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Lethal and sublethal body residues for PCB intoxication in the oligochaete, *Lumbriculus variegatus*

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Aims and Scope

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Lethal and sublethal body residues for PCB intoxication in the oligochaete, *Lumbriculus variegatus*

Susan W. Fisher [a,*], Stephen W. Chordas III [a], Peter F. Landrum [b]

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Abstract

The oligochaete, *Lumbriculus variegatus*, was used to examine the utility of critical body residues in describing lethal and sublethal chronic endpoints during polychlorinated biphenyls (PCB) exposure. *L. variegatus* was exposed to four ^{14}C-PCB congeners and 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene (DDE) on algal cells. Accumulation and resulting effects were monitored in 10-day acute and 35-day chronic exposures. *L. variegatus* was resistant to the acute lethal narcotic effects of these contaminants and no mortality was obtained in 10-day exposures. However, mortality that was significantly different from unexposed controls occurred for four compounds in 35-day assays; average body residues for chronic mortality were consistent among contaminants (0.88–1.35 mmol kg^{-1}). Kinetic studies showed that failure to generate mortality in some exposures was due to rapid elimination. Mono-2-chlorobiphenyl, for instance, had a K_d of 0.22 h^{-1} which was seven to 44 times faster than for the other contaminants. Sublethal reductions in body mass and reproduction occurred at lower body residues than were needed to produce mortality (0.34–0.56 mmol kg^{-1}). The consistency of the sublethal data suggests that they may offer a means of interpreting residue data for PCBs in the environment. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Critical body residues; *Lumbriculus variegatus*; PCBs; Narcosis; Sublethal endpoints

1. Introduction

It is estimated that 60% of environmental contaminants are narcotic chemicals (Veith et al., 1983) which accumulate in lipid membranes in proportion to their lipophilicity as defined by log K_{ow} (Lipnick, 1995). Narcotics exert toxicity by disrupting the lipid bilayer resulting in loss of selective permeability. In nerve cells, these changes may impair ability to maintain membrane potential and for conducting action potentials (Franks and Leib, 1994; Mullins, 1984; Cantor, 1997). Symptoms of narcotic intoxication range

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from general slowness of gait, loss of balance and reduced response to external stimuli (Sijm et al., 1993) to death (McKim et al., 1987). In addition, narcotic chemicals may impact a variety of ecologically important, sublethal processes including organism growth rate, reproductive rate, and length of development time (Nebeker and Puglisi, 1974; Keilty et al., 1988; Kukkonen and Landrum, 1994).

Because the environmental behavior of narcotic contaminants such as polychlorinated biphenyls (PCBs) is related to their ability to be dissolved in lipid membranes, their partitioning behavior can often be described by simple parameters such as log K_{ow}. The predictability of the relationship between PCB hydrophobicity, fate and effects, has made it possible to base regulatory decisions about risk on environmental concentrations of contaminants (Ankley et al., 1992). However, if comparisons are made between species or classes of contaminants, the linear relationship between contaminant hydrophobicity and fate or effects begins to break down. For instance, it is frequently observed that different organisms, living in identical media, will show significantly different levels of bioaccumulation of the same contaminant (Phipps et al., 1993; Wilcock et al., 1993; Crommentuijn et al., 1994). The differences in bioaccumulation occur because organisms which interact with sediment do so in vastly different ways (Meyers et al., 1993; Harkey et al., 1994). Thus, contaminant bioavailability changes as the feeding type and mode of interaction with sediment changes. Importantly, these differences are not reduced by normalization to sediment organic carbon or organism lipid content.

The use of environmental contaminant concentrations in risk assessment are, in fact, surrogates for the target site concentration of contaminant needed to produce a given effect. While environmental concentrations of narcotic contaminants necessary to produce acute or chronic toxicity may vary by orders of magnitude, the contaminant concentration at the target site in the organism should vary much less across contaminant classes with identical modes of action or between different organisms. This is because estimates of effect that are based on environmental concentrations, e.g. LC_{50}, LOEC, describe bioaccumulation potential as well as intrinsic toxicity. In contrast, target site concentrations needed to produce toxicity reflect only toxic potential (van Wezel, 1995). Thus, the only variation in target site toxicity that should occur is variation due to genetic differences of the organisms in susceptibility to the contaminant. In fish, the tissue concentration needed to produce acute lethal narcosis varies only between 2 and 8 mmol kg^{-1} while chronic mortality occurs between 0.2 and 0.8 mmol kg^{-1} (Van Hoogen and Opperhuizen, 1988; McCarty and Mackay, 1993; Sijm et al., 1993). This same consistency in tissue residues needed to produce narcosis has been observed in unrelated organisms ranging from mussels (Donkin et al., 1989) to rats and mice (Kaiser and Palabrica, 1991). Thus, the use of tissue-based estimates of effect has the potential to eliminate variability due to changes in bioavailability, routes of exposure and feeding type.

