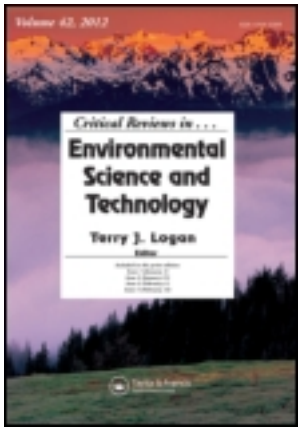

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Sediment Toxicity Testing of Organic Chemicals in the Context of Prospective Risk Assessment: A Review

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Sediment toxicity tests play an important role in prospective risk assessment for organic chemicals. This review describes sediment toxicity tests for microorganisms, macrophytes, benthic invertebrates, and benthic communities. Current approaches in sediment toxicity testing are fragmentary and diverse. This hampers the translation of single-species test results between freshwater, estuarine and marine ecosystems and to the population and community levels. A more representative selection of species and endpoints as well as a unification of dose metrics and exposure assessment methodologies across groups of test species, constitutes a first step toward a balanced strategy for sediment toxicity testing of single organic compounds in the context of prospective risk assessment.

Supplementary materials are available for this article. Go to the publisher's online edition of Critical Reviews in Environmental Science and Technology *for the supplemental material.*

KEY WORDS: benthic community, benthic invertebrates, macrophytes, microorganisms, prospective sediment toxicity testing

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1. INTRODUCTION

1.1 History of Sediment Toxicity Testing

Chemical contamination of aquatic sediments is a worldwide issue and may lead to toxic effects in aquatic organisms.[1–3] Sediment is a complex heterogeneous matrix, in which biota may be exposed to sediment-associated contaminants by a number of routes.[4] Historically, toxicity testing mainly used aquatic animals, whereas aquatic plants were used only occasionally.[5,6] It has been recognized, however, that by testing animals in the aquatic phase, the role of sediment as an exposure route is neglected and these tests are not sufficient to assess environmental hazards to benthic invertebrates, plants, and microorganisms.[6,7] Consequently, there is an urgent need to evaluate the role of toxicity tests with benthic species in sediment risk assessment procedures including toxicity tests with macrophytes[8] and microorganisms.

Early sediment toxicity testing methods and regulatory instruments were developed in North America,[1] due to dredging concerns and the recognition of widespread contamination of sediments.[9] The development of whole-sediment tests with sediment-related test species has gone through many changes (Figure 1). Originally, aquatic species (e.g., *Daphnia* sp.) were tested in the aqueous phase. These species, which predominantly dwell in the water column, cannot be used to test the toxicity of the solid phase directly, which is why they have been used as a surrogate measure of the toxicity to benthic species by testing them in pore water and elutriate. Pore water contains the bioavailable fraction and therefore is important for exposure to infaunal species.[10,11] Elutriate tests provide information on the leaching capacity of sediment-associated contaminants[4] and were used to mimic the open water disposal of dredged material,[12] thus representing the potential adverse effects to aquatic organisms due to sediment disturbance.[13,14] Nevertheless, simulation of in situ exposure of organisms to contaminated sediments is most realistic when whole-sediment samples are used.[15,16] Whole-sediment tests allow different exposure routes (e.g., via pore water or ingestion of particles)[10] and can be conducted under more realistic sediment physicochemical conditions.[17] Hence, sediment was introduced as an extra compartment. The existence of multiple exposure routes, however, increases the complexity and unpredictability of exposure, which may differ for different chemicals tested, sediment types, and species with different living and feeding strategies.

After the early phases of sediment toxicity testing, benthic organisms were introduced in pore water, elutriate or sediment tests with and without an overlying water phase. Macrophytes and soil species (e.g., earthworms) were mainly tested in sediment without overlying water.[18] The first standard protocols for whole sediment tests with benthic invertebrates were developed in the 1990s.[19] So far, however, no standard protocols are available for sediment-rooted macrophytes and sediment-related microorganisms.[20–22]

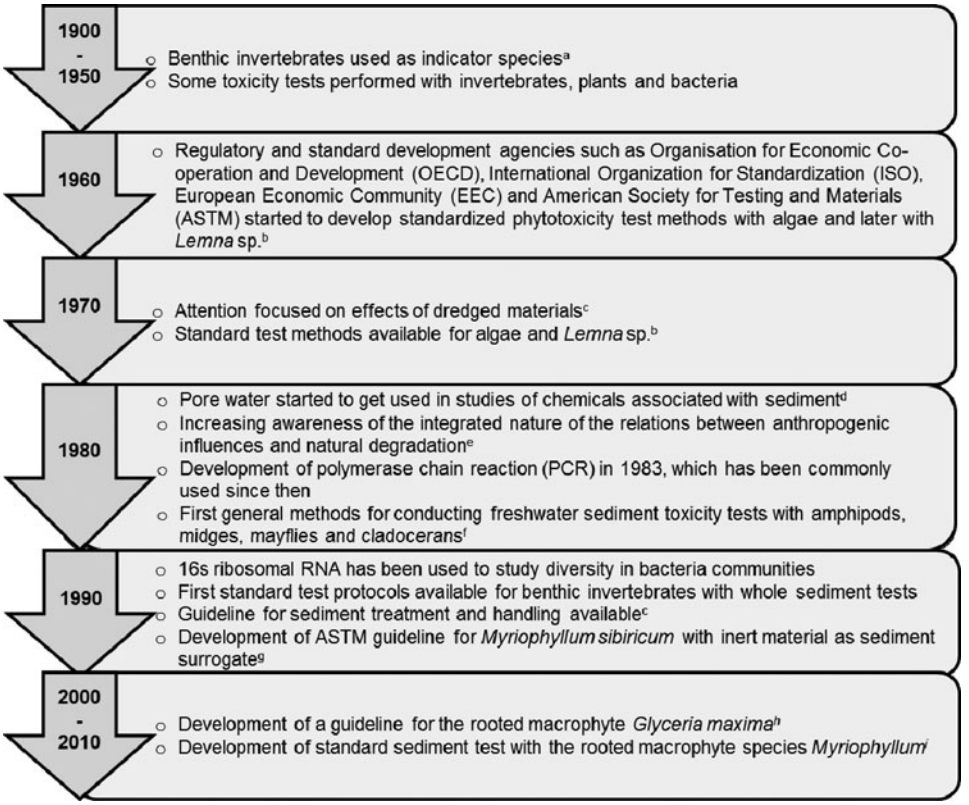

FIGURE 1. Timeline of the development of sediment toxicity testing with microorganisms, animals and plants. References: [a][214], [b][5], [c][19], [d][10], [e][20], [f][215], [g][99], [h][203], [i][8].

This raises the main question addressed in this review: Which test species and test methodologies should be recommended to fill this gap? Compared to freshwater sediment tests, marine and estuarine tests have received much less attention.[17,19]. Furthermore, sediment tests have been developed mainly in North America and Europe, indicating a need to develop test methods that are suitable for subtropical, tropical, and Australasian organisms.[18] For aquatic microorganisms, the focus today is on how they degrade organic contaminants rather than on how natural microbial populations in water and sediment could be impacted.[21]

Despite the level of sophistication that single-species whole-sediment laboratory tests may have reached, they cannot capture all processes at the population and/or community level. To some extent, community level tests (micro- and mesocosm experiments) have been developed to increase ecological realism.[23] Still, they cannot fully account for the natural complexity of ecosystems. Micro- and mesocosm experiments typically lack the presence of top predators and realistic recolonization by certain species, for instance

semivoltine or univoltine species that lack insensitive life-stages (e.g., eggs) and/or well-developed dispersal abilities (e.g., aerial stages).[24]

1.2 Regulatory Frameworks

Contaminated sediment testing has received most attention within the framework of retrospective risk assessment (RRA). RRA is defined by the Environmental Protection Agency (EPA) as an evaluation of the causal linkages between observed ecological effects and a stressor in the environment. In, RRA, sediment toxicity tests are used to identify the cause of adverse effects of a stressor already present in the environment and has been used to screen contaminated field sites and rank contaminated sediments, and plan and monitor remedial actions.[18,25] Less effort has been invested in the development of sediment toxicity tests in the framework of criteria setting and prospective risk assessment (PRA) in the context of market authorization of existing and new chemicals.[19] PRA is defined by the EPA as an evaluation of the future risks of a stressor(s) not yet released into the environment or of future conditions resulting from an existing stressor(s). Prospective risk assessment schemes are mandatory in many industrialized countries for a large number of potentially toxic substances used commercially. This has resulted in a number of regulatory instruments such as the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Control Act (FIFRA) in the United States, the Canadian Environmental Protection Act (CEPA) and the Pest Control Products Act (PCP Act) in Canada, Australian Pesticides and Veterinary Medicines Authority (APVMA) in Australia and Regulation EC No 1907/2006, commonly known as REACH (Registration, Evaluation, Authorization and Restriction of Chemical Substances), and Regulation EC/1107/2009 (plant protection products) and Directive 98/8/EC (biocides) in the European Union. In all of these laws and regulations, lower-tier effect assessment procedures should be based on protocol tests, but standard protocols are not widely available for sediment toxicity testing. Ideally, such standard protocols would be used in the context of a risk assessment scheme that unifies exposure metrics, enables read-across between freshwater, estuarine, and marine environments, as well as read across between different species and trophic levels, and accounts for interactions at the community level.

