Development of a New Bioaccumulation Testing Approach: The Use of DDE as a Challenge Chemical to Predict Contaminant Bioaccumulation

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PURPOSE: This technical note describes the continued development of an alternative approach to bioaccumulation testing. It employs an effects-based approach to assess contaminant bioaccumulation in organisms while limiting the analytical chemistry requirements associated with traditional bioaccumulation tests.

BACKGROUND: Federal regulations (Clean Water Act Section 404(b)1 and MPRSA Section 103) require that biological evaluations be conducted to determine the suitability of dredged material for placement in open water. These biological evaluations include an assessment of the biological effects resulting from the presence as well as the extent of bioaccumulation of the chemical contaminants. Specific regulations (40 CFR § 227.6) require:

> *Bioassay results on the solid phase of the wastes do not indicate occurrence of significant mortality or significant sublethal effects….*

> *…no significant undesirable effects will occur due either to chronic toxicity or to bioaccumulation of [contaminants of concern].*

Short-term and longer-term toxicity tests and bioaccumulation tests have been developed and are currently used to assess the suitability of dredged sediments for aquatic placement as required by the CWA ("Inland Testing Manual," U.S. Environmental Protection Agency (USEPA) 1998) and MPRSA ("Ocean Testing Manual," USEPA 1991). However, these testing approaches have limitations and undesirable characteristics. Short-term toxicity tests may under predict the "real" biological effects associated with a contaminant present in sediment. Longer-term toxicity tests address chronic effects, but must be conducted for 28 days or longer. Due to the long exposure duration and personnel time, these tests typically cost greater than $5,000 per sample. Bioaccumulation tests address the requirement to assess the potential for the contaminants to bioaccumulate in organisms. However, these tests have similar challenges to the chronic test (i.e., time and cost). Bioaccumulation tests also have additional cost due to the expensive analysis of small quantities of tissue for a large number of contaminants. Furthermore, the interpretation of bioaccumulation test results is often complex and may require statistical modeling and probabilistic risk assessment techniques.

In order to improve the quality of the assessment of bioaccumulation, an effects-based bioaccumulation test is being developed where the organism is exposed to a "challenge" chemical during a traditional bioaccumulation test. The approach is based on the critical body residue (CBR) theory that non-polar organic contaminants acting via non-polar narcosis (anesthesia) produce acute toxicity when the total tissue concentration of all organic compounds exceeds 2-8 mmol/kg (McCarty and Mackay 1993). This approach assumes the compounds acting by non-polar narcosis act jointly in an additive manner. Knowledge of this mechanism of action may be utilized to

estimate the potential effects associated with the bioaccumulation of non-polar organic contaminants in sediment.

An effects-based method is being developed for determining how close an organism is to a body burden toxicity threshold following exposure to contaminated sediment. The body burden toxicity threshold is the concentration in the tissues of an organism associated with a specific response (i.e., mortality). Proximity to a body residue threshold will be measured using a toxicological challenge or exposure to a known concentration of a second chemical, the "challenge" chemical. CBR theory predicts that for surviving test organisms challenged with a second chemical in a bioassay, the amount of "challenge" chemical required to produce a toxic response would be proportional to the total load of organic contaminants the organism acquired, i.e., amount of "challenge" chemical plus compounds acquired in the original bioassay (Van Wezel et al. 1996). However, when the relative potencies of the two compounds differ and strict mass additivity (moles of A plus moles of B) does not describe the dose response, response additivity, can be determined using a toxic unit approach. Toxic units (TU) are a means of expressing the toxicity of a mixture of compounds as a portion of its threshold effect concentration (Sprague 1970). The toxicity of a mixture of compounds is expressed as a sum of the ratios of the exposure (water or sediment concentration, tissue concentration) and threshold effect concentrations (LC_{50}, lethal body burdens, LR_{50}) of the individual chemicals in the mixture (Equation 1).

Equation 1. Toxic unit equation

$$SumToxicUnits = \left(\frac{ConcentrationX}{LR_{50}X} \right) + \left(\frac{ConcentrationY}{LR_{50}Y} \right)$$

If compounds in a mixture are acting additively, the sum TU required to result in 50-percent mortality of the exposed population will equal one. However, if sum TU is greater than one, then the compounds will result in a less than additive effect. Conversely, if sum TU is less than one, the mixture would be expected to have an effect that was greater than additive. Measuring the TU of the "challenge" chemical will provide an indicator of the level of accumulation of organic chemicals associated with dredged material. Therefore, the closer an organism's body burden of toxicants is to a toxicity threshold, the smaller the amount of "challenge" chemical required to produce an effect from the challenge, resulting in a lower TU of the "challenge" chemical.