In this study, we investigated the use of tissue residues to describe sublethal narcotic impairments of growth and reproduction in an invertebrate, *Lumbriculus variegatus*. *L. variegatus* is frequently used in field studies as a bioindicator species (Lauritsen et al., 1985). It has also been proposed for use in standardized freshwater assays (USEPA, 1992) which can be used for regulatory decision making. In addition, *L. variegatus* is known to demonstrate measurable differences in behavior and feeding rate (Keilty et al., 1988; White and Keilty, 1988) as well as growth and reproduction (Wiederholm et al., 1987) during narcotic intoxication. Thus, *L. variegatus* appears to be well suited to an investigation of the spectrum of effects that may be produced by common environmental contaminants such as PCBs.

2. Materials and methods

2.1. Organisms

Brood stock of *L. variegatus* was acquired from USEPA, Duluth, MN. The worms were cultured in aerated 40-l aquaria using shredded brown paper towels as substrate. Hard standard refer-

ence water (HSRW; pH 8.3–8.5; alkalinity 115 mg l^{-1} as $CaCO_3$; hardness 160 mg l^{-1} as $CaCO_3$) was used as culture water and in all tests (USEPA, 1975). The worms were held at a temperature of 20–22°C under ambient light. Three times weekly, 50% of the culture water was replaced with fresh HSRW. Cultures were fed 1 g of crushed Tetramin at each water change.

Pure inocula of the algae, *Chlorella vulgaris* and *Ankistrodesmus* spp. were purchased from Carolina Biological Supply (Burlington, NC). Monocultures of each alga were grown in Bold's basal media (Nichols and Bold, 1965) in an environmental chamber set at 20 ± 2°C under a 14-h:10-h light:dark cycle. Lipid levels in each alga were determined to be 9–11% for *C. vulgaris* and 23–27% of dry weight for *Ankistrodesmus* spp. using the colorimetric method of van Handel (1985).

2.2. Contaminants

Analytical grade PCB congeners were purchased from Chemservice (West Chester, PA): 2-monochlorobiphenyl (MCBP); 4,4′-dichlorobiphenyl (DCBP); 2,2′,4,4′-tetrachlorobiphenyl (TCBP); 2,2′,4,4′5,5′-hexachlorobiphenyl (HCBP). The organochlorine, 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene (DDE) was purchased from Aldrich (Milwaukee, WI). Radioactive (^{14}C) samples of each compound were purchased from Sigma (St. Louis, MO). The specific activities were as follows: DDE, 12.7 mCi $mmol^{-1}$; MCBP, 12.1 mCi $mmol^{-1}$; DCBP, 13.8 mCi $mmol^{-1}$; TCBP, 18.5 mCi $mmol^{-1}$; HCBP, 12.6 mCi $mmol^{-1}$. All labeled compounds were determined to be greater than 98% pure by thin layer chromatography (TLC) on silica gel F-254 plastic-backed TLC plates developed in a moving phase of hexane/benzene 80:20. Parent compounds were identified by comparison to unlabeled analytical grade standards, the R_f values for which were: MCBP, 0.45; DCBP, 0.50; TCBP, 0.56; DDE, 0.58; and HCBP, 0.59. The section of the developed TLC plate corresponding to parent compound was excised and placed in a vial for scintillation counting. The remainder of each column was subdivided into 2-cm sections and each section, including the origin, was analyzed by scintillation counting to screen for the presence of metabolites.

2.3. Preparation of media

Contaminants were delivered to *L. variegatus* on algal cells. Stock solutions of algae were prepared consisting of 90% *C. vulgaris* and 10% *Ankistrodesmus* spp. in HSRW. The concentration of each cell type in the stock solution was determined using a Coulter counter. The algal stock solution (approximately 2.5–3.0 l in volume) was divided into six aliquots. One served as the unspiked control. The remaining five aliquots were dosed with varying amounts of each unlabeled contaminant and ^{14}C-contaminant added in trace amounts. The spiking procedure consisted of adding a predetermined amount of contaminant (labeled and unlabeled), dissolved in 10–25 μl acetone, to 25 ml of HSRW. Thereafter, the solution was mixed with an aliquot of the mixed algal cells and placed on an orbital shaker at 75 rpm for 12 h. Specific activities were recalculated to reflect the contributions of both labeled and unlabeled contaminant. Recalculated specific activities are denoted adjusted SAs and were: 0.22, 0.25, 0.41, 0.35 and 0.56 μCi μmol^{-1} for MCBP, DCBP, TCBP, HCBP and DDE, respectively. Algal contaminant concentrations were then determined by removing two 10-ml aliquots of spiked algal solution from each concentration of spiked algae. One 10-ml sample was filtered through a preweighed filter to obtain a measurement of algal cell mass. The other 10-ml aliquot was spun in a centrifuge at 3000 rpm for 3 min. The supernatant was decanted and the pellet was put into a scintillation vial to measure radioactivity. The concentration of PCB in algae was calculated from these measurements and the adjusted SA values. The algal cell mass varied less than 5% at each concentration.

2.4. Ten-day acute toxicity tests

Ten *L. variegatus*, 7–10 mg each, were used in each of three replicates for each concentration. Worms were added to 1-l glass beakers containing 500-ml HSRW without substrate at 21°C. Water

was changed completely and new spiked algae were added every 