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Development of a Biological-Effects-Based Approach to Assess the Significance of Contaminant Bioaccumulation

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PURPOSE: This technical note describes the development of an alternative approach to evaluate chronic toxicity and the significance of contaminant bioaccumulation in dredged material assessments. It describes potential approaches and outlines the experimental progress of a project focusing on an effects-based approach to assess bioaccumulation.

CURRENT APPROACH TO ASSESS SEDIMENT TOXICITY AND BIOACCUMULATION: Federal regulations (Clean Water Act 404b1 and Marine Protection Research and Sanctuaries Act 103) require that biological evaluations be conducted to determine the suitability of dredged material sediment for placement in open water. These biological evaluations include an assessment of the biological effects resulting from the presence of chemical contaminants as well as an assessment of 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].

Guidance to implement the regulations for biological evaluations is offered through two documents, the "Inland Testing Manual" (U.S. Environmental Protection Agency (USEPA) 1998) and the "Ocean Testing Manual" (USEPA 1991). These documents outline a tiered process whereby the sediments are evaluated using, in order of complexity, background and historical data, analytical chemistry and screening methods, toxicity and bioaccumulation tests, and risk assessment. In the third tier, the biological tests are conducted to assess the toxicity and bioaccumulation of contaminants in dredged sediments. Acute toxicity associated with the sediments is assessed using two different species of organisms exposed to sediment for a period of 10 days. Endpoints in these tests include survival and growth of the organisms. In addition to the toxicity tests, bioaccumulation tests are conducted using two different species of organisms for a period of 28 days to assess the bioavailability and concentration of contaminants in the proposed dredged sediment. At the end of the exposure period, organisms are collected and tissues are analyzed for the contaminants of concern.

Results of the toxicity and bioaccumulation tests are used to determine the suitability of the dredged sediments for aquatic placement. Toxicity and bioaccumulation results for the proposed dredging site and a reference site are compared to make this determination. Sediments proposed for dredging

are considered to have an unacceptable potential for adverse effects if statistical analysis reveals significant toxicity compared to the reference site and the difference is a magnitude of at least 10 percent. Results of the bioaccumulation test are compared for statistical significance and then interpreted based on several factors listed in the guidance manuals. These factors include the toxicological importance of the chemical, potential for biomagnification, magnitude of bioaccumulation compared to reference, and the number of chemicals that were observed in the test organisms. Very often, these factors are used subjectively to make regulatory decisions when the bioaccumulation test provides no clear evidence.

Chronic, long-term tests may be used when acute toxicity and bioaccumulation tests do not provide clear evidence regarding the potential for adverse effects associated with sediment. Chronic toxicity tests are typically conducted for 28 days or longer and involve the assessment of sublethal endpoints such as growth and reproduction of the test organisms. Endpoints such as growth and reproduction are considered more sensitive to contaminant exposure than acute effects on mortality. Currently several tests are available for chronic toxicity testing including the *Hyalella azteca* 42-day test, the *Chironomus tentans* life-cycle test (USEPA 2000), and the *Leptocheirus plumulosus* 28-day test (USEPA 2001).

Short-term, chronic, and bioaccumulation tests, as described above, have limitations in regard to making regulatory decisions and demonstrating that a sediment will not cause undesirable effects. Acute toxicity tests evaluate adverse effects associated with a short-term exposure that are measured as dramatic differences in mortality associated with exposure to the sediment. These acute toxic responses may underpredict the "real" long-term biological effects associated with a contaminant present in sediment. Chronic toxicity tests address the long-term exposure and associated effects (i.e., changes in growth and reproduction) but must be conducted for 28 days or longer. Due to the long exposure duration and personnel time, these tests typically cost more than $1,000.00 per sample. Bioaccumulation tests address the requirement to assess contaminant bioavailability and 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 have additional cost due to the expensive analysis of small quantities of tissue for a large number of contaminants. Futhermore, the interpretation of bioaccumulation data is subjective and small differences in tissue concentrations are difficult to interpret (U.S. Army Corps of Engineers (USACE) 1996).

