

TIME-DEPENDENT TOXICITY OF DICHLORODIPHENYLDICHLOROETHYLENE TO
HYALELLA AZTECA

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Abstract—Temporal effects on body residues of dichlorodiphenyldichloroethylene (DDE) associated with mortality in the freshwater amphipod *Hyaella azteca* were evaluated. Toxicokinetics and body residues were determined from water-only exposures that varied from 4 to 28 d, and DDE concentrations ranging from 0.0013 to 0.045 $\mu\text{mol L}^{-1}$. Uptake and elimination parameters were not affected significantly by the various temporal and concentration treatments. Uptake rate coefficients ranged from 134.3 to 586.7 $\text{ml g}^{-1} \text{h}^{-1}$, and elimination rate coefficients ranged from 0.0011 to 0.0249 h^{-1} . Toxicity metric values included body residue for 50% mortality at a fixed sample time (LR50) and mean lethal residue to produce 50% mortality from individual exposure concentrations (MLR50) for live organisms and dead organisms. A twofold increase occurred in the MLR50 values calculated using live organisms compared to MLR50 values using dead organisms. Toxicity and kinetic data were fit to a damage assessment model that allows for the time course for toxicokinetics and damage repair, demonstrating the time-dependence of body residues to toxicity. The DDE appeared to act through a nonpolar narcosis mode of action for both acute and chronic mortality in *H. azteca*. Furthermore, the temporal trend in the toxic response using body residue as the dose metric is steep and found to be similar to another chlorinated hydrocarbon, pentachlorobenzene, but was more potent than that found for polycyclic aromatic hydrocarbons (PAHs).

Keywords—Dichlorodiphenyldichloroethylene *Hyaella azteca* Lethal residue Temporal response

INTRODUCTION

The use of body residue, or concentration of chemical in tissues, to determine exposure as opposed to using the external media concentrations shows great promise to improve our ability to interpret the significance of bioaccumulated contaminants and decrease the uncertainty associated with estimating ecological risk. The body residue concept was formulated to help overcome the obstacles of multiple routes of exposure and differences in chemical bioavailability when using the external concentration as the dose metric [1]. The use of body residues to assess exposure also would address the temporal issue presented by Sprague [2] that bioassays should be run such that the influence of time be minimized and the asymptotic (threshold or incipient) toxicity estimates should be reported. From the initial work of McCarty [3], the temporal response to toxicity was thought largely to be governed by the toxicokinetics and that the internal body residue essentially would be a constant value (a threshold), particularly for nonpolar narcotic (anesthetic) compounds. The concept of a constant threshold for nonpolar narcotic compounds continues to be espoused in the literature [4]. Several studies of body residue response relationships appear to support an apparent constant threshold [5–7].

However, temporal changes also have been observed with some nonpolar narcotic (anesthetic) compounds in crabs [8], fish [9,10], and amphipods [11,12], generally showing a decline in the body residue required to produce a toxic response (mortality) as exposure duration increases. Three mechanisms

might account for a temporal response, biotransformation, change in mechanism of action, and build up of damage. The impact of biotransformation has not been well studied. However, for polycyclic aromatic hydrocarbons (PAHs) there is a hypothesis that the phase I metabolites essentially should be as toxic as the parent compound [13]. In one study, the metabolites of fluoranthene were examined in aqueous exposures and, though most of the metabolites were substantially less toxic, the 9-hydroxyfluoranthene essentially was found to be equally toxic to *Daphnia* [14]. Assuming that the toxicokinetics are not substantially different between fluoranthene and 9-hydroxyfluoranthene, this work would suggest that similar body residues would be required for toxicity. In another study, the metabolites of PAH, specifically naphthalene, were estimated to be substantially less toxic than the parent compound [11]. Thus, the issue of the role of metabolites in the body residue response relationship remains in question. However, unless a toxic metabolite is formed, the concentration of the total residue required to produce a toxic response should remain the same or increase with biotransformation.

No body residue response studies have attempted to address the potential change in the toxic response as a change in mechanism of action when there was no biotransformation and the response endpoint is the same. Nor is there any easily formed hypothesis to support a change in mechanism of action; however a change in mechanism of action is not required if there is a build up of damage with continual exposure. For compounds where the interaction with the receptor essentially is irreversible, there is a clear build up of damage and the temporal response was modeled as the integrated exposure to the toxicant [15,16]. In this case, damage repair is negligible.

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However, nonpolar contaminants are expected to produce reversible interactions with the receptor. Thus, the build up of damage for nonpolar narcotics in the absence of significant biotransformation, will depend on the rate of elimination and the rate of damage repair. For a series of PAH congeners where biotransformation was found to be small [11] and for pentachlorobenzene with no detectable biotransformation [12], the body residue response relationship was found to be variable temporally and the pentachlorobenzene was more potent than the PAH congeners. The temporal response was described by a damage assessment model that described the change in body residue response as a function of both the toxicokinetics and the toxicodynamics, specifically the rate of damage repair [17].