1.3 Aim of the Review

In the present article we critically review the state of science with regard to protocol sediment toxicity testing of single organic compounds in the context of PRA. This includes discussing the aforementioned knowledge gaps; providing recommendations for optimum sediment toxicity test designs for

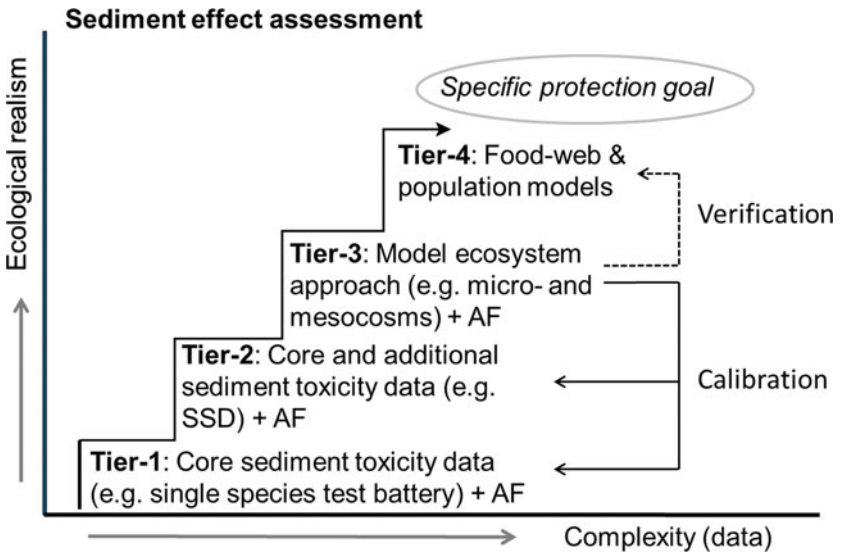

FIGURE 2. Schematic overview of a tiered approach as used in prospective risk assessment. In each tier an assessment factor (AF) may be necessary to derive a predicted no effect concentration (PNEC). The higher tiers could be used to calibrate the lower tiers (adapted from Brock and Van Wijngaarden[216]) (Color figure available online).

microorganisms, macrophytes, benthic invertebrates, and benthic communities; and identifying new research priorities. Although our focus is on freshwater, estuarine, and marine systems in the temperate zone, we also offer a comprehensive view of other climate zones. Finally, a first outlook is provided on how the recommendations could be used in the framework of PRA in a regulatory context. The fact that this review focuses on organic chemicals implies that metal testing is not covered. Moreover, literature on assessment of effects in the field or on testing with natural or field contaminated sediments is considered only if relevant for chemical test development in the context of PRA.

2. PROSPECTIVE SEDIMENT TOXICITY TESTING

2.1 The Tiered Approach in PRA

Tiered approaches often form the basis of environmental effect assessment schemes that support prospective effect assessments. In this context, a tier is defined as a complete effect assessment resulting in an appropriate assessment endpoint (e.g., the PNEC [predicted no effect concentration]). The concept of tiered approaches involves starting with a simple conservative assessment and only doing additional, more complex work when necessary for refinement of the risk assessment (Figure 2). Within a tiered effect assessment scheme all tiers aim to assess the same well-defined specific protection

goal, but going from lower to higher tiers the problem is addressed with higher accuracy and precision. Consequently, lower tiers are more conservative than higher tiers.[25–27] The first tier of the effect assessment usually starts with toxicity data from standard tests and assessment factors (AFs) that are prescribed by the relevant legislation. The next tier usually is based on the combination of laboratory toxicity data from standard and additional test species. The highest effect tiers may comprise model ecosystem experiments and ecological models.

A logical consequence of the principles of the tiered approach is that higher tiers can be used to calibrate the lower tiers. In the prospective effect assessment for toxic chemicals in sediments the PNECs derived from appropriate micro-/mesocosm tests may be the most appropriate tier to calibrate the other effect assessment approaches (Figure 2). Note that in the prospective risk assessment, the toxic chemical may not yet be placed on the market so that effect assessments based on field monitoring programs are not an option as a reference. Furthermore, the advantage of microcosm and mesocosm studies over field monitoring studies is that due to increased control over confounding factors, causality between exposure to a sediment-bound contaminant and effects is easier to demonstrate. In addition, micro/mesocosms with artificially contaminated sediments allow to study different contaminant levels, replication, and real controls (contaminant not present), which normally is not possible in a field study. It is, however, important to note that the biological and environmental conditions in a specific micro-/mesocosm test represent only one of the many possible conditions for sediment communities. This variability should be accounted for in the effect assessment (e.g., by applying an appropriate AF for spatiotemporal extrapolation of the concentration-response relationships observed in micro-/mesocosm tests). The height of this AF may be based on the observed variability in threshold concentrations for effects on sediment organisms derived from different micro-/mesocosm tests and of which the sediment was polluted with the same chemical. Whether in these tests, multiple stressors should be investigated to derive an appropriate AF depends on the specific protection goals defined by risk managers.

2.2 General Guidelines From a Regulatory Perspective

For an optimal toxicity test, both in the lower as higher tiers, many factors need to be considered (Figure 3). This section reviews the recommendations on these factors described in the literature. The ideal sediment toxicity test provides accurate and reproducible results. This requires standardized tests with well-defined endpoints that are linked to the related protection goals. Hence, test guidelines produced by international (e.g., OECD, ISO) and national (e.g., U.S. E.P.A., ASTM) bodies are highly appreciated. These test guidelines are preferably ring tested. In a ring test, the performance of a

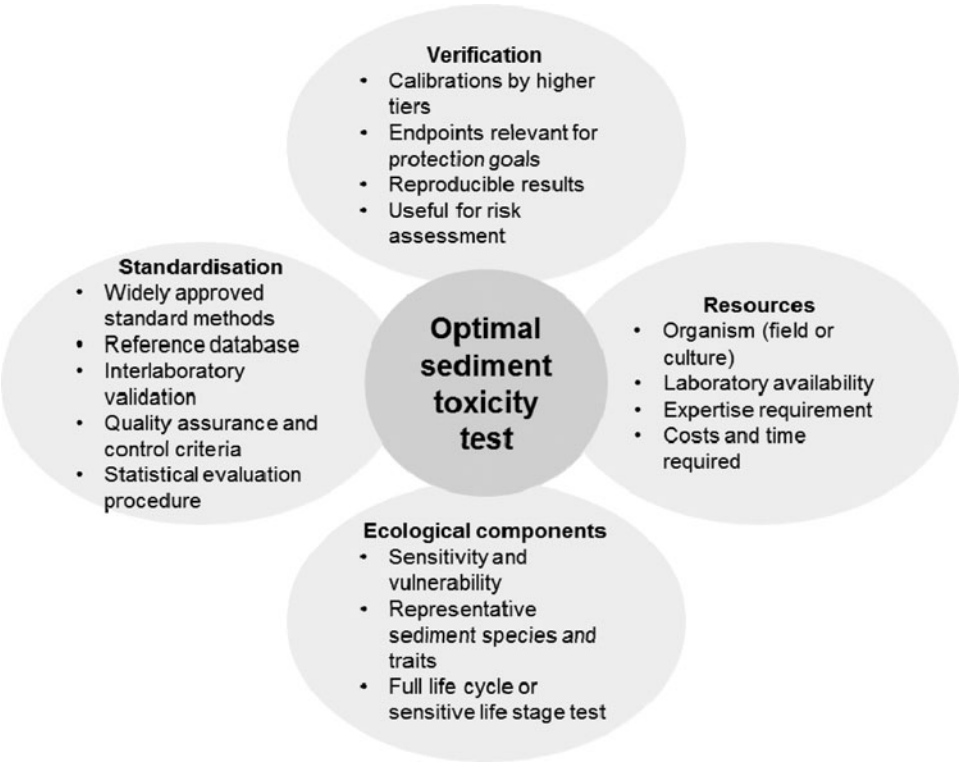

FIGURE 3. Factors to be considered in designing an optimal sediment toxicity test (after Burton and Scott[19] and Chapman[28]).

method is evaluated across different laboratories and countries. Such a ring test is required (e.g., by the OECD) to approve the test as a guideline. Another regulatory requirement is that the standard sediment species to be used should be easy to obtain or culture, should be ecologically and ecotoxicologically relevant and should represent specific trophic levels or taxonomic groups that allow extrapolation to the wider array of sediment organisms occurring in the field. Battery testing, using species that differ in biological traits and taxonomy should be used to get a more complete view of a compound's toxicity.[19,28–31] A read across can be used, with the data from the test battery, as a method to fill data gaps for a substance or species by extrapolating data from one substance or species to another substance (usually with a similar toxic mode of action) or species (that are usually taxonomically related or have similar traits with respect to sensitivity). A fundamental assumption in every sediment toxicity test conducted for prospective purposes is that an exposure-response curve can be derived. In addition, sediment toxicity tests should be designed in such a way that the measurement endpoints can be evaluated with sufficient statistical power. Ideally, the statistical power of the test should be known.

The duration of the test should be long enough to allow the relevant effect to be fully expressed. Ideally, the incipient should be reached or it should be possible to extrapolate responses observed in time by means of an appropriate assessment factor or model.[27] For sediment organisms and sediment-associated chemicals, the relevant exposure regime is usually chronic, which calls for chronic testing. Chronic toxicity tests with sediment-dwelling organisms focus not only on lethal endpoints but also on sublethal endpoints (e.g., related to reproduction and growth). Endpoints must be as sensitive and ecologically relevant as possible to allow the effects to be extrapolated from the individual level to the population level. A chronic toxicity test is generally defined as a study in which the species is exposed to the toxicant for at least one full life-cycle, or the species is exposed to the toxicant during one or more critical and sensitive life stages. Assessing potential effects of endocrine-disruptive contaminants in the sediment may require multigeneration tests. Consequently, what is considered chronic or acute depends on the species and endpoint considered.[32,33]

In the ideal case, the time-to-onset of the effect and preferably the maximum effect should be recorded, however this may be difficult in practice. Ideally, the effect estimates derived from the toxicity test avoid NOECs[34,35] and include EC_x values (e.g., EC_{10}; EC_{50}). As external exposure is only a surrogate for internal dose, the exposure concentration in the course of the laboratory test should be well controlled and characterized, either by measurements or by exposure modeling (more details in the next section). This allows maximum flexibility in selecting the best dose metric (the ecotoxicologically relevant concentration [i.e., the C in EC_x]), such as the mean or time-weighted average bioavailable concentration during the toxicity test.[26,36] As the effects observed may be modified by intrinsic factors, the history, origin and life-history stage of the test species/individuals should be appropriately described. As exposure in the lab test may also be modified by extrinsic factors, it is important to appropriately describe sediment properties (e.g., organic carbon content, clay content, pH, cation-exchange capacity, grain size), ambient test conditions (e.g., temperature, salinity, light conditions), and exposure duration (including changes in exposure concentrations during the test).