APPROACH TO IMPROVE THE ASSESSMENT OF DREDGED MATERIAL: The existing approaches are amenable to further development to improve the quality of bioaccumulation assessment and the potential effects associated with contaminant exposure. Improved approaches include reductions in time and effort while providing more accurate and precise estimates of the toxicity and bioaccumulation of dredged material. Several modifications have been suggested to improve the existing methodology. One approach is to include multiple organisms in a single test chamber so as to reduce the efforts associated with a toxicity test (Ho et al. 2000). This approach uses the standard sediment bioassay with the exception of multiple species present in a single test chamber. This approach minimizes the quantity of sediment necessary and provides toxicity endpoints for several organisms from a single bioassay. A second approach is to combine the chronic toxicity test and bioaccumulation test. Combining these tests would provide valuable information regarding the toxicity associated with a long-term exposure while assessing bioaccumulation. A challenge with this approach is to select an organism that will respond to toxicant exposure but

provide an estimate of contaminant bioavailability and bioaccumulation. A third approach employs an effects-based bioaccumulation test 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 narcosis produce acute toxicity in a broad range of aquatic taxa when the total concentration of all organic compounds present in tissue exceeds 2-8 µmol/kg (McCarty and Mackay 1993). This approach assumes that the compounds acting by this mechanism of toxicity act jointly and in a dose-additive fashion. 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. The remainder of this technical note will focus on the last approach.

Narcosis is the general and nonspecific depression of neuronal activity, produced by a number of physical and chemical agents, usually resulting in stupor.

(Stedman's Medical Dictionary (Dirckx 1987))

EFFECTS-BASED BIOACCUMULATION TEST: Research focusing on the CBR approach has demonstrated that toxicity in aquatic organisms is observed when the load of bioaccumulated contaminants exceeds specific body residues. For example, non-polar organic contaminants (i.e., PAHs and PCBs) acting via narcosis produce acute mortality in a broad range of aquatic taxa when the total molar concentration of all non-polar organic compounds in tissue exceeds 2-8 µmol/kg. Knowledge of this dose additive mechanism of toxicity can be employed to assess the significance of contaminant concentrations in an organism's tissues during a bioaccumulation test. CBR theory and the knowledge of dose additivity predicts that for surviving test organisms challenged during or at the conclusion of a bioassay with a known chemical, the amount of challenge chemical required to produce a toxic response would be proportional to the total load of non-polar organic contaminants the organism acquired, i.e., amount of challenge chemical plus compounds acquired in the original bioassay (van Wezel et al. 1996). This approach is shown in Figure 1. The closer the contaminant concentration in the tissue of an organism is to the toxicity threshold, the smaller the amount of challenge chemical required to produce an effect from the challenge. Given

