

Contaminant Effects: Investigations on the Utility of Body Residue as the Dose Metric

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Overview

Because determining the exposure of aquatic organisms to contaminants via multiple routes of exposure is difficult particularly in the field and the continued inability to assess bioavailability from some compartments, particularly sediments, establishing the utility of the body residue as a measure of the dose metric allows for integration of exposure. This project has focused on the temporal relationship between the body residue required to produce a toxic effect and the exposure duration. This temporal relationship was formalized into a new model for contaminant response and published. Two aspects of this approach, interpreting the role of biotransformation, specifically the role of metabolite toxicity, using the body residue approach and the impact of pulsed exposure were the focus of last year's work. This work is also included the potential for delayed effects after the exposure terminates as a part of the role of pulsed exposures. Such delayed effects are the result of the time required to eliminate the toxicants and repair the damage created by the toxicants. From the studies, a new model was developed as an extension of the previous damage assessment model that permits interpretation of mixtures for the body residue analysis. The model was applied to the biotransformation of select PAH and the role of metabolites contributing to the observed toxicity. Studies were also completed that evaluated the impact of pulsed exposures with specific amounts of recovery. These led to an improved understanding of delayed effects post exposure.

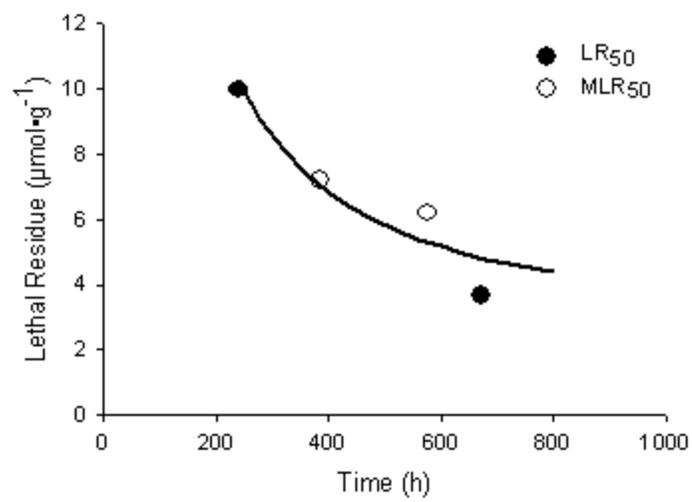
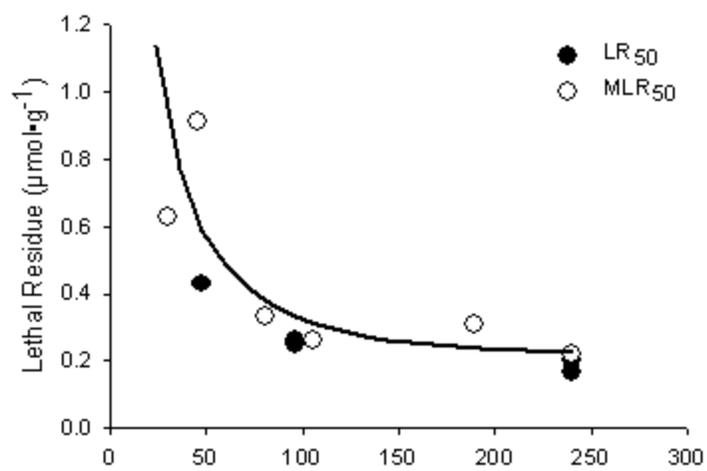
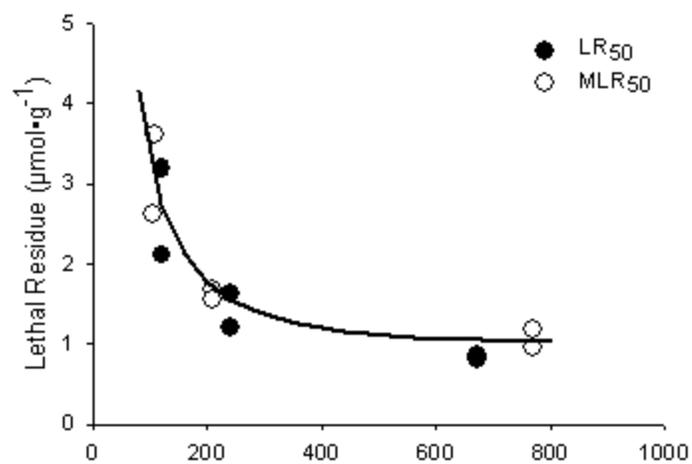
Plans

Examine the role of biotransformation on the interpretation of body residues as the dose metric for toxicity evaluations will continue with a focus on chronic effects. A manuscript on the use of body residue to describe the chronic toxicity of persistent contaminants to fish will be drafted. An experiment examining the impact of mixture toxicity will also be completed this year.

2005 Accomplishments

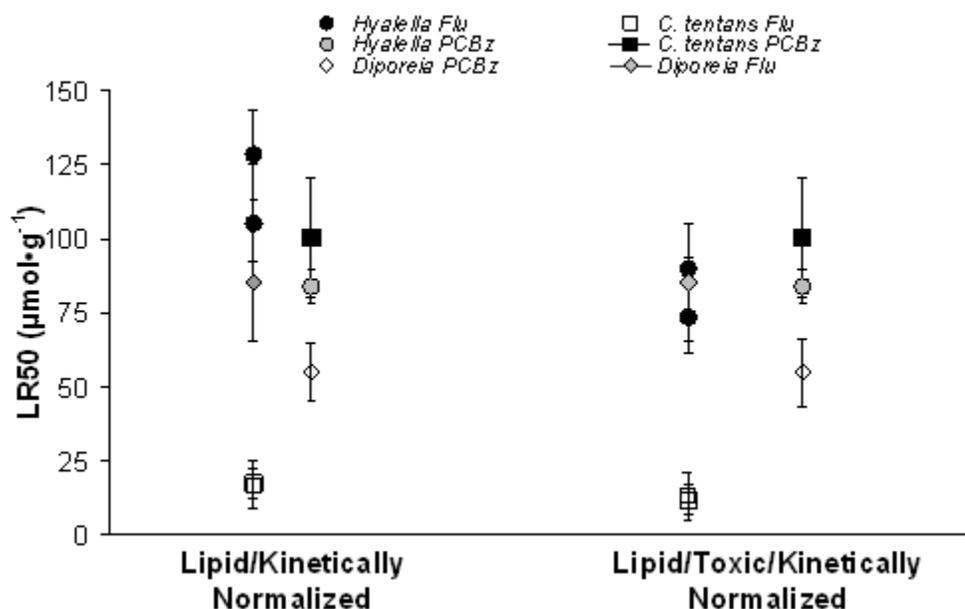
The time-dependent toxicity of fluoranthene was examined for *Hyalella azteca*, *Chironomus tentans*, and *Diporeia* spp. *C. tentans* appeared to be the most sensitive species and *Diporeia* was the least sensitive. Incipient LC_{50} values, the concentration at which the LC_{50} reaches an asymptote and does not change with increasing duration of exposure, for *H. azteca* and *C. tentans* were approximately 60 and 40 $\mu\text{g L}^{-1}$, respectively. Incipient levels were not reached for *Diporeia*; however, the 28-d LC_{50} concentration was 95.5 $\mu\text{g L}^{-1}$. There was a temporal relationship with respect to lethal body residues for each of the test species. For *H. azteca*, the LR_{50} , the median lethal residue at an identified exposure time required to cause 50% mortality,

based on total fluoranthene equivalents (parent + metabolite compounds) decreased from 3.19 $\mu\text{mol g}^{-1}$ at 5 d to 0.80 $\mu\text{mol g}^{-1}$ at 28 d. For *C. tentans*, the LR_{50} decreased from 0.43 to 0.17 $\mu\text{mol g}^{-1}$ from 2 to 10 d. The 10-d LR_{50} for *Diporeia* was 9.97 $\mu\text{mol g}^{-1}$ and the 28-d value was 3.67 $\mu\text{mol g}^{-1}$. The toxicokinetics are not sufficient to address the temporal changes in LR_{50} values. Thus, the data were fit to a Damage Assessment Model that also accounts for toxicodynamic processes (FY 05 Fig. 1). This analysis provides estimates of the incipient lethal residues for *H. azteca*, *C. tentans* and *Diporeia*, 0.84, 0.21 and 3.00 $\mu\text{mol g}^{-1}$, respectively. When comparing the relative sensitivity among species using lethal body residues, special attention should be given to ensure that comparisons are made at a common point in relation to exposure duration (i.e. time to steady state, T_{ss}). When the $\text{LR}_{50 (\text{lipid})}$ values among the three species were compared at steady state, *C. tentans* is more sensitive than *H. azteca* and *Diporeia* spp.; however, there are no significant differences between the amphipod species. The greater sensitivity of *C. tentans* to fluoranthene compared to the amphipods may be due, in part, to a potential toxic metabolite.



FY05 Figure 1: The above three plots demonstrate the time dependence of *Hyalella azteca* (top), *Chironomus tentans* (middle) and *Diporeia* spp. (bottom) to fluoranthene total residues.

A follow up study examined the temporal component of pentachlorobenzene lethal body residues among three freshwater invertebrates. Also, using previous fluoranthene data allowed a more detailed examination of the role of biotransformation in lethal body residues and comparisons of lethal residues across chemical classes. Time-dependent toxicity of fluoranthene and pentachlorobenzene were compared among *Hyalella azteca*, *Chironomus tentans*, and *Diporeia* spp. Lethal body residues required for 50% mortality (LR_{50}) was not constant and decreased with exposure time for all species. Fluoranthene was most toxic to *C. tentans* with LR_{50} values of $0.38 \mu\text{mol g}^{-1}$ at 2 d to $0.15 \mu\text{mol g}^{-1}$ at 10 d and least toxic to *Diporeia* spp. with values of $9.97 \mu\text{mol g}^{-1}$ at 10 d to $3.67 \mu\text{mol g}^{-1}$ at 28 d. The LR_{50} values for *H. azteca* were intermediate and ranged from $2.25 \mu\text{mol g}^{-1}$ at 5 d to $0.56 \mu\text{mol g}^{-1}$ at 28 d. Pentachlorobenzene LR_{50} values were less variable among species and ranged from $1.20 \mu\text{mol g}^{-1}$ at 4 d to $0.81 \mu\text{mol g}^{-1}$ at 10 d for *C. tentans*, $5.0 \mu\text{mol g}^{-1}$ at 20 d and $2.75 \mu\text{mol g}^{-1}$ at 28 d for *Diporeia* spp., and $1.51 \mu\text{mol g}^{-1}$ at 4 d and $0.71 \mu\text{mol g}^{-1}$ at 28 d for *H. azteca*. When LR_{50} values for fluoranthene and pentachlorobenzene were compared at steady state, the lethal residues for the amphipod species were within the range expected for nonpolar narcotic chemicals (anesthetics); however, *C. tentans* was more sensitive to fluoranthene than pentachlorobenzene confirming our previous hypothesis that biotransformation of fluoranthene likely produces a metabolite(s) acting by some specific mechanism of action. The information collected from this study allows a greater understanding of residue-response relationships, specifically relative species sensitivities (FY05 Fig 2).



FY05 Figure 2: Comparison of the body residues causing 50% mortality in *Hyalella azteca*, *Chironomus tentans*, and *Diporeia* spp. for fluoranthene (FLU) and pentachlorobenzene (PCBz)

corrected for kinetic status and lipid content in the left panel and additionally corrected for the toxic fraction of fluoranthene equivalents as well in the right panel.

