

Critical Review

MEASURING BIOACCUMULATION OF CONTAMINANTS FROM
FIELD-COLLECTED SEDIMENT IN FRESHWATER ORGANISMS:
A CRITICAL REVIEW OF LABORATORY METHODS

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Abstract—To be effective, decision-making frameworks require data from robust and reliable test methods. Using standard methods allows for more effective comparison between studies and application of data, and it reduces unnecessary duplication of efforts. Laboratory methods to assess the toxicity of sediment have been standardized and extensively used; however, procedures for measuring the bioaccumulation of contaminants from sediment into aquatic organisms need further standardization. Bioaccumulation methods using freshwater invertebrates and fish exposed to field-contaminated sediment were reviewed to identify important similarities and differences in method protocols, test conditions that need to be controlled, and data gaps. Although guidance documents are available, great variation still exists in exposure techniques used in tests, which may potentially affect the estimation of bioaccumulation. The techniques most consistent across studies include the use of *Lumbriculus variegatus* as a test species, test temperatures between 20 and 25°C, and a 28-d exposure with no addition of food, followed by purging of organisms. Issues that were inconsistent between studies or remained unspecified, which should be addressed, include the bioaccumulation potential of other test species, loading density of organisms, and sediment-to-water ratio. In addition to proper evaluation of the various exposure techniques and conditions, a need exists for more consistent inclusion of quality control procedures during testing. Environ. Toxicol. Chem. 2010;29:2391–2401. © 2010 SETAC

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Contamination of sediment in freshwater ecosystems, caused by the historical and current release of persistent and toxic substances, is considered a major environmental concern for aquatic systems [1,2]. The physicochemical properties and persistent nature of many contaminants of concern (e.g., PCBs, dioxins and furans, DDT, and mercury) has resulted in their accumulation within sediment. Not only may these substances be toxic to benthic organisms, many can be transferred from the sediment into benthic organisms and fish and further up the food chain to fish-eating birds, wildlife, and humans through bioaccumulation and biomagnification. Although emissions to water and the atmosphere have been reduced because of environmental regulations, and the production and use of some substances is banned (e.g., PCBs, DDT, some polybrominated diphenyl ethers), the sediment now serves as a source of many of these contaminants [2]. In addition, new and emerging contaminants such as polybrominated diphenyl ethers and perfluorinated substances, which have not historically or routinely been measured or detected in the sediment, are of concern. Although exposure to environmentally relevant concentrations of these contaminants may not be acutely lethal, they have the potential to induce sublethal effects such as reproductive and developmental impairment, endocrine disruption, tumor formation, and cancer [3–6].

In addition to the physicochemical characterization of sediment, toxicity testing, and benthic surveys, an assessment of the biomagnification potential of contaminants has been identified

as crucial to the risk assessment process [7,8] and has now been incorporated into decision-making frameworks for contaminated sediment [9,10]. Bioaccumulation of contaminants from sediment can be measured directly through the collection of organisms from the field, transplant studies, and laboratory tests, or it can be predicted using models. Each tool has applications and limitations based on the questions being addressed, the levels of certainty required, and the stage of the risk assessment or site remediation, as noted by a number of authors when discussing the approach to bioaccumulation assessment [4,11,12]. As the demand for robust and cost-effective tools to use in ecological risk assessment and regulatory decision-making increases, a need has arisen to standardize procedures for assessing bioaccumulation to ensure that studies are comparable. Laboratory methods to assess the toxicity (i.e., impact on growth, survival, and reproduction) of sediments have been standardized and extensively used [13–16]; however, laboratory methods for measuring the bioaccumulation of contaminants from sediment have not achieved the same level of standardization.

Numerous laboratory studies have examined the bioaccumulation of contaminants from field-contaminated sediment, using a variety of test organisms. Some studies are research-based and focus on a specific organism, compound, route of exposure, or hypothesis [17–21]. Other studies are more applied and include the development and evaluation of methods applied broadly to regulatory and monitoring programs [22–25] and subsequent environmental monitoring [26–28]. General guidance for conducting a 28-d bioaccumulation test with the Oligochaete *Lumbriculus variegatus* is offered by the American Society for Testing and Materials (ASTM; Annex 8 [29]) and the U.S. Environmental Protection Agency (U.S. EPA; [16]). However, even these protocols note that additional research is

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needed on the standardization of bioaccumulation procedures with sediment [16,29]. The identification of additional species for bioaccumulation methods and development of these methods is also considered a high priority with respect to research needs [30].

Although bioaccumulation data are frequently generated and applied on a site-specific basis, the use of standardized laboratory methods enables contaminated sites to be compared with each other on a scientifically defensible basis and accurately ranked with respect to priority of cleanup and effectiveness of remediation. Standard methods that have undergone proper development and validation are likely to improve the translation of laboratory data to the field, thereby strengthening ecological risk assessments and regulatory decision-making. As part of its commitments to the Canada–Ontario Agreement, the Ontario Ministry of the Environment has undertaken work on the development and standardization of laboratory bioaccumulation methods with freshwater organisms and field-contaminated sediments. The first step in this process was to evaluate the current and historical application of methods for measuring bioaccumulation for the purposes of research or field assessments, and this forms the basis of this review.

Within method development, a number of factors related to experimental design should be considered to ensure that data of the highest quality and consistency are produced. Many of these factors have been assessed and standardized in sediment toxicity methods and also are relevant to methods for measuring bioaccumulation [31]. General guidance for determining the bioaccumulation of sediment-associated contaminants in benthic invertebrates (freshwater and marine) has been included in an ASTM standard [29]. The ASTM guide includes discussion of the collection, storage, and preparation of sediment, the selection and handling of test organisms, experimental design (replication, duration, and sampling), and test procedures (including purging vs nonpurging). It provides specific recommendations regarding test species, exposure conditions, and test duration but lacks sufficient guidance on amounts of sediment to use, sediment-to-water ratios, and assessment of survival and growth in organisms as performance-based measures of test acceptability. Much of the guidance is based on techniques used in successful bioaccumulation studies and expert opinion rather than experimental comparisons of different techniques [29].