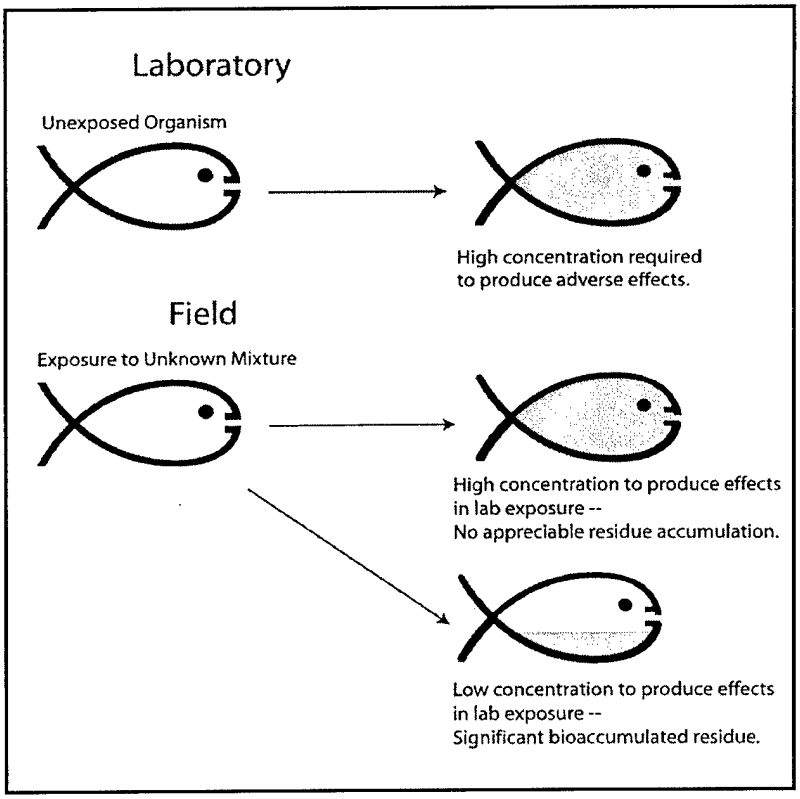

Figure 1. Approach to assess contaminant bioaccumulation (adapted from A. van Wezel (1995))

that such a relationship between toxicity and residue levels exists, an effects-based method for determining how close an organism is to a body burden toxicity threshold following ex-posure to contaminated sediment could be developed. The proximity to a body residue threshold could be determined using a toxicological challenge. This challenge would be applied to test organisms when exposure and bioaccumulation are insufficient to produce toxicity. Such a challenge could be implemented during or after a chronic exposure (i.e., toxicity or bioaccumulation tests) to determine the biological significance of the contaminants accumulated by the organisms in test sediment compared to the reference sediment. This effects-based challenge simultaneously answers two questions: (a) did organisms bioaccumulate biologically significant amounts of non-polar organic contaminants, and (b) 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.

Several approaches could be considered to deliver the challenge chemical to aquatic organisms exposed in sediment (Figure 2). These include delivery of the challenge chemical by spiking the sediment prior to conducting the test, adding the chemical to the overlying water during the test, delivery to the experimental chamber via food laced with the challenge chemical, or removal of the organisms at the end of the experiment and exposing them for less than 48 hr to the challenge chemical. Challenge compounds have been delivered through water-only and spiked sediment exposures. In addition, recent studies have demonstrated that even very water-insoluble compounds such as hexachlorobiphenyl can be delivered in toxic concentrations through dietary exposure to aquatic organisms resulting in mortality and chronic toxicity (Hwang, Fisher, and Landrum 2001). The difference in delivery methods can have significant consequences on the rate and extent of challenge chemical delivery to the organism. Therefore, delivery methods will be chosen based on the specific question addressed and whether acute or chronic effects are the endpoint of the challenge.

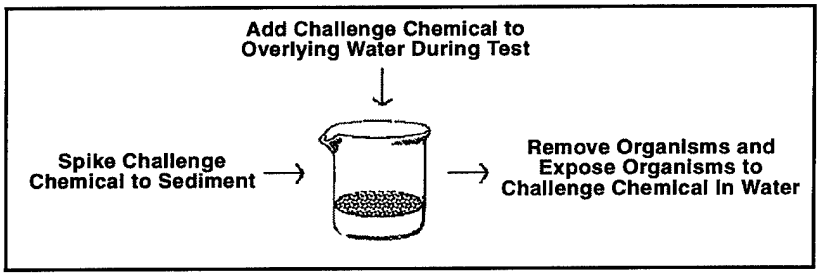

Figure 2. Delivery approached to assess contaminant bioaccumulation

Currently a work unit funded through the Long-term Effects of Dredging Operations (LEDO) Program is focusing on the proof of concept, development, and validation of the approach described above. The project, in the first year, focused on the identification and toxicological assessment of a potential challenge chemical using two model organisms. The proof of concept was assessed through the determination of a CBR for the model compound and demonstration that the model organisms would respond at concentrations predicted by chemicals acting through non-polar narcosis. In addition, methods for toxicant delivery were assessed. Results of the first year of the project are described below.