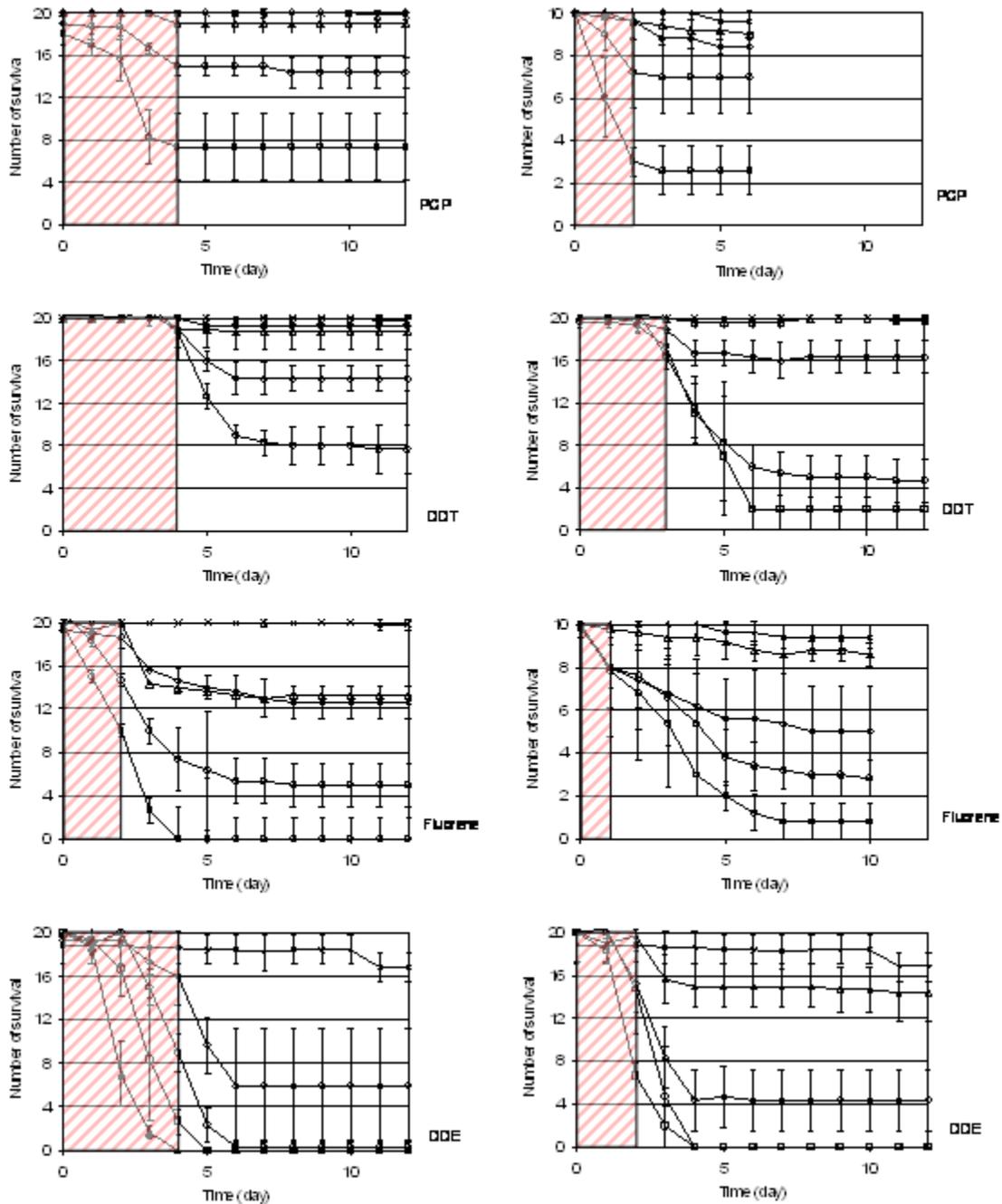
A second project examined the delayed effect, recovery, and tolerance induction in *Hyalella azteca* exposed to organic compounds with different modes of toxic action. The delayed effect (toxicity that occurs post exposure) and recovery (repair of damage occurring post exposure) as well as adaptation/tolerance are challenging issues in higher levels of ecological risk assessment for time-varying exposure conditions in aquatic systems. For several taxa of aquatic organisms, it has been reported that the toxicity of different types of compounds after the end of exposure showed delayed mortality, or the sub-lethal toxicity recovered rapidly or slowly depending on the compound. Both laboratory and field studies have shown that intermittent exposures exerted more toxic effect than the continuous exposures on the basis of the time-averaged concentration. Pre-exposure to some toxicants can induce the tolerance. Post-exposure observation should be, therefore, an essential part of experimental design to determine the intrinsic toxicity of a compound. A pulsed exposure experiment can provide additional information such as the potential for recovery, cumulative effects, or resistance by induction of detoxification or biotransformation enzymes.

In this study, two narcotic compounds (fluorene and DDE) and two other organic compounds with a specific modes of toxic action (PCP and DDT) were exposed to pulses and delayed toxicity after the exposure ended was followed for 7 - 10 days in *Hyalella azteca*. The delayed effects were separately measured for narcosis (loss of balance or immobility), and mortality (stop of heart-beating). In addition, the effect of the pre-exposure to the same compound was investigated by comparing the time-to-death curve with and without pre-exposure. Body residues of the organic compounds in dead animals were analyzed to establish body residue response relationships.

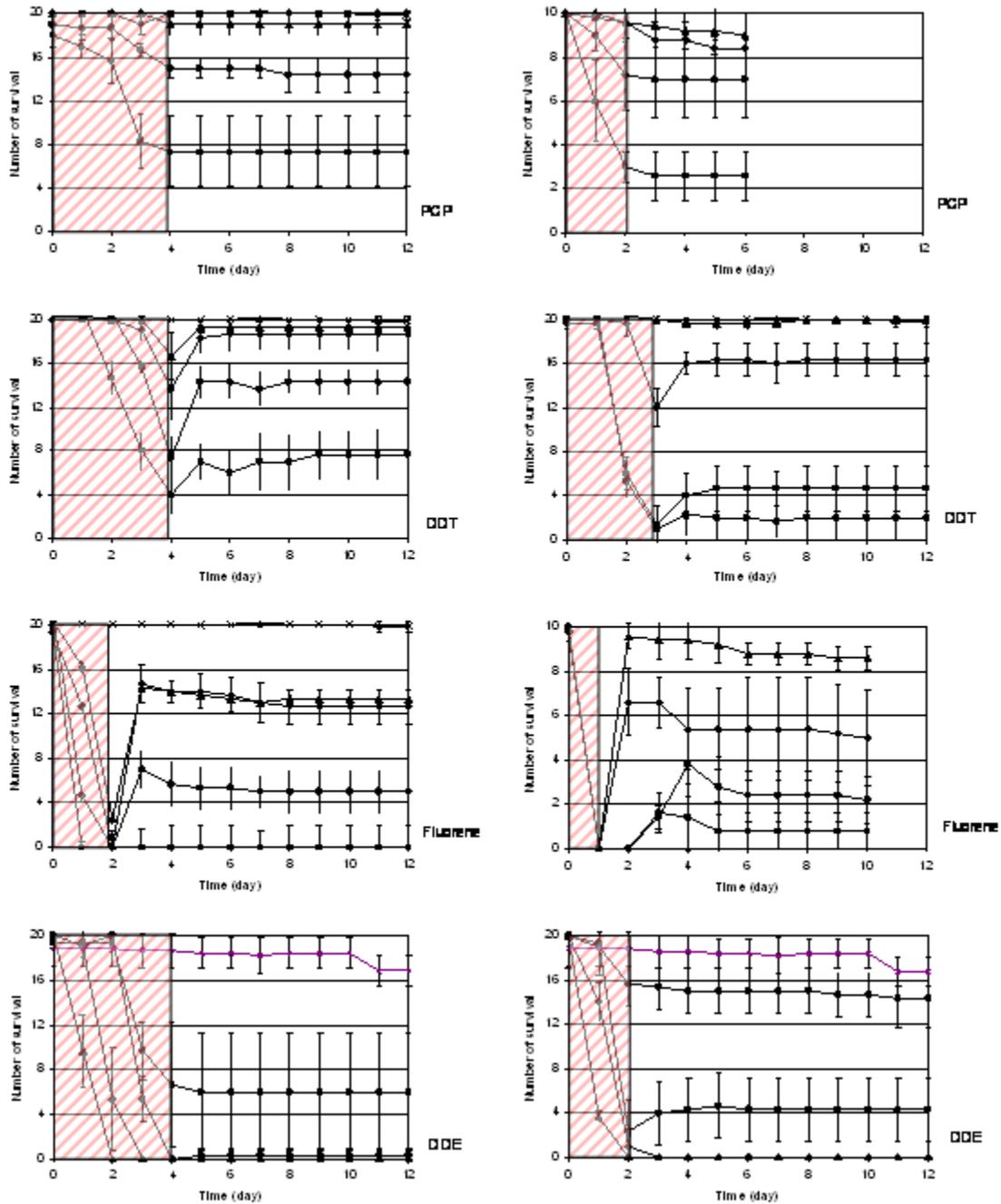
For fluorene, DDE, and DDT, delayed effects were observed after the exposure ended, whereas for PCP exhibited no delayed effects (FY05 Fig. 3). Since PCP showed time-independent toxicity and very fast elimination of body residue (the apparent elimination rate constant, 0.19 h^{-1}), no delayed effect would be expected because of the toxicokinetics. However, the delayed effect for narcotic compounds with very fast elimination rate constant (1.65 h^{-1}) such as fluorene was not expected but was similar to that for DDE with very slow elimination rate constant (0.01 h^{-1}).

In the case of fluorene and DDT, within about three days after the end of exposure, a fraction of the narcotized animals were recovered, and the fraction of recovery increases with decreasing of exposure concentration (FY05 Fig. 4). Other remaining narcotized animals were no longer recovered and continued to die over the next 2 - 6 d. In contrast, narcotized animals exposed to DDE did not recover during the depuration period (Figure 4). In all of cases, the active and recovered test animals did not die during the recovery period. In this study it was apparent that the death process was not an instantaneous reaction, but a multi-step process including loss of balance, immobility, irreversibly damaged status, and finally death. Meanwhile, acute exposure to PCP known as an uncoupler of the oxidative phosphorylation resulted in such a rapid onset of mortality that there was no delayed effect.

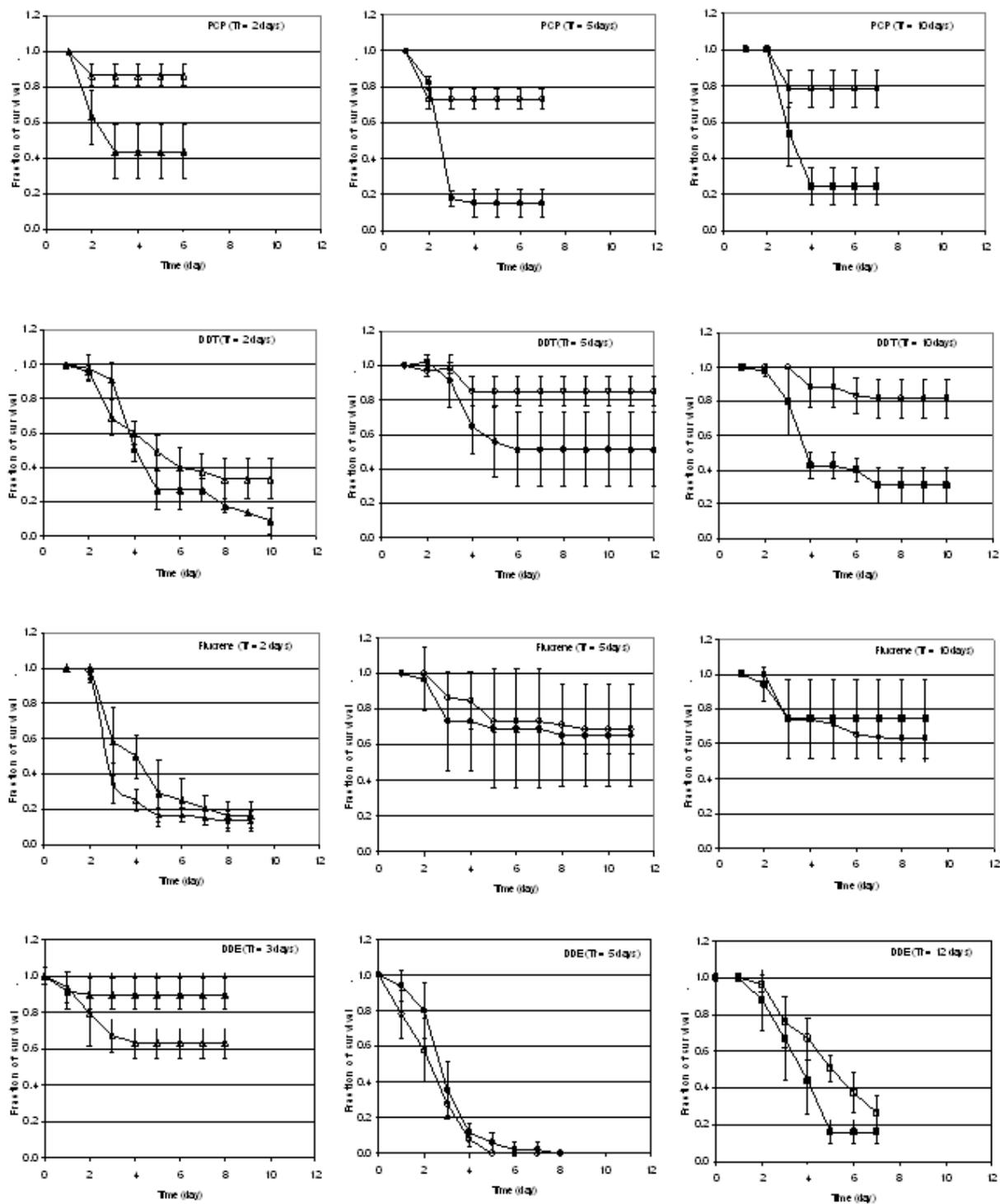
Pre-exposure to narcotic compounds such as fluorene did not induce the tolerance when organisms were subsequently exposed to the same exposure condition after a period of recovery (FY05 Fig. 5). In the case of DDE, the longer the recovery time, the more tolerant the pre-exposed test animals compared with those were not pre-exposed. However, PCP and DDT, with specific modes of toxic action, showed an apparent induction of tolerance after the recovery times, when the mortality did not increase after the end of exposure. Further studies are needed to investigate the influence of pre-exposure to toxicokinetics as well as toxicodynamics, and the influence of mode of toxic action on tolerance induction.



