryo test.

These results emphasize the importance of combining tests into a tiered battery and of integrating various approaches, such as chemical analyses and evaluation of *in situ* community structure, to assess sediment toxicity. Such a strategy allows validation (Long & Chapman, 1985; Cairns, 1988c) and calibration of methodologies. It can also potentially simplify subsequent monitoring in the area of concern by reducing the sediment assessment to a small battery of monospecies bioassays. Becker et al. (1990) recommend the most sensitive test (here Microtox^R) to be used as a screening tool. Subsequently, the most effective test (here the oyster embryo bioassay) should be applied to the subset of sites diagnosed initially as potentially impacted. The second tier, therefore, would identify those sampling stations with the highest priority for remedial action. In the above case, consequently, the *Rhepoxynius* bioassay could be eliminated from the test battery. Microtox^R has been also recommended for freshwater sediment analyses as a screening tool to be used in combination with macroinvertebrate bioassays (e.g. Giesy et al., 1988). Inclusion of macroinvertebrates is justified not only to increase the ecological relevance of the test, but also because Microtox^R is less sensitive to certain toxic compounds than macroinvertebrates (references in Giesy et al., 1988). The greater sensitivity and discriminatory power of test-batteries over single bioassays has been demonstrated quantitatively for marine and freshwater sediment tests (Becker et al., 1990; Giesy et al., 1988).

Although rankings of sensitivity are sometimes used to determine the composition of bioassay test batteries, as well as the order of exposure of the different test organisms, a cautionary note results from evidence suggesting that sensitivity is a highly complex and situation specific variable (e.g. Williams L.G. et al., 1986; Giesy et al., 1988; Long et al., 1990; Schlekat et al., 1990; West et al., 1993). West et al. (1993) compared the sensitivity of some test organisms to sediments contaminated with copper in a 10 d survival (LC50) assay with a flow-through, water renewal system. Culture methods -described in detail by West et al. (1993) - were based on Nelson et al. (1991) for *H. azteca* and *C. tentans*, and on Phipps et al. (1993) for *L. variegatus*. *Hyalella azteca* was more sensitive to copper than *Chironomus tentans* and *Lumbriculus variegatus*. However, *C. tentans* survival was affected at one site at which the other two species were not affected. Thus, protection strategies based on the use of only the most sensitive species may be underprotective for (apparently) less sensitive organisms (West et al., 1993). Survival of *L. variegatus* was not affected by exposure to contaminated sediments but its reproduction was. The results contrast greatly with the relative sensitivities shown by these species when tested in water-only copper exposures or in exposures to natural sediment (Dermott & Munawar, 1992).

Bioaccumulation of toxicants

Biotransformation, bioaccumulation and bioconcentration of toxicants in benthic organisms, e.g. in bivalve molluscs (Alden & Butt, 1988; Bauer et al., 1989; Berthet et al., 1992; Strømgren et al., 1993; ASTM 1994b), is one way of documenting bioavailable contamination of aquatic systems. Studies with benthic metazoa which have measured bioaccumulation in contaminated sediments are indicated by a footnote in Tables 2 and 3. The uptake and accumulation of some sediment contaminants by oligochaetes has been well documented (e.g. Mac et al., 1984; Oliver, 1984). Data on bioaccumulation, however, do not allow a direct inference about the quality of sediments as such because (1) the relationship between bioaccumulation and toxicity is not simple (Berthet et al., 1992) and (2) because the source of contamination in the case of bivalves is typically the overlying water or phytoplanktonic food.