2.3 Sediment Preparation and Exposure

This section briefly reviews the key mechanisms determining the exposure of organisms to sediment-bound organic chemicals in a sediment toxicity test. Subsequently, we provide recommendations for exposure assessment in such a test (overview is given in Table 1). This has substantial links and overlap with recommendations for bioaccumulation testing, on which a review was recently published.[37]

TABLE 1. General recommendations for standard prospective sediment toxicity testing under laboratory conditions

Recommended principles for prospective sediment testing under laboratory conditions
• Test single chemicals.
• Use artificial sediment and artificial test water, matching habitat of test organism (salinity). Consider including a black carbon surrogate.
• Mix sediment, food and spiked test compound in suspension in test water.
• Pre-equilibrate 3–4 times the adsorption half-life.
• Allow two weeks for settling and incubation prior to exposure.
• Keep biomass <5% of mass of sediment organic matter plus food.
• Use static exposure with high water-to-solids ratio and minimum periodic water renewal.
• Monitor exposure at start and end of test using passive sampling and/or mild extractions.
• Monitor oxygen, pH, ammonium, sulfide (redox electrodes).

Exposure in a sediment accumulation or toxicity test is best understood using a mass balance approach where the time-course of the concentration in the organism is the net result of chemical uptake and depuration fluxes between the organism and its environment.[38–42] Uptake may take place through fluxes from pore water, overlying water, and particle ingestion.[40,43] Transport to water takes place through desorption from the bulk sediment. If uptake through particle ingestion takes place, particle or diet composition is important. Depuration may include passive elimination, defecation, transformation and exudation. Organism concentration may also be reduced by growth dilution. Uptake is a complex time-dependent process, as the relative importance of the individual processes differs among chemicals and organisms, and vary with environmental and life-stage changes over time.[40]

It is impossible to obtain accurate dose-response relationships in the kinetic phase of uptake, or if exposure varies due to nonequilibrium between sediment and water. Test results may also be obscured by mixture toxicity or other stress responses during exposure. Consequently, prospective sediment toxicity tests should be designed to (a) sufficiently approach steady state in exposure, (b) be in a state of sediment-water sorption (pseudo-) equilibrium, and (c) avoid mixture toxicity, unless testing a mixture is required for other reasons. Finally, (d), actual exposure should be monitored throughout the test. Subsequently, we describe how this can be achieved at the bench.

(a) Steady state can be achieved using prolonged exposure times, which is also the concept of chronic testing. Existing guidelines for invertebrates usually prescribe exposure periods of 28 days, which should suffice to achieve >80% of steady state for hydrophobic organic chemicals (EPA/OECD).[44–47] Ionized chemicals can be assumed to reach steady state earlier, because their adsorption to the sediment surface is generally faster than retarded intraparticle diffusion driving hydrophobic organic chemicals sorption kinetics. Although some scattered information

is available on the uptake kinetics of aquatic macrophytes in water-only test systems without bed-sediment,[48] guidelines are not yet available for this functional group.

(b) The requirement of sorption equilibrium relates to the bioavailable fraction only, that is, the pore water concentration and/or the concentration of fast desorbing compounds from sediment.[49–51] These concentrations will remain more or less constant during a 28-day test, once the first (fast) stage of adsorption of the (spiked) test compound has passed and turned into a much slower stage of further adsorption. This second stage should be so slow that its effect on exposure is expected not to occur during the 28 days of the actual test, or at least to stay below a predefined difference between the start and end of exposure. In practice, this can be achieved by pre-equilibrating the sediment for at least three to four times the adsorption half-life.[52] Based on known kinetic data for hydrophobic organic chemicals, a pre-equilibration time of up to 28 days in suspension is recommended, followed by two weeks of incubation in bed sediment.[31,53] However, this time may need to be shorter for rapidly degradable compounds. Furthermore, the biomass should not exhaust the concentration of rapidly desorbing compounds from the sediment in the test.[50] This can be roughly achieved by keeping the total lipid mass below 5% of the amorphous sediment organic matter. Pre-equilibration in suspension also causes the pore water and overlying water to have identical electrolyte compositions at the start of the test.

(c) The problem of multiple causation of effect (i.e., mixture toxicity) should be avoided by using a standardized water composition and standardized sediments, spiked with the (single) chemical of interest. Toxic macroconstituents (ammonium, hydrogen sulfide) should be avoided. Natural sediments would be less suitable because effects of unknown background chemicals or differences due to food quality should be ruled out first.[54,55] This is why current protocols generally recommend artificial, formulated sediments for testing.[45,56] Guidelines for the preparation of freshwater sediment and the provision of food throughout the test have been provided by the OECD,[57–59] however, similar guidelines for artificial marine sediments are not yet available. The OECD suggests that food can best be mixed in with the sediment and co-equilibrated with the test chemical prior to exposure,[57–59] an approach also applied in recent method development studies.[53] The OECD guidelines, however, do not yet recommend including condensed carbon[60,61] in the standardized sediment, although such condensed carbon (e.g., black carbon [BC]) has been shown to be a sediment component with crucial effects on the bioavailability of organic compounds.[40,60–63] Two types of effects of BC have been suggested: a reduction of exposure due to strong sorption of BC[61] and a reduction of exposure due to a lower absorption efficiency of chemicals bound to ingested BC particles.[40,64] The question whether

sediment toxicity tests should include a standardized, nontoxic BC phase still needs to be addressed. Improvements with respect to other carbon phases also need to be considered. The *Sphagnum* moss particles generally recommended might not adequately represent the organic matter found in field sediment, whereas dissolved organic carbon is often poorly taken into account. In general, the quality of sediment toxicity testing would be improved if a sediment standard would be developed that best represents natural sediment. This could be either an artificial sediment prepared in the laboratory from standardized components, or a noncontaminated natural sediment, which is made available to all users as a certified reference material. Different sediments may be developed to represent different habitats, like high or low organic content or freshwater versus marine sediment.

(d) There are three categories of methods to assess the exposure of hydrophobic organic chemicals. The first method is to estimate exposure from chemical concentration in the bulk sediment and to calculate the available fraction based on sediment parameters, like organic carbon content. This approach uses equilibrium partitioning theory (EPT) and is considered inaccurate due to the fact that state of equilibrium and magnitude of the equilibrium partition coefficient are unknown or uncertain.[61] The second category measures the freely dissolved concentration in the pore water or overlying water using direct solvent extraction, or passive samplers in the case of very low aqueous concentrations.[53,60,63,65,66] Frequently used samplers are POM-SPE (PolyOxyMethylene Solid Phase Extraction)[63] and SPME (Solid Phase Micro Extraction).[65] The samplers are often equilibrated with the water phase in a suspension of the sediment.[60,63] In the framework of a toxicity test with bed sediment, this would mean that exposure conditions could be substantially altered. Alternatively, samplers can be inserted into the sediment.[53,66] This may require equilibration times of days to weeks. Consequently, the use of sediment-inserted passive samplers in 28-day sediment tests is not straightforward. The third category uses mild sediment extraction to measure the concentration in the sediment that is available for uptake, the so-called fast desorbing concentration.[38,50,51] These mild extractions with XAD, Tenax, or cyclodextrin are also used in a suspension of the sediment. Fast desorbing concentrations, however, are not assumed to change when the sediment is taken into suspension. Consequently, exposure may best be assessed by a stirred passive sampler in the overlying water layer, close to the sediment water interface, and by passive samplers inserted into the sediment, which are analyzed at regular time intervals. This may be complemented by mild extractions of sediment sampled at 0 and 28 days. To accurately determine fast desorbing concentrations, these mild extractions should be based on at least four time points.

2.4 Benthic Invertebrates

This section provides an overview of current approaches for benthic invertebrate tests. It discusses which species are used most often, the selection of a set of recommended species, and recommendations for preferred endpoints, origin and density of test animals, feeding during the test, and test apparatus.

Benthic invertebrate species are often highly abundant in ecosystems and differ in morphological, physiological, behavioral, and ecological characteristics (i.e., traits). These traits influence the uptake potential, metabolic capacity, exposure routes, and bioaccumulation, and thus the sensitivity of invertebrate species to contaminants.[67] Moreover, benthic invertebrates provide important ecosystem functions,[68,69] which underlines the importance of protecting the biodiversity and functionality of benthic communities. As the sensitivity of species is determined by the biological and ecological traits of taxa, a test battery should be developed that takes into account the trait range within a community.[30]

Many retrospective tests are available in which contaminated field sediments are tested with single species in the laboratory or in situ, and a large variety of prospective tests have also been described. Prospective tests are generally conducted with freshwater or marine species, leaving true estuarine species underrepresented.[17] Tests mainly focus on single species and short-term effects, with exposures of 4–10 days,[19] which seems insufficient to detect effects at the population level[70,71] (and references therein), and to reach a steady state in exposure. Tests regarding long-term effects, full life-cycles, multiple generations or their implications at population level are less well developed.[70] Full life-cycle and multigeneration tests are more useful for risk assessment and setting quality standards for sediment-dwelling organisms, since they include all sensitive life stages of an organism. However, these tests are time-consuming and expensive.[33,71] Various short- and long-term standard methods have been validated using ring tests, and are internationally accepted (Table 2). Standard methods may vary in terms of test conditions, such as water renewal versus static condition, exposure time, amount of food, and the use of sediment and endpoints (Table S2).