FY05 Figure 3: Survivorship in *Hyalella azteca* exposed to of PCP, DDT, fluorene, and DDE for two different exposure periods (shaded zones) and post-exposure period. Error bars: standard deviation, $n = 3$. Exposure concentrations for the different compounds were: DDT : 0.270 ± 0.026 , 0.304 ± 0.019 , 0.387 ± 0.019 , 0.552 ± 0.023 , 0.776 ± 0.035 PCP : 181 ± 9 , 289 ± 9 , 488 ± 15 , 798 ± 20 FLU : 627 ± 27 , 748 ± 35 , 909 ± 26 , 1183 ± 37 DDE : 40.9 ± 3.5 , 24.6 ± 1.4 , 16.2 ± 1.9 , 11.1 ± 0.7 , 9.4 ± 0.7 , 5.9 ± 0.5



FY05 Figure 4: Number of active animals in *Hyaella azteca* exposed to of PCP, DDT, fluorine, and DDE for two different exposure periods (shaded zones) and post-exposure period. Error bars: standard deviation, $n = 3$. Exposure concentrations are the same as those for Figure 3.



FY05 Figure 5: Comparison of survivorship in *Hyalella azteca* with pre-exposure (closed markers) and without pre-exposure (open markers) to PCP, DDT, fluorene, and DDE. Tr: recovery time between the first and second exposure periods. Error bars: standard deviation, $n = 3$.

2004 Accomplishments

In a continuation of work to evaluate the challenge approach to develop a method to assess bioaccumulated residues, the experimental organism was changed to the midge *Chironomus tentans* because it had not been possible to establish reproducible exposure of the amphipod *Hyalella azteca* to sediment-associated contaminants. The studies included, 10-d water-only exposures to pentachlorobenzene (PCBZ) and pyrene (PY). These two chemicals were also used in 10-d sediment-water exposures where PY was dosed into the sediment and PCBZ was dosed in the water as the challenge chemical. Mortality was the effects endpoint of choice and the toxicokinetics were examined. *C. tentans* were also exposed to a low PY concentration via water-only study to examine the toxicokinetics and biotransformation.

PCBZ Water-Only Exposure

The 10 d LR₅₀ value for PCBZ water-only exposure was 234 nmol g⁻¹ (219 - 256, 95% CI). In a duplicate PCBZ water-only exposure, a 10-d LC₅₀ of 106 ug L⁻¹ (96.2 - 129.7), with corresponding LT50 of 196 h (175 - 228) and a 10-d LR₅₀ 412 nmol g⁻¹ (341 - 616) were determined. In both these experiments the organisms were housed as 10 organisms per replicate. The 95% confidence limits for the LR₅₀ values for the two studies do not overlap suggesting some difference in the response, but the two values are less than a factor of 2 different indicating reasonable replication. Thus, the expected 10-d LR₅₀ is estimated to lie between the two values.

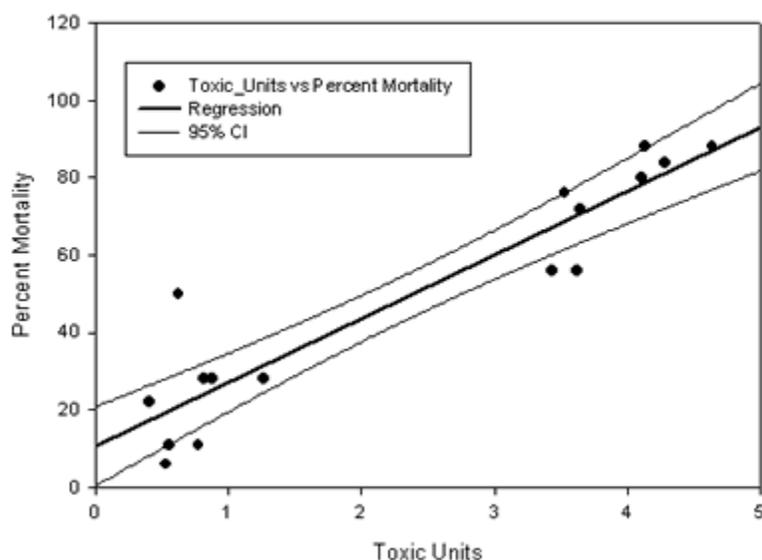
Pyrene Water-Only Exposure

The 10-d LR₅₀ value for PY, as total Py equivalents, was 411 nmol g⁻¹ (303 – 743) with a 10-d LC₅₀ 26.92 ug L⁻¹ (18 – 49). In a second study, in which the animals were housed individually in smaller beakers, the beaker size was reduced from 150 mL down to 23 mL, the 10-d LR₅₀ value was 236 nmol g⁻¹ (180 – 350 nmol g⁻¹) and the corresponding LC₅₀ value was 36 ug L⁻¹ (31 – 45). As with the PCBZ experiments, the two values were less than a factor of two different and the 95% confidence intervals of the LR₅₀ values of the two experiments overlapped.

Sediment/Water Exposure

The concentration in the organisms (nmol/g) was calculated for both PY and PCBZ in dead and live organisms. While the PY is expected to be biotransformed and is expressed as total PY equivalents, the PCBZ should be resistant to biotransformation. In the PY-control 0.1 umol g⁻¹, the 10-d concentration was 133 ± 24 nmol g⁻¹ for organisms found alive. For the PY-control at 0.2 umol g⁻¹, a concentration of 169 ± 49 nmol g⁻¹ was found for the live organisms and 161 ± 26 for the dead organisms. At higher PY sediment concentrations, 0.5 umol g⁻¹ and 1.0 umol g⁻¹ PY, the midges were found to contain 1068 ± 154 nmol g⁻¹ and 1065 ± 146 nmol g⁻¹ for live organisms and 892 ± 234 nmol g⁻¹ and 832 ± 200 nmol g⁻¹ for dead organisms, respectively.

There were insufficient data from the two experiments, the high and low PY treatments to calculate LR₅₀ values from individual treatments. In the PY at 0.1 $\mu\text{mol g}^{-1}$ and 0.2 $\mu\text{mol g}^{-1}$, even in the presence of the challenge compound, the mortality was low and was a result of failure to achieve expected concentration of PCBZ in the test organisms. For the high treatments, the amount of PY was sufficient that the mortality was high even at low challenge concentrations. However, if one combines the two data sets, calculates the number of toxic units, and assumes additivity then there is a relationship between mortality and the number of toxic units (FY04 Fig. 1). A toxic unit is the ratio of the concentration observed divided by the concentration for 50% mortality. A toxic unit of one indicates that the observed concentration represents that which would produce 50% mortality.



FY 04 Figure 1: The toxic units for the combined toxicity of Pyrene and pentachlorobenzene to *C. tentans* assuming additivity

The data was scattered between very low mortality and very high mortality so the shape of the dose response curve is not well known. However, the estimate for 50% mortality was 2.1 ± 0.44 TU. While this is greater than one it is not sufficiently greater that an additivity model should be rejected at this time.

PAH Toxicokinetics and Biotransformation

The uptake clearance coefficient, k_u , for PY at a water concentration of $10 \mu\text{g L}^{-1}$ was $40.6 \text{ mL g}^{-1} \text{ h}^{-1}$ and the elimination rate constant, k_e , was 0.028 h^{-1} . The biotransformation of PY by *C. tentans* was assessed over-time at seven time points during the uptake using a modification of a standard lipid extraction technique. Using this method, metabolites are extracted into three phases: aqueous soluble, organic extractable, and bound residue phases. The aqueous soluble phase exhibited the greatest percent fraction of the metabolites (FY04 Table 1). The production of metabolites peaked at 72 h and then declined out to 96 h (Table 1). The aqueous metabolites are expected to be conjugated phase one biotransformation products. The organic extractable

non-parent metabolites are expected to be phase one biotransformation products. Because the concentration required to produce the observed body residue is small relative to that expected for non-polar narcosis then it is expected that the biotransformation products may be contributing to the total toxicity.

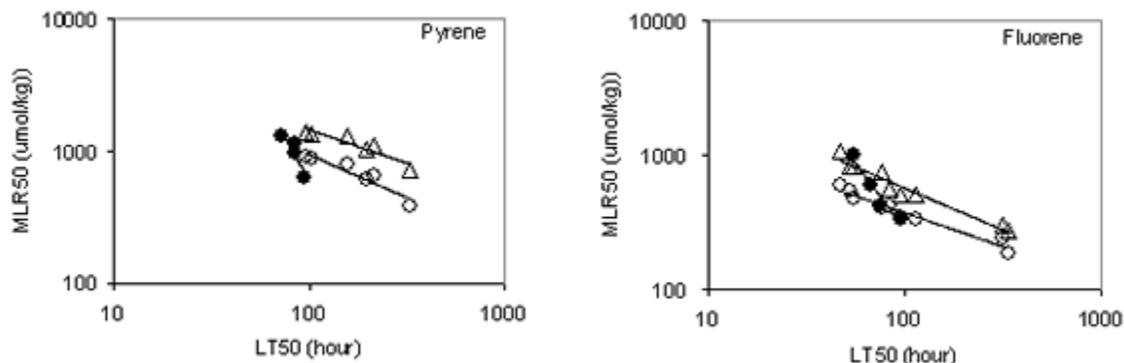
FY04 Table 1: The biotransformation of pyrene by the midge, *Chironomus tentans*, over a 96 h exposure.

Exposure Duration	2h	4h	8h	24h	48h	72h	96h
% Parent	71	56	42	32	26	32	36
% Total Metabolite	29	44	58	68	75	68	64
% Aqueous Soluble Metabolites	18	25	45	55	58	54	52
% Bound Metabolites	3	3	2	4	1	3	2
% Non-parent Organic Metabolites	8	16	11	10	16	11	10

The second portion of this project further examined the biotransformation and its impact on both bioconcentration and toxicity of PAH in *Hyalella azteca*. Biotransformation toxicokinetics and the time-dependent toxicity of pyrene and fluorene were investigated in the presence and absence of a biotransformation inhibitor (piperonyl butoxide, PBO, 0.3 mg L⁻¹). *H. azteca* metabolized pyrene and fluorene actively and the amount of metabolites at steady state in the absence of PBO was 43% and 58%, respectively. According to the body residue approach, the mean lethal residue for 50% mortality (MLR₅₀

(t)) in the presence and absence of PBO should be the same, if the contribution of PBO to the total toxicity is negligible and toxic potency of the parent compound and metabolites of PAH are similar. However, MLR₅₀(t) values based on total body residue and parent compound of pyrene in the presence of PBO were smaller than in the absence of PBO (FY04 Fig. 2). These results indicated that the metabolites in the absence of PBO were likely non-toxic because the body residues of metabolites in the absence of PBO were greater than in the presence of PBO. In addition, the difference between MLR₅₀(t) values in the presence and absence of PBO could not be explained only by body residue of the PAH and metabolites. Therefore, it was apparent that PBO contributed significantly to the toxic effect. The contribution of PBO ranged from 0.35 toxic units at day 4 to 0.78 toxic units at day 10 to the mixture toxicity. Meanwhile, metabolites of

fluorene were significantly toxic, because $MLR_{50}(t)$ values in the presence of PBO were greater than $MLR_{50}(t)$ for parent compound in the absence of PBO (Figure 2).



FY04 Figure 2: Time-dependent toxicity of pyrene and fluorene in *Hyalella azteca* in the presence and absence of the biotransformation inhibitor, piperonyl butoxide (PBO, 0.3 mg L^{-1}). The mean lethal residue (MLR_{50}) for total body residue (\circ) and parent compound (\bullet) matched with the median lethal time (LT 50) in the absence of PBO, and the MLR_{50} for total body residue in the presence of PBO (\triangle). In the presence of PBO, MLR_{50} s for parent compound were omitted because most of the values for the parent compound overlapped with those for total body residue.

Efforts are ongoing to better formulate the previous damage assessment model to better reflect the role of metabolites and to accommodate mixtures so that improved interpretation of bioaccumulated residue will result.

Toxicity of Fluoranthene

The third part of this research investigated the difference in the time-dependent toxicity of fluoranthene to the midge, *C. tentans*, and two amphipods, *H. azteca* and *Diporeia* spp. The two amphipods were selected for comparison because *Diporeia* is not capable of biotransforming PAH while *H. azteca* is well known to biotransform PAH. The toxicity of the two amphipods once corrected for differences in lipid content produced essentially equivalent toxicity based on total fluoranthene equivalents. The values for the amphipods were similar ($152 \text{ } \mu\text{mol g}^{-1} \text{ lipid}$ and $121 \text{ } \mu\text{mol g}^{-1} \text{ lipid}$ for *H. azteca* and *Diporeia* respectively at 28 d) to those expected for non-polar narcosis as a mechanism of action. The midge however was much more sensitive with toxicity requiring about 2 to 3 fold lower concentrations ($56 \text{ } \mu\text{mol g}^{-1} \text{ lipid}$). The midge concentration is at the lower end of the values considered to represent non-polar narcosis. The difference between the midge and the amphipods may be a result of differences in biotransformation pathway and not the extent of biotransformation as *H. azteca* exhibited the most extensive biotransformation while *Diporeia* could biotransform PAH the least.