The purpose of the current review is to provide a critical evaluation of laboratory methods used for measuring the bioaccumulation of sediment-associated contaminants in aquatic organisms. The goal is to identify important similarities and differences between methods, test parameters that are and need to be controlled, and data gaps, so that robust, effective, and standardized test methods can be developed for routine use in environmental monitoring programs and ecological risk assessment of contaminated sediments. Herein we specifically review bioaccumulation methods using freshwater organisms exposed to field-contaminated sediments, because these are the intended applications of the method to be used by the Ontario Ministry of the Environment. Standard methods for assessing the toxicity of sediments are discussed only in terms of their relevance to bioaccumulation methods.

METHODS

A thorough search of the literature was conducted to obtain bioaccumulation methods using freshwater organisms exposed to sediment from North American and European publications

between 1980 and 2008. More than 150 studies were identified. The focus of the literature search and thus criteria for inclusion of studies for the review were those that used laboratory methods to measure bioaccumulation of persistent organic pollutants or heavy metals (i.e., As, Cd, Hg) from field-contaminated sediments into freshwater invertebrates or fish. This resulted in the selection of 22 studies for inclusion and detailed review. Many of the bioaccumulation studies found in the literature used spiked sediment. These studies typically use very specific analytical procedures designed to answer specific research questions that may not easily translate to methods applied in a regulatory context or to routine monitoring of environmental samples. Therefore, studies using spiked sediment are discussed only to elaborate on different aspects of experimental design.

In the current review, we focus on a number of factors that could potentially affect estimation of bioaccumulation, particularly those related to test species and techniques for exposing test organisms. A thorough discussion of procedures carried out on sediment before exposure, statistical design, and both abiotic and biotic sampling is found in the ASTM guide [29] and has therefore not been included in this review. In addition, these considerations may be specific to each study. However, considerations of quality control procedures, experimental controls, and treatment of data are included, because these are more universally applied across studies, and some discrepancy remains as to how these are addressed in the ASTM guide [29]. Each study was reviewed for information regarding test species, exposure techniques and test conditions, physical and chemical analyses, test endpoints, and treatment of the data (Supplemental Data; Table S1) that were judged to be important for the characterization of bioaccumulation (and are typically covered in standard test methods). The most important test conditions of each method are listed in Supplemental Data, Table S2; the trends are summarized in Figure 1, and have been grouped according to major influencing factors (capitalized headings on the y axis of Figure 1) to help organize the ensuing discussion.

Factors influencing characterization of bioaccumulation

Test organism. The success, ecological relevance, and interpretation of a bioaccumulation test can be greatly influenced by the choice of test species [29]. The selection of freshwater species for use in sediment toxicity testing has been reviewed extensively [31–33], and many of the selection criteria also apply to organisms used in bioaccumulation tests. These include ease of culture or maintenance in the laboratory and availability for testing at any time, contact with sediment (through feeding or behavior) to assess the appropriate route of exposure, ecological importance, tolerance of a range of sediment physicochemical characteristics (particle size and organic carbon), and response confirmed with that of benthic populations. The ASTM guide lists two required characteristics for a bioaccumulation test organism as the ingestion of sediment, because this is often the major route of uptake for hydrophobic compounds, and the ability to survive the exposure [29]. This latter requirement limits the use of some sensitive species routinely used in sediment toxicity testing (*Hyalella azteca* is sensitive to metals), particularly for sediments with high concentrations of contaminants. Desired characteristics are that organisms should be amenable to the long-term exposures required to reach equilibrium conditions (e.g., chironomids with short life cycles may not be appropriate) and should supply sufficient biomass, either as individuals or as a logistically reasonable number of