CURRENT INVESTIGATIONS: The effects-based approach to assess bioaccumulation requires the identification and toxicity characterization of a challenge chemical. Characteristics of an ideal challenge chemical include: (a) the chemical should act through a narcosis mechanism, (b) the

Challenge Chemical Properties:
- **Acts through narcosis mechanism**
- **Not readily metabilized**
- **Toxic within water solubility limits**
- **Bioaccumulates in organisms**
- **Has low volatility**

chemical should not be readily metabolized by the organisms of study, (c) the chemical should produce a toxic effect within its aqueous solubility limit, (d) the chemical should have relatively high log K_{ow} to permit substantial bioaccumulation, and (e) the chemical should not be volatile. While many compounds fit one or more of the criteria, it is nearly impossible to find one that fits all criteria. After surveying the literature for available compounds, pentachlorobenzene (PCBZ) was chosen for the initial evaluation of the effects-based approach. Based on the chemical structure and properties, PCBZ should act as a non-polar anesthetic, should not be metabolized, and the water solubility (0.55-0.831 mg/L) and log K_{ow} (5.17) indicated that it should be possible to attain sufficient body residues to produce mortality from aqueous exposures. Although it had many of the ideal characteristics, the low vapor pressure of the compound (0.00101 torr) indicated a significant potential for volatilization during the exposure. In addition to the characteristics identified above, it was also important for the purposes of developing this approach that the compound be available in a radiolabeled form. Use of a radiolabeled compound allowed the early developmental work to proceed during the development of PCBZ analytical methodology.

Two aquatic organisms were selected to develop the effects-based approach for bioaccumulation. The freshwater amphipod, *Hyalella azteca,* was chosen because it is routinely used for assessing the toxicity of dredged materials for aquatic disposal and standard methods have been described by the USEPA (2000). *Leptocheirus plumulosus*, an estuarine amphipod, was chosen for comparison purposes to assess the bioaccumulation challenge approach in a saltwater environment. Guidance for the *L. plumulosus* chronic toxicity test used for evaluating dredged material for ocean disposal was published by the USEPA (2001).

Initially, the studies that were conducted to assess the challenge chemical included two 10-day, water-only PCBZ uptake and toxicity experiments and a 28-day PCBZ uptake and toxicity experiment. These studies were conducted to characterize the uptake (K_u) and elimination (K_e) of the challenge chemical as well as establish the tissue concentration at which toxicological effects occur (survival for 10 days and growth for 28 days).

28-day Experimental Results. The 28-day experiment was conducted to assess the chronic toxicity and characterize the uptake and elimination of ^{14}C-PCBZ. Organisms were exposed to concentrations ranging from 10 to 100 µg/L. Exposure chambers consisted of a 200-mL beaker with a small square of cotton gauze in the bottom containing 150 mL of test solution and 10 juvenile *H. azteca*. Approximately 75 percent of the water was changed daily and the organisms were fed 0.25 mL of a mixture of YCT (Yeast, cerophyll, and trout chow). After 7 days, the organisms were transferred to fresh beakers, as there was substantial growth on the walls of the beakers. The concentration of PCBZ was measured by liquid scintillation counting before and after each water change to determine the loss from the water column. The beakers were sacrificed in triplicate on days 1, 2, 4, 7, 10, 17, and 28. Each sampling day, mortality was determined and the amphipods were weighed for growth and the amount of accumulated PCBZ determined by liquid scintillation counting. The concentrations of PCBZ in each matrix were determined based on the calculated specific activities of the stock solutions.