2003 Accomplishments

Pyrene and Pentachlorobenzene Exposures

Hyalella azteca had previously been exposed to mixtures of pyrene and PCBZ in aqueous exposures and the result was evidence that additivity could be demonstrated on a toxic unit basis. Thus, to continue the work for evaluating benthic organisms exposed to sediments and subsequently challenged with PCBZ, *H. azteca* were exposed to sediments containing pyrene and challenged with PCBZ in overlying water. The light regime was varied. In the dark and under yellow light there was little evidence that the method would work because the response was not different from exposures containing only PCBZ as the concentration for 50% mortality required 0.8 ± 0.4 toxic units (TU). This was the result of *H. azteca* failing to accumulate the pyrene (Table 1). Two likely causes were investigated. The first was that the sediment bioavailability was too limited. The first sediment (approximately 1% organic carbon) was replaced with a low organic carbon sediment (0.4% OC), but the result was the same with very low pyrene in the organisms. The bioavailability was examined with exposure of *Lumbriculus variegatus* to the same sediment and the use of Tenax® resin extraction. Both measures exhibited good evidence of bioavailability (Table 1). Thus, the problem was thought to be the light regime under which the tests were performed. It appeared that the *H. azteca* were only exposed to the overlying water particularly in the presence of PCBZ. Since *H. azteca* are negatively phototrophic, white light should encourage the organisms to stay in the sediment.

Table 1: Examination of the bioavailability of pyrene from Lake Michigan sediment.

Sediment Conc. (nmol/g)	BAF <i>Hyalella</i> 10 d	BAF <i>Lumbriculus</i> 10 d	Fraction Tenax® Ext. (6 h)	Ratio <i>L.v.</i> -BAF /Tenax Ext
750	ND	0.71 ± 0.21	0.33 ± 0.04	2.2
2500	ND	0.54 ± 0.07	0.19 ± 0.01	2.8

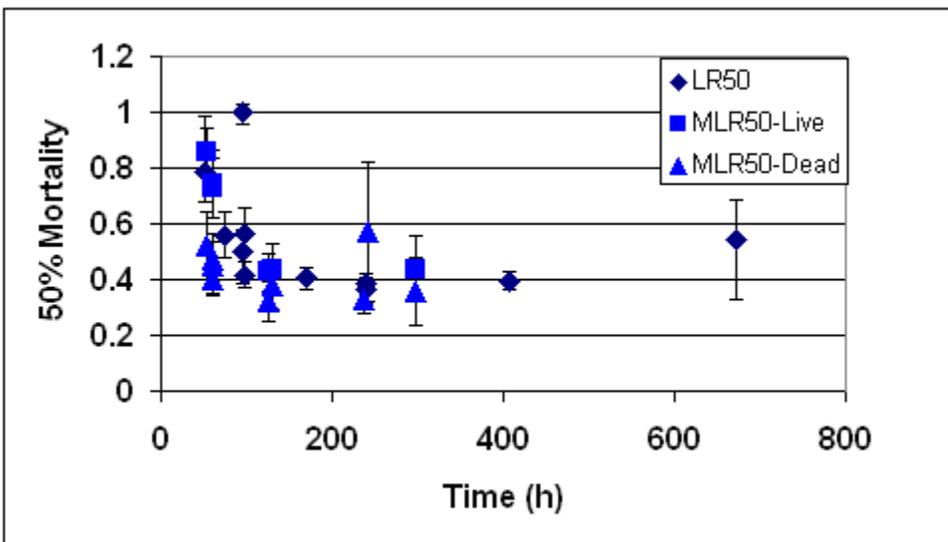
While the initial thought was to avoid white light which will force the *H. azteca* into the sediment due to the potential for photoinduced toxicity, previous studies with fluoranthene and various light regimes suggests that the toxicity of the PAH is not enhanced because the organisms stay buried in the sediment when the light is on. Thus, an experiment with two concentrations of pyrene using three concentrations of PCBZ was employed to study the challenge problem. In this case, pyrene was accumulated by the amphipods but the extent of toxicity was much greater than anticipated. Even in the absence of PCBZ there was substantial mortality. In fact, the extent of mortality was greater in these studies than previously observed in water only exposures at the same body residue levels. Water only exposures under yellow light yielded 2 d LR₅₀ estimates of 4.14 mmol g^{-1} . In this study, the estimate in the absence of PCBZ was 56.6 nmol g^{-1} and between the response of *H. azteca* under fluorescent and UV enhanced light

observed at 10 d exposure. This suggests that the *H. azteca* were likely experiencing photoinduced toxicity or there is some additional toxicant in the system that is not evident based on the control mortality which was >5%. The estimate for the LR₅₀ is not very strong because the data were either zero and near 100% mortality. There was no statistical difference between the estimated body residue required to produce mortality in the presence of PCBZ dosed at 250 $\mu\text{g L}^{-1}$ with is likely the result of having the very high mortality. At least by using white light, *H. azteca* did accumulate pyrene and it was toxic. This experiment will need to be repeated with lower pyrene concentrations to determine whether the overall approach will work. So far we have found both ends of the spectrum, in the case of no accumulation of pyrene the toxicity was only due to PCBZ and in the case of high toxicity by pyrene the amount of PCBZ required for toxicity was minimal (approximately 0.2 TU of PCBZ).

Time Dependent DDE Toxicity in *H. azteca*

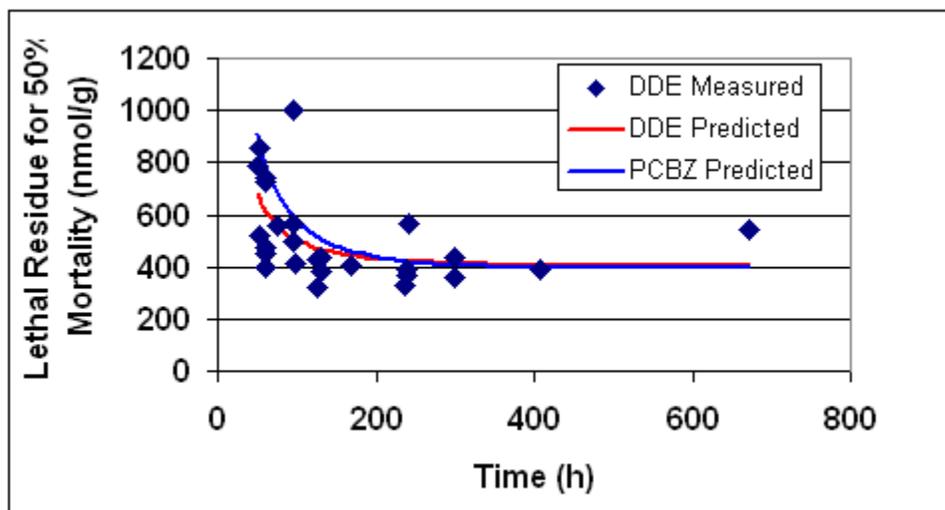
Hyalella azteca were exposed to DDE solutions with 24 h renewal of the water concentration. As with the PCBZ, the water concentration declined during the 24 h exposure by approximately 50%. However, as with PCBZ, the elimination is slow $0.009 \pm 0.006 \text{ h}^{-1}$ (mean \pm SD, $n = 14$) measured across all concentrations and all experimental time frames. This elimination rate is somewhat slower than that found for PCBZ (0.014 h^{-1}) but not statistically different. Unlike PCBZ, the uptake rate for DDE is much faster ($347 \pm 88 \text{ mL g}^{-1} \text{ h}^{-1}$) leading to much higher bioaccumulation factors $44,962 \pm 23,523$ where the PCBZ BCF was about 500 - 2200. There was also no apparent impact of increasing concentration on the BCF for DDE. The exposure range for the DDE was much lower ranging from 0.40 to $14.1 \mu\text{g L}^{-1}$ while that for PCBZ ranged from $66.5 \mu\text{g L}^{-1}$ to 1 mg L^{-1} which reflects the relative solubility limits for the two compounds.

The body residues required for 50% mortality were however very similar for the two compounds. For DDE as with PCBZ, the method for determining the body residue did not affect the time dependence (FY03 Fig. 1).



FY03 Figure 1: Time dependent toxicity of *Hyalella azteca* exposed to DDE in aqueous solution for exposures approaching 30 d.

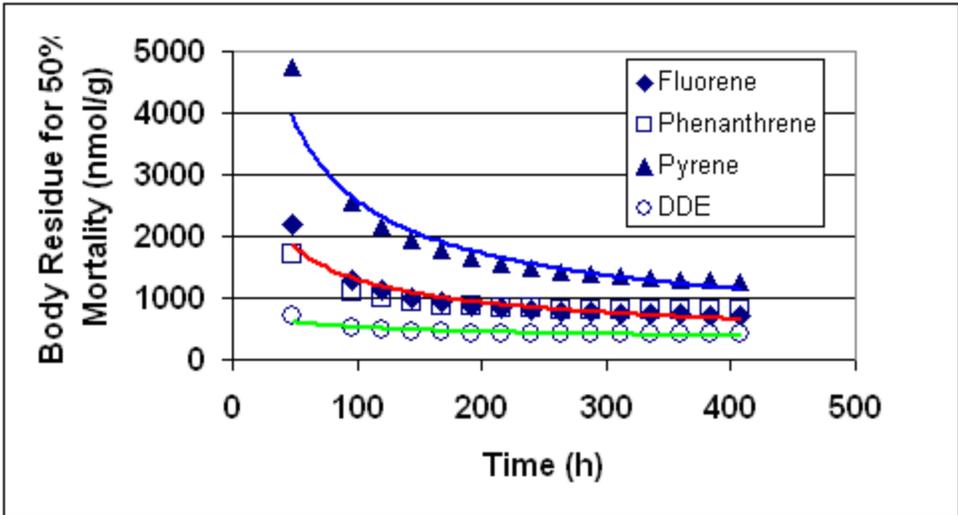
The temporal change in the concentration required to produce toxicity shows a steep time response. This was essentially the same temporal response found for PCBZ (FY 03 Fig. 2).



FY 03 Figure 2: The time dependent response of *Hyalella azteca* predicted from the damage assessment model (Lee et al. 2002a) incorporating both toxicokinetics and toxicodynamics and the measured values for DDE exposures.

Thus, both compounds are equipotent on a molar basis suggesting that they have the same mechanism of toxicity in *Hyalella azteca*. When the data is fit to the damage assessment model, it is possible to obtain an estimate for the rate of damage repair (k_r), which was found to be 0.038 h^{-1} . This rate is essentially the same as that for PCBZ. With the above data it is possible to obtain interpretation of the toxicity of DDE with any duration of exposure for *Hyalella azteca*. These data expand our ability to interpret body residue data and to better understand the impact of multiple pulsed exposures as might occur in the field.

The toxicity of DDE to *Hyalella azteca* was substantially different from previously reported temporal toxic responses to PAH congeners (FY 03 Fig. 3).

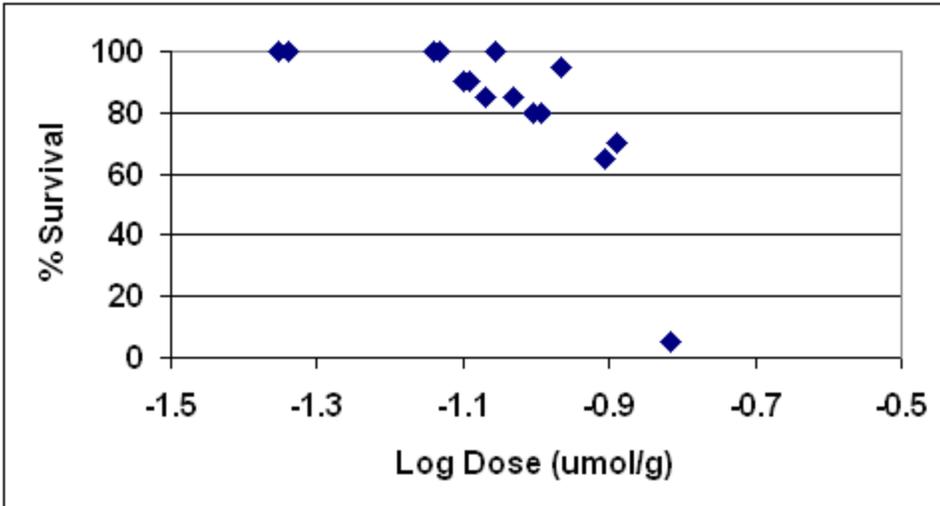


FY 03 Figure 3: Temporal response of *Hyalella azteca* to selected PAH (Lee et al. 2002b) and to DDE.

On this scale, the temporal response of DDE is minimal relative to that for Pyrene and at long exposures the body residue values required to produce a toxic response tend to converge. Thus, if one is going to assess the toxicity of a mixture of PAH with DDE as a challenge compound in *Hyalella azteca* then the evaluation cannot be done strictly on an additive molar basis but must be done on a toxic unit basis. If the exposures for the challenge experiment are performed within the range of 4 to 28 d exposure then essentially DDE will exhibit a constant value for the LR₅₀ against which to compare the response in the mixture. 10 d DDE and PAH

Exposures in *Leptocheirus plumulosus*

Leptocheirus plumulosus were exposed to DDE and mixture of PAH in sediment exposures for 10 d. The experimental design was set to establish both the toxic response for DDE only and DDE in the presence of differing concentrations of PAH mixture. The LR₅₀ based on measured DDE concentrations was 0.13 (0.142-0.122) μmol g⁻¹ DDE. In the presence of the lowest concentration of PAH, the LR₅₀ was estimated to be 0.118 (0.129 - 0.111) μmol g⁻¹(fw) DDE suggesting that the DDE contributed approximately 0.90 TU to the mixture and not significantly different than DDE only exposures.



FY 04 Figure 4: The dose response relationship for *Hyalella azteca* exposed to DDE in sediment for 10 d.