A survey of currently available test species for freshwater, estuarine and marine sediments is presented as supplemental information (Table S2). Based on the available information, we have selected species by following the guidelines presented in the above section on general guidelines from a regulatory perspective. Criteria were (a) presence in freshwater, estuarine, and/or marine environment; (b) diversity of feeding modes; (c) direct contact with sediment; (d) global distribution; and (e) availability of standard methods. The selection (Table 3) is intended as a proposed test battery to compare and read across sensitivities of freshwater, estuarine and marine species for chemicals in prospective testing in European countries. Chronic test protocols are available for most of the selected test species, either as

TABLE 2. Overview of test species used in internationally accepted sediment toxicity tests with benthic invertebrates (ASTM, EPA, ISO and OECD)

Taxonomic group	Guideline	Species	F/E/M[a]	Test type	Endpoints[b]	Reference
Insecta (ephemeroptera)	ASTM E1706	*Hexagenia spp.*	F	Short term (10 days)	S, G	[192]
Insects (diptera)	OECD 218	*Chironomus riparius*	F	Long term (28 days)	E	[57]
		Chironomus dilutus	F	Long term (65 days)	E	[57]
		Chironomus yoshimatsui	F	Long term (28 days)	E	[57]
	OECD233	*Chironomus riparius*	F	Life-cycle (44 days)	E, TE, SR, No, E.R., F	[59]
		Chironomus dilutus	F	Life-cycle (100 days)	E, TE, SR, No, E.R., F	[59]
		Chironomus yoshimatsui	F	Life-cycle (44 days)	E, TE, SR, No, E.R., F	[59]
	EPA2000	*Chironomus dilutus*	F	Short term (10 days)	S, G	[45]
		Chironomus dilutus	F	Life-cycle (50–56 days)	S, W, E, No, E., HS	[45]
	ASTM E1706	*Chironomus dilutus*	F	Short term (10 days)	S, G	[192]
		Chironomus riparius	F	Short term (10 days)	S, G	[192]
		Chironomus dilutus	F	Life-cycle	S, G, R, E	[192]
Annelida (oligochaeta)	OECD225	*Lumbriculus variegatus*	F	Long term (28 days)	S, R, W	[58]
	EPA2000	*Lumbriculus variegatus*	F	Bioaccumulation (28 days)	B	[45]
	ASTM E1688	*Lumbriculus variegatus*	F	Bioaccumulation (28 days)	B	[47]
	ASTM E1706	*Tubifex tubifex*	F	Short term (10 days)	S, G	[192]
Nematoda (chromadoria)	ISO 10872:2010	*Caenorhabditis elegans*	F	Short term (4 days)	F, G, R	[193]
Annelida (polychaeta)	ASTME1611	*Neanthes arenaceodentata*	E/M	Short term (10 days)	S	[194]
			E/M	Long term (20–28 days)	S	[194]
			E/M	Short term (10 days)	S	[194]
Crustacean (amphipoda)	ASTM E1706	*Diporeia spp.*	F	Short term (10 days)	S, G	[192]
	EPA2000	*Hyalella azteca*	F/E	Short term (10 days)	S, G	[45]
		Hyalella azteca	F/E	Long term (42 days)	S, G, R, RFM	[45]
	ASTM E1706	*Hyalella azteca*	F/E	Short term (10 days)	S, G	[192]
		Hyalella azteca	F/E	Long term (42 days)	S, G, R	[192]
	EPA1996	*Eohaustorius estuarius*	E	Short term (10–28 days)	S, Reb	[195]
	ASTM E1367	*Eohaustorius estuarius*	E	Short term (10 days)	S, Reb	[196]
	EPA1996	*Leptocheirus plumulosus*	E	Short term (10–28 days)	S, Reb	[195]
	EPA2001	*Leptocheirus plumulosus*	E	Long term (28 days)	S, G, R	[195]
	ASTM E1367	*Leptocheirus plumulosus*	E	Short term (10 days)	S	[196]
		Leptocheirus plumulosus	E	Long term (28 days)	S, G, R	[196]
	EPA1996	*Ampelisca abdita*	M	Short term (10–28 days)	S	[195]
	ASTM E1367	*Ampelisca abdita*	M	Short term (10 days)	S	[196]
	EPA1996	*Rhepoxynius abronius*	M	Short term (10–28 days)	S, Reb	[195]
	ASTM *E1367*	*Rhepoxynius abronius*	M	Short term (10 days)	S, Reb	[196]
	ISO 16712	*Corophium volutator*	E/M	Short term (10 days)	S	[197]

Note. F = freshwater; E = estuarine; M = marine; B = bioaccumulation; E = emergence; F = fertility; G = growth; HS = hatching success; No E = number of eggs; No ER = number of egg ropes; R = reproduction; Reb = reburial; RFM = female/male ratio; S = survival; SR = sex ratio; TE = time to emergence; W = weight or biomass.

TABLE 3. Selection of benthic invertebrate species and endpoints in freshwater, estuarine, and marine habitats to compare sensitivity of species along a salinity gradient

Fresh	*Chironomus riparius* (insect)	Emergence[57]
	Hyalella azteca (crustacean)	Reproduction[45,192]
	Lumbriculus variegatus (annelid)	Reproduction,[58,199] growth,[3,58] bioaccumulation[44,47]
	Pisidium sp. (mollusk)	Reburial (to be developed based on[199,200]) bioaccumulation and feeding rate (to be developed)
	Corophium volutator (crustacean)	Reproduction[81]
Estuarine	*Arenicola marina* (annelid)	Growth and/or bioaccumulation (to be developed based on[58])
	Macoma balthica (mollusk)	Reburial (to be developed based on[199,200]) bioaccumulation and feeding rate (to be developed)
Marine	*Corophium volutator* (crustacean)	Reproduction[81]
	Arenicola marina (annelid)	Growth and/or bioaccumulation (to be developed based on[58])
	Macoma balthica (mollusk)	Reburial (to be developed based on[199,200]) bioaccumulation and feeding rate (to be developed)
	Echinocardium cordatum (Echinoderm)	Burrowing activity and/or bioaccumulation[85,201]

Note. This selection focuses mainly on temperate species. A similar selection can be made for other regions (e.g., *Chironomus yoshimatsui* for Asia). The endpoints mentioned are additional to survival.

standard protocol (Table 2) or in the scientific literature (see Table S2). The selection includes where possible (internationally) standardized tests and involves representatives of three taxonomic groups of freshwater and estuarine/marine test species with similar feeding modes, behavior, and exposure pathways to enable a read across of results and sensitivity to chemicals from freshwater to estuarine and marine environments. This results in a read across for crustaceans (*Hyalella azteca* [fresh] – *Corophium* sp [estuarine/marine]), annelids (*Lumbriculus variegatus* [fresh] – *Arenicola marina* [estuarine/marine]), and a preliminary suggestion for bivalves (*Pisidium* sp [fresh] – *Macoma balthica* [estuarine/marine]). The selected estuarine and marine species possess a high salinity tolerance, which implies they can be used for estuarine and marine prospective testing systems by adaptation of the salinity in the tests. Additionally for freshwater, a representative species of the taxonomic groups of insecta (*Chironomus riparius*) was selected to be able to assess the sensitivity of a predominantly freshwater taxonomic group. This was also done for an exclusively marine species with the taxonomic group of echinoderms (*Echinocardium cordatum*). By selecting both similar and specific species for a certain environment covering different taxonomic groups, we feel that a sufficient assessment of the sensitivity of benthic invertebrates to chemicals in fresh, estuarine and marine environments can be made. Concerning the group of bivalves, standardized tests have been developed focused on the embryonic development of bivalves, bioaccumulation

and for field situations using caged bivalves. However, acute and chronic standard protocols for laboratory toxicity tests are still lacking for freshwater, estuarine and marine bivalves.[72] A suitable bivalve species for estuarine and marine environments appears to be *Macoma balthica*, in view of its wide salinity tolerance, extensive distribution in the northern hemisphere and easy use in handling for instance in sediment bioaccumulation testing.[73] A suitable freshwater species may be *Pisidium* sp., based on its comparable place in the sediment, its distribution and feeding mode. Sediment-dwelling nematodes are currently not selected as test species, however, they do show a high potential. Nematodes are widely spread in the environment. They are easy to culture, have a short generation time[74] and may tolerate a high salinity range.[75] However, single-species experiments with spiked sediments have been scarce. *Caenorhabditis elegans*, which is well known for its use in soil toxicity tests, has also been used for sediment toxicity testing.[76,77] If additionally standardized estuarine and marine tests with nematodes can be developed, this group may complement the currently selected benthic invertebrates for freshwater, estuarine and marine environments (*L. variegatus* and *A. marina*).

Most single-species tests focus on alterations within organisms (e.g., biomarkers), their physiology, life history variables, behavior, and mortality.[18] Current chronic tests focus on survival, growth,[78–80] reproduction,[45,78,80,81] behavior,[82,83] and, for *Chironomus* species, emergence and male: female ratio.[33,56,59,71] Ideally, endpoints for prospective testing focus on parameters that allow extrapolation from single species to populations and communities, such as reproduction, taking into account a full life-cycle of a species. However, such a full life-cycle often takes too long to complete to be used as a cost-effective test. Therefore, if coverage of a full life-cycle is not feasible, other sublethal parameters are recommended, such as emergence, changes in burrowing behavior and growth, the latter providing more time integrated information on the conditions of an organism during exposure. Even though bioaccumulation does not give information on an effect level at the organism level, it does provide information on the bioaccumulation potential of a chemical. Suitable endpoints for the test species selected in prospective testing are given in Table 3.