To continue to explore the features important for the utility of body residue as a dose metric, this effort examines the temporal variation in the toxicity of dichlorodiphenyldichloroethylene (DDE) body residues in the aquatic benthic invertebrate *Hyalella azteca* and compares the results to that of other compounds. Dichlorodiphenyldichloroethylene is one of the main metabolites of DDT, and the environmental concentrations often are higher than those of the parent compound at contaminated sites [18]. Although the toxicity of DDE is far lower than that of DDT and another metabolite, dichlorodiphenyldichloroethane [19], it persists in natural systems for years potentially posing a threat to biota [20]. Thus, evaluating the temporal trend in the body residues required to produce mortality in *H. azteca*, an important sediment bioassay species [21], is expected to lead to improved risk assessment in circumstances where the bioavailability of the contaminant may be complicated.

MATERIALS AND METHODS

Chemicals

The *p,p'*-dichlorodiphenyldichloroethylene (DDE, uniformly ring labeled, 13.4 mCi mmol⁻¹) was purchased from Sigma Chemical (St. Louis, MO, USA). The toluene solvent was evaporated under a stream of nitrogen and the DDE dissolved in acetone. The radiolabeled compound was purified of an unknown contaminant on a silica gel column using 90:10 hexane:benzene solvent. The radiopurity was determined by thin-layer chromatography on silica gel plates using hexane:benzene (95:5, v:v) as the eluting solvent, followed by liquid scintillation counting of silica gel scraped from the plate in sections in 3a70B scintillation cocktail (Research Products International, Mount Prospect, IL, USA) and counted on a Packard Tri-Carb liquid scintillation analyzer model 2500 TR (Packard Instruments, Meriden, CT, USA). Samples were corrected for quench using the external standards method after subtracting background. The radiopurity was determined as the amount of activity from the silica gel sections corresponding to a standard versus all remaining activity and was >98%.

Exposure water

All exposures were carried out in water from the Huron River, collected at the Hudson Mills MetroPark (Dexter, MI, USA), and filtered through a glass fiber filter (934-AH, Whatman, Clifton, NJ, USA). Water was spiked with ¹⁴C-DDE and the appropriate amount of additional acetone (<100 μL L⁻¹). The concentration of acetone was maintained constant across doses, and the solvent control received the same amount of acetone as the treatment concentration. The concentration of acetone varied from 38 to 70 μL L⁻¹ for the different experi-

ments. After mixing the dosed water thoroughly, the water was allowed to sit for 1 h prior to use. Dissolved oxygen was measured periodically throughout the experiments and averaged 5.4 ± 1.0 mg L⁻¹.

Toxicity experiments

The duration of the exposures varied from 4 to 28 d at 23 ± 1°C, and the water concentrations ranged from 0.0013 to 0.045 μmol L⁻¹ (0.4 to 14.2 μg L⁻¹, molecular weight 318.03 g mole⁻¹). The exposures were performed below the mean water solubility 43.5 ± 56 μg L⁻¹ [22] and the use of Huron River water, which only minimally (<10%) binds DDE [19], suggests that the water concentrations can be considered dissolved for the purpose of the toxicokinetics. The exposures were performed with four or five doses per experiment, in addition to a solvent control. *Hyalella azteca* (10- to 14-d old) were purchased from Aquatic Biosystems (Fort Collins, CO, USA) and exposed in 200-ml beakers filled with 150 ml water and 10 amphipods along with a small piece (2–4 cm²) of cotton gauze to serve as a substrate. The animals were transferred daily to fresh exposure water and fed 0.25 ml yeast-cerophyll-trout chow mixture [21]. The dead organisms were removed each day, blotted dry, weighed, and counted by liquid scintillation counting for body residue. The organisms were placed in 6 ml of xylene-based scintillation cocktail (which serves as the extracting solvent), mixed, and allowed to stand for 24 h prior to liquid scintillation counting. Water samples (2 ml) were taken for each concentration before and after each transfer, placed in a 12-ml scintillation cocktail, and counted for radioactivity. On each sampling day three beakers per dose were removed, the organisms removed and counted, and live organisms taken for determination of body burden as described above for the dead organisms. Body burden was calculated using the measured activity and the specific activity of the DDE. The sampling regime was 1, 2, and 4 d for the 4-d exposures; 2, 4, 7, and 10 d for the 10-d exposures; and 4, 7, 10, 17, and 28 d for the 28-d exposures. Lipid content was determined for organisms removed from the controls on day 1 for the 4-d exposures and days 4 and 17 for the 28-d exposure using the spectrophotometric method of Van Handel [23].

Modeling

The accumulation and loss kinetics were calculated using a two-compartment model with water as the source and the organism as a sink:

$$\frac{dC_a}{dt} = k_u C_w - k_e C_a \quad (1)$$

where C_a is the concentration in the organism (μmol g⁻¹), k_u is the uptake clearance rate (ml g⁻¹ h⁻¹), C_w is the concentration in the water (μmol ml⁻¹), k_e is the elimination rate constant (h⁻¹), and t is time (h). Because DDE concentrations in the water decreased over the period between transfers to fresh exposure solution (55.6 ± 3.3%), the time-weighted average water concentration was used as the source concentration. This is acceptable because the elimination half-life is substantially longer than the exchange period [12].