The measured concentration of PAH in the organisms was $0.01 \pm 0.002 \mu\text{mol g}^{-1}$ measured as the sum of PAH. Assuming that all the PAH were as sensitive as fluoranthene, the number of TU represented by the PAH would be 0.04. Thus the toxicity was largely contributed by the DDE. Thus it is clear that PAH accumulation was limited and did not contribute significantly to the toxic response.

2002 Accomplishments

The challenge approach is predicated on the additivity of toxicity of the challenge compound with the other compounds. To initially address this issue with PCBZ, *Hyalella azteca* were exposed in water-only exposures. The toxicity of these two compounds was previously demonstrated to be time dependent (Figure 02-1).

Two exposures were performed with two different concentrations of PY and several concentrations of PCBZ. The body residues for the LR_{50} values and the MLR_{50} values were determined along with the respective pyrene body residues (Figure 02-2). Using the temporal relationships in Figure 02-1 and the temporal concentrations in Figure 02-2, toxic units (body residue divided by the LR_{50} for the appropriate time) were calculated for each set of exposure conditions. 1.1 ± 0.2 TU across all exposure durations and for both levels of pyrene exposure.

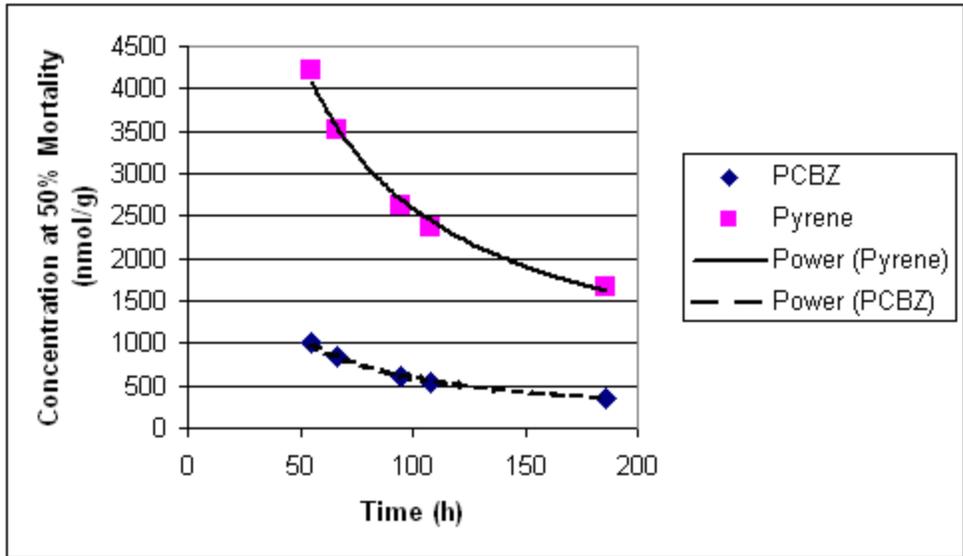


Figure 02-1: This shows the time dependence and difference in potency for pentachlorobenzene and pyrene in the amphipod *Hyalella azteca*.

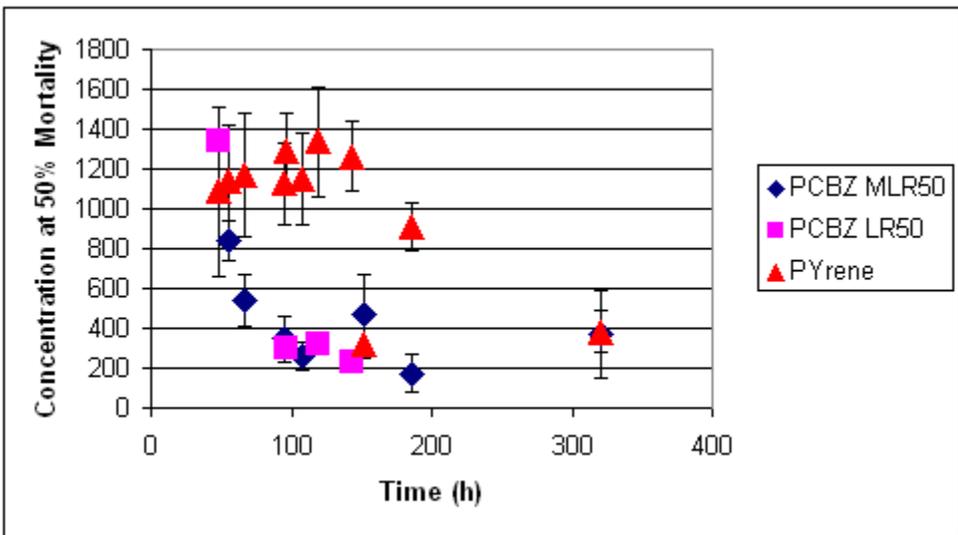


Figure 02-2: The concentrations of PCBZ and PY in organisms corresponding to 50% mortality for a range of exposure durations for two different levels of PY.

The toxic units were then added, since the values above represent concentration at 50% mortality then the sum of toxic units should be unity if the compounds are acting additively (Figure 02-3). The average number of toxic units was found to be 1.1

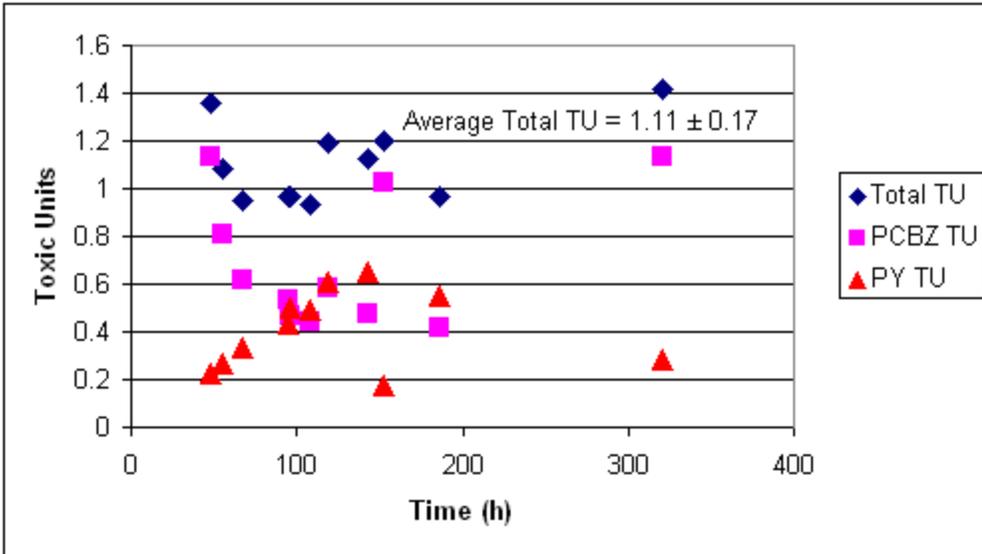


Figure 02-3: The contributions of the PY and PCBZ to the sum of toxic units vary with exposure duration and concentration.

While the contribution of PY and PCBZ varied the summation of the number of toxic units required for 50% response was not statistically different from unity. Thus, the compounds are acting additively. This can be more clearly seen with a plot of the relationship between the contribution of the two compounds and their fit to a 1:1 isobole (Figure 02-4). The deviation of the points near 0.2 TU of PY from the strict 1:1 line represent the sensitivity of the bioassay. Thus, it is not clear that this approach would be able to detect contributions from other compounds at much less than 0.2 toxic units. Further, since additivity was found between PY and PCBZ, it is likely that additivity will occur with all the PAH congeners since for this purpose they are expected to act additively as has been previously demonstrated.

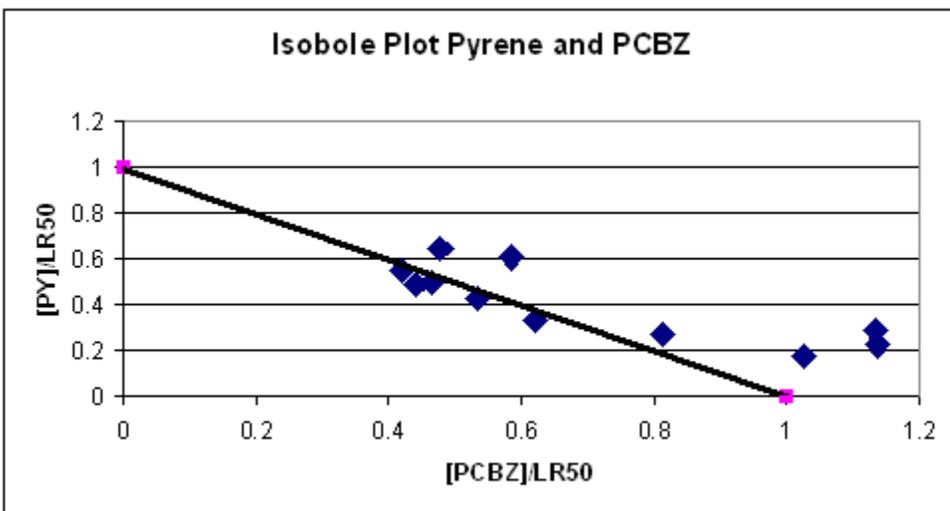


Figure 02-4: An isoboles plot of PY and PCBZ relative to a line of strict additivity.

Since additivity was established and it was previously demonstrated that the challenge could be applied in water when the animals inhabited sediment with a similar sensitivity, a set of experiments were designed to better establish the appropriate methodology for the challenge bioassay. What was critical was to consider the toxicokinetics of the exposure in sediment and the temporal toxicity of the challenge chemicals. If the organisms were exposed to the challenge before they had come to steady state with the sediment, then it was likely that the method would be less sensitive. Two experiments one with a 1 d pre exposure and one with a 10 d pre exposure to PY contaminated sediments ($0.8 \text{ } \mu\text{mol g}^{-1}$) were performed. The concentration of the PY in the sediment was set to yield approximately 1 mmol kg^{-1} in the *H. azteca* based on equilibrium partitioning calculations. However, the amount of PY accumulated was found to be below detection limits while the number of toxic units was found to be 0.77 ± 0.44 . The experiment was replicated with higher levels of radioactive PY to enhance the detection limit and with two PY concentrations (0.75 and $2.5 \text{ } \mu\text{mol g}^{-1}$). Again the PY was not detected in *H. azteca* by radioactivity, but GC analysis indicated that the concentration was approximately 0.08 and $0.11 \text{ mmol kg}^{-1}$ for the sediment exposures respectively. This gave a very small fraction of a toxic unit such that the value could be considered zero. In addition, the range of toxic units found in the two sediment experiments ranged from about 0.5 TU to 1.1 TU with the larger value at the longer exposure duration. The variability may be due to the behavior of the *H. azteca* during the exposure suggesting that sediment exposures may decline with duration of exposure as the organisms become intoxicated and migrate into the water column. The experimental design was repeated with a 3 d pre exposure but the data remain to be analyzed.

In addition to working with PCBZ, studies were performed with DDE to establish the body residue required to produce mortality in water-only exposures. The exposure duration ranged from 4 to 28 d and the toxicity for 50% mortality was essentially constant at about $0.53 \pm 0.17 \text{ } \mu\text{mol g}^{-1}$ with no temporal trend. If one looks at the data for PCBZ, most of the temporal change in concentration occurs within the first 4 days of exposure. However, with DDE it is not possible to load the organisms fast enough to obtain shorter term responses because of solubility limits for DDE. The magnitude of response for DDE is similar to that of PCBZ.

Finally, this year, several quality control experiments were performed. The first was one to demonstrate that the presence of light would not enhance the toxicity of PCBZ to *H. azteca* as the body residue data had been developed in the dark. There was no difference in the response when organisms were exposed under yellow light and in the dark. Yellow light was chosen so that there could be a light cycle without the interference of photoinduced toxicity when PAHs were chosen as test compounds. The second experiment examined the biotransformation of PCBZ and PY. There was no measurable biotransformation of PCBZ. However, the PY exhibited about 30% biotransformation in 10 d. This may account for the difference observed in the relative potency for the two compounds (Figure 1). Since the toxicity was based on measured radioactivity for both compounds as parent compound equivalents.

2001 Accomplishments

1. The temporal variability in the use of internal concentrations to produce a toxic response has recently been recognized as critical to developing hazard assessment for long term and pulsed exposures to contaminants. A damage assessment model was developed to provide improved prediction of time-dependent internal residue effects concentrations. The time-dependent internal lethal concentration (ILC_{50}) data for the modeling exercise were collected previously (See below FY 2000). The model accounts for both the toxicokinetics and the toxicodynamics of contaminants in *Hyalomma azteca* to predict the body residue that will yield 50% mortality. The model is derived from a first order toxicokinetics model linked to a first order toxicodynamics model, which predicts that damage is formed in proportion to the amount of toxicant in the organism and is repaired by a first order process in proportion to the amount of damage. This simple model has several underlying assumptions, including constant exposure and coefficients not affected by the damage process.