Such a challenge could be integrated into any long-term exposure (i.e., chronic toxicity or bioaccumulation tests) to determine if organisms exposed to dredged material bioaccumulated significantly more contaminant than reference exposed organisms by comparing the amount of challenge chemical required to produce a response in the two test groups. This effects-based challenge simultaneously answers two questions: 1) did organisms bioaccumulate significant amounts of contaminants, and 2) what is the potential for adverse effects from that bioaccumulation. The fact that these questions are addressed without the need to analyze tissues for a wide range of contaminants means that this method can be used as a cost-effective screen for both potential effects and bioaccumulation.

CURRENT INVESTIGATION: Previous studies in this research effort focused on the selection and characterization of the chemical used for the challenge. Initially these efforts focused on the chemical pentachlorobenzene (PCBZ). The kinetics and toxicity of pentachlorobenzene were assessed using a freshwater (*Hyalella azteca*) and marine amphipod (*Leptocheirus plumulosus)*. The results of these studies demonstrated the additive toxicity of PCBZ with other organic chemicals (pyrene). However, one disadvantage for using PCBZ was the high volatility of the chemical (vapor pressure 0.001 mmHg). As a result, PCBZ was rapidly lost from the bioaccumulation exposures and was difficult to recover during chemical analysis.

The second year of the research effort focused on selecting a more suitable chemical for use in the challenge and developing the appropriate exposure approach. The challenge chemical selected for use in the effects-based approach was DDE (1,1-dichloro-2,2-bis(chlorophenyl) ethylene). DDE has the ideal characteristics outlined by Steevens and Landrum (2002) of an appropriate challenge compound, which includes the following: 1) the compound acts by non-polar narcosis (anesthesia), 2) the compound is not biotransformed by the organism of the study, 3) the compound has sufficient water solubility to produce mortality within its aqueous solubility limit, 4) the compound has a relatively high log K_{ow} to permit substantial bioaccumulation, and 5) the compound has a low vapor pressure so it is not highly volatile. For development of the effects-based approach in the saltwater environment the estuarine amphipod, *L. plumulosus*, was chosen for the study organism. *L. plumulosus* was selected because it is routinely used for dredged material evaluations for marine sediments (USEPA 2001).

Three approaches were evaluated for integration of the challenge chemical within the currently used bioaccumulation test design. The approaches considered must expose the organism to the challenge chemical before the organism has eliminated the contaminants bioaccumulated from the test sediment. Furthermore, the approach should be easily adapted within the existing test methods. The three challenge approaches, outlined in Figure 1, include exposure to the challenge chemical in the overlying water during exposure to the sediment (approach 1), exposure to the challenge chemical in water after exposure to the sediment (approach 2), and exposure to the challenge chemical spiked directly into the test sediment (approach 3).

The first approach evaluated was to deliver the challenge chemical through the overlying water with sediment present as a substrate for the organisms. Organisms, *L. plumulosus*, were exposed to DDE in the water for 96 hr without any additional chemicals added to the sediment. DDE was delivered through the overlying water with a dose range approaching water solubility limits (5, 25, and 50 µg/L DDE). Survival of the organisms and bioaccumulation of DDE was assessed after 24, 48, and 96 hr. Significant mortality was not observed at any of the doses. Furthermore, tissue analysis indicated the organisms did not bioaccumulate significant quantities of DDE (< 0.1 µmol/g). The poor water solubility and high lipophilicity of DDE (log K_{ow} 6.51) suggests that the mass of the organic carbon contained in the sediment (1.38 percent total organic carbon) outcompetes the organism for the compound. As a result, the DDE was sequestered rapidly in the sediment and unavailable to the test organisms. Therefore, it was concluded that exposure to the challenge chemical through addition to overlying water was not appropriate because the challenge chemical is sequestered in the sediment and results in limited exposure of the organisms to the challenge chemical.
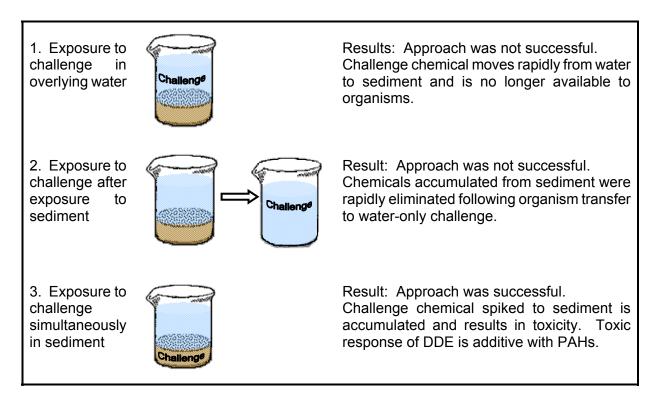
1. Exposure to challenge in overlying water		Results: Approach was not successful. Challenge chemical moves rapidly from water to sediment and is no longer available to organisms.
2. Exposure to challenge after exposure to sediment		Result: Approach was not successful. Chemicals accumulated from sediment were rapidly eliminated following organism transfer to water-only challenge.
3. Exposure to challenge simultaneously in sediment		Result: Approach was successful. Challenge chemical spiked to sediment is accumulated and results in toxicity. Toxic response of DDE is additive with PAHs.