Lethal body residues (LR50) of DDE were determined for each exposure period using logit analysis of response versus log concentration of the chemical in live organisms at the corresponding exposure time [17,24]. Median time to mortality (LT50) was calculated for each dose where mortality exceeded 50% by the end of the exposure period. Logit analysis of

Table 1. Toxicokinetics of dichlorodiphenyldichloroethylene toxicity in *Hyaella azteca*

Exposure (d)	Target dose ($\mu\text{g L}^{-1}$)	Actual dose (T wtd avg) ^a	Concn. (% decline) ^b	k_a (ml g ⁻¹ h ⁻¹) ^c	k_e (h ⁻¹) ^d	Survival ^e (%)
4	1	0.58	56.1 (7.0)	504.8 (153.9)	0.0171 (0.0103)	97 (6)
	2	1.23	56.1 (2.6)	335.0 (89.4)	0.0050 (0.0069)	97 (6)
	5	3.19	54.7 (1.2)	472.1 (55.8)	0.0249 (0.0047)	100 (0)
	10	6.90	55.5 (6.9)	364.6 (90.8)	0.0121 (0.0075)	77 (23)
	20	14.17	52.2 (9.3)	251.1 (30.1)	0.0104 (0.0041)	13 (15)
10	0.5	0.40	52.5 (3.9)	436.7 (113.1)	0.0145 (0.0051)	97 (6)
	1	0.81	54.1 (3.6)	309.1 (50.3)	0.0069 (0.0022)	97 (6)
	2	1.60	54.9 (5.4)	313.1 (61.1)	0.0069 (0.0027)	90 (0)
	5	4.24	52.1 (2.6)	209.6 (46.5)	0.0047 (0.0027)	100 (0)
	10	8.32	51.9 (9.9)	134.3 (32.8)	0.0011 (0.0025)	60 (17)
28	1	0.61	57.3 (4.9)	300.5 (51.4)	0.0063 (0.0015)	93 (6)
	2	1.26	60.4 (3.7)	413.5 (79.7)	0.0097 (0.0023)	83 (15)
	5	3.12	61.4 (4.2)	341.0 (57.6)	0.0070 (0.0015)	77 (12)
	10	6.12	60.8 (4.7)	266.0 (34.5)	0.0024 (0.0008)	7 (12)
	2	1.08	54.0 (7.7)	516.5 (82.4)	0.0169 (0.005)	100 (0)
4	5	3.07	51.4 (3.5)	551.9 (83.8)	0.0207 (0.005)	100 (0)
	10	6.16	54.4 (3.7)	586.7 (113.2)	0.0231 (0.007)	40 (10)
	15	9.06	52.1 (3.2)	539.9 (135.6)	0.0173 (0.009)	5 (6)
	20	15.59	53.5 (2.0)	413.1 (146.5)	NS ^f	0
	2	1.27	52.1 (3.7)	372.9 (65.4)	0.0055 (0.0055)	96 (8)
17	5	3.28	55.2 (4.1)	389.2 (92.3)	0.0082 (0.0026)	33 (27)
	7	4.77	50.0 (4.5)	352.4 (119.3)	0.0084 (0.0046)	0
	10	6.36	55.9 (7.1)	295.3 (115.0)	0.0095 (0.0055)	0
	12	7.26	57.8 (8.9)	ND ^g	ND	0

^a Actual dose in $\mu\text{g L}^{-1}$ as the time-weighted average (T wtd avg) over the entire exposure.

^b Mean percent water concentration decline between water exchanges (standard deviation).

^c Estimate (standard deviation [SD]) for uptake clearance rate (k_a).

^d Estimate (SD) for elimination rate (k_e).

^e Mean percent survival (SD) of *H. azteca* at termination of the exposure.

^f NS = Not significant.

^g ND = Not determined, insufficient data.

response versus log time was used to calculate the LT50. Mean lethal residue values (MLR50) are calculated as the mean concentration of chemical in dead organisms for a given water exposure concentration associated with the LT50 [9,10]. Another estimate for MLR50 values was determined from the mean body residue concentration in live organisms post-LT50 to the end of the exposure associated with the LT50 [12]. The MLR50 values were associated with each LT50 to provide a temporal body-residue response value to augment the LR50 determinations [12].