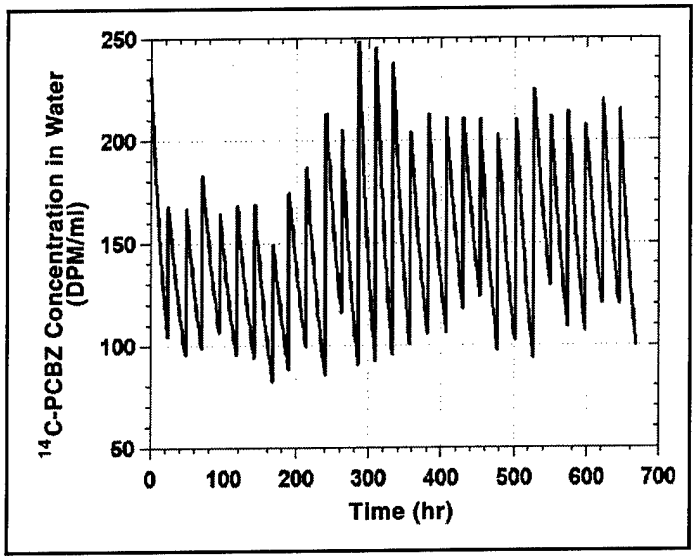

Figure 3. Daily PCBZ concentration in water for 10 µg/L concentration over the 28-day exposure period

No significant mortality was observed at any of the doses ranging from 10 to 100 µg/L. The water concentration varied substantially on a daily basis but the time-weighted average was fairly constant (Figure 3). Toxicokinetics of PCBZ were determined from the initial doses using all of the water data (Figure 4) but kinetics based on the time-weighted average water concentration gave the same values and were simpler to calculate (Table 1). There was considerable variability in the concentration of PCBZ in the individual animals. However, the variability was thought to be due in part to errors and limitations in weighing very small organisms. The toxicokinetics were nearly constant across the doses, resulting in proportional accumulation

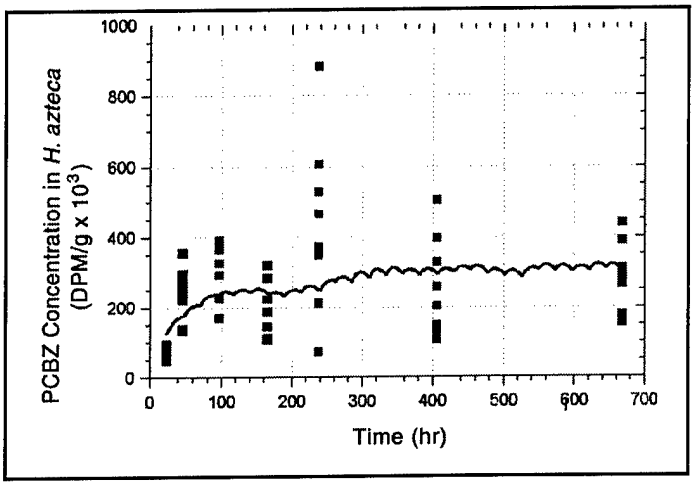

Figure 4. Toxicokinetics for the accumulation of PCBZ by *H. azteca* at the nominal 10 µg/L dose (Note variability in bioaccumulation of individual organisms)

Table 1
Toxicokinetics of PCBZ in *H. azteca* Determined in a 28-day Bioassay

Concentration (µg/L)	k_u (mL/g/hr)	k_e (hr^{-1})
10	44.8 ± 13.1	0.023 ± 0.007
20	57.1 ± 10.3	0.022 ± 0.005
40	43.6 ± 15.3	0.023 ± 0.009
60	40.7 ± 16.0	0.019 ± 0.009
100	35.3 ± 11.0	0.016 ± 0.006

as the dose in the water increased. The only observed toxicity in the 28-day study was a decrease in growth rate compared to the control (Figure 5). The growth rate based on body residue was not as strong a relationship as that based on the concentration in the water due largely to the variability in determining the body residue concentration.