Test organisms are most commonly collected from clean local sites. Certain species (e.g., *Chironomus riparius*, *Hyallella azteca*, *Corophium volutator*,[84] and *Echinocardium. cordatum*[85]) can be cultured in the laboratory. However, there may be differences in the sensitivity of cultured and field organisms. For instance, Schipper et al.[85] found that field urchins showed higher sensitivity than cultured urchins. Cultured organisms are more favorable for prospective testing, as the origin of the test species is known and their quality is more standardized as long as proper protocols are applied to prevent inbreeding. If no cultures exist, organisms should be collected from clean field sites. In all cases, the chemical to be tested should be analyzed,

prior to testing, to establish the background concentration of the specific chemical in the test organism. Another laboratory-field issue is the animal density since toxic effects can be density dependent. Laboratory tests may overestimate effects in natural environments since they use low densities, while field populations often have high densities. This could have important consequences for risk assessment.[86] Hence, it is important to use optimum densities—depending on the organisms—for lab conditions. For practical reasons, these do not necessarily equal field conditions. An actual comparison with the field situation is more suitable for mesocosm studies and/or field experiments.

The ingestion of contaminated sediment may be an important exposure pathway especially for highly sorptive substances.[40,43,61] Long-term tests without food are possible for some species, but only with sediments having a high organic carbon content.[87] Usually, food is added either as fresh food or mixed with the sediment at least 48 h prior to spiking.[56,88] Adding food however also adds organic carbon to the system affecting bioavailability of the chemical and hence the uptake of chemicals through sediment ingestion.[88] On the other hand, fresh food addition is more ecologically relevant for certain species but might exclude the exposure route through the sediment (due to food avoidance or preference). Food source and feeding regime may influence organic carbon, ammonium concentrations and physicochemical parameters.[31,87,88] In static systems, water quality could decrease to unacceptable levels in the course of the test, while maintaining constant exposure conditions is also difficult with a semistatic system (recommended by OECD and EPA).[31,87] As an alternative, Borgmann and Norwood[89] recommended a static test with larger water-to-sediment ratio (67:1, as compared with the normal 4:1 ratio). For practical reasons, static systems are recommended for prospective testing. However, the water-to-sediment ratio used should be as high as possible to keep the water quality at an acceptable level and reduce the need to change the water on a regular basis. Additionally, ammonium (especially unionized ammonia) needs to be measured regularly during the test to avoid toxic effects. These recommendations are summarized in Table 4.

2.5 Aquatic Macrophytes

This section reviews the literature on testing with macrophytes and discusses current types of tests, species used, choice of medium and sediment, chemical spiking method and endpoints.

Aquatic macrophytes fulfill several critical structural and functional roles in aquatic ecosystems.[90] They are at the base of the aquatic food web, and may accumulate and translocate chemicals and enhance or decrease their bioavailability.[22,52,91] Consequently, these organisms and the ecosystem services that they provide must be protected at both local and global scale.[92] The

TABLE 4. General recommendations for standard prospective sediment toxicity testing with benthic invertebrates under laboratory conditions

Recommended principles for prospective sediment testing of benthic invertebrates under laboratory conditions
• Focus on full life-cycle tests and multigeneration tests or tests that cover the most sensitive life stage.
• Select species based on traits (e.g., ingesters, facultative suspension feeders).
• Source of test species: preferably cultured, if not possible from field.
• If food is needed for the test, mix it into the sediment for a period of 48 hr prior to spiking.
• Mix organic carbon into the sediment simultaneously with food to a standardized percentage, prior to spiking the sediment.
• Test with sufficient densities for laboratory conditions.
• Use a static system with water-to-sediment ratio as high as possible.
• Monitor water quality.

availability of standardized methodologies to assess the environmental risks of organic chemicals to nontarget freshwater plants is currently limited. Test guidelines are only available as water-only tests for algae and *Lemna* (duckweed; e.g., guidelines from ASTM, EPA, OECD), while the existing ASTM *Myriophyllum* protocol without sediment was never officially accepted. A new *Myriophyllum*-sediment protocol has recently been ring-tested.[8] In risk assessment, submerged rooted macrophytes are not addressed in any standard procedure. Sediment-testing guidelines for sediment-rooted macrophytes have not been standardized (Table S3). Limited literature is available on sediment toxicity testing of rooted freshwater macrophytes[8,93] and rooted estuarine and marine macrophytes.[22,91,94] As rooted aquatic macrophytes are mostly tested over a period of 14–28 days (see Table S3), these tests are considered long-term. Macrophytes are usually tested as vegetative shoots in their growth phase, while tests covering a full life-cycle and seed emergence tests have not been reported for aquatic macrophytes within the context of environmental risks of toxicants.

The standard freshwater test species, *Lemna*, is a free-floating, non–sediment-rooted macrophyte and therefore is not representative of sediment-rooted emergent and submerged macrophyte species, especially when chemicals partition to the sediment.[8,95,96] Where sediment exposure is a concern, Maltby et al.[8] proposed to test a sediment-rooted macrophyte species. This approach takes into account the different pathways by which rooted macrophytes take up chemicals, viz. by roots and shoots.[97,98] The considerable current knowledge about and experience gained with *Myriophyllum* sp.[99,100] and its physiological properties as a sediment-rooted and dicot species were reasons to recommend it as an additional test species.[8,101] *Elodea* sp. and *Glyceria maxima* are used for toxicity testing especially when monocot species are required.[102,103] For the estuarine and marine environment, coastal wetland species (emergent species including mangrove

TABLE 5. Suggested selection of macrophyte species and endpoints in freshwater, estuarine and marine habitats to compare sensitivity of species along a salinity gradient. This selection focuses mainly on temperate species. A similar selection could be made for other regions (e.g., *Zostera capricorni* or *Thalassia testudinum* as a tropical marine submerged species). The endpoints mentioned are additional to biomass based on growth

Fresh	*Myriophyllum spicatum*.[a]	Shoot length, shoot weight (updated protocol from[8]), total fresh weight[93]
	Elodea sp.	Total length main shoot, weight[103]
	Glyceria maxima	Shoot length, shoot weight, shoot number[202]
	Scirpus sp.	Growth, peroxidase activity, peroxidation products, chlorophyll,[203] length, germination
Estuarine	*Vallisneria* (sp. or americana)	Leaf to root ratio[115,122]
	Ruppia (sp. or maritima)	Rel. growth rate, oxygen production[94]
	Stuckenia pectinatus (previously *Potamogeton pectinatus)*	Weight, rhizome tips,[204] length
	Scirpus sp.	Growth, peroxidase activity, peroxidation products, chlorophyll,[203] length, germination
	Ruppia (sp. or maritima)	Relative growth rate, oxygen production[94]
Marine	*Zostera* (sp. or marina)	Photosynthesis,[205,206] chlorophyll, pigments[105]

[a]Tests are under development as standard test for the OECD.

species) or submerged macrophytes (mainly seagrass species) have been recommended[22,91,104,105] (Table S3). The estuarine species cover a broad salinity range, from low to high values. Table 5 gives an overview of recommended test species, suitable to be used in a test battery in the laboratory. No standardized methods are available for any of the rooted macrophytes, as these are only available for the floating macrophyte *Lemna* sp. Instead, the literature was screened for available but not standardized test protocols. Selected macrophyte test species are widely distributed in the northern hemisphere. Moreover, they are representative of different sediment-rooted growth forms (submerged and emergent), are specific for different habitats (freshwater and marine) and allow comparison between freshwater, estuarine and marine habitats to determine whether sensitivity to tested chemical may differ between these habitats and vice versa. An important question is to what extent such a read-across is feasible.

For prospective risk assessment, protocols are available for testing rooted freshwater macrophyte species[99] but these tests include the water medium only. An adapted test approach based on this protocol[99] has recently been ring-tested for *Myriophyllum spicatum*.[106] As such tests might suffer from microbial and algal development, they are mostly performed as axenic tests (which is further discussed subsequently). In order to sustain macrophyte growth, the test medium in these tests includes sucrosis.[106–108]

Test protocols including sediments and water medium are under development.[8] The test protocols proposed by Maltby et al.[8] are currently being ring-tested for the sediment-rooted macrophytes *Myriophyllum aquaticum* and *M. spicatum*. Protocols for estuarine and marine sediment tests have neither been standardized nor involved in a ring-testing procedure.[105] Consequently, experimental techniques are varying considerably.[22] Sediment toxicity tests with estuarine and marine macrophyte species have rarely been conducted.[94]

In retrospective risk assessments, standard protocols are available.[93,109,110] They include contaminated sediments, but lack the overlying water layer.[93,109–112] These methods are not directly applicable to sediment toxicity testing where a water layer is included in the test setup.