The time-dependent toxicity data were then fit to a damage assessment model that accounts for the toxicokinetics and the toxicodynamics [17]. The time-dependent model couples a first-order toxicokinetics model (see above) with an estimate of the formation of damage. Damage takes time to occur in an organism once the contaminant reaches the site of toxic action and damage takes time to repair once the receptor is no longer occupied. Thus, the dynamics for the damage is modeled as though it were a first-order process as first approximation of the damage formation and repair processes.

$$\frac{dD}{dt} = k_a C_a - k_r D$$

where k_a ($\mu\text{mol}^{-1} \text{h}^{-1} \text{g}$) is the rate constant for damage formation, D (unitless) is the amount of damage, and k_r (h^{-1}) is the rate constant for damage repair. Because damage cannot be measured directly but its effect (mortality in this case) is determined, then it is not possible to determine separate estimates for D or k_a ; however, if we assume that there is a value D_L that represents the amount of damage to produce 50%

mortality, then a limiting condition that lumps D_L and k_a can be estimated in the following equation. In this case, estimates for k_r can be estimated as well without having to make specific measures of the amount of damage.

$$\text{LR50}(t) = \frac{\frac{D_L}{k_a}}{\frac{1}{(1 - e^{-k_e t})} \times \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right)}$$

This model assumes a net amount of damage as the result of the rate of damage formation, which is the product of rate constant k_a and the concentration of the contaminant in the organism, and a rate of damage repair, which is the product of k_r and D , the amount of damage.

Regression analysis was performed using Scientist[®] Version 2.01 (MicroMath, St. Louis, MO, USA). Logit analysis was performed using Systat[®] 10.0 (SPSS, Chicago, IL, USA). All values were considered significant at $p < 0.05$ and significantly different if the 95% confidence intervals did not overlap.

RESULTS

Toxicokinetics

The uptake and elimination constants showed no clear relationship with increasing DDE concentration in the water (Table 1). However, the uptake and values in the second 4-d study were elevated by approximately a factor of two over the rate constants derived from the other test exposures. While elevated concentrations might have occurred if the animals were small-

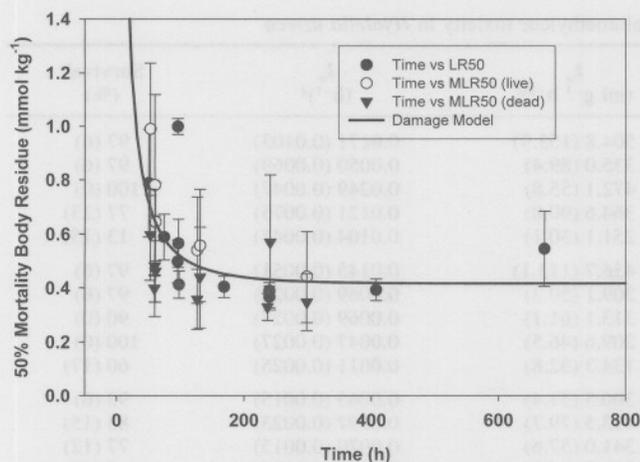


Fig. 1. Time-dependent body residue for 50% mortality of dichlorodiphenyldichloroethylene (DDE) in *Hyalella azteca* and the fit of the damage assessment model to the temporal data. This relationship includes the data from Lotufo et al. [19]. The LR50 is the body residue for 50% mortality determined at a fixed time across exposure concentrations. The MLR50 is the mean body residue for 50% mortality determined from an individual exposure and applied at the LT50. The LT50 is the exposure time to 50% mortality.

er, which leads to more rapid uptake as observed for PCB congeners in *Diporeia* [25], there was no observed difference in organism weight among the experiments. Despite the scatter in both the uptake and elimination coefficients, the elimination rate constant did track directly with the estimated uptake coefficient, which leads to a relatively constant estimate for the bioconcentration factor (BCF). The resulting bioconcentration factor (ml g^{-1} wet wt), calculated as the ratio of k_u/k_e , was $45,546 \pm 26,444$ (mean \pm standard deviation, $n = 22$), which is within the range reported in the literature for DDE in other aquatic organisms [22].

Toxicity

The calculated lethal body residues of DDE for 50% mortality in *H. azteca* exhibited a temporal response (Fig. 1). The measures of the body residue to produce a toxic response were similar for a given exposure duration regardless of the method used to calculate the body residue for 50% mortality. All of the data was fit to the damage assessment model that allows for a time course for repair of damage [17]. The resulting fit had a coefficient of determination of 0.56, and estimates of D_t/k_a (the critical damage level for 50% mortality divided by the rate constant for damage formation) was $11.24 \pm 2.47 \mu\text{mol h g}^{-1}$ and k_r was $0.037 \pm 0.012 \text{ h}^{-1}$. These values are similar to those found for *H. azteca* with pentachlorobenzene (PCBZ) where $D_t/k_a = 18.1 \pm 2.7 \mu\text{mol h g}^{-1}$ and $k_r = 0.021 \pm 0.007 \text{ h}^{-1}$ [12].