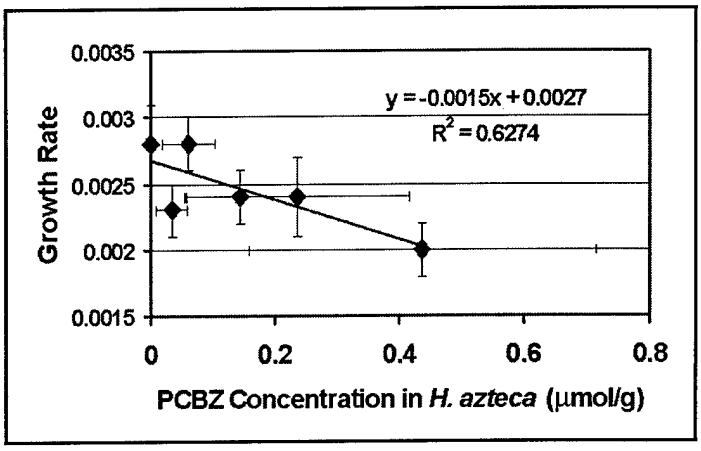

Figure 5. Reduction in growth rate as a function of PCBZ in *H. azteca*

10-day Experimental Results. Two 10-day mortality studies were performed in a manner similar to that of the 28-day study except all of the water was exchanged daily by moving the organisms to fresh exposure media. Mortality was assessed on days 2, 4, 6, 8, and 10. Dead organisms found on the other days were removed and the body residues determined. Due to the substantial difficulty in determining the individual weights of *H. azteca*, the organisms were composited and the PCBZ concentrations in the tissues were determined. Toxicity was determined by logit analysis to estimate the LR_{50} (median lethal residue for a fixed exposure duration) or the LT_{50} (median time to 50-percent lethality for a given exposure concentration). The LR_{50} is calculated from the concentrations in the live organisms at a given exposure concentration and the percent response at that exposure concentration for a fixed exposure time. The mean lethal residue (MLR_{50}) is calculated at the mean body residue in dead organisms for a given dose and is the residue concentration to produce 50-percent mortality at a given LT_{50} (Chaisuksant, Yu, and Connell 1997). If both of these measures of body residue effects are employed to define the toxicity of PCBZ to *H. azteca*, a strong change in body residue with increasing duration of exposure is found (Figure 6).

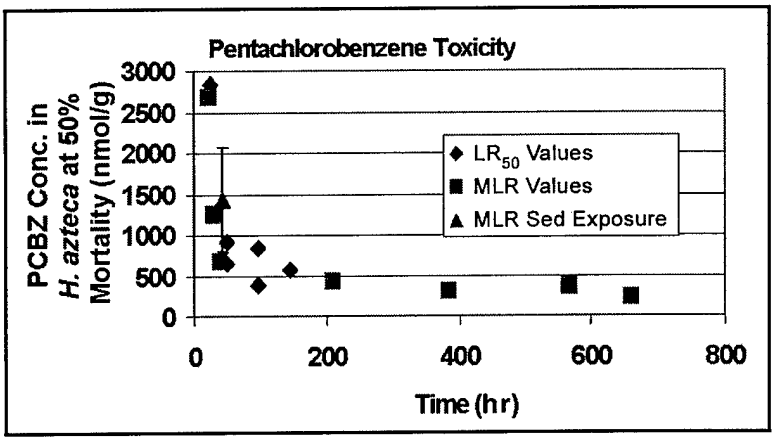

Figure 6. The body residue producing 50-percent mortality with increasing duration of exposure

The estimates of the body residue associated with 50-percent mortality, whether estimated as LR_{50} values or MLR values, yield essentially identical results for the same duration of exposure. Thus, the residue required to produce a given percent mortality in a population is the same whether determined from the PCBZ concentration in live or dead organisms. This is consistent with a dose response relationship where a portion of the population responds while another portion does not for a fixed dose, e.g., at the LD_{50}, 50 percent of the population dies while the other 50 percent survives. This is critical to the challenge approach, since in most tests concentrations will be determined in live organisms. In addition to testing *H. azteca* in water-only exposures, it was important to demonstrate that the challenge approach would work when organisms were in sediment. In a simple

demonstration, *H. azteca* were allowed to burrow into sediment and then the challenge chemical was added to the water and 75 percent of the water was exchanged daily. Since there was only one dose, the LT_{50} was determined and the MLR was measured. The resulting toxic dose on a body residue basis was the same as that observed in water-only exposures.