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

The $LR_{50}(t)$ is the body residue for 50% mortality at time t , D_I is the amount of damage required to produce 50% mortality, k_a is the rate of damage formation, k_r is the rate of damage repair and k_e is the elimination rate constant for the compound. This model was useful for predicting the toxicity of PAH congeners to *Hyalomma azteca* over time and was better than other models in the literature including the constant critical body residue model and the constant area under the curve model (Figure FY01-1)

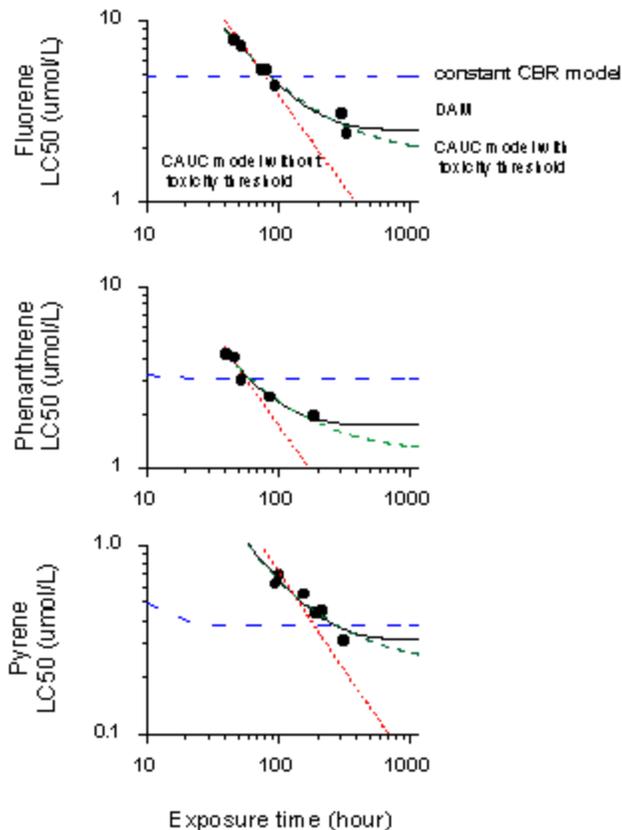


Figure FY01-1: Comparison of the damage assessment model (DAM) for predicting the time dependent toxicity of PAH to *Hyalella azteca*.

2. A joint project with the U. S. Army Corps of Engineers and investigators from the USGS Great Lakes Science Center was initiated to develop an effects-based method to screen for the potential hazard associated with bioaccumulated residue. The approach was to apply a challenge chemical to organisms and determine the amount required to produce a specific response, e.g. 50% mortality. Those organisms with little bioaccumulated residue would respond at the same internal concentration as naive organisms while those with appreciable bioaccumulated residue would respond at lower internal concentrations. This assumes that the toxicants act in an additive manner. The criteria for selecting the challenge compound included that the toxicant 1) should be toxic within the water solubility of the compound, 2) should act through a non-polar narcotic (anesthetic) mechanism of action, 3) should not be readily biotransformed, 4) should not be volatile, and 5) should have a relatively large log Kow so there will be substantial bioaccumulation. It would also be useful for the compound to be available in a radio labeled form to ease method development. After surveying the literature and available radio labeled compounds, pentachlorobenzene (log Kow 5.17, water solubility 0.55-0.83 mg/L) was selected as the initial challenge compound. The only drawback to this compound is its volatility (vapor pressure 0.00101 torr). Several bioassays of differing durations out to 28 d were performed with *Hyalella azteca* and the data fit to the DAM model (Figure FY01-2).

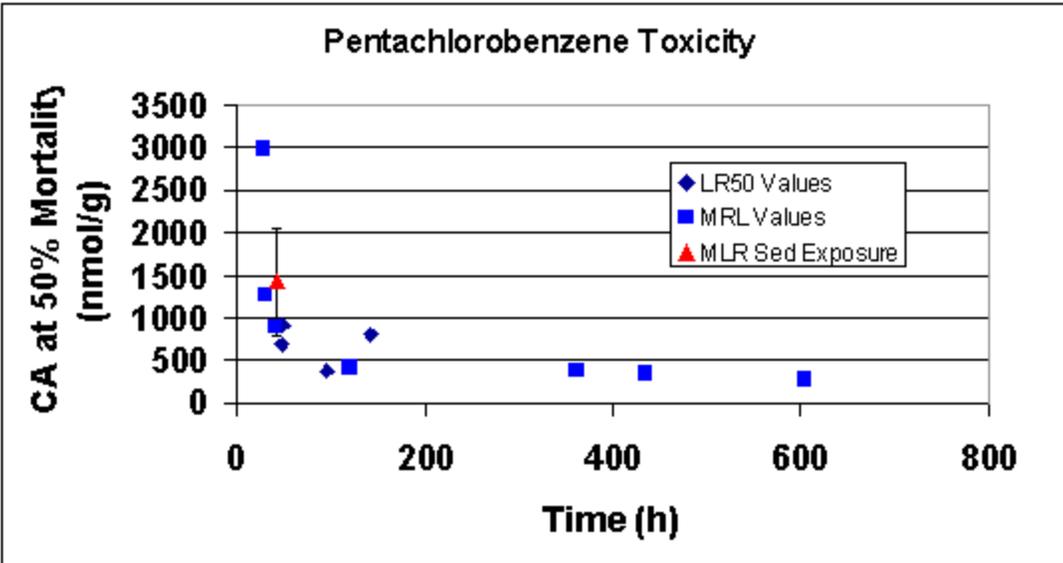


Figure FY01-2: Time dependent toxicity of pentachlorobenzene to *Hyaella azteca* showing the relationship between the body residue required for 50% mortality versus exposure time.

Because it is necessary to perform the challenge application while the organism is still being exposed to contaminated sediment, a test was performed exposing the *H. azteca* in the presence of sediment. The results fit with the water-only exposures (red triangle in Figure FY01-2). Tests are underway to perform the challenge experiment in the presence of a single compound, pyrene.³ Two experiments were performed in conjunction with Dr. Allen Burton and Matti Leppanen at Wright State University to investigate the effect of PCBs on the bioturbation of sediments by the oligochaete, *Lumbriculus variegatus*. The experiments were performed at 23° C and 10° C. The reworking rate was measured as the rate of burial of a ¹³⁷Cs marker layer. Oligochaetes bury the marker layer by feeding at depth and defecating on the sediment surface. The biological burial rate declined with increasing concentration in the sediment (Figure FY01-3) and the rate was also measured for a contaminated sediment.

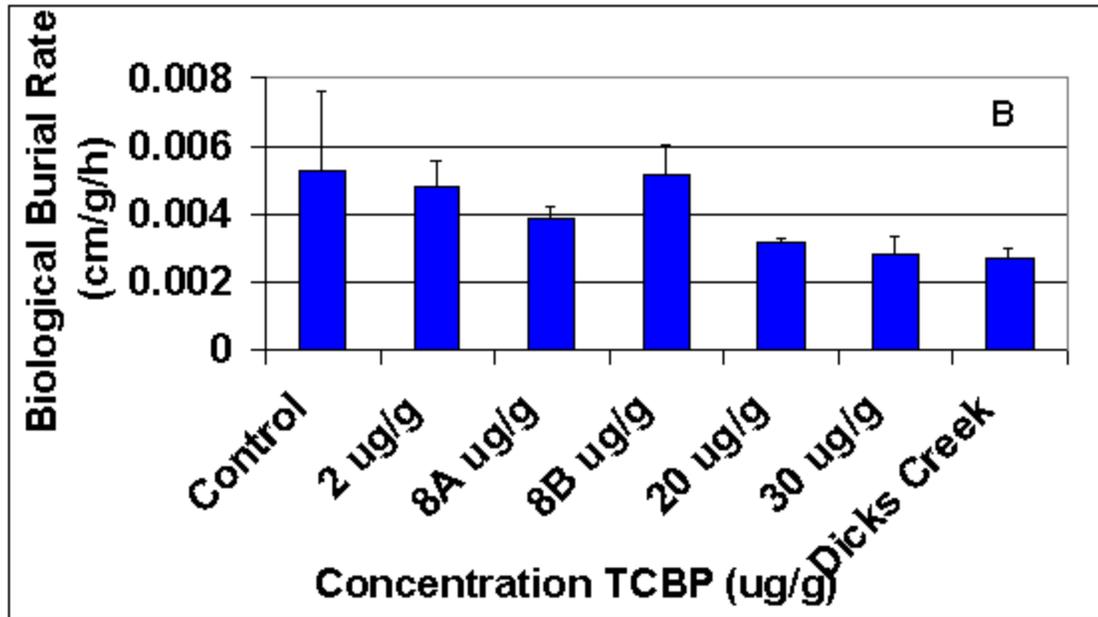
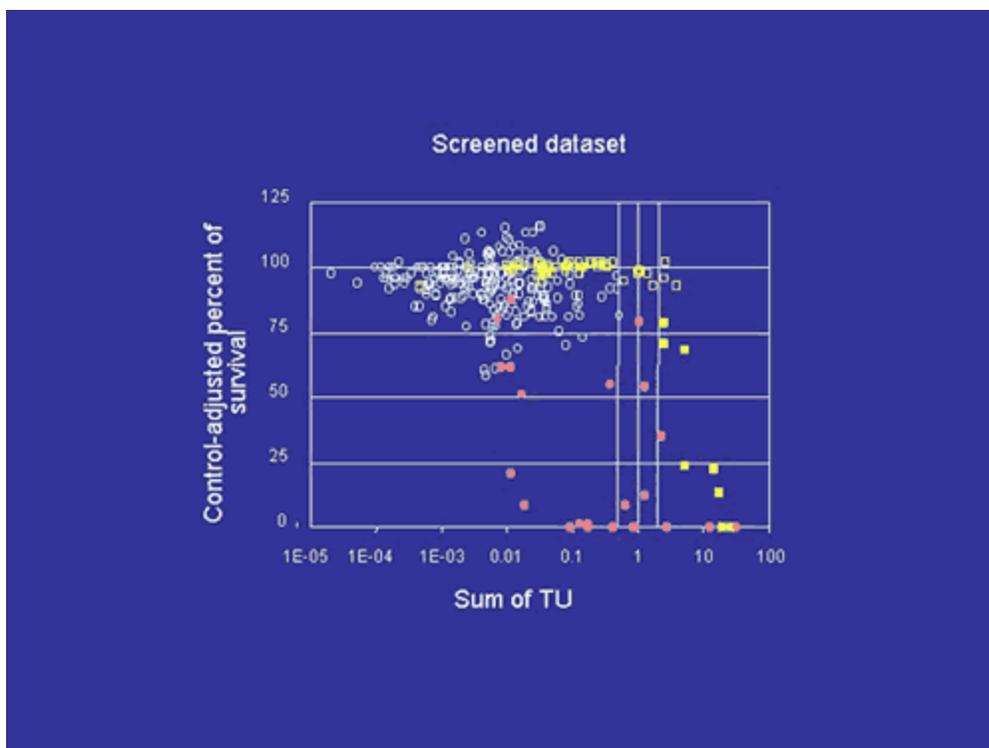


Figure FY01-3: The biological burial rate at 10° C as a function of 3,4,3',4'-tetrachlorobiphenyl concentration in sediment. The worms were not fed during the experiment except for the 8B dose. The control was sediment taken from upstream of the contaminated site on Dicks Creek.

The results from the reworking studies will be compared with the outcome of more conventional bioassays that were performed on the same batches of sediment at Wright State University including toxicokinetics determination, feeding rate determination and 10-d toxicity studies with midges and amphipods. The data have been collected and data analysis is underway.

2000 Accomplishments

1. The Sum PAH model to assess the toxicity of polycyclic aromatic hydrocarbons (PAH) assumes additivity of toxicity. A model was developed for *Hyalella azteca* by developing the relationship between the aqueous LC_{50} and the log Kow. The toxicity of a sediment was estimated as the number of interstitial water toxic units (concentration in the interstitial water divided by the LC_{50}) estimated from the measured PAH concentrations in the sediment using equilibrium partitioning theory to calculate the interstitial water concentration. The toxicity of sediments was predicted for sediments that had been tested in 10 and 14 d studies with *H. azteca*. The database for these sediments was developed as a cooperative effort involving NOAA, EPA, USGS, Macdonald Environmental Sciences Ltd., Environment Canada and the states of Minnesota and Washington. The data was then screened to remove sediments that were likely toxic due to contaminants other than PAH. The resulting plot (FY00-Figure 1) shows that the extent of toxicity increases with increasing toxic units.