When comparing the MLR50 determined using live or dead organisms, larger values were obtained when live organism residues were used as compared to the values from residues in dead organisms (Fig. 2). This difference between the estimate using residues from live versus dead organisms is different than that observed for PCBZ [12] and for dodecylbenzene sulfonate [26] where the two methods for determining the mean lethal residue resulted in similar values. No direct method exists for evaluating the relationship between the LR50 values and the two methods of measuring the MLR50 because the exposure times differ. Nevertheless, visual examination of

the data in Figure 2 suggests that the LR50 values fall between the MLR50 estimates derived using live and dead organisms.

In one measurement, the LT50 was 241 h, and the MLR50 was reported to be $0.930 \mu\text{mol g}^{-1}$ (Table 2). This value was estimated for organisms that were alive, and the body residues were measured at essentially the same time as the LT50. If all values post-LT50 had been used to estimate the MLR50 value, it would have been much larger at $1.2 \pm 0.37 \mu\text{mol g}^{-1}$. Because the LT50 occurred at 241 h and steady state would have required over 1,000 h based on the toxicokinetics for the $10 \mu\text{g L}^{-1}$ exposure in the 28-d study, the organisms that did survive were not near steady state and thus continued to accumulate contaminant. Thus, the approach of using the body residues post-LT50 for the estimate in live organisms has some limitations when steady state is not achieved by the LT50. When PCBZ was used, this was not an issue because the organisms reached steady state in a much shorter timeframe of about 240 h [12]. Thus, the kinetic limitation accounts in large part for the difference in the estimates for the MLR50 from live organisms and dead organisms. Therefore, for contaminants that have very slow elimination kinetics, the timeframe used for estimating body residue likely is critical and should be matched with the estimate for response as is done with the LR50 estimates.

DISCUSSION

Toxicokinetics

The uptake coefficients for DDE for all tests except the second 4-d test were similar to those observed in previous work with this organism [19] and exhibited a general decline with increasing DDE concentration as observed previously. The data collected for the second 4-d test were larger and did not show the same decline (Table 1). Overall, there was no relationship with DDE concentration. Currently, there is no explanation for the substantially larger uptake in the second 4-d test as compared to the other tests or other previous studies. The elimination rate constants also were of similar magnitude and matched with the previously measured elimination rate for DDE from *H. azteca* [19]. We did not determine the potential for biotransformation with *H. azteca* as it was determined previously that essentially there is no biotransformation of DDE by *H. azteca* [19]. Thus, if kinetic information (k_u/k_e) were used to estimate the bioconcentration factor, there would be no need to account for biotransformation in such calculations. The magnitude of the resulting estimate for the bioconcentration factor matched that in Lotufo et al. [19]. Thus, from a toxicokinetics perspective, *H. azteca* behaved similarly to previous work.

Temporal toxicity

Previous work has shown that the LR50 values are the same whether calculated from body residues measured in live or dead organisms [12,26]. This is likely the case because the organisms have similar toxicokinetics until death and demonstrated by similarities in the bioconcentration factors in live and dead organisms for pentachlorobenzene [12]. This is important for environmental measurements because it generally will not be possible to collect dead organisms in the field because they degrade rapidly. To apply the body residue method to field-collected organisms it is important to relate the concentrations in live organisms to the toxic response, mortality in this study. Additionally, the MLR50 values for each

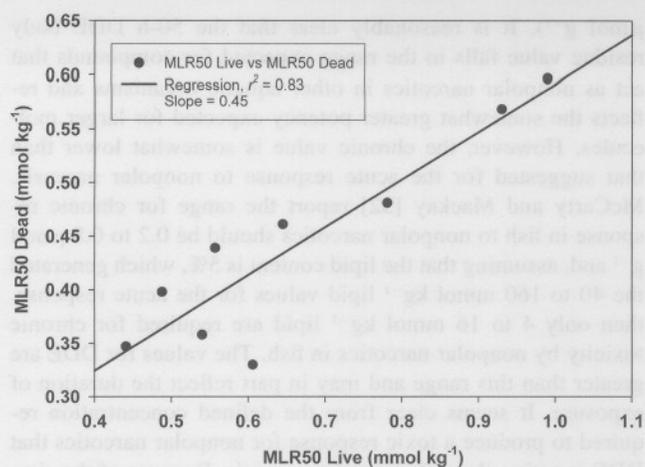


Fig. 2. Relationship between the estimates for the mean lethal residue (MLR50) using the body residues found in dead versus live organisms from Table 2. The MLR50 is the mean body residue for 50% mortality determined from an individual exposure and applied at the LT50. The LT50 is the exposure time to 50% mortality.

exposure concentration provide an estimate of the body residue responsible for 50% mortality. When these residues are associated with the LT50, the combination provides the temporal relationship between the toxic response and the body residue for each exposure concentration. Thus, these values in combination with other measures help define the temporal response of the body residue. These measures have more variability associated with them than the LR50 values, as there are uncertainties with both the body residue measurement and the associated timeframe. However, these values are not different statistically from the LR50 values where the timeframes match based on overlap of confidence intervals.