In the Context of Bioaccumulation Testing: Interpretation of CBR Toxicity Data. Finally, to use the toxicity information for the purposes of a challenge it is necessary to have a predictive model for the time-dependent data. The data in Figure 6 were fit to a damage assessment model that was previously developed at the Great Lakes Environmental Research Laboratory, NOAA. The model assumes first-order kinetics for the accumulation and loss of the contaminant from the organism. This is appropriate for exposures to aqueous solutions where the compound is not biotransformed by the organism as is expected for PCBZ. This is coupled to a damage repair model that assumes damage and repair in the organism are first-order processes. In this case, the damage accrues with increasing concentration of contaminant in the organism and damage is repaired in response to increasing damage up to a critical level where the organism can no longer function. While it is likely that this damage assessment model is somewhat simplistic, it provides a first approach to evaluate the results of the 10-day and 28-day studies. If there exists a critical damage level (D_L) that produces 50 percent mortality, then the LR_{50} has the following relationship:

$$LR_{50}(t) = \frac{D_L / k_a}{\frac{1}{\left(1 - e^{-k_e \cdot t}\right)}} \times \left(\frac{e^{-k_r \cdot t} - e^{-k_e \cdot t}}{k_r - k_e} + \frac{1 - e^{-k_r \cdot t}}{k_r} \right)$$

Where k_r is the rate of damage repair, k_a is the rate of damage accrual, and k_e is the elimination rate constant.

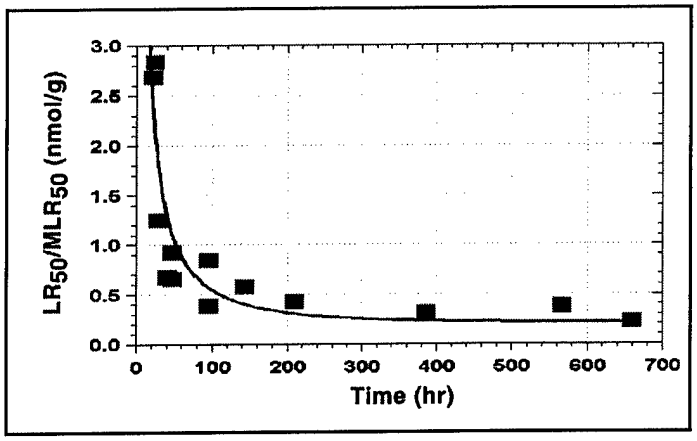

Figure 7. Damage assessment model fit to the accumulated data for PCBZ toxicity to *H. azteca*

Fitting the mortality data generated above provides us with a relationship that describes the data with a coefficient of determination of 0.80 (Figure 7). With this relationship, the challenge exposure can be conducted for the duration that is required to produce a toxic response and the observed data can be compared to the data generated in the absence of a competing contaminant. Sufficient data have been generated that PCBZ can now be tested in *H. azteca* for its use as a challenge chemical when the amphipods are exposed to other compounds. The one complication to the use of PCBZ found during these studies is the volatility of the compound as demonstrated in the variation in the water concentration. While the development of this potential compound will continue for the next year, additional efforts to find a more suitable compound will be pursued.

SUMMARY: Because the challenge method provides information about the amount and toxicological significance of contaminant bioaccumulation without the need for extensive analytical chemistry, this method can be used as an effects-based screen for bioaccumulation testing. Most of the time and cost associated with bioaccumulation testing is due to the extensive chemistry performed on tissue samples at the conclusion of the exposure. Coordinating the challenge with Tier III toxicity testing could eliminate the need for bioaccumulation testing and its associated costs for a measurable portion of test sediments. In addition, the challenge method offers the means to reduce the uncertainty associated with a chemical-specific bioaccumulation assessment. The challenge method, once validated, would provide an effects-based measure of the level of contamination experienced by the test organisms and account for the bioavailability of all organic contaminants in the test sediment. Development of the effects-based approach has the potential for substantial cost and time savings as well as improved assessments of the risks associated with contaminant bioaccumulation.

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