Measures of bioaccumulation, however, could be specifically adapted to assess sediment quality by calibrating bioaccumulation with toxic effects and by choosing organisms from deep sediment layers, such as e.g. certain nematodes or oligochaetes, which have a reduced relative exposure to overlying water. Finding a suitable benthic species for such a test may not be simple, however. In choosing an organism for a bioaccu-

mulation bioassay for freshwater sediments, Mac et al. (1984) rejected several benthic invertebrates because of size limitations (e.g. *Chironomus*) or intolerance to certain sediment types (e.g. *Hexagenia*). The species finally recommended for that bioassay were the fathead minnow (*Pimephales promelas*) and the earthworm (*Lumbriculus terrestris*). In a combined bioassay using *Daphnia* and benthic organisms, Nebeker et al. (1984) recommend *Gammarus* and *Hexagenia* when toxicity and bioaccumulation are of interest, for they yield greater biomass for tissue analyses than *Chironomus* or *Hyalella*. Nebeker et al. (1984) describe bioaccumulation tests for these organisms. The amphipod *Diporeia* has been used in bioaccumulation studies of sediment associated organic toxicants in the Great Lakes (e.g. Landrum, 1989; Landrum et al., 1992).

Nalepa & Landrum (1988) reviewed the bioaccumulation of organic contaminants and heavy metals in macrobenthic freshwater organisms (for gammaridean amphipods see Reish, 1993). Adequate data of contaminant uptake rate, depuration rate, biotransformation rate and their toxicity are a prerequisite to develop sediment diagnosis charts based on bioaccumulation. Such data, unfortunately, are still missing for many benthic species and toxic compounds. Nevertheless, a body of knowledge about benthic bioaccumulation is growing from studies on oligochaetes (see review in Nalepa & Landrum, 1988; Keilty et al., 1988b, c). Oligochaetes have already been recommended as monitoring tools for metal pollution of aquatic systems (e.g. Chapman et al., 1979; Chapman et al., 1985). This group is, therefore, a good candidate for the development of guidelines for sediment toxicity assessment based on bioaccumulation. Such a procedure could become incorporated into test strategies as an additional source of information on the quality of sediments. Advantageous starting points in this endeavour are, firstly, that bioassays and standardised procedures for measuring bioaccumulation of toxic substances from sediments have been already developed (Mac et al., 1984; US EPA, 1994b) and, secondly, that considerable progress has been recently made in the theory underlying patterns of bioaccumulation of sediment-associated pollutants (Lee, 1992).

Future perspectives

The usefulness of bioassays to assess and/or predict toxicity in sediments is indisputable, but the basis for an interpretation of bioassay results is still weak (Luoma & Carter, 1993). One important future avenue in sediment ecotoxicology is the study of mechanisms that control toxicity under field conditions, because a better understanding of these processes would reduce many of the uncertainties currently associated with results of bioassay and benthic community analyses. In addition, sediment ecotoxicology could benefit from progress in the use of biomarkers, mutagenic responses, and energetic measures of organism health, as well as from the development of bioassays with additional test species.

Biomarkers are defined as biochemical, physiological or pathological responses of single organisms with information about exposure to toxicants and/or sublethal consequences resulting from such exposure (Benson & Di Giulio, 1992). Most biomarkers have been so far identified in phytoplankton (e.g. Berglund & Eversman, 1988), benthic fishes (e.g. McMahon et al., 1988; Huggett et al., 1992) and in some invertebrates upon exposures to single-contaminants (review in Giesy & Graney, 1989). This technique is promising for applications in meio- and macrobenthic organisms exposed to contaminated sediments. Giesy & Graney (1989) recommend the use of endpoints reflecting effects on bioenergetics and of the RNA/DNA ratio as a measure of growth. They conclude, however, that biochemical effects following acute exposures are not yet sufficiently calibrated with chronic effects on survival, growth and reproduction to allow replacement of chronic studies. The biochemical measures may nevertheless contribute to our understanding of modes of action in the laboratory or may serve as early warning measures to determine probable causes of field toxicity (Giesy & Graney, 1989; Depledge, 1994).

No tests of marine sediment mutagenic or promutagenic toxicity is thus far widely established. At present, the best candidates for such a standardized test are the polychaete *Neanthes arenaceodentata* and echinoderm embryos, of which cytological and cytogenetic abnormalities have been already used as bioassay endpoints (Pesch et al., 1981; Hose, 1985; Long et al., 1990). The *Strongylocentrotus purpuratus* bioassay, for example, indicated mutagenicity in several sediment samples with high hydrocarbon concentrations (Long et al., 1990). Mutagenicity could become a useful endpoint to document the toxicity of some compounds which escape detection in tests of acute mortality (Long et al., 1990). A further potential development in the assessment of sediment toxicity can be the standardized application of stress protein bioassays with benthic organisms (see Bradley, 1990).