The temporal data generated in this work are similar to those generated with *H. azteca* in a previous study [19] except that the LR50 for 96 h as determined through that study was larger. The temporal change in the body residue resulting in 50% mortality is a curve that rapidly declined to reach a relatively constant body residue after 100 to 200 h despite the relatively slow elimination coefficient. In fact, the curve flattens faster than would be predicted from the toxicokinetics where steady state would be expected only after about 312 h. The long time to steady state is reflected in the continued drop in the LC50 values. The damage assessment model thus shows how the balance between the toxicokinetics and the toxicodynamics plays out to yield a relatively constant body residue response before the organism actually reaches steady state. This may be in part because of the relatively rapid apparent damage repair with a half-life of about 18 h. This rate is comparable to that observed for PCBZ in *H. azteca* of about 33 h [12].

This work, like most other aquatic toxicology studies, has employed essentially constant exposure with no opportunity for recovery. Recent work has suggested that, after the exposure has reached a critical level, additional time is required for full manifestation of a potentially delayed response [27,28]. The damage assessment model attempts to incorporate the dynamics that dictate the response in that there is direct evaluation of the time course of the toxicodynamics. However, this model uses a simplified structure for the toxicodynamics assuming two linear processes. This simplification does allow estimation of the time-dependent phenomena and permits the

Table 2. Toxicity of dichlorodiphenyldichloroethylene measured using three measures of body residue as the dose metric

LT50 ^a (h)	LR50 ^b ($\mu\text{mol g}^{-1}$)	MLR50 ^c (live) ($\mu\text{mol g}^{-1}$)	MLR50 (dead) ($\mu\text{mol g}^{-1}$)
50.2	0.789 (0.682–0.993) ^d		
53.1		0.990 (0.247) ^e	0.597 (0.169) ^e
59.6		0.488 (0.137)	0.398 (0.050)
59.9		0.781 (0.145)	0.480 (0.114)
61.4		0.675 (0.145)	0.462 (0.114)
74.4	0.589 (0.477–0.644)		
96	0.498 (0.416–0.606)		
96.8	0.565 (0.502–0.649)		
98	0.412 (0.374–0.460)		
126.5		0.540 (0.197)	0.358 (0.111)
130.9		0.557 (0.208)	0.439 (0.188)
168.6	0.404 (0.366–0.444)		
237.1		0.606 (0.21)	0.330 (0.053)
239.9	0.367 (0.314–0.409)		
241		0.93 (0.23) ^f	0.569 (0.249)
298.5		0.441 (0.168)	0.347 (0.105)
407.6	0.393 (0.361–0.432)		
672	0.545 (0.408–0.737)		

^a LT50 is the exposure time to 50% mortality.

^b LR50 is the body residue for 50% mortality determined at a fixed time across exposure concentrations.

^c MLR50 is the mean body residue for 50% mortality determined from an individual exposure and applied at the LT50.

^d 95% confidence interval.

^e Standard deviation.

^f The estimate for the concentration in live animals associated with 50% mortality came from organism concentrations measured in the same time frame as the LT50.

evaluation of the time required for recovery based on k , after elimination is complete. For nonpolar narcotics, the recovery will be both a function of the elimination rate, which is slow for DDE, and the damage repair, which appears relatively rapid based on the estimate of k_r . Further, this steep temporal response suggests that if traditional approaches that generally use 96-h or 10-d exposures were employed, the body residue for the toxic response likely could not be distinguished for the two timeframes because of the usual variability in toxicity data. However, the temporal curve for the damage assessment model allows interpretation of the response for pulsed exposures as might occur during runoff events better than the selection of a single toxic response value that would result from selecting a 96-h or 10-d exposure response.

With the damage assessment model, it becomes possible to interpret the impact of body residues at exposure timeframes of 28 d, which is the timeframe for the bioaccumulation tests. It is equally possible to interpret bioaccumulation data if appropriate 28-d bioassays are performed to develop relationships between body residue and response. However, clearly it is not sufficient to assume that the body residue response is constant temporally and uses results from short-term tests to interpret responses from 28-d bioaccumulation.

The toxicity of DDE, based on body residue concentration, essentially was the same as that observed for PCBZ ([12], Fig. 3). Thus, the toxic responses of the two compounds on a body residue basis have the same potency on a molar basis and would be expected to produce additive toxicity based on both compounds acting as nonpolar narcotics. Both of the chlorinated hydrocarbons required substantially lower body residues than the PAHs to produce 50% mortality [11]. There also was a difference between pyrene and the other two PAHs, fluorene and phenanthrene, which appeared to require the same body