The advantage of sediment tests is that nutrients can be mixed through the sediment, thereby limiting nutrient-availability in the water layer and therefore limiting algae growth. Nonaxenic tests do include microorganisms. If this is not desired, axenic, artificial sediments may be used to overcome this problem. However, axenic tests are time-consuming. Therefore, in general, the addition of sediment obviates the need for axenic cultures[113] and offers many other advantages, such as increased macrophyte growth,[103,114], decreased endpoint coefficients of variation, and increased ecological realism. Artificial[8,115–117] as well as natural sediments[115,118,119] have been used in macrophyte toxicity tests. From Table S3 it can be concluded that the available information is scattered and applied test protocols are very different in all kind of aspects including growth media, test duration, macrophyte species, assessed endpoints, and chemicals considered. Only the artificial sediments, if applied, were similar in their composition. Sediment spiking is not common practice in macrophyte toxicity tests that include sediment and an overlying water layer. It has been applied by Burešová et al.[98] (herbicide) and is currently part of the *Myriophyllum* sediment ring-test.[8]

A wide range of endpoints is used, and these do differ considerably between tests (Table 5). A combination of morphological and physiological endpoints represents macrophyte fitness better than biomass and growth only.[120] Although macrophyte length and biomass endpoints are characterized by low coefficients of variation,[103,114] macrophyte main shoot length is not a sensitive indicator in all cases, but should be replaced by total shoot length. Total shoot length also takes into account the length of the newly formed side shoots. Root endpoints (e.g., root length) on the other hand are sensitive endpoints both in water-only tests and in sediment tests, although they show high intrinsic variability.[112,120,121] The leaf-to-root surface area has been suggested as a sensitive and robust endpoint in macrophyte tests with sediment and water medium.[122] In general, growth based on biomass can be used as an indication of effects on macrophytes, which can easily be linked to the population level, where a decreased biomass might

TABLE 6. General recommendations for standard prospective sediment toxicity testing with sediment-rooted macrophytes under laboratory conditions

Recommended principles for prospective sediment testing of sediment-rooted macrophytes under laboratory conditions
• Use artificial sediment.
• Add nutrients to the sediment to avoid algae growth in the water.
• Add growth medium to the water layer to support maximum photosynthesis.
• Optimize light conditions for the different test species.
• Choose experimental conditions to support exponential/steady growth in the controls.
• Use field or culture stock populations, which can easily be grown from vegetative cuttings and acclimatized, in the laboratory.
• Use macrophyte endpoints that combine toxicological sensitivity, low coefficients of variation, and ecological relevance.
• Take account of hormesis in the evaluation of effects.
• Mimic natural conditions as closely as possible for marine and estuarine species.

directly influence the survival potential of a macrophyte population. Appropriate endpoints combine toxicological sensitivity with low coefficients of variation and ecological relevance.[120] For sediment tests, these include belowground and aboveground macrophyte endpoints. It should be noticed that hormesis could stimulate growth in the lower concentration range and should, therefore, be taken into account in the calculation of effect concentrations.[123] An overview is given of the above-mentioned recommendations in Table 6.

Macrophytes can take up organic compounds by roots and shoots.[48] Uptake and elimination studies and sorption models with aquatic macrophytes, and *Myriophyllum* in particular, often disregard the sediment compartment.[97,124–127] However, sediment is an integral part of experiments and models, which describe accumulation of sediment-bound chemicals in aquatic food webs.[50,52,128,129]

2.6 Microorganisms

This section presents an overview of current approaches to microorganism tests, including endpoints and methods for single-species tests and a wide variety of molecular methods that can be used at the community level.

Sediment microbial communities, including benthic bacteria, archaea, algae, fungi, and protozoans, perform crucial ecosystem functions like nutrient cycling, primary production and decomposition[130] and form an important food source for many sediment-dwelling organisms.[131] Interactions between different microorganisms and with higher organisms range from mutually beneficial symbiosis to purely antagonistic (pathogenic) relationships, all of which contribute to shaping the ecosystem functioning at different trophic levels. Hence, microbial communities constitute a relevant endpoint

in sediment quality assessment. Depending on the regulatory framework, the specific protection goal for microorganisms may concern the population, functional group or community level.[9]. The majority of bacteria grows in biofilms on surfaces of submerged substrata or sediments, rather than in suspension, although it should be noted that suspended microorganisms are especially important in degrading highly soluble chemicals.[132] Biofilms are complex communities that besides bacteria, comprise algae, protozoa and fungi embedded in a matrix of extracellular polymeric substances,[133] and are consumed by deposit-feeding invertebrates.[134] Various compounds are effectively adsorbed into the matrix, resulting in increased or decreased bioavailability. However, their role in the bioaccumulation of organic contaminants has been poorly investigated,[135] and most tests focus on suspended microbial cultures. It should be noted that considerable work has been done on the evaluation of biocides on biofilms, however, focusing largely on systems relevant to the prevention of growth of microbial pathogens such as those found associated with medically relevant environments as well as drinking water distribution systems.[136,137] Furthermore, biofilms have been studied with respect to their role in the degradation of environmentally adverse pollutants.[138,139]

The uptake of chemicals from the sediment by microorganisms is more direct than that by higher organisms. Uptake is diffusion-driven and fast due to the much higher surface-to-volume ratio of microbial cells, implying that freely dissolved pore water concentrations are the most relevant dose metric for microbial testing. Some bacteria (e.g., *Bacillus cereus*) have a hydrophobic surface, which further facilitates the direct uptake of chemicals and may enhance bioavailability.[16]

Various microorganisms have the capability to accumulate, detoxify or metabolize chemicals[21,131,140,141] and are therefore used for bioremediation in polluted soils and sediments. Hence, many studies have focused on microbial degradation of contaminants rather than on impact on the composition and functioning of natural microbial communities. Toxicity data in the open literature on organic contaminants involving microorganisms, however, are limited[21,142] although it should be noted that freshwater protozoans such as *Tetrahymena pyriformis* have been extensively used in toxicity testing.[143] Only a few studies have addressed the effects of chemicals on structural and/or functional responses of microbes[21,144,145]. The wide variety of size classes, morphology, reproductive strategies, growth rates, and metabolism results in a wide range of sensitivities of microorganisms to chemicals.[21] Nevertheless, if the metabolism of a bacterial cell is disturbed, this may also indicate potential toxicity to other organisms.[146] Effects on microorganisms, both negative and positive, may have direct and indirect impacts at higher trophic levels and therefore may change ecosystem functions.[21,135]

Many different methods are available to test effects of sediment-bound chemicals on microorganisms.[147] However, although some are commercially available, none of them have so far been ring-tested and described as standard tests. Ecologically relevant community assessments have been used in RRA, where characteristics of contaminated field sediment have been correlated to microbial activity.[148] In PRA, mixed communities can be much more easily exposed to spiked, artificial, sediments than single species.[7] To improve the microbial component of artificial sediments, it has been suggested[135] that a microbial extract from natural sediment could be added in the sediment preparation procedure. However, it is also possible to introduce pure cultures of microbes into spiked field or artificial sediment. Such tests are relatively cheap and easy to perform, use species that can be easily cultivated, and are useful for rapid screening. As they represent principal functions, they relate to an integral part of the ecosystem and are more sensitive than animal and plant tests for a number of compounds.[7,16,135,149] However, very few studies have investigated the microbial communities of artificial sediments and compared these with natural sediments. Hence, further knowledge is needed to assess how microbes govern the fate of test compounds in standardized tests and ultimately affect toxicity test results.[135]

The available microbial tests can be divided into single-species tests; community-level assessments based on functionality, biomass or processes[7] and molecular methods (Figure 4). Tests depending on single-species microbial culture fall into the following categories: population growth, substrate

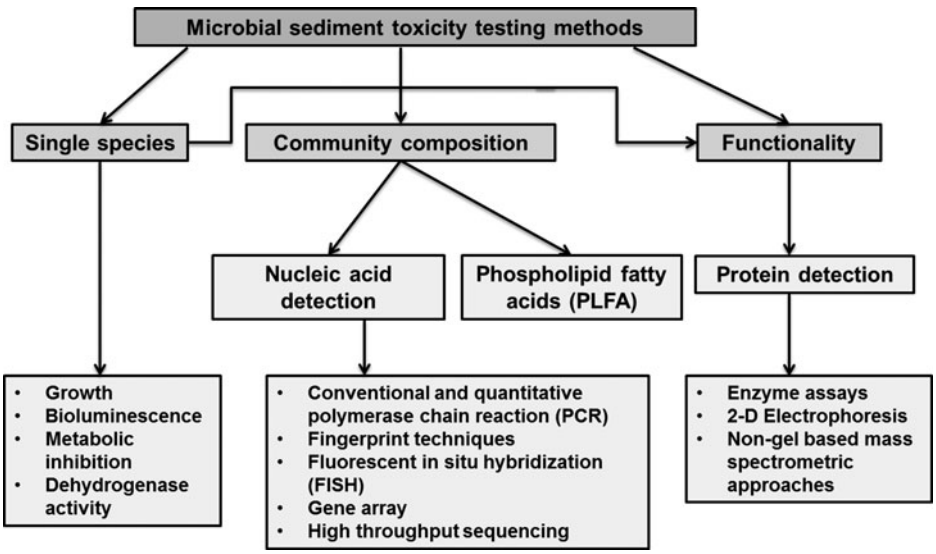

FIGURE 4. Overview of methods for prospective sediment toxicity testing with microorganisms.

consumption, respiration, and adenosine triphosphate (ATP) luminescence and bioluminescence inhibition assays. Species used for bioluminescence inhibition assays include *Vibrio fischeri* (formerly *Photobacterium phosphoreum*), *Vibrio harveyi*, and *Pseudomonas fluorescens*. Although bioluminescence inhibition assays were originally applied to aqueous or extracted samples, a modified solid phase assay has been developed for the analysis of soil and sediment toxicity.[150] Metabolic inhibition tests use the species *Escherichia coli* and *Pseudomonas putida*.[149] Test duration usually varies from 24 to 96 hr.[21] The solid phase bioluminescence inhibition test with *Vibrio fischeri* (marine gram-negative bacterium) is one of the most commonly used single-species tests.[7] It is an acute toxicity test with a sublethal endpoint. Several commercial test kits (i.e., Microtox, LUMIStox, and ToxAlert) are based on this strain.[151] This is the most sensitive microbial test available, is cost-effective, easy to operate[152–154] and takes 5–30 min. Other single-species tests are associated with higher costs (ATP luminescence) or low investment cost but high operational costs (nitrification inhibition assay).[153] An interlaboratory precision study of the solid phase Microtox test showed that the method has acceptable precision and can be developed as a standard method.[155,156] Despite its easy operation, however, there are several pitfalls in interpreting the test results. Direct sediment contact increases the exposure to potential toxicants. Moreover, sediment composition can affect the test response since bacteria can bind to sediment particles, which results in a reduction of the intensity of luminescence and/or a loss of bacteria by sediment extraction for the test suspension.[157–159] For example, a high proportion of silt or clay in the sediment samples is found to reduce the EC_{50} values, thereby indicating higher toxicity than expected. Moreover, it remains difficult to distinguish between inherent chemical sensitivity and mediating sediment factors. This issue could be circumvented by the use of sediment correction. Bioluminescence tests require normalization to account for the adsorption of the bacteria to the sediment particles.[158,160] Additionally, sediment properties such as pH, sulfide content, redox potential, and oxygen saturation play an important role and may interfere with toxic effects. Consequently, it has been recommended to match organisms with appropriate sediment as well as associated physicochemical conditions.[7]