Figure 1. Approaches tested for exposure to challenge chemical

A second challenge approach tested the method of challenging the surviving organisms removed from the sediment at the end of a bioaccumulation test. This approach combined a 10-day sediment exposure to a PAH spiked sediment, using fluoranthene (FLA) as the model PAH, followed by a 48-hr aqueous exposure to a single concentration of DDE (75 µg/L). The concentration of DDE was determined from previous experiments and at a concentration where mortality would be expected if the organisms were exposed to other organic chemicals (i.e., FLA). At the end of the exposure no mortality was observed, even in treatments where organisms were exposed to high concentrations of fluoranthene in the sediment. The low mortality observed with the DDE challenge is likely the result of rapid elimination of the fluoranthene from the sediment bioaccumulation test. Previous studies have demonstrated the half-life of PAHs to range from 0.5 to 5.5 hr in freshwater amphipods (Lee, Landrum, and Koh 2002). Therefore, it is likely the organisms eliminated the accumulated FLA within 18-22 hr of removal from the sediment. Furthermore, the rate of DDE uptake is sufficiently slow, ranging from 491-653 ml/mg/hr, suggesting that equilibrium would not be reached during the short exposure time (24-48 hr) after the bioaccumulation test (Lotufo, Landrum, and Gedeon 2000). Therefore, the rapid elimination rate of FLA combined with the slow uptake rate of DDE eliminated this approach for delivering the challenge chemical.

The third approach that was evaluated delivered the challenge chemical to the organisms simultaneously during the bioaccumulation test. Sediments spiked with DDE and *L. plumulosus* were exposed to the sediment for 10 days. At the end of the exposure, surviving organisms were counted and the concentration of DDE in their tissues was determined. Using this approach, DDE was found to be bioavailable to the organisms, resulted in a dose-dependent toxic response, and could be measured in the tissues of the organisms. Therefore, this third approach will be used for

exposure to the challenge chemical and in further development of the bioaccumulation challenge design. The remainder of this technical paper addresses additional experimentation of the combined exposure approach and future implications of this method of challenge chemical delivery.

DEVELOPING THE CHALLENGE APPROACH: Following the identification of the appropriate exposure design, the next goal of the research effort was to address two main questions:
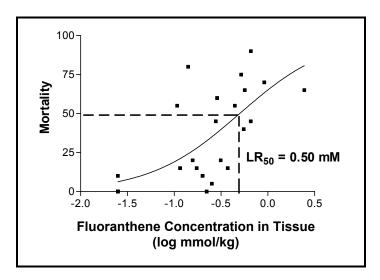
1. Are the chemicals acting additively? If the two chemicals are acting additively, the concentration of unidentified organic contaminants bioaccumulated from the test sediment can be predicted. This must be demonstrated by evaluating the toxic response (mortality) associated with the concentration of DDE and the model PAH, FLA, that is bioaccumulated.

2. What concentration of DDE in the tissue of *L. plumulosus* is necessary to cause an increase in mortality when organisms are exposed to the model PAH, FLA, in sediment? The result of the experiment should provide valuable information for developing the practical aspects of the bioassay design such as the DDE sediment spiking concentration and sensitivity of the bioassay.