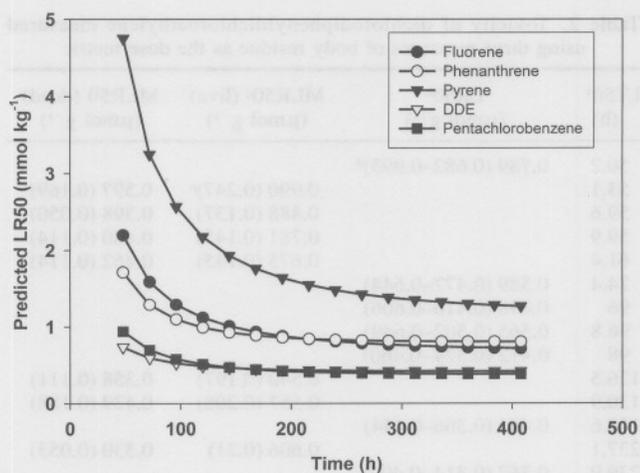


Fig. 3. The damage assessment model predictions of the body residue required for 50% mortality for fluorene, phenanthrene, pyrene [17], pentachlorobenzene [12], and dichlorodiphenyldichloroethylene (DDE). The LR50 is the body residue for 50% mortality.

residues. The difference among the PAH and between the PAH and the chlorinated hydrocarbons may be due to biotransformation, as the body residues are based on total radioactive residue and not parent compound. Although biotransformation was not observed for PCBZ [12] or for DDE [19], the biotransformation of PAH by *H. azteca* is recognized [11,29,30]. Based on the reported biotransformation, the extent of difference in the body residues required for 50% mortality between the PAH and chlorinated compounds or among the PAH, assuming that the metabolites are considered nontoxic and the compounds are acting as nonpolar narcotics, could not be fully accounted for. The differences in the potencies among the PAH congeners appears to suggest 20 to 50% biotransformation assuming that the metabolites are not toxic, while the difference between pyrene and the chlorinated hydrocarbons is nearly a factor of four. The apparent differences in potencies between the chlorinated hydrocarbons and the PAH congeners may be a result of the limited information on the biotransformation of PAH under varying conditions of concentration and duration of exposure. Based on the time-dependent predicted body residues for 50% mortality (Fig. 3), a mixture of PAH and chlorinated hydrocarbons would not act additively on a simple molar additivity basis. The data also suggest that simple additivity would not occur within the PAH for *H. azteca*; however, there is supporting data for molar additivity for PAH in another amphipod, *Diporeia* spp., where biotransformation is not an issue [7]. Clearly, the details of the biotransformation rate and the role of metabolites in the toxicity of compounds like the PAH need to be better defined specifically for interpretation of body residue response data.

Does DDE act through narcosis?

One of the issues of importance in developing and using body residues as a dose metric is to understand the mechanism of action of the chemicals that are being compared [31]. Nonpolar narcotics (anesthetics) require a concentration of 40 to 160 $\mu\text{mol g}^{-1}$ lipid for acute response [5]. Based on the measured lipid concentrations in *H. azteca* ($6.25 \pm 2.2\%_{\text{dw}}$ with a dry to wet weight ratio of 0.276 ± 0.53 , $n = 61$), the concentration for 50% mortality was $46.4 \mu\text{mol g}_{\text{lipid}}^{-1}$ at 50 h (LR50 $0.789 \mu\text{mol g}^{-1}$) and $23.1 \mu\text{mol g}_{\text{lipid}}^{-1}$ at 400 h (LR50 0.393

$\mu\text{mol g}^{-1}$). It is reasonably clear that the 50-h DDE body residue value falls in the range expected for compounds that act as nonpolar narcotics in other aquatic organisms and reflects the somewhat greater potency expected for larger molecules. However, the chronic value is somewhat lower than that suggested for the acute response to nonpolar narcosis. McCarty and Mackay [32] report the range for chronic response in fish to nonpolar narcotics should be 0.2 to 0.8 $\mu\text{mol g}^{-1}$ and, assuming that the lipid content is 5%, which generated the 40 to 160 mmol kg^{-1} lipid values for the acute response, then only 4 to 16 mmol kg^{-1} lipid are required for chronic toxicity by nonpolar narcotics in fish. The values for DDE are greater than this range and may in part reflect the duration of exposure. It seems clear from the defined concentration required to produce a toxic response for nonpolar narcotics that DDE is acting through nonpolar narcosis. Because of the similar responses on a molar basis to DDE, PCBZ also likely acts through the nonpolar narcosis mechanism.

In comparison to DDE and PCBZ, it is likely that PAHs also act through nonpolar narcosis. However, PAHs require higher molar concentrations to produce the same level of response. This suggests that they are not as potent at the site of toxic action. As stated above, the potential role of biotransformation has not been addressed directly. Based on the highest reported biotransformation rates for PAHs (i.e., pyrene), PAHs should have higher body residues, particularly for parent pyrene than the chlorinated hydrocarbons. Therefore, even when biotransformation is considered, the PAH seem to be somewhat less potent than the chlorinated hydrocarbons via the nonpolar narcosis mechanism of action.