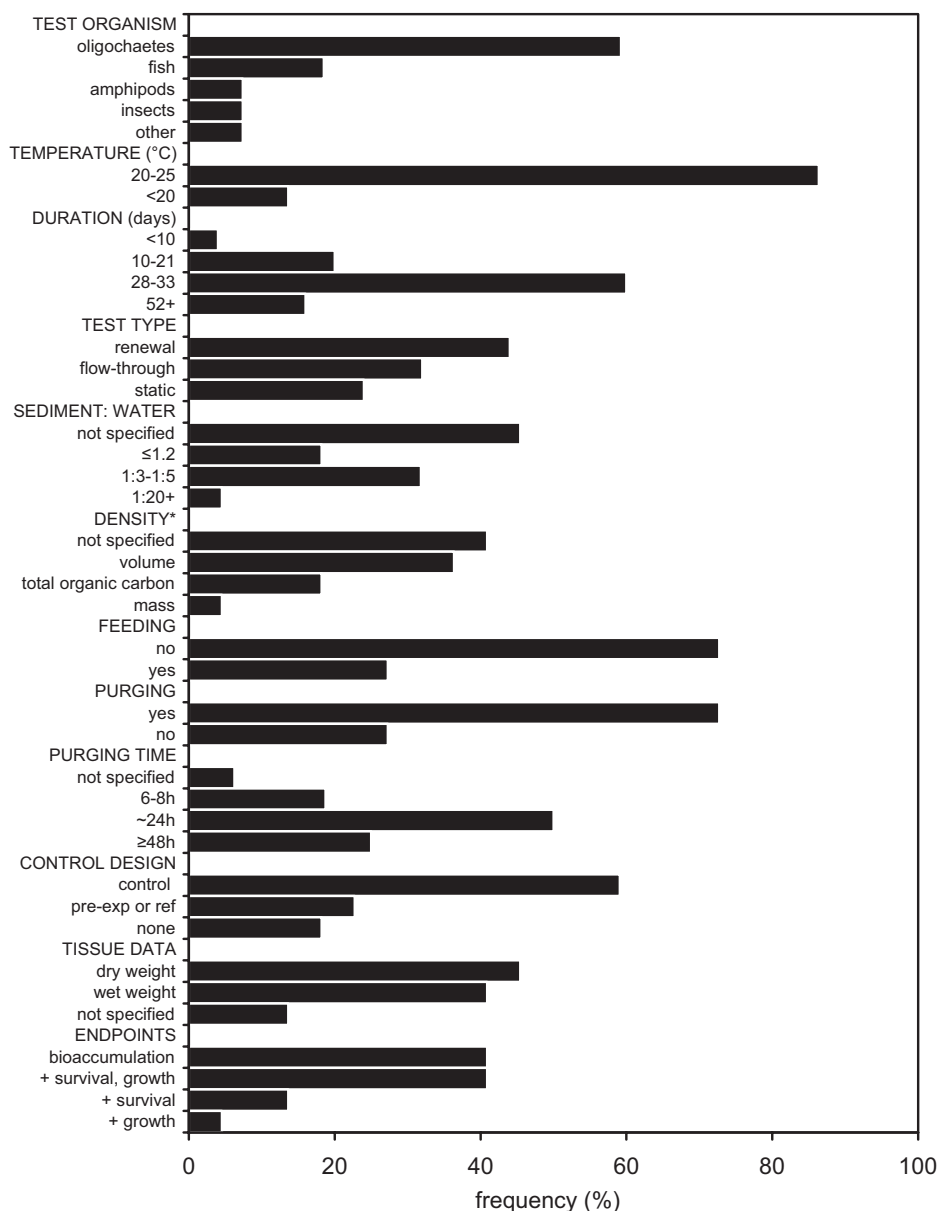


Fig. 1. Analysis of test conditions used in reviewed bioaccumulation methods. *Standardized to sediment volume, total organic carbon, or mass. Numbers under each heading (capitalized) sum to 100%.

individuals, for chemical analysis [29]. The guide also recommends using species with high bioaccumulation potential. That is, organisms with high lipid content will typically accumulate a greater amount of hydrophobic contaminants, and those with minimal ability to metabolize and biotransform contaminants (e.g., PAHs) are preferred. However, the ASTM guide also notes that an insufficient number of tests have been performed that compare multiple species in individual exposures to adequately characterize the bioaccumulation potential of a range of species over a range of contaminants.

A variety of freshwater species have been rated by Ingersoll et al. [33] and the ASTM [29] with respect to the selection criteria or characteristics of the organism as discussed. Both recommend the oligochaete *Lumbriculus variegatus* as a primary organism for bioaccumulation tests, because it meets many of the selection criteria. Other organisms such as mollusks, midges, mayflies, amphipods, cladocerans, and fish are considered secondary species because of indirect routes of exposure, size, sensitivity, or short life spans. Many of these

organisms are represented in the bioaccumulation studies included in the current review (Table 1).

Invertebrate species. Oligochaetes represent almost 60% of the test organisms used in bioaccumulation tests, with *L. variegatus* being the most frequent. This oligochaete meets many of the requirements of a test organism, because it is exposed to contaminants through burrowing and ingestion of sediment, is easily cultured, and is tolerant of a range of sediment characteristics. *Lumbriculus variegatus* has been observed to biotransform PAHs, such as pyrene and benzo[*a*]pyrene, albeit slowly [34–36], and to a much lesser degree than *H. azteca* and *Chironomus dilutus* (formerly *C. tentans*) [35]. *Lumbriculus variegatus* are small (5–12 mg), thereby requiring large numbers of organisms to attain sufficient biomass for chemical analysis. They reproduce rapidly and asexually through fragmentation, which is advantageous for culturing; however, ingestion of sediment and active uptake of contaminants may not take place continuously throughout a test while portions of the body are regenerated. *Lumbriculus variegatus* is

Table 1. Organisms used in reviewed bioaccumulation studies with field-contaminated sediment

Organism	No. of studies	% of total
Oligochaetes		59
<i>Lumbriculus variegatus</i>	13	
<i>Limnodrilus hoffmeisteri</i> and <i>Tubifex tubifex</i> (mixed)	1	
<i>Lumbriculus terrestris</i>	1	
<i>Octolasion tyrtaeum</i>	1	
Amphipods		7
<i>Hyalella azteca</i>	2	
Insects		7
<i>Hexagenia</i> spp.	2	
Other		7
<i>Corbicula fluminea</i>	2	
Fish		19
<i>Pimephales promelas</i>	4	
<i>Perca flavescens</i>	1	

the species of choice in the ASTM [29] and U.S. EPA [16] bioaccumulation protocols on which a number of other studies have been based [20,37,38].

Other, less frequently used invertebrate species include *H. azteca*, *Hexagenia* spp., and *Corbicula fluminea* (Table 1). The amphipod *H. azteca* is a standard test species in sediment toxicity methods [13,15,16] because of its ecological importance and sensitivity to contaminants, particularly metals. However, the sensitivity and small size of this epibenthic species make it difficult to use in bioaccumulation testing. Studies using *H. azteca* included in the current review only examined the uptake of metals, which has much smaller sample size requirements for analytical purposes than do organic compounds.

Hexagenia spp. mayfly nymphs reflect an appropriate route of exposure, because they burrow into and ingest sediment. In addition to providing adequate biomass, nymphs naturally inhabit the sediment for a year or more and are thus suited for long-term exposures. Many researchers have avoided using *Hexagenia* spp., because they cannot be continuously cultured in the laboratory, and field-collected nymphs are not available year round. However, eggs have been collected in the field from emerged adults, stored, then hatched and reared in the laboratory with good success [39,40]. *Hexagenia* spp. nymphs have been shown to demonstrate a preference for finer-grained sediments in which burrows can be maintained [41]. Sampling of organisms in the field has shown that *Hexagenia* spp. nymphs have higher body burdens of contaminants (PAHs and PCBs) than dreissenid mussels, amphipods, and crayfish [42].

Freshwater mollusks are also suited for bioaccumulation testing in that they provide sufficient biomass, are easy to handle, and are relatively long-lived. These features and their sedentary lifestyle have led to their extensive use in field studies as part of environmental monitoring programs. Continuous cultures cannot be maintained in the laboratory; therefore, adult mollusks must be collected from the field and held in the laboratory for testing, or juveniles may be propagated by collection of larvae from gravid females. Exposure may be uncertain because of valve closure, and filter-feeders, such as *C. fluminea*, typically accumulate much lower concentrations of contaminants than other organisms [23,43,44].

Although small invertebrates including midges, snails, isopods, and other amphipods have life history and practical characteristics that limit their utility in bioaccumulation assessments with field-contaminated sediments, radiolabeled

analytical techniques that require much smaller sample sizes have enhanced their use in assessments with spiked sediments [45–47].