Experimental Design to Test Additivity and Challenge Approach. To address these two questions, an experiment was designed to expose *L. plumulosus* to DDE and FLA in a 10-day sediment exposure. The purpose of the experiment was to determine if DDE (challenge chemical) and FLA (model PAH) would act additively in a 10-day mixture experiment and to determine the concentration of DDE resulting in a significant difference in survival associated with uptake of FLA. The experiment was conducted with juvenile *L. plumulosus* exposed to uncontaminated Sequim Bay sediment (Sequim Bay, Washington, USA) spiked with a range of 20 mixture treatment concentrations of DDE and FLA. To simplify the chemical analysis ^{14}C-DDE and ^{3}H-FLA radiolabeled chemicals were used. Ranges of DDE and FLA were selected based upon preliminary experiments conducted to determine the level of contaminant required to be available to organisms in a sediment exposure and to cause an effect associated with a detectable level of accumulated compound. Sediments were spiked with DDE and FLA following the treatment design shown in Table 1. At the completion of the 10-day experiment, survival was assessed and surviving organisms were analyzed using Liquid Scintillation Counting (LSC).

Table 1
Treatments for Combined DDE and Fluoranthene Experiment

DDE Treatment (µg/g)	Fluoranthene Treatment (µg/g)				
	0	**50**	**100**	**150**	**200**
1,500	1,500 DDE 0 FLA	1,500 DDE 50 FLA	1,500 DDE 100 FLA	1,500 DDE 150 FLA	1,500 DDE 200 FLA
1,000	1,000 DDE 0 FLA	1,000 DDE 50 FLA	1,000 DDE 100 FLA	1,000 DDE 150 FLA	1,000 DDE 200 FLA
500	500 DDE 0 FLA	500 DDE 50 FLA	500 DDE 100 FLA	500 DDE 150 FLA	500 DDE 200 FLA
0	0 DDE 0 FLA	0 DDE 50 FLA	0 DDE 100 FLA	0 DDE 150 FLA	0 DDE 200 FLA

Results of 10-Day DDE and Fluoranthene Experiment. In the bioaccumulation experiment, the contaminant residue accumulated in organisms resulting in 50-percent mortality (lethal residue, LR_{50}) was calculated. The lethal residue is calculated by log transformation of the tissue residues (log mmol/kg) from live organisms associated with the specific effect (mortality) using nonlinear sigmoidal dose response curve analysis. In the combined exposure experiment, the LR_{50} for both FLA (0.50 mmol/ kg, 0.28 - 0.90 95-percent CI) (Figure 2) and DDE (1.42 mmol/ kg, 0.006-322 95-percent CI) (Figure 3) were calculated. As a result of spiking inconsistencies at the higher doses of DDE, large variability in the higher treatments (1,000 and 1,500 µg/g) were observed, creating a larger-than-optimum 95-percent confidence interval for the LR_{50} value calculated. To further characterize the dose response, the DDE exposures will be repeated in future experiments.

Figure 2. Dose response curve generated from log transformed fluoranthene residue mmol/kg and percent mortality of juvenile *Leptocheirus plumulosus* exposed for 10 days to fluoranthene
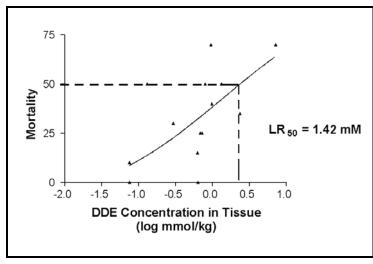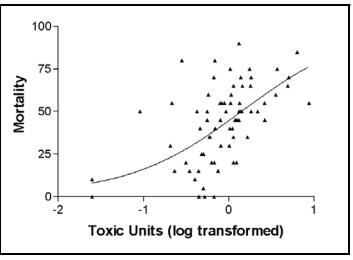

Figure 3. Dose response curve generated from log transformed DDE residue mmol/kg and percent mortality of juvenile *Leptocheirus plumulosus* exposed 10 days to DDE

The relative potency of the two compounds, DDE and FLA, differed by nearly a factor of 3, as demonstrated by the LR_{50} values. Thus, the response additivity was explored using the toxic unit approach. The number of toxic units for each individual data point (replicate) was calculated and analyzed by nonlinear regression analysis (variable slope sigmoid dose-response curve). The sum TU resulting in 50-percent mortality in this data set is 1.47 toxic units (0.50-4.28 95-percent CI) (Figure 4). The sum TU value calculated from the tissue residues is not statistically different from

1.00 so that it can be concluded that DDE and FLA act additively. Additional graphical analysis of the interaction data is shown in the surface response plot of the data obtained from the mixture experiment (Figure 5). The surface response plot was performed through weighted average smoothing. The 50-percent effect boundary represents the LR_{50} estimated in the mixture of DDE and FLA.