Individual organism tolerance

Recently, the role of individual tolerance as the concept to explain the probit dose-effect model was questioned with a set of data suggesting that the response may be due to stochasticity [33]. One of the utilities of examining the dose response based on body residue rather than on external concentration is that some of the factors producing the response can be investigated. In our previous study with pentachlorobenzene there was no difference between determining the MLR50 values using live or dead organisms [12]. However, in this data set, there was a difference in the response estimated based on the body residues in live organisms (MLR50 live) and on dead organisms (MLR50 dead) (Fig. 2). In this case, the MLR50 values estimated from live organisms are larger than those for dead organisms at each timeframe where both estimates could be made (Table 2). Unlike the pentachlorobenzene case where the organisms post-LT50 generally were at steady state, the live organisms exposed to DDE were not at steady state and continued to accumulate DDE substantially above the estimate for 50% mortality, particularly for long duration exposures (Fig. 4). This occurs because the organisms do not reach steady state for DDE over the course of the exposures used in this study, and surviving animals with greater tolerance for the toxicant will continue to accumulate body burden even after the less tolerant organisms die. A similar difference in individual sensitivity was observed for some exposures with PCBZ [12] but was not as clear as that observed for DDE. Thus, the use of body residue as the dose metric demonstrates that individual tolerance is a viable mechanism for the dose-response relationship.

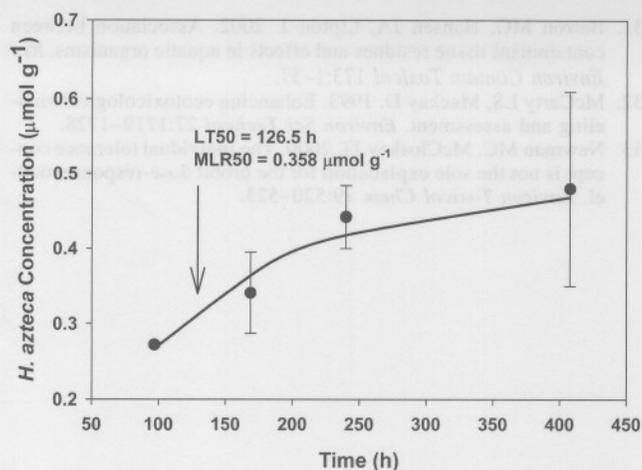


Fig. 4. Example of the increasing dichlorodiphenyldichloroethylene concentration of surviving *Hyalella azteca* beyond the LT50 showing accumulation in more tolerant organisms beyond the estimated mean lethal concentration at the exposure concentration of $3.28 \mu\text{g L}^{-1}$. The MLR50 is the mean body residue for 50% mortality determined from an individual exposure and applied at the LT50. The LT50 is the exposure time to 50% mortality.

CONCLUSION

Overall, DDE appears to act as a nonpolar narcotic for both acute and chronic mortality in *H. azteca*. The temporal trend in the toxic response using body residue as the dose metric rapidly reaches a plateau after 100 to 200 h exposure and essentially is the same as the response to PCBZ. Both DDE and PCBZ appear to be more potent than PAH even when accounting for PAH biotransformation. Finally, the body residue determined to produce 50% mortality demonstrates the concept of individual tolerance as surviving organisms can survive substantially higher concentrations than dead organisms.

Acknowledgement—This is joint work between the National Oceanic and Atmospheric Administration and the U.S. Army Corps of Engineers through an interagency agreement. This work was funded in part through the Cooperative Institute of Limnology and Ecosystems Research under Cooperative Agreement (NA67RJ0148) from the Office of Oceanic and Atmospheric Research, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and the U.S. Army Corps of Engineers Civil Works Long-Term Effects of Dredging Operations Research Program. Great Lakes Environmental Research Laboratory Contribution 1312.

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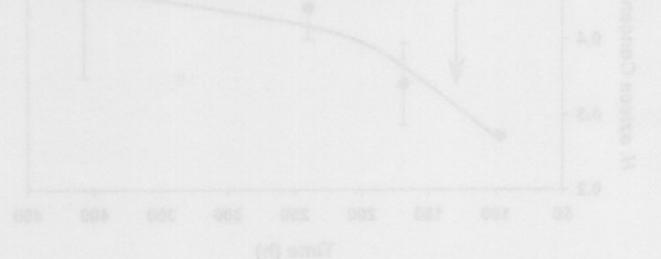


Fig. 4. Example of the increasing fish body concentration of a chemical over time. The concentration of a chemical in a fish body increases over time, reaching a peak of approximately 350 ng/g at 100 h. The concentration then declines over time, reaching approximately 100 ng/g at 400 h. The arrows indicate individual exposures and applied at the L10. The L10 is the mean body residue for 10% mortality determined from an individual exposure and applied at the L10. The L10 is the exposure time to 10% mortality.

CONCLUSION

Overall, DDE appears to act as a neurotoxicant for both acute and chronic mortality in *H. azteca*. The temporal trend in the toxic response using body residue as the dose metric rapidly reaches a plateau after 100 to 200 h exposure and essentially is the same as the response to PCB. Both DDE and PCB appear to be more potent than PAH even when accounting for PAH bioaccumulation. Finally, the body residue determined to produce 50% mortality demonstrates the concept of individual tolerance as surviving organisms can survive substantially higher concentrations than dead organisms.

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