Fish. The fathead minnow, *Pimephales promelas*, was the second most frequently used test organism in the bioaccumulation studies we reviewed (Table 1). This species has been used extensively in toxicity testing and is one of the fish species listed in an ASTM guide for bioconcentration tests [48]. Although most fish typically interact with sediment to a much lesser extent than do burrowing invertebrates, they do offer some unique advantages, as suggested by Mac and Schmidt [49]. Fish often resuspend sediment, increasing availability of contaminants. They may integrate several routes of exposure by accumulating contaminants dissolved in the water (through gills and skin) and through the ingestion of food and sediment. Fish provide adequate tissue mass with high lipid content, are easily collected or caged in the field for comparison, and are of direct concern regarding the transfer of contaminants to higher trophic levels. When exposed to sediment, minnows often accumulate much different concentrations of contaminants from those acquired by oligochaetes [23,24,50]. In addition to the route of exposure and lipid content, and in contrast to invertebrates, fish also may readily metabolize or transform certain compounds (PAHs [51]) or require more time to reach steady-state conditions. In a study by Ankley et al. [24], larval fathead minnows, exposed in the laboratory for 30 d, accumulated PCBs to a much lesser extent than did field-collected black bullheads. This was probably attributable to the different life stages, and thus behavior and relative exposure, of these fish. However, this was not considered in the authors' discussion of differences in bioaccumulation, and they suggested that, under these conditions, laboratory bioaccumulation tests with fish could significantly underestimate exposure of species in the field. This stresses the importance of the selection of an appropriate type of test organism, as well as the appropriate life stage, based on the route of exposure to be assessed.

As in toxicity testing, no one species is best suited to assess all possible environmental conditions encountered in routine bioaccumulation testing. Different species may vary in their bioaccumulation potential, which may be influenced by contaminant and sediment characteristics. Pauwel and Sibley [12] noted that many studies assessing sediment toxicity do not justify their selection of test species and often base their choice on recognized protocols and guidelines, convenience and availability, and experience with the organism. They suggest that the use of a few widely adaptable species would allow for comparison of toxicity or bioaccumulation under different environmental conditions. The literature offers much support for a battery of tests with multiple species representing different taxa, trophic levels, and routes of exposure [29,31,32,52]. The most comprehensive assessment of bioaccumulation includes both benthic invertebrates and fish species that have some association with sediment [49]. The use of two or more species from different major taxa increases the probability of measuring maximum tissue residues and has been recommended for assessing moderate to large discharges or dredging operations [29] and in regulatory testing [30]. Currently, only bioaccumulation methods with *L. variegatus* have been standardized (by the ASTM [29] and U.S. EPA [16]), and many researchers have used *L. variegatus* based on these protocols. Yet, this raises the question of whether the risk characterization of bioaccumulative compounds should be based on one organism, particularly when the bioaccumulation potential between species has not been sufficiently compared. This need should be addressed,

because toxicity and bioaccumulation data from comparative testing of several species may be essential to provide greater ecological relevance to assessments of sediment quality and potential for bioaccumulation.

Exposure techniques

A number of factors related to exposure, including duration of exposure, test temperature, quality of the overlying water, feeding, loading density of organisms, and purging, have the potential to influence the interpretation of results from bioaccumulation studies. Herein we discuss these key factors, placing special emphasis on exposure techniques.

Duration of the test. The exposure duration of a bioaccumulation test is considered a critical aspect of experimental design [30] and may vary, depending on the objectives. Ten-day tests were previously deemed sufficient for estimating the bioavailability of contaminants in dredged material for ocean disposal [53]. However, 28-d tests are now required in these assessments if organic and organometallic compounds are present. Estimates of steady-state concentrations in tissue are necessary for evaluating risks to wildlife and human health, including advisories for consumption of fish. The time required to achieve steady-state can vary with compound, sediment quality parameters (e.g., organic carbon), and the metabolic capacity of the organism. Twenty-eight days has been proposed as a standard exposure period, because this typically results in tissue residues within 80% of steady-state concentrations and provides a much better estimate than 10-d tests [29]. Steady-state conditions will not necessarily be reached in 28 d, and a longer exposure or kinetic approach may be necessary for estimates that are more accurate [49]. However, long-term tests may be influenced by a change in concentration of the contaminant, reduction of water quality, and loss of biomass or lipid in the test organisms. Kinetic experiments are also more costly, because they require greater laboratory and analytical resources or the use of radiolabeled material, of which the latter is not applicable to field-collected sediments.

Of the bioaccumulation studies reviewed, most of the tests were 28 to 33 d in length (Fig. 1). Other tests were typically between 10 and 21 d or 50 d or longer. Exposure period was not necessarily related to the contaminant of concern (i.e., metals vs organic compounds). The appropriateness of an exposure in representing steady-state can be assessed using time-series sampling or field validation. Concentrations in tissues of laboratory-exposed organisms can be compared with organisms collected in the field with the sediment, which are assumed to be at steady-state. However, differences between laboratory- and field-exposed organisms may be attributable not only to the length of exposure, but to changes in sediment composition because of sampling, processing, and storage, differences in temperature affecting metabolic rates, and differences between species and their behavior.

Drouillard et al. [18] used a kinetic approach to evaluate the uptake of 14 PCB congeners and several other organochlorine compounds in *Hexagenia limbata* nymphs. They found that tissue concentrations of all the chemicals reached 95% of steady-state concentrations within 32 d. In a 14-d test with *L. variegatus*, Van Hoof et al. [19] observed three different patterns of PAH accumulation. Bioaccumulation of low-molecular-weight PAHs was rapid initially, peaked between 2 to 4 d, and then declined, with only a few compounds approaching steady-state. Other PAHs peaked at 7 d, followed by a gradual decline, and high-molecular-weight PAHs had a sigmoidal uptake curve with no peak or approach to steady-state observed.

Similarly, Harkey et al. [17] observed a peak at 2 to 4 or 14 d for low- and high-molecular-weight PAHs, respectively, followed by a decline in tissue concentrations in *L. variegatus*, without reaching steady-state in 28 d. Ingersoll et al. [37] conducted a 56-d bioaccumulation test with *L. variegatus* exposed to sediments contaminated with PAHs and DDT and its metabolites. They also observed a peak in low-molecular-weight PAHs on d 3, followed by a decline and plateau. Other PAHs, as well as DDE and DDD, appeared to reach steady-state between 14 and 28 d. This study also compared steady-state concentrations from laboratory exposures with field-collected oligochaetes and found that concentrations in tissue were similar. Other comparisons of 28- to 30-d laboratory exposures with field-collected oligochaetes found that organisms accumulated similar concentrations of PAHs [54] and PCBs [24]. However, it was observed that concentrations of highly chlorinated PCB congeners (hepta to deca) were slightly higher in field organisms, suggesting that 30 d was insufficient for these very hydrophobic compounds ($\log K_{OW} > 7$) to reach steady-state in the laboratory [24].