Figure 4. Dose response curve generated from log transformed toxic units and percent mortality of juvenile *Leptocheirus plumulosus* exposed for 10 days to a mixture of DDE and fluoranthene
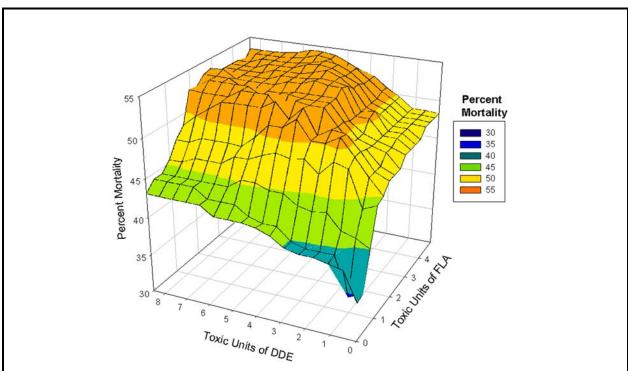
Figure 5. Surface response plot of the toxic units of DDE and fluoranthene versus percent mortality of juvenile *Leptocheirus plumulosus* exposed for 10 days to a mixture of DDE and fluoranthene

The sediment receiving no DDE and the lowest concentration of DDE (500 mg/kg) resulted in a dose-dependent increase in mortality when *L. plumulosus* were exposed to varying concentrations of FLA (Figures 6 and 7). Significant mortality occurred at FLA body residues of 0.507 mmol/kg in

the absence of DDE (Figure 6) and at 0.401 mmol/kg in the presence of 500 mg/kg DDE (Figure 7). Furthermore, less FLA residue was required to yield significantly higher mortality than detected in the 0 mg/kg FLA treatments (Figure 7). The difference in body residue and associated effect is the result of response additivity as predicted from Figure 4. The increase in mortality observed at lower FLA tissue residues is the direct result of the DDE challenge. It is this difference that will be used to exploit the use of a challenge chemical.

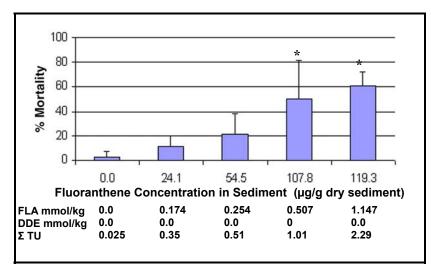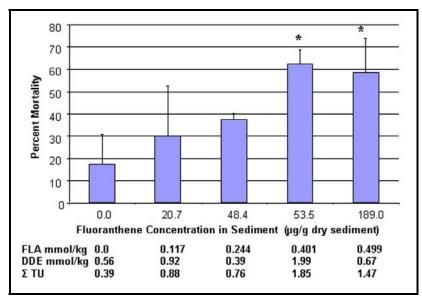|					
FLA mmol/kg	0.0	0.174	0.254	0.507	1.147
DDE mmol/kg	0.0	0.0	0.0	0	0.0
Σ TU	0.025	0.35	0.51	1.01	2.29

Figure 6. Percent mortality in treatments with fluoranthene spiked in the sediment. X-axis represents exposure sediment concentrations of FLA. Below figure are FLA and DDE tissue residues and sum TU (FLA TU + DDE TU) associated with the X-axis exposure concentrations. * Indicate significantly different from control (FLA 0.0 µg/g) p < 0.05 (ANOVA)

Figure 7. Percent mortality in treatments with mixture of approximately 500 µg/g DDE and fluoranthene present in the sediment. X-axis represents exposure sediment concentrations of FLA. Below figure are FLA and DDE residues and sum TU (FLA TU + DDE TU) associated with the X-axis exposure concentrations. * Indicate significantly different from control (FLA 0.0 µg/g) p < 0.05 (ANOVA)

FLA mmol/kg	0.0	0.117	0.244	0.401	0.499
DDE mmol/kg	0.56	0.92	0.39	1.99	0.67
Σ TU	0.39	0.88	0.76	1.85	1.47

PROJECTED APPLICATIONS: The effects-based bioaccumulation challenge approach has been proposed as a means of predicting the potential for contaminant accumulation from sediments potentially contaminated with unknown contaminants, particularly non-polar organic chemicals. By combining the theory of non-polar organic mediated narcosis and additivity of compounds acting via similar mechanisms of toxicity, it is believed that the effects-based mixture approach can replace the traditional bioaccumulation tests presently conducted following toxicity tests. The effects-based approach addresses both endpoints required by Federal Regulations: Is there potential for effects? And what is the bioaccumulation potential of the contaminants present? The combined mixture

approach will also be evaluated for its capability to assess the significance of bioaccumulation of additional classes of compounds such as pesticides, metals, and PCBs.