Communities can be used to assess growth inhibition[149] and loss of functionality or processes, the latter of which can be measured either by activity tests or by means of biomolecular proxies (see subsequent discussion). However, measuring functionality alone may cause shifts in microbial composition to be overlooked because tolerant microbes could compensate for the loss of functions of the more sensitive groups (i.e., functional redundancy).[131]

The development of culture-independent molecular methods to analyze microbial communities provides new opportunities to detect pollutant-induced changes in the composition of natural communities.[161,162] To this

end, it is important to realize that, to date, the overwhelming majority of microorganisms cannot be cultured as pure culture isolates by routine methodology in the laboratory, but rather can only be maintained in the context of more or less complex defined or natural microbial communities.[163,164] Molecular methods target a range of cellular biomarkers that provide information with respect to microbial identity and function, and have been developed especially to allow for the analysis of complex mixed microbial communities. Biomarkers that are frequently used include proteins, phospholipid fatty acids (PLFA),[165] and nucleic acids. Whereas proteins can be assessed using enzyme activity assays, as well as proteomics methods such as 2-D gel electrophoresis and non–gel-based mass spectrometric techniques, nucleic acids are the biomarkers of choice in most applications. Microbial identity and community composition are routinely determined by targeting ribosomal RNA (rRNA) or its encoding gene, using fluorescent in situ hybrization (FISH), DNA oligonucleotide microarrays, conventional and quantitative polymerase chain reaction (PCR), and a number of different fingerprint techniques.[166,167]. Information about metabolic potential as well as activity can be obtained by analyzing functional genes, their transcripts, and/or corresponding proteins, largely using the previously mentioned approaches.[164,168]

The three categories of tests show that endpoints for microorganisms are primarily in terms of functions (e.g., nitrogen fixation), processes (e.g., luminescence), or quantitative data (e.g., rRNA; Figure 4 and Table S1). However, most reported EC_{50} values relate to the endpoint of growth rate (e.g., cell counts or optical density).[21] A combination of endpoints relating to functioning (enzyme activity, functional genomics) and microbial composition (rRNA) will offer a more complete overview of the toxicity effects. Single-species tests can be used for rapid screening, whereas higher-tier testing should focus on the level of functions, processes and communities. Hence, a test battery for microorganisms should be focused on the functional diversity of a community rather than on tests with various single species. Therefore, proposed selected methods for prospective sediment toxicity testing with microorganisms on community level are (a) high throughput sequencing for community composition and (b) quantitative PCR assays targeting selected functions for specific functions. Recommended test principals are summarized in Table 7. Moreover, combining test outcomes in a species sensitivity distribution (SSD) would significantly improve the PRA.[7]

2.7 Community Level Tests

Micro- and mesocosm experiments are carried out to study the effects of chemicals at the population level, the recovery of affected species, and to include interactions between species and/or evaluate more realistic exposure patterns than those used in single-species laboratory tests.[23,169] Only a few

TABLE 7. General recommendations for standard prospective sediment toxicity testing with microorganisms under laboratory condition

Recommended principles for prospective sediment testing of microorganisms under laboratory conditions
• Focus on community functionality, using culture-independent proxies.
• Include solid surfaces in test systems, allowing biofilm testing.
• Use field communities to mimic complex interactions in situ.
• Inoculate the artificial sediment with microorganisms from natural sediments.
• Use proper oxic state.
• Use sediments with low clay content and sediment correction for the loss of microbes, when artificial sediments are used.
• Use static/dynamic systems, depending on target ecosystem (e.g., lake vs. stream).
• Use a combination of endpoints on microbial composition and functioning.

micro- and mesocosm studies were found that had evaluated the effects of single, organic contaminants on sediment-associated macroinvertebrates or macrophytes in multispecies test systems (Table 8). Twelve studies were retrieved, six of which had been performed in Europe, five in North America and one in Australia (Table 8).

Although the difference between micro- or mesocosms is often based on their size (a criterion used rather loosely by different authors) both should

TABLE 8. Characteristics of the micro- and mesocosm studies evaluated in this review

Reference	Invertebrates or Macrophytes	Size (m^3)	F/E/M	Geographic region	Chemical
Fletcher et al.[171]	Inv.	(25 × 25 cm)	F	North America	Pesticide
Rand[208]	Inv.	31	F	North America	Pesticide
Brock et al.[95]	Inv.	60	F	Europe	Pesticide
Pablo and Hyne[208]	Inv.	1.05	F	Australia	Pesticide
Roessink et al.[209]	Inv./Macr.	0.84	F	Europe	Pesticide
Bouldin et al.[172]	Inv./Macr.	0.047	F	North America	Pesticide
Roessink et al.[52]	Macr.	0.847	F	Europe	PCB/PAH
Tessier et al.[210]	Macr.	0.144	F	Europe	Antifouling
Thorsson et al.[211]	Inv.	0.0025	E	Europe	PCB
Cunningham et al.[212]	Macr.	0.7	E	North America	Pesticide
Farke et al.[173]	Inv.	13	M	Europe	Oil
Frithsen et al.[174]	Inv.	13	M	North America	Oil

Note. For further details, see Table S4. F = freshwater; E = estuarine; M = marine.

comprise bounded systems that are constructed artificially with samples from, or portions of, natural ecosystems, or consisting of enclosed parts of natural ecosystems. Although these model ecosystems are usually characterized by reduced size and complexity when compared with natural ecosystems, they have to include an assemblage of organisms representing several trophic levels to allow realistic food-web interactions. Moreover, the micro-/mesocosms require an acclimatization period long enough to allow the establishment of a community that is recovered from the construction-stress and adapted to the conditions in the test system.[170]

Out of the 12 studies, eight had been performed in freshwater, two in a marine and two in an estuarine setting (Table 8). Regardless of system size, experimental studies show a preference for block designs involving, for instance, control, low and high exposure conditions, instead of a regression design. Eight of the 12 studies had evaluated the impact of a pesticide on benthic communities, with the test compound actively added to the systems, while the other studies were based on oil, PCBs or PAHs that were usually already present in the sediment. All 12 studies used natural sediment for testing. With the exception of the studies by Fletcher et al.[171] and Brock et al.,[95] all studies include analytical verification of the contaminants of interest in the sediment compartment (Table S4).

The invertebrate organisms studied comprised mostly benthic invertebrates and nematode meiofauna. Test organisms were always chronically exposed to the contaminants and endpoints studied always included abundance and, in the case of PCBs and PAH, also biomass and bioaccumulation (Table 8 and Table S4). Most studies performed with macrophytes monitored the bioaccumulation of these chemicals after spiking them to the water or the sediment, and sometimes evaluated mediation of effects on invertebrates by the presence of macrophytes[172] (see also Table 8 and Table S4). If effects were studied, threshold concentrations were only expressed as concentrations in sediment in those studies examining the effects of oil addition.[173,174]

It is clear that if micro- and mesocosms are to be used more routinely in the higher tier risk assessment of sediment-mediated exposure of chemicals, further standardization is needed. Therefore, further guidelines need to be developed on the conduct (i.e., which standard sediment to use and in which matrix to measure the used compound), interpretation of micro-/mesocosm tests that focus on sediment effect assessment as this is not sufficiently addressed in guidance documents.[170,175] Moreover, it would be helpful to gain more experience in the use of spiked artificial sediment, to study the bioaccumulation and biomagnification of the chemicals through the food web and direct and indirect biological effects on the various biological levels of organization.[50,52]

3. USE OF STANDARDIZED SEDIMENT TOXICITY TESTS IN RISK ASSESSMENT

While the previous sections reviewed the technical details of single chemical tests for single species and communities of species, this section describes how such tests (with different species and environments) could be integrated in one risk assessment framework, and which research priorities would emerge from this integration.

Depending on the protection goals in legislation, results of laboratory toxicity tests with benthic organisms may be used in a regulatory context for deriving predicted no effect concentrations and setting sediment quality standards in both retrospective and prospective effect assessments.[176–180] Currently, the protection goals for benthic organisms are defined in general terms only (e.g., no unacceptable effects). These protection goals could be made operational by using the ecosystem services concept to derive specific protection goals.[92] To date, however, this remains a research objective for benthic organisms in freshwater, estuarine and marine sediments. Note that specific protection goals may differ for different types of benthic organisms. For example, the European Food Safety Authority[181] defined specific protection goals for microorganisms at the functional group level to assess environmental risks of pesticides, whereas they were defined at the population level for invertebrates and macrophytes. A future dialogue between stakeholders is required to define which specific protection goals should be adopted for benthic organisms, depending on the regulatory context. Whatever the outcome of this dialogue will be, a separate tiered decision scheme may be necessary for each specific protection goal that will be defined for sediment key drivers (i.e., main taxonomic groups relevant for a specific ecosystem service) in order to derive sediment quality standards. This derivation usually follows a hierarchy depending on the amount of data available (see Figure 2).