An apparent strength of the studies we reviewed is that length of exposure was similar (60% were 28–33 d), thus reducing the influence of an important variable between studies. This is of particular importance, because bioaccumulation data generated through environmental and regulatory monitoring programs may be used for comparing different sites. However, some uncertainty remains as to how representative a 28-d exposure is of steady-state conditions, particularly for high K_{OW} compounds or organisms such as fish. None of the studies assessed kinetic uptake in fish, and recent research has tended to focus on benthic invertebrates in bioaccumulation tests with sediment. Elimination rate is considered the key factor determining the time to steady-state for a compound in an organism. Few of these data had been generated for benthic invertebrates at the time the ASTM method was initially developed, and the selection of 28 d appears to be based on contaminant uptake (see Table 4, p. 1096, in ASTM [29]). Recent studies have added to our understanding of kinetics by measuring both uptake and elimination for compounds such as polybrominated diphenyl ethers [38,55] and PAHs [34,56] in *L. variegatus*, and DDT in amphipods [46] from spiked sediments. These and the previously discussed studies, however, are limited to a few species and few compounds (and do not include dioxins and furans). In general, 28 d appears to be an appropriate standard (or minimum) duration for conducting routine bioaccumulation tests with invertebrates. A series of kinetic studies assessing the major classes of contaminants with a few distinct groups of species would support or improve the selection of a standard duration for bioaccumulation tests.

Temperature. Temperature is generally strictly controlled under laboratory conditions to limit its influence on test results and facilitate comparison between studies. Temperature not only affects the bioavailability of a compound via partitioning processes, but also metabolic activity, and thereby uptake and elimination rates, of an organism. However, the selection of a single temperature at which to conduct toxicity or bioaccumulation tests is arguably arbitrary and ecologically irrelevant, because organisms under natural conditions are exposed to daily and seasonal temperature fluctuations. Test temperatures should correspond to the average spring–summer temperature of a study site to represent the most biologically active season [29], but this approach is difficult to apply in monitoring programs covering a wide range of geographical locations. More importantly, the temperature must meet the physiological

requirements of the organism, and often the temperature at which maximum growth or reproduction rates occur is selected for culturing and testing. More than 80% of the studies we reviewed were conducted between 20 and 25°C (Fig. 1), with 13°C being the lowest temperature used. The ASTM and U.S. EPA standard methods for measuring bioaccumulation in *L. variegatus* are conducted at 23°C. Standard methods for assessing the toxicity of sediments to *H. azteca* and *Chironomus* spp., and effluent to *P. promelas*, are also typically conducted between 20 and 25°C. The test species used in bioaccumulation tests discussed in the previous section are not considered cold-water species and likely function effectively at these temperatures. Very few, if any, of the studies we reviewed justified the selection of a test temperature, and choices were likely based on existing methods for toxicity testing. Continued use of the test temperature recommended in the ASTM and U.S. EPA bioaccumulation methods will facilitate comparison between studies, whether methods are research-based or applied. However, the uncertainty associated with the extrapolation of laboratory data to the field may be greater when temperatures are different. Studies comparing bioaccumulation in the laboratory and field at the same temperatures also should be considered to address this uncertainty.

Quality of the overlying water. Maintaining an acceptable quality of overlying water in sediment tests is extremely important. The biological oxygen demand of sediment can deplete DO, and concentrations of ammonia and hydrogen sulfide can increase, adversely affecting survival. This may confound the true toxicity of sediments and potentially affect estimates of bioaccumulation. Attempts to maintain water quality include the use of flow-through or renewal conditions, aeration, and large volumes of overlying water, as discussed below. The ASTM [29] and U.S. EPA [16] standard methods support the use of both flow-through and renewal methods.

The most widely accepted approach to maintaining water quality is to use flow-through conditions. However, the apparatus required for this (e.g., diluter) can be expensive, and the quality (temperature and DO) of the inflowing water must be controlled. Depending on flow rates, large volumes of wastewater may need to be collected and treated, and contaminants could be flushed from the system, particularly when sediment is suspended via bioturbation. This may be less of a concern for highly hydrophobic compounds that do not readily partition into water, unless these compounds are also associated with suspended particulates. Flushing of contaminants may be more important for metals and organic compounds with low K_{OW} (<4). Static tests have no introduction of clean water and require aeration of overlying water in each vessel. Water quality, including concentration of the chemicals of interest, can change during the exposure, but chemical concentration is more likely to reach equilibrium between water and sediment in the closed system. With either of these two methods, flow or aeration should be sufficient to maintain levels of DO above 2.5 mg/L or 40% saturation and ammonia less than 20 µg/L [29]. A third option to maintain water quality is intermittent water renewal, an approach that limits the amount of turnover and flushing of contaminants from the exposure system. The frequency of water exchange ranges from 400% per d to 50% water change once per week, but it is suggested to be minimal to reduce changes in exposure of organisms [57]. However, this intermittent addition of clean water has the potential to prevent a system, and thus the test organisms, from reaching equilibrium [58].

The frequency of use of static, flow-through, or renewal conditions in the methods we reviewed was 24, 32, and 44%, respectively. In addition, the exposure system used was not based on the contaminant of concern being measured (i.e., metals vs hydrophobic organic compounds). From a practical standpoint, static tests provide the greatest flexibility with test setup and require the least maintenance. Because a static test is a closed system, exposure to contaminants from sediment or overlying water through diffusion from sediment cannot be separated, which reflects a potentially worse-case exposure scenario. Flushing of contaminants from sediment in flow-through or renewal systems may decrease exposure of test organisms; however, tissue residues may better reflect bioaccumulation from sediment as the sole route of exposure. Whether this results in significant differences in bioaccumulation is uncertain and may be more of a concern for interfacial species, because burrowing organisms typically receive a greater proportion of their exposure from sediment or interstitial water [16].