A potential approach for the utilization of the bioaccumulation challenge test would include several decision points to determine the potential for significant bioaccumulation (Figure 8). The effects-based bioaccumulation test could be conducted by exposing organisms within the scope of a traditional 28-day bioaccumulation test. In addition to the reference sediment and test sediment, an additional set of test sediments would be included for the challenge determination. After 18 days of exposure, organisms from the additional set of test sediments would be recovered and transferred into the same sediment that has been spiked with various concentrations of the challenge chemical. Transferred organisms would be exposed to the material for an additional 10 days. At the end of the 28-day exposure (18 days + 10 days), survival would be assessed and the concentration of the challenge chemical would be measured. Results of the analysis in combination with the demonstrated knowledge of additivity (in the current study) would be used to quantify the toxicity associated with the unknown chemicals (toxic units) bioaccumulated from the test sediment. If the quantity of unknown chemical is less than 0.2 TU, or indistinguishable from background, it is unlikely the toxicity is the result of bioaccumulated contaminants. If the quantity of unknown chemical is greater than 0.2 TU, then tissue from the traditional bioaccumulation test should be collected and the risk associated with those body residues should be assessed.

NEXT STEPS: The demonstrated additivity in the present study will be further evaluated with additional characterization of the challenge chemical (DDE). By properly assessing the effect of DDE individually we will be able to evaluate the toxic units of FLA contributed to the body burden and resulting in mortality in a mixture treatment design. At the low body burden residues of FLA, organisms are expected to require more toxic units of DDE to elicit an effect. Conversely, at higher concentrations, fewer toxic units of DDE are expected to result in an equivalent effect. Overall, in the challenge approach the shift in the dose response curve suggests that less DDE accumulation is required to result in an equivalent effect than required when organisms are exposed to DDE individually.

Long-range goals of this effort include additional characterization of the bioaccumulation challenge approach. In addition to the spiked sediment studies, the demonstrated additivity in the present study will be further evaluated with field-collected sediments that are contaminated with a mixture of PAHs. The combined mixture approach will also be used to assess significance of bioaccumulation of additional classes of compounds such as pesticides, metals, and PCBs. In addition, it will be necessary to evaluate the effectiveness of the approach with a variety of sediment characteristics and the potential for sediments to interact with the challenge chemical.

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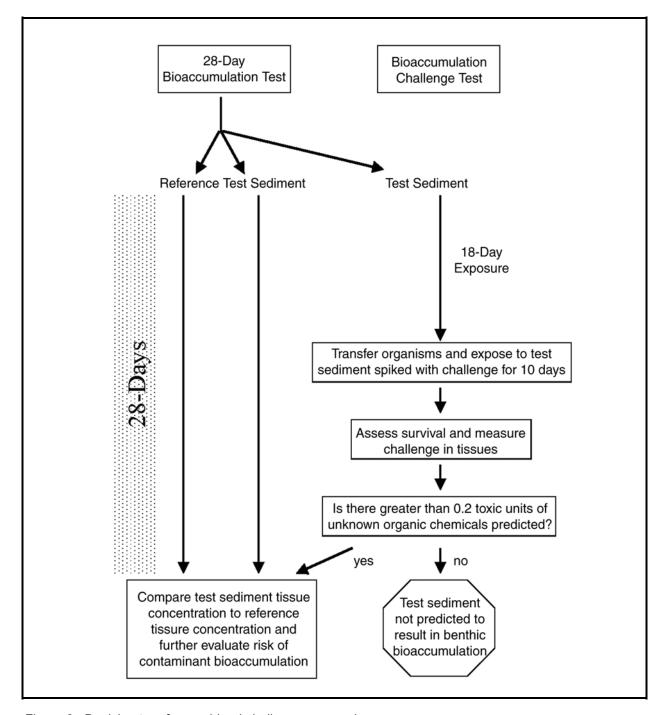

Figure 8. Decision tree for combined challenge approach

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