Scope for growth, an energetic measure of an organisms health, has been measured in various marine taxa (e.g. polychaeta: Johns et al., 1985) and in the freshwater amphipod *Gammarus pulex* as an indicator of environmental pollution (review in Maltby, 1992). The advantage of measuring scope for growth instead of growth and fecundity is that its components (i.e. energy intake and expenditure) can be measured over short time scales (hours/days). The *G. pulex* scope-for- growth bioassay has been used to test toxicity of effluents and of single contaminants in water (Naylor et al., 1989; Maltby et al., 1990 a, b), but rarely to analyse contaminated sediments (e.g. Roddie et al., 1992). The use of sophisticated equipment to measure scope for growth, has been meanwhile overcome by simplifying measures of energy intake and respiration to an estimate of feeding rate (Maltby, 1992; Roddie et al., 1992). This bioassay, which can be used in the laboratory and *in situ,* could be potentially developed into a standard bioassay to test the chronic toxicity of freshwater sediments.

Benfield & Buikema (1980) list miscellaneous aquatic invertebrates, which have been used in toxicity studies and for which standardized test protocols could be potentially developed for sediment toxicity analysis. The benthic metazoans mentioned therein are: (1) Gastropoda, *Goniobasis livescens, Lymnaea emarginata, Nitrocris* sp., *Physa heterostropha, Physa integra*; (2) Bivalvia: *Mytilus planatus, Neotrigonia margaritacea, Elliptio* sp., *Plectomerus* sp., *Musculium* sp., (3) Crustacea: *Asellus brevicaudus, Orconectes nais, Palaemonetes kadiakensis;* (4) Insecta: *Callibaetis* sp., *Ephemerella cornuta, Limnephilus* sp., *Enallagma* sp., *Lestes congener, Libellula* sp., *Pteronarcys californica;* (5) Oligochaeta: *Lumbricillus rivalis, Aelosoma headleyi;* and (6) Turbellaria: *Dugesia tigrina.* In addition to species mentioned in Table 3, the US EPA/US ACE (1991) cites the following marine organisms as potential test species in whole sediment bioassays of dredged material: (1) Bivalvia: *Tapes japonica,* (2) Polychaeta: *Glycera* sp., *Abarenicola* sp., *Nephthys* sp., (3) Crustacea: *Neomysis* sp., *Holmesimysis* sp., *Pandalus* sp., *Callinectes sapidus* and *Cancer* sp.. Other potential test species for sediment ecotoxicology are listed in Table 4.

Although meanwhile a range of organisms have become established in routine sediment toxicity testing, the search for additional bioassay organisms is still a worthwhile endeavour. Despite the fact that aquatic pollution is a world-wide problem, development of sediment bioassays, with few exceptions, has been thus far centred around North American and European faunas. A selective review of the distribution of 30 species contained in Tables 2 and 3, and for which ranges were indicated in publications cited therein, shows that there is an urgent need to develop more sediment bioassays with tropical and austral organisms. Meanwhile, sediment toxicity analyses in those regions can include cosmopolitans for which bioassay procedures are already well established (e.g. for freshwater sediments the oligochaetes *Tubifex tubifex* and *Limnodrilus hoffmeisteri,* and for marine sediments sea urchins (Dinnel et al., 1988) or the polychaetes *Nereis arenaceodentata* and *Capitella capitata*). The optimal organism for bioassays is the most sensitive, locally important species, but, as noted by Benfield & Buikema (1980) "...the *most sensitive and locally important species* is not known for most areas of the world, and thus the *most convenient and well tested* is generally selected. While the *most convenient and well tested* approach may optimize efficiency, it may retard progress toward achieving the optimum choice."

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