In prospective effect assessments, the basic dossier requirements may comprise chronic toxicity data for a limited set (e.g., 3–4) of standard sediment organisms that represent different taxonomic/trophic groups (e.g., benthic arthropod, benthic annelid, rooted macrophyte) and the application of an appropriate assessment factor. Although in chronic Tier-1 effect assessments usually an AF of 10 is applied to derive a PNEC, the height of this AF needs to be scientifically underpinned (e.g., on the basis of comparisons with SSD curves or micro-/mesocosm tests for a sufficient number of sediment contaminants). Ideally, candidate standard sediment test species, for which internationally accepted test protocols are available, should be selected as soon as possible, to harmonize the lower tier effect assessment procedure across different laws and regulations. Note that the same benthic test species (e.g., *Lumbriculus variegatus* or *Echinocardium cordatum*), which are recommended for toxicity assessment, may be used as well to

assess risks due to bioaccumulation and subsequent transfer of the chemical to higher trophic levels (e.g., to assess risk due to secondary poisoning of predators that have sediment organisms on their diet). Laboratory tests that include a full life-cycle of the test species are considered most suitable, as these cover all sensitive life stages. In addition, results of full life-cycle tests are more appropriate to extrapolate to the field.[182] Examples of full life-cycle tests are chronic protocol tests with *C. riparius*, *H. azteca*, *L. variegatus*, and *C. volutator* (Table 3). Often, however, the life cycle of test species takes too long to complete in order to design a cost effective full life-cycle laboratory test (e.g., for macrophytes and some macroinvertebrates). Therefore, good alternatives are tests that include the most sensitive part of the life cycle and/or the most sensitive parts or tissues (e.g., new shoots) of the sediment test species and focus on the endpoints survival and growth (e.g., tests with *E. cordatum*, *Myriophyllum* sp., *Stuckenia pectinatus*). Tests exclusively focusing on activity (of the sensitive life stage) and/or functional endpoints, such as burrowing activity or feeding rate, (e.g., *L. variegatus*), photosynthesis, (e.g., macrophytes), and luminescence (e.g., *Vibrio fischeri*) may be sensitive (and useful for early-warning) but harder to extrapolate to community-level effects.

The data and recommendations presented in this review suggest that the invertebrate and macrophyte taxa presented in Table 9 are the most promising. Note, however, that harmonized test protocols are available or under development only for the set of freshwater taxa mentioned in Table 9. Consequently, an important future activity is the development of such test protocols for candidate estuarine and marine standard test species. For chemicals with a specific toxic mode of action, e.g., pesticides and biocides with an insecticidal or herbicidal mode-of-action, it is the taxonomic group rather than the place in the food chain or food web (trophic level) that determines sensitivity.[183] Therefore, an important research question is whether specific taxonomic groups that exclusively occur in the marine environment (e.g., Echinodermata) are sufficiently covered by the traditional taxonomic groups tested (Annelida, Crustacea, Insecta). As this information is lacking, a comparative study that evaluates the relative sensitivity of different taxonomic groups of sediment dwelling organisms to a suit of chemicals that differ on mode-of-action is a research priority. Such a comparative study may trigger the development of a standard test protocol for relevant sediment organisms not yet covered by the traditional taxonomic groups tested. For example, this might theoretically be the case for marine Echinodermata and if so *E. cordatum* might be a candidate test species. Furthermore, cross-linking results from sediment toxicity tests, such as those for microorganisms, invertebrates, macrophytes, and sediment micro- and mesocosm tests, requires a unification of dose metrics and exposure assessments in these tests, such as those summarized in Table 1. This involves development of artificial or standardized sediment, to better represent natural sediment. Current standardized test

TABLE 9. Possible suitable species for the first-tier assessment

		Species	Motivation
Fresh	Insecta	*Chironomus riparius* or *C. dilutus*	Specific for freshwater, OECD test
	Crustacea	*Hyalella azteca*	Comparable across environments, ASTM test
	Annelida	*Lumbriculus variegatus*	Comparable across environments, OECD test
	Dictoyledonous	*Myriophyllum spicatum*	Wide distribution, standard test is being developed
	Proteobacteria	*Pseudomonas fluorescens*	Rapid and cheap test
Estuarine	Crustacea	*Corophium volutator*	Comparable across environments, ISO test
	Annelida	*Arenicola marina*	Comparable across environments
	Monocotyledonous	*Stuckenia* (pectinatus) / *Ruppia* (sp. or maritima) / *Vallisneria* (americana)	Wide distribution, easy to culture
	Proteobacteria	*Vibrio fischeri*	Rapid and cheap test
Marine	Crustacea	*Corophium volutator*	Comparable across environments
	Echinodermata	*Echinocardium cordatum*	Specific for marine water
	Monocotyledonous	*Zostera* sp. *(noltii)*	Wide distribution
	Proteobacteria	*Vibrio fischeri*	Rapid and cheap test

Note. Species were selected from the species recommended for a test battery (Tables 3, 5, and Figure 4).

protocols recommend the use of artificial sediments and aim at the closest possible match with natural conditions in the field. If sediment toxicity assessment is to be as realistic as possible in terms of exposure, test designs may need to include condensed carbon phases (i.e., black carbon) as a part of artificial sediment,[61,184] particularly if the chemical becomes bioavailable when sediment particles are ingested (i.e., increased bioavailability of the chemical in the gastrointestinal tract).[40] Omitting a condensed carbon phase such as BC from artificial sediment could lead to an overestimation of the bioavailability and risk.

The uncertainties and possible risks indicated by the first-tier assessment can be used by risk assessors and risk managers to decide which organisms and methods they should focus on in the higher-tier effect assessment. Appropriate intermediate tiers may be developed based on additional toxicity data for potentially sensitive sediment organisms. Suitable additional test

species may be selected from the species mentioned in Tables 2, 5, and 7. It is anticipated that the test conditions for additional test species will not fully comply with the specific testing guidelines for standard test species. Any deviations in terms of test conditions and the properties of the test organisms should, however, be documented in detail. If this leads to additional toxicity data becoming available for the relevant taxonomic groups of sediment organisms, an approach might be to calculate the geometric mean of the chronic toxicity values (e.g., EC_{10} values addressing the same measurement endpoint) within taxonomic groups and to apply the assessment factor (e.g., 10) that is also used in the first tier when the basic set of standard test species is complete. This approach was suggested by the European Food Safety Authority Panel on Plant Protection Products and their Residues, as an intermediate effect assessment tier for pesticides and water organisms[182] and may also be an option for the effect assessments for a wider array of chemicals and sediment organisms. Note, however, that the predictive value of this Geomean approach needs to be calibrated (e.g., with focused micro- and mesocosm tests).

If enough chronic toxicity data for sediment-dwelling organisms become available, the SSD concept may be used for prospective risk assessment by using the HC_5 (hazardous concentration to 5% of the species tested) to derive the sediment quality standard (e.g., by applying an appropriate assessment factor). For aquatic species, at least toxicity data on 8–10 different taxa are usually recommended to apply the SSD approach within a regulatory context.[176,185] Toxicity data used in the SSD need to be expressed in terms of equivalent exposure conditions and dose metrics, as was discussed previously. To date, this number of appropriate chronic toxicity data is usually not available for sediment organisms and one particular chemical. If future research demonstrates that the chronic toxicity data for freshwater, estuarine and marine sediment organisms could be combined in a single SSD, there might be an increased scope for effect assessment based on the SSD approach.

As discussed already, appropriate community-level (micro-/mesocosm) experiments that address the concentration-response relationship for sediment organisms may in the near future be used as an appropriate higher tier test (e.g., by selecting the most sensitive endpoint for sediment-dwelling organisms and an appropriate assessment factor or modeling approach for spatiotemporal extrapolation) and to calibrate the risk assessment on the basis of laboratory toxicity tests with sediment organisms. Current guidance documents[170,175] focus on effect assessment and water exposure. Consequently, guidance for conducting and interpreting sediment micro- and mesocosm tests is required. Another research need is to study the possible variability in threshold concentrations of population and community-level effects for sediment organisms in different model ecosystem experiments in order to

derive an appropriate AF for spatiotemporal extrapolation if only one appropriate micro-/mesocosm test is available for the sediment contaminant under evaluation.

Note that the prospective effect assessment tiers described previously can also be evaluated and verified by means of the extensive information gained from the development of sediment quality guidelines in North America and Europe within the context of retrospective risk assessment.[1,186–191]

4. CONCLUSIONS

In this review, we have summarized the technical literature on whole-sediment toxicity tests for microorganisms, benthic invertebrates, macrophytes, and benthic communities. We have presented recommendations based on earlier papers and reviews, and have identified knowledge gaps and priorities for further research. All in all, despite the observed progress in individual fields of sediment toxicity testing over the past two decades, the approaches are currently still too heterogeneous to allow unification in risk assessment frameworks. Consequently, we have proposed a balanced selection of species that seem to be most suitable for future frameworks for the prospective assessment of risks associated with single chemicals. Together with optimized standard test protocols, these selected species could form the basics of the first tier of sediment toxicity risk assessment. Consequently, the formal selection and approval of species and tests in regulatory contexts is an important priority. Within this domain of prioritized protocol development, a second distinct priority is the development of standardized test protocols for estuarine and marine species, microorganisms and macrophytes, as these are still less well developed than freshwater benthic invertebrate tests. A further question is whether specific taxonomic groups that exclusively occur in the estuarine and marine environment are sufficiently covered by the traditional test species, which may call for the development of tests for species that characteristically occur in the estuarine/marine environment. In addition, guidance for conducting and interpreting higher-tier sediment micro- and mesocosm tests needs to become available in the near future, as such tests are crucial for the calibration of tests in lower tiers of the risk assessment.

Ultimately, results from sediment toxicity tests focusing on microorganisms, invertebrates, macrophytes and communities (in micro- and mesocosm tests) may be combined in higher tiers of prospective risk assessment such as the SSD approach. This, however, requires unification of dose metrics and exposure assessment methodologies across the groups of test species. We have therefore proposed recommendations for exposure assessment and sediment preparation.

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