The use of larger volumes of overlying water, particularly within static tests, can also minimize the degradation of water quality. In addition, this allows the collection of water for chemical analysis and can reduce crowding if water-column species such as fish are used. Differences in the ratio of sediment and overlying water can potentially influence partitioning and bioaccumulation, and a consistent ratio should be maintained between tests. Standard toxicity methods with freshwater sediment use a sediment-to-water ratio of 1:1.75 (v/v) [13–16], although this is under review (Minutes of the 2008 Inter-Governmental Ecotoxicological Testing Group annual meeting; Saskatoon, SK, Canada). A 1:4 ratio is used in Ontario Ministry of the Environment sediment methods [40], as well as standard marine methods [59]. However, no discussion of sediment-to-water ratios is found in the bioaccumulation methods of the ASTM [29] or U.S. EPA [16]. In the bioaccumulation studies we reviewed, the ratio of sediment to water used ranged from 1:1 to 1:1,000, with approximately 30% of studies using a ratio between 1:3 and 1:5 (Fig. 1). Some studies specified a particular ratio, whereas, for others, this was calculated a posteriori from the volumes of sediment and water used. In 45% of the studies, this ratio could not be determined because the volume of water was not specified. This is a major limitation in terms of repeatability of the experiments and comparison with other studies. Some studies have examined the effects of different ratios of sediment and water on toxicity to *H. azteca* ([58] and T. Watson-Leung, Ontario Ministry of the Environment, Etobicoke, ON, Canada, 2005, unpublished data), but not for bioaccumulation testing. Although somewhat arbitrary, a 1:4 ratio in our experience provides a sufficient amount of overlying water to minimize degradation of water quality and for chemical analysis. It also enables practical volumes while working within the confines of widely available test vessels. Using a consistent ratio of sediment to overlying water is another simple step toward the standardization of methods.

Feeding. The addition of food is a standard practice in toxicity tests with *H. azteca* and *Chironomus* spp. to ensure acceptable survival and growth in controlled exposures [60]. In contrast, feeding has been discouraged in bioaccumulation tests, because organisms may preferentially ingest the food instead of sediment, reducing the uptake of contaminants [29]. Harkey et al. [61] observed that fed *H. azteca* accumulated higher concentrations of fluoranthene from spiked sediment than unfed organisms after 96 h at all concentrations and up to 30 d in exposures to low concentrations. However, the health of the

unfed organisms may have been compromised, because essentially no growth occurred throughout the 30-d test. Organisms were not fed in most (73%) of the studies we reviewed; those receiving food included non-sediment-ingesting species, such as *P. promelas*, *H. azteca*, and *C. fluminea*. Feeding these organisms may be necessary to prevent them from entering a state of starvation metabolism, thereby affecting rates of bioaccumulation. The necessity of feeding relates back to the selection of a test species most appropriate for assessing uptake of contaminants from sediment, in that organisms that ingest sediment are best suited to assess this route of exposure. However, if study objectives include the use of these non-sediment-ingesting species, feeding should be at a minimum to prevent high growth rates potentially resulting in growth dilution or fouling of water.

Amount of sediment/organism loading density. Sediment serves as both a habitat and a food source for benthic species. In bioaccumulation tests, the depth and surface area of sediment should allow normal feeding and burrowing behavior, which may depend on the requirements of different-sized species. The amount of sediment should ensure a sufficient supply of food and that contaminants are not depleted over the time of the test. This amount should exceed, by twofold to fivefold, the total amount of sediment processed by a species over the test duration [29]. However, this requires knowledge of the processing rates of particular species, which can vary with sediment grain size and content of organic carbon [62,63]. Kukkonen and Landrum [64] unexpectedly observed that bioaccumulation of pyrene by *L. variegatus* increased with increasing density of animals, suggesting that the animals were not depleting the available contaminant in 14-d tests. Lyytikäinen et al. [36] transferred *L. variegatus* into fresh sediment at regular intervals over 12 d to investigate the depletion of the rapidly desorbing (labile) fraction of PAHs from sediment and pore water and found that this had a minor effect on bioaccumulation compared with organisms that were not transferred to fresh sediment. Therefore, a need remains to standardize the amount of sediment that should be used in a bioaccumulation test.

In the studies we reviewed, we assessed whether sufficient information was provided to determine the loading density of organisms based on biomass. The amount of biomass is important because of the requirement of obtaining a sample size adequate for tissue analysis. In studies that listed the number of organisms exposed, but gave no indication of the size of organisms, loading density could not be determined. Additionally, loading density could not be determined in studies that did not specify a mass or volume of sediment. Loading density was then classified on the basis of sediment volume, mass, or TOC. For these reasons, loading density could not be determined in 41% of the studies reviewed. The classification of the remaining studies was as follows: 36% based the density on sediment volume, 18% on TOC, and 5% on mass of sediment.

Differences in the physical characteristics of sediments may influence the outcome of toxicity tests as well as bioaccumulation studies by affecting the survival, feeding rates, and growth of benthic organisms. Many studies, including standard toxicity tests, standardize organism density to sediment volume, which enables initiation of tests without physical characterization of sediment. The two standard test species, *H. azteca* and *Chironomus* spp., typically forage in the surficial layer of sediment and are less likely to be impacted by the total amount of sediment or organic carbon. The addition of supplementary food in tests with these species also minimizes their dependence on the sediment as a source of food, which could further bias the

estimate of uptake of contaminants from sediment. Burrowing species, however, receive most of their exposure from ingestion of and interaction with sediment. They are therefore more dependent on, and their response potentially more influenced by, the physical characteristics of sediment such as organic carbon.

Sediment organic matter represents a sink for many hydrophobic contaminants, and a food source for sediment-ingesting species. From their study, Kukkonen and Landrum [64] suggest that a standard density of organisms relative to sediment organic carbon should be established to ensure that exposures are similar among tests. They comment that an optimal density is uncertain because the mechanism of differential accumulation is unknown, but that perhaps a 50:1 ratio of sediment TOC to organism dry weight is appropriate. As a result, the ASTM [29] and U.S. EPA [16] bioaccumulation methods with *L. variegatus* have recommended using a 50:1 ratio to ensure the availability of sufficient food and to minimize depletion of contaminants. However, in either case, no rationale was provided for the selection of this ratio, and no experimental evidence suggests how appropriate it is for a 28-d exposure. Other ratios of TOC to organism dry weight have been used with this species, ranging from 10:1 to 100:1 [17,65,66]. Even when the density of organisms is standardized to TOC, little consensus exists on which ratio is appropriate, and research is needed to obtain a better understanding of whether or how this affects bioaccumulation in burrowing species. An optimal loading density may not exist, but a range of TOC to organism dry weight ratios should be tested across standard conditions to identify one that provides sufficient carbon/food to prevent significant weight loss and starvation metabolism in the test organisms and facilitates easy retrieval of organisms from the volume of sediment used. Not only is it important to standardize loading density between the sediments tested for a particular species, but using a common approach for multiple species will allow for more improved interspecies comparisons.

Purging of the gut. Contaminants associated with sediment remaining in the guts of organisms potentially leads to artificially high estimates of tissue concentrations. Purging organisms in clean conditions before analysis is a means of reducing or eliminating this bias. However, depuration and metabolism of compounds can occur during purging, leading to an underestimate of tissue concentrations. A number of errors associated with gut sediment and purging are summarized in the ASTM guidance document (Table 5, p. 1101, in ASTM [29]). Purging of *L. variegatus* in clean sediment has been shown to enhance depuration, possibly leading to the dilution of total body burden with uncontaminated sediment [34,37,64,65]. Increased depuration in sediments with higher TOC also has been observed in sediment- and soil-ingesting species [67,68]. Purging appears to have less effect in fish than invertebrates because of the smaller contribution of gut sediment to total body weight [23].

Organisms were purged in more than 70% of the studies we reviewed, half of which purged for approximately 24 h (Fig. 1). Purging times varied from 6 h [20] to approximately 24 h [17,27] or 48 to 72 h [21,23]. Brooke et al. [69] determined that the inorganic contents of the gut represented approximately 10% of the whole body dry weight in unpurged *H. limbata*, *C. tentans*, and *L. variegatus*, and that these species lost 75, 90, and 100%, respectively, of their gut contents in the first 12 h of water-only purging. Mount et al. [70] evaluated purging of sediment in *L. variegatus* and found that only 6 h were required to eliminate more than 98% of gut contents. As a result, a 6- to 8-h purge for *L. variegatus* is recommended in the ASTM [29]

and U.S. EPA [16] methods. Many of the compounds of interest in bioaccumulation studies have log K_{OW} values greater than 5, in which case Mount et al. [70] predict that 90% accuracy in estimates of tissue concentrations, with little or no bias from gut contents, would be observed in purges as long as 24 h. The selection of a purging period may need to consider the accuracy of estimates and the contaminants being measured. As an alternative to purging, corrections for the contribution of gut contaminants to total body burden can be applied using the mass of gut content and concentration of the contaminant in sediment (suggested by Chapman [71] and Neumann et al. [72]).

A number of situations that arise when purging may not be necessary or should not be conducted. These include laboratory–field comparisons, or using bioaccumulation data to determine trophic transfer of contaminants. Under environmental conditions, a predator eats the whole prey and is therefore exposed to contaminants associated with gut sediments. Purging is also not recommended with low-molecular-weight compounds, such as PAHs, which may be quickly depurated [29].

Experimental controls and treatment of data

Control, reference, or pre-exposure conditions. The objective of a bioaccumulation test is to quantify the accumulation of contaminants in organisms exposed to sediment. However, potential for error exists from uncharacterized contamination. Before conducting any bioaccumulation or toxicity test, one must measure the background concentrations of potential contaminants. This may include analysis of water, food, sediment, or substrate used in the culturing and holding of organisms. Analysis of a sample of pre-exposure organisms incorporates all of these routes of exposure to an extent and may be sufficient as a routine analysis when conducting bioaccumulation tests, particularly when the culturing sources (of water, food, substrate) are relatively consistent.

The use of control sediment that ideally contains no or low concentrations of the compounds of interest and supports good survival and growth of test organisms is necessary to assess the extent of bioaccumulation from contaminated test sediment. Comparison of tissue residues between control and pre-exposure organisms provides an indication of the functioning of the test system and whether contamination has occurred. A reference sediment also may be used in addition to a control treatment. Reference sediment is typically collected in a similar location as the test sediment and is meant to reflect its physical characteristics while having low concentrations of the contaminants of concern. Comparison with reference conditions is often used in toxicity tests when the approach is to permit no further degradation at a particular site or area of concern [29]. Approximately 40% of the studies we reviewed did not use a control treatment (Fig. 1), although half of these measured bioaccumulation from pre-exposure or reference conditions. In some circumstances, comparison with only pre-exposure organisms could be suitable; however, this does not assess the functioning of the test system.

Assay endpoints. Bioaccumulation is typically the only ecologically significant endpoint investigated in laboratory bioaccumulation methods, because other tests with more sensitive species and endpoints have been designed to assess the toxicity of sediment [13–16]. Although the species used in bioaccumulation methods are often selected for their relative tolerance of contaminants, many field-contaminated sediments still have the potential to elicit moderate to high toxicity to these organisms. This has prompted the recommendation to conduct a short-term test to screen the toxicity of a sediment before use in a

bioaccumulation test [16,29]. Even if toxicity is not observed in this preliminary test, survival and growth are still important to measure in definitive bioaccumulation tests for quality control purposes and to assist in the interpretation of bioaccumulation data. Mortality and signs of stress, including loss of biomass and avoidance or lack of burrowing in sediment, may indicate altered exposure, thereby affecting the estimate of bioaccumulation. Mortality or stress occurring in control exposures indicates that the health of test organisms may have been compromised initially or that the test system was contaminated. Bioaccumulation was the only endpoint measured in approximately 40% of the studies we reviewed. Of the remaining studies, 14% also measured survival, 5% measured growth, and 40% measured both survival and growth of organisms. Regardless of the test objectives, survival and growth should be considered essential measures with regard to quality control and should be included in bioaccumulation tests.

Reporting of data. Various analytical methods have been used to determine the concentration of a compound, on a wet or dry weight basis, in tissue samples. The reporting of results on a wet or dry basis without a conversion factor, or an indication of which is used, makes it very difficult to compare results between studies. The studies we reviewed were equally split in whether concentrations in tissue were normalized to wet or dry weight; however, only two studies actually reported a conversion factor or water content of the organisms. Regardless of whether reported data has been adjusted from the analytical results, reporting whether the data are normalized to wet or dry weight and including a conversion factor opens up the opportunity for broader comparisons between bioaccumulation studies.

One method of reporting and analyzing bioaccumulation data is to calculate a ratio of the concentration of the contaminant in the organism relative to the concentration in the sediment. Ankley et al. [24] commented that these sediment-based bioaccumulation factors had been named inconsistently, with terms such as bioavailability index, accumulation factor, and biota-sediment factor being used. They proposed using a new term, the biota-sediment accumulation factor (BSAF), to be most analogous with the water-based bioaccumulation factor (BAF). However, the common practice of expressing bioaccumulation from sediment has led to the term BSAF incorporating normalization to lipid and organic carbon, while still referring to non-normalized ratios as BAFs [16,29,73]. The formal definition of a BAF is a ratio of the concentration of a contaminant in the tissue of an organism relative to that in water when the organism is exposed through water and food [30,74]. Technically all accumulation factors that relate bioaccumulation to the concentration of a contaminant in sediment are BSAFs and should be referred to as such. Part of the reason for treating these separately in the past may have to do with developing sediment quality guidelines using equilibrium-partitioning theory, in which it was assumed that the major route of uptake in benthic invertebrates was through pore water. However, bioaccumulation of contaminants through diet and the ingestion of sediment has been shown to have an equal or even greater contribution than pore water in many benthic species [75,76]; therefore, BSAF is now more appropriate. Most of the studies we reviewed did use the term BSAF; however, some inconsistency may be found in the literature, even within and between U.S. EPA documents [16,30].

In its simplest form, a BSAF is calculated by dividing the concentration of a contaminant in tissue by that in the sediment. It is preferably determined using dry weight concentrations, but these units must still be reported [29]. This form of the ratio is

typically used for metals [77–79] and contaminants other than nonionic organic compounds. Many nonionic organic compounds preferentially partition into lipids; hence, normalizing concentrations of contaminants to lipid concentration in the tissue is the accepted practice to reduce the variability both within and between species. The concentration of TOC in sediment also may influence the bioavailability of contaminants through sorption/desorption, causing increased variability in BSAFs between sites. Many researchers now calculate a BSAF in which the concentration of the organic contaminant is normalized to both lipid and sediment organic carbon [37,44,54]. The resulting BSAF has the units organic carbon/lipid and is theoretically independent of species or sediment type. Regardless of whether BSAFs are determined using normalized concentrations, this information must be specified and cannot be assumed based on the contaminant of study.

Conclusions and research needs

Bioaccumulation is now becoming an important and routine part of environmental assessments. Guidance on conducting a bioaccumulation test with the oligochaete *L. variegatus* is offered by both ASTM [29] and U.S. EPA [16]. However, as noted in the ASTM and U.S. EPA documents and discussed in the current review, procedures for measuring bioaccumulation require further standardization. Even with guidance documents available, great variation is found in the techniques of exposure (duration, renewal conditions, and loading) used in various studies. These differences, even with a particular species, potentially have significant implications regarding the estimation of bioaccumulation. Many of the studies we reviewed sufficiently specified test conditions and procedures; however, a number of studies cannot be considered reproducible, because they have omitted information regarding size of organisms and volumes of sediment or water. Even when test conditions are specified, they are rarely justified. As has been suggested (in ASTM [29]), most exposure techniques for the assessment of bioaccumulation are based on those used in successful studies and expert opinions rather than experimental evidence.

Various needs regarding measurement of bioaccumulation and interpretation for the purpose of sediment quality assessment have been discussed and ranked for priority by the U.S. EPA. In terms of methods for assessing bioaccumulation, one of the greatest needs is to identify additional species and develop these methods [30]. To date, failure to do this with freshwater species other than *L. variegatus* may be attributable to culturing difficulties, inappropriate length of life cycles, insufficient biomass, sensitivity of the organism, or lack of ecological relevance [33]. An insufficient number of multispecies comparative tests with different sediments and contaminants have been done to adequately assess the bioaccumulation potential of various test species [29]. Another priority is to continue to validate laboratory methods with field data [30] to reduce the uncertainty associated with laboratory-to-field extrapolation and strengthen ecological risk assessments and regulatory decision-making. Ankley et al. [24] commented on the importance of field validation of any test species or exposure regimen before using quantitative estimates in ecological risk assessment.

In addition to those needs specified by the U.S. EPA [30], one of the most important conditions that should be standardized is loading density of the test organisms. Evidence suggests that in bioaccumulation tests, standardizing the loading density to sediment TOC rather than volume is more appropriate. As previously discussed, and observed in

L. variegatus [64], loading density has the potential to affect bioaccumulation and should be examined in more detail. The techniques used to maintain the quality of overlying water also may require further standardization. Uncertainty remains as to whether and how static, flow-through, or renewal conditions affect bioaccumulation in both water-column and burrowing species. Both a specific and a sufficient ratio of sediment to overlying water also need to be selected for standard bioaccumulation methods. Also needed is kinetic information regarding the uptake and elimination of various compounds, to both improve predictive models and evaluate the appropriateness of exposure duration in a test. This information has been generated for a select number of compounds, mostly in *L. variegatus*, and needs to be expanded to include other organisms that are appropriate for bioaccumulation testing (insects, amphipods, fish). Both research-based and applied methods were included in this review, and differences in study objectives could have a strong influence on the methods used. Various regulatory agencies may have different mandates and requirements for their data quality objectives. Even with these differences, employing a standard and effective bioaccumulation method will allow for greater sharing of data and reduce unnecessary duplication of efforts.

Finally, developing and standardizing a robust and effective method for routinely measuring bioaccumulation requires a number of key components. It includes proper evaluation of the various exposure techniques and conditions, but also the incorporation and use of quality control procedures throughout the process. Development of a method to an accepted set of standards (e.g., International Standard for Organization [80]) can simplify and improve its adoption as a standard. Adequate experimental (and statistical) design and ecological representation are of course important for a standard bioaccumulation method, but the practicality of the method, including cost, time, and effort, will also greatly determine its use and ability to be applied for various purposes.

SUPPLEMENTAL DATA

Table S1. Review criteria of bioaccumulation methods.

Table S2. Summary of important test conditions in reviewed bioaccumulation methods with field-contaminated sediment.

Table S3. Standard methods without bioaccumulation end point.

Supplemental references. (195 KB DOC)

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