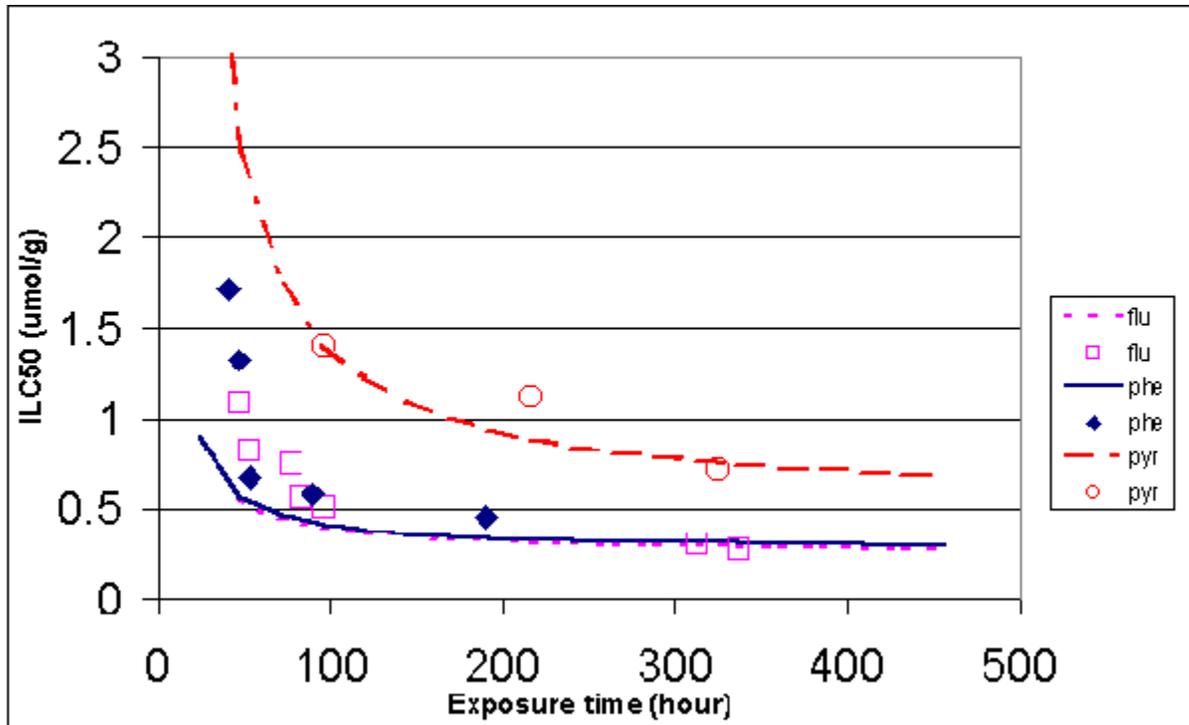


FY00-Figure 1: Plot of the screened bioassay *Hyalella azteca* bioassay data with increasing number of toxic units of PAH in the sediments. The filled symbols are toxic sediments based on their respective controls.

1. In theory, 50% mortality should occur at 1 toxic unit. Thus, this plot reveals several interesting features. First, not all sediments are toxic at PAH concentrations that should have produced significant mortality (Open Yellow Squares) suggesting that bioavailability of the PAH is likely limiting in some cases. Second, it is expected that other contaminants in the sediment whether measured or not can contribute to the observed toxic response (Filled in circles at less than 0.1 toxic units). Finally, reports in the literature suggest that using only the 13 priority PAH, as was done in this study, can underestimate the toxicity potential of sediments. If such a correction for unmeasured PAH were made then the model would have severely over-predicted the toxicity of sediments. It is clear that issues of bioavailability must be addressed before more accurate predictions can be made. A logistic regression model was applied to this data. The model predicted a 50% probability of finding a sediment toxic in this bioassay required 1.3 (0.6 - 3.9 95%CI) toxic units. A manuscript on this work was completed.

2. The use of internal body residues as a metric of dose have been proposed to overcome the problems of interpreting the bioavailability particularly of sediment associated contaminants. The toxicity of fluoranthene (FLU), pyrene (PY) and phenanthrene (PHE) were measure in *Hyalella azteca* with exposure times ranging to 14 d (336 h). The internal lethal concentrations for 50% mortality (ILC_{50}) were determined. While a constant threshold model has been proposed for the toxicity of non-polar contaminants such as PAH, the data were better fit by a model considering the integrated exposure (FY00-Figure 2). This exposure model assumes that the area under the exposure curve is constant. This model requires that either the receptor be irreversibly

occupied, constantly occupied as a result of constant exposure thus mimicking the irreversible binding, or the damage in the organism must be irreversible. A new damage assessment model is currently being developed that will permit improved interpretation of or the time dependence of body residue data.



FY00-Figure 2: The temporal variation in the body residue required to produce 50% mortality in *Hyalella azteca*. The lines represent the fit of the constant area under the exposure curve model to the data.

3. Exposure of the oligochaete, *Lumbricus variegatus*, to sediments dosed with 3,3',4,4'-tetrachlorobiphenyl were performed to examine changes in reworking rate. In addition to reworking rate, the toxicokinetics were determined as well to improve interpretation of the reworking data. The sediment used in these studies was very high in organic carbon content (22.6%). As a result of the high organic carbon content, the organisms did not accumulate any of the tetrachlorobiphenyl and thus did not show any change in reworking rate compared to the control. However, the reworking rate of the worms in a field contaminated material showed a significantly lower reworking rate. These studies will be repeated in the next fiscal year using a sediment with less organic carbon.

In addition to the work with tetrachlorobiphenyl, a control study was performed to examine the utility of Fe59 as a tracer for reworking rate studies. This tracer performed just as well as the Cs137 dosed clay as a tracer of reworking rate for sediments. The main difficulty in using this material is that it must be generated at a local reactor just prior to experimental use. It does have the advantage of having a shorter half life so will be less persistent when disposed of and in the event of a spill.

The data from the reworking study with fluoranthene was examined. Over the range of concentrations used, 50 - 300 ug/g, the reworking rate per worm increased with increasing dose but the number of organisms surviving in the cells decreased with increasing dose. The toxicokinetics still need to be calculated.

4. The main objective of this three-year project is to determine the feasibility and potential environmental impacts associated with using the biocide, glutaraldehyde, for treating the ballast water of ocean-going vessels that enter Great Lakes' ports. This annual summary reviews work accomplished during the second year of this project, extending from September 1999 through August 2000.

The three main areas of research during this time period include: 1) 24-hour acute toxicity bioassays using multiple aquatic organisms; 2) chronic bioassays using fish embryos and a cladoceran species; and 3) degradation experiments to evaluate rates of glutaraldehyde decay. Acute, 24-hour toxicity bioassays were conducted in both water-only and water-sediment exposures to better characterize potential glutaraldehyde effectiveness in ballast tanks. Water-only exposures employed the marine bacterium, *Vibrio fischeri*, and the cladocerans, *Daphnia magna* and *Ceriodaphnia dubia*. Of these three organisms, the bioluminescent bacterium was the most sensitive, with a 15-minute IC83 of 11 mg/L glutaraldehyde (Figure FY00-3).

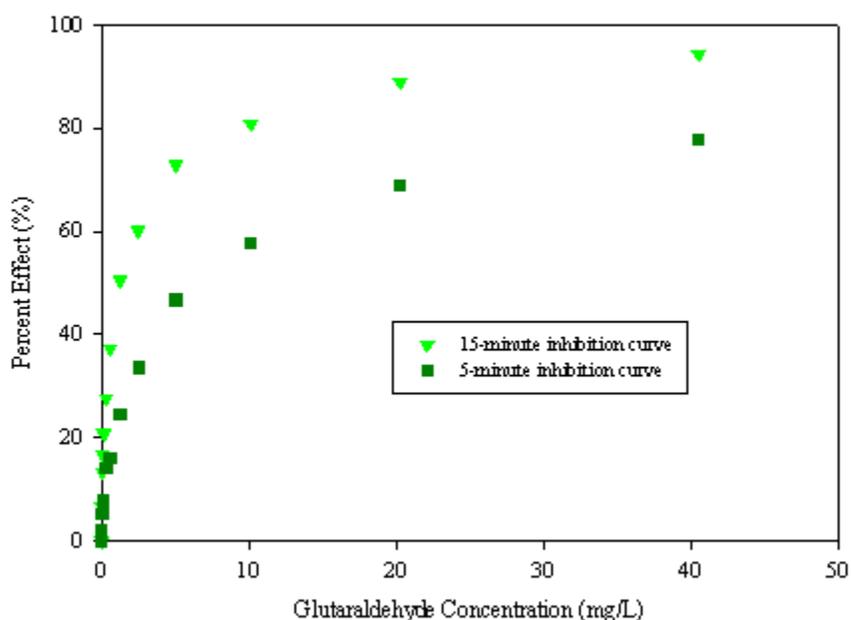


Figure FY00-3: Inhibitory concentrations of glutaraldehyde to the marine bacterium, *Vibrio fischeri*, as determined using the Microtox® test. The effects of glutaraldehyde were measured at both a 5-minute and 15-minute interval, with a significantly larger increase in

inhibition occurring with the longer contact period. The estimated 15-minute IC83 for this experiment was 11 mg/L (95% C.I. 8-14 mg/L).

Sediment-water exposures were also conducted in order to assess the effect of sediment on the exposure-response relationship. These experiments employed the oligochaete, *Lumbriculus variegatus*, and used three different sediment:water ratios. The data indicate that the presence of sediment substantially alters concentrations required to achieve 90% mortality rates. Chronic toxicity bioassays were also conducted to characterize the potential impacts of the release of low concentrations of glutaraldehyde into the environment. These experiments included 7-day reproduction bioassays using the cladoceran, *Ceriodaphnia dubia*, and a 72-day survival and growth bioassay using embryos of the rainbow trout, *Oncorhynchus mykiss* (Figure FY00-4).

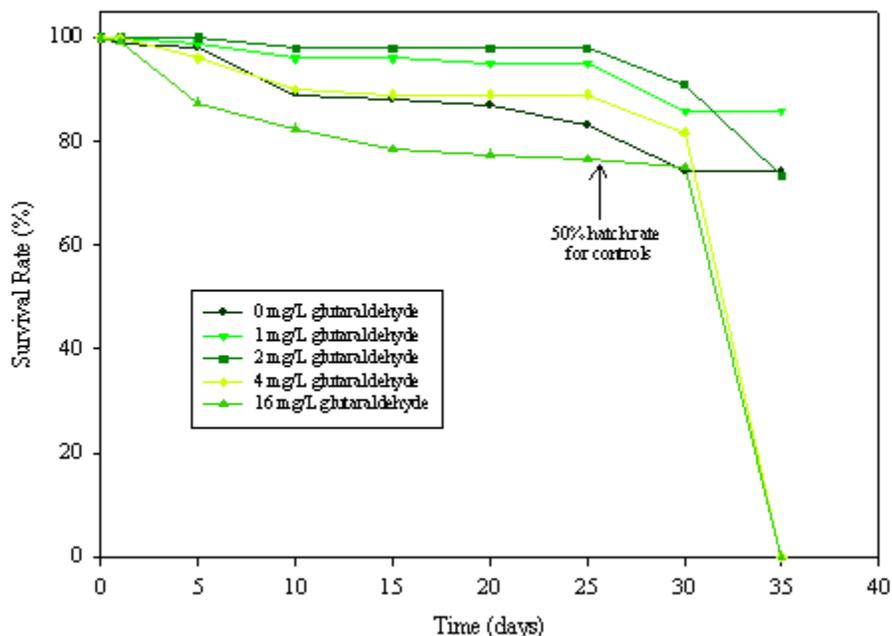


Figure FY00-4: Survival rate for rainbow trout embryos. Data are shown for 10 days after 50% of the controls reached the alevin stage (sac-fry). After this point, all treatments failing to hatch were eliminated from the experiment. The only concentrations with embryos surviving through the hatching period were 0 mg/L, 1 mg/L, and 2 mg/L glutaraldehyde.

To evaluate the potential rate of decay of glutaraldehyde in both ballast tanks and the natural environment, biodegradation experiments were performed in water-only and sediment-water environments. These degradation experiments have been conducted at different sediment-water ratios and at different temperatures in order to evaluate the effect of these variables on degradation rates. Preliminary data indicate that the presence of sediments alters degradation rates, depending on the water:sediment ratio. Finally, the results from the laboratory

experiments are being used to plan a preliminary field trial of glutaraldehyde treatment of a NOBOB (no ballast on board) vessel. This trial is currently scheduled to occur in late October 2000.

1999 Accomplishments

1. The manuscript on the toxicity of DDT and its metabolites from water only exposures by *Hyalella azteca* and *Diporeia* spp. was completed and is in press. The data analysis was completed for the sediment exposure of the two amphipods and the manuscript drafted. The manuscript will be submitted in early FY2000.

2. The toxicity of selected polycyclic aromatic hydrocarbons was determined for *Hyalella azteca* in water-only studies. The Sum PAH model was applied to determine the potential for this approach to predict the toxicity observed in the ARCS data and for a data set collected by NOAA's Office of Response and Restoration. The Sum PAH model was developed using 10-d and 14-d *Hyalella azteca* water-only LC_{50} values. For field sediments, a logistic regression model was derived for assessment of concentration-response relationship using toxic units on predicted interstitial water concentrations. A toxic unit is the concentration in the interstitial water divided by the LC_{50} concentration from water only exposures. When the concentration in the interstitial water is the same as the LC_{50} concentration the ratio results in 1 toxic unit and an expectation of 50% mortality in the bioassay. The model showed that one toxic unit would result in the 50% probability that the sediment would be toxic (Figure FY99-1). For a sediment to be sufficiently toxic to produce 50% mortality nearly 3 toxic units was required. Thus the Sum PAH model was applicable but the bioavailability issue remains an issue.

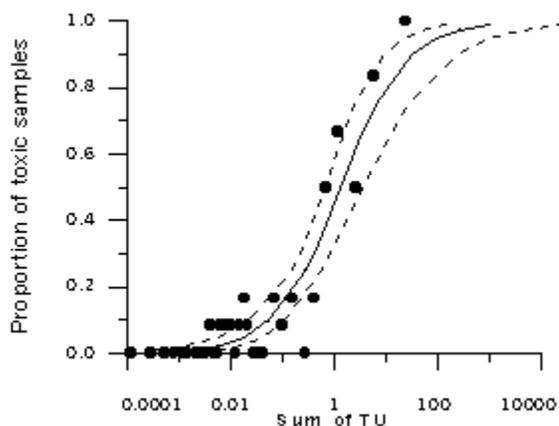


Figure FY99-1: Probability of a sediment containing PAH exerting a toxic response to *Hyalella azteca* based on the sum of the toxic units in the sediment.

To compare the toxicity of pollutants, several benchmarks such as LC_{50} , EC_{50} , LOEC, etc. have been developed. However, this comparison is possible only if the gradient parameter (the slope of effect to external concentration of pollutants), time-dependency of toxicity and the mechanism of toxic action are the same. For three PAHs, we examine the toxicity mechanism using the ILC_{50} -ln LT50 plot (ILC_{50} : the average internal lethal concentration, (Figure FY99-2). This plot presented the gradient parameter and time-dependency of toxicity at the same time. For

phenanthrene and fluorene, the decreasing pattern of ILC_{50} showed a steep initial slope and then gradual slope. In the case of pyrene, ILC_{50} values were not available in the shorter time than four days, which showed the slope different from those of the other PAHs. Since the ILC_{50} values were presented as a 14C equivalency, toxicity of the metabolites of pyrene may be lower than those of the other PAHs. The study on role of biotransformation in PAH toxicity will be continued in the next step of the research.

Relationship between the average Internal Lethal Concentration (ILC_{50}) and the median lethal time

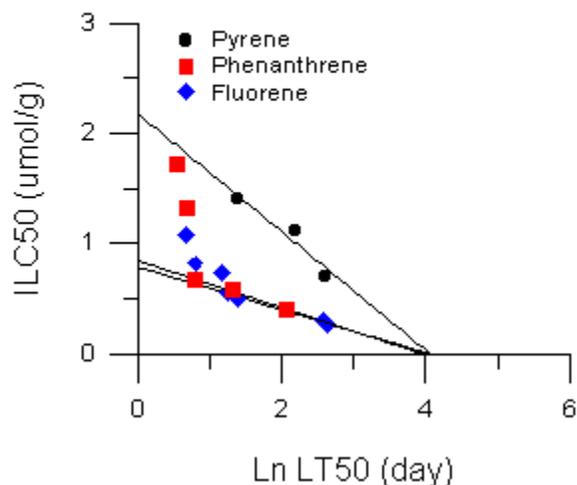


Figure FY99-2: Relationship between internal body residue for 50% mortality and time to 50% mortality.

3. An investigation of the response of *Lumbriculus variegatus* to fluoranthene was performed as the initiation of work on collaboration with Wright State University to separate the factors that influence the response of benthos to contaminated sediment. This work examined changes in reworking rate, toxicokinetics, and measured the body residue required to produce these results for a series of fluoranthene concentrations in sediment. It had been noted previously that the accumulation of contaminants exhibited complex toxicokinetics thought to be a result of changes in the bioavailable fraction of contaminants in sediment. This work identified that such changes occurred with a cessation of the reworking by the organisms. Additional, analysis of the data needs to be completed to evaluate the role of feeding on the bioassay. In addition to investigation of the effect of fluoranthene, this study was used to explore a substitute isotope for the Cs-137 that is used as a tracer for the reworking. The purpose was to use a shorter-lived isotope for additional long-term safety and disposal. Iron-59 was employed for one control but did not seem to be effective. This will be retested in a more comprehensive experiment.

4. Additional data analysis was required and so the manuscript was not completed this past year.

5. In the collaboration with Dr. Fisher at Ohio State, the midge, *Chironomus riparius* was exposed to contaminated water with trace amount of linear alkylbenzenesulfonate, a surfactant. The midges were collected in predetermined time intervals and analyzed for total LAS and metabolites. A toxicokinetics model that incorporates changing water concentration and metabolism was developed. However, the metabolism portion was removed since it was later found that the midges do not metabolize LAS. The parameters calculated using the model are as follows:

- Uptake rate constant, $k_u = 9.10$ (7.73 - 10.47) $\text{mL g}^{-1}\text{h}^{-1}$
- Elimination rate constant, $k_e = 0.050$ (0.042 - 0.057) h^{-1}
- Bioconcentration factor, $\text{BCF} = 182$
- Half-life, $T_{1/2} = 13.8$ h

Toxicity tests were divided into 3 portions; 4-d, 10-d, and partial life cycle tests. Dead midges were collected twice a day and analyzed for body residues by LSC. At the end of the experiment, all midges, dead or alive, were collected and body residues were determined. Four day LC_{50} (95 % CI) was 2.16 (1.76 - 2.55) mg L^{-1} and 4-d LR_{50} body residues to kill 50 % of test organisms, was 2.16 (1.76 - 2.55) mmol/Kg , while ten day LC_{50} was 1.25 (0.49 - 3.05) mg L^{-1} and 10-d LR_{50} was 0.40 (0.14 - 1.10) mmol Kg^{-1} (Figure 3). The partial life cycle test was initiated by putting 2nd instar midges into serially dosed water with algae and a commercial trout chow as food and a burrowing material. The significant changes in developmental time are observed at body residues of 0.022 mmol Kg^{-1} for females, 0.024 mmol Kg^{-1} and 0.029 mmol Kg^{-1} for male developmental time increase (Figure FY99-3). The mortality occurred at 0.022 mmol Kg^{-1} for 34-d, 0.40 mmol Kg^{-1} for 10-d, and 1.17 mmol Kg^{-1} for 4-d.

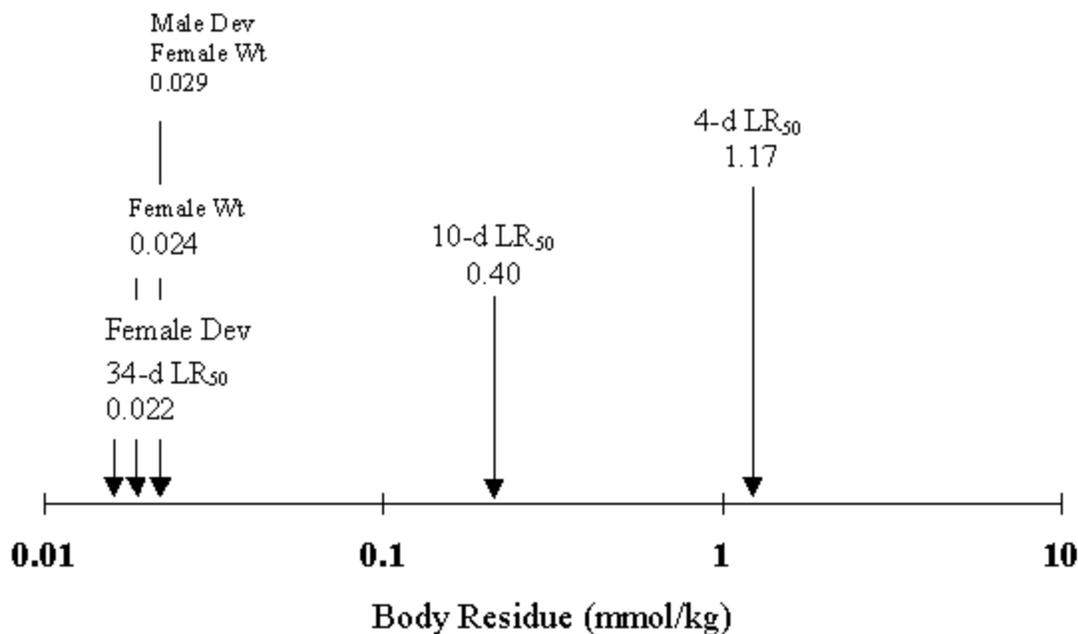


Figure 3 Response spectrum of midges, *Chironomus riparius*, LAS in relationship to body residues. Arrows represent the body residues at which significant effects are first detected.

6. The main objective of this three-year project is to determine the feasibility and potential environmental impacts associated with using the biocide, glutaraldehyde, for treating the ballast water of oceangoing vessels that enter Great Lakes' ports. During the first year of study, this project has focused on three key issues related to biocide use: (1) determining the approximate concentrations of glutaraldehyde required to achieve 90% eradication rates of representative aquatic organisms; (2) assessing the possible environmental risks associated with intermittent release of low concentrations of glutaraldehyde; and (3) evaluating the potential decay rates of glutaraldehyde under varying environmental conditions. Preliminary data from toxicity bioassays indicate that organism sensitivity to glutaraldehyde varies substantially among species. For example, we estimated a 24-hour 90% lethal concentration (LC90) for *Lumbriculus variegatus* (an aquatic oligochaete) of 5 parts-per-million (ppm: 95% confidence interval, 4-7 PPM). In contrast, the estimated 24-hour LC90 for *Hyalella azteca* (a freshwater amphipod) was 510 PPM (95% confidence interval, 451 - 601 PPM). The majority of organisms tested, however, were relatively sensitive to small amounts of glutaraldehyde, indicating that concentrations less than 100 PPM may eliminate most target species. In addition to these acute toxicity studies, chronic toxicity bioassays have been conducted using the green algal species, *Selenastrum capricornutum*, and the cladoceran, *Ceriodaphnia dubia*. Preliminary data from these experiments indicate that glutaraldehyde concentrations of less than 1 PPM may not adversely affect either of these two organisms.

7. Although not formally part of this project, investigations into the mechanism for the decline of *Diporeia* spp. in Southern Lake Michigan were undertaken in conjunction with the benthic

survey work. *Diporeia* survived exposure to sediments taken from the southern basin in 30 d bioassays indicating that the sediments were not overtly toxic. However, *Diporeia* avoided the sediments from the St. Joseph area but no avoidance was found for sediments from Muskegon, Grand Haven or Saugatuck. The avoidance was eliminated for the St. Joseph sediments when fresh diatoms were added as a food source suggesting food limitations in those sediments despite a high organic carbon content. A manuscript covering these findings was drafted and follow up bioassays were performed to confirm the previous findings.

1998 Accomplishments

Because prediction of bioavailability is not accurate for benthic organisms exposed to sediment-associated contaminants, chemical measures of the concentration of contaminants in sediments will not necessarily reflect exposure. As a result, developing the relationship between measured contaminant residues in organisms and the effects of those contaminants should lead to more accurate risk assessments. Developing these relationships will also lead to better interpretation of risk from bioaccumulation data. Investigations with DDT, DDD, and DDE were completed. These studies examined the residue-effects data for these compounds from water-only exposures and subsequently confirmed the data for exposure in contaminated sediment. The amphipods, *Hyalella azteca* and *Diporeia* spp., were exposed to each of the contaminants at 20°C and 4°C respectively. After 10 d exposure for *H. azteca*, the LR₅₀ (residue concentration resulting in 50% mortality) was lowest for DDT (0.006 m mol g⁻¹ wet weight) followed by DDD (0.047 m mol g⁻¹) and the least toxic was DDE at 0.389 m mol g⁻¹. *Diporeia* spp. appeared less sensitive when comparing the 10 d values, DDT 0.085 m mol g⁻¹, DDD 0.362 m mol g⁻¹ and DDE no detectable toxic dose when exposed to concentrations up to aqueous saturation. The apparent differences in sensitivity are the result of differences in the storage capacity of the two organisms. *H. azteca* has relatively low lipid (fat) levels (7% of dry weight) in which to store contaminants and is near steady state within 10 days. However, *Diporeia* has very high lipid levels (24% of dry weight) and is not near steady state after only 10 days. When the differences in storage capacity are adjusted, *H. azteca* is more sensitive by about a factor of two. Evaluation of toxicity of mixtures of these contaminants must consider the relative toxicity of the various biotransformation products to properly assess the observed toxicity based on body residue. The exposures to contaminated sediments have been completed and data analysis is continuing.

The residue concentrations of selected chlorinated biphenyl congeners and DDE were evaluated for the oligochaete, *Lumbriculus variegatus* (Fisher et al. 1998). The worms were exposed to these contaminants through contaminated food, and the toxic response was determined based on dose received as body residue. Mortality, reproduction, and growth were the endpoints examined. No LR₅₀ could be measured out to 28 d exposure, but statistically significant reductions in survival were observed at body residues in the range of 0.88 - 1.35 m mol g⁻¹ for the various contaminants. None of the contaminants was particularly more toxic than another. For reproduction and growth, the concentration required to produce statistically significant reductions was 0.34 - 0.56 m mol g⁻¹ again with no particular difference among compounds. For this organism, mortality occurs within the range that is generally expected for non-polar narcosis. This organism is recommended for use in bioaccumulation studies from

contaminated sediments. Thus, establishing these residue effects levels for non-polar narcotics in this species will permit improved interpretation of bioaccumulation data.

The toxicity of selected polycyclic aromatic hydrocarbons (PAH) was determined for *Diporeia* spp. In water-only studies. These results were then used with EPA's Equilibrium Partitioning Approach to examine the utility of the SUM PAH Model (which predicts the effects of mixtures of sediment-associated PAH as additive based on the proportion of the contaminant freely dissolved in interstitial water). Using data from US EPA Assessment and Remediation of Contaminated Sediments (ARCS) database, the model had limited ability to predict observed toxicity of field collected sediment. This may be due to the limited data available for *Diporeia* or to the observation that in freshwater systems the Equilibrium Partitioning Approach has not been successful in prediction exposure of *Diporeia* to PAH contaminated sediment. This is a major reason for continued studies of the factors that affect the bioavailability of sediment-associated contaminants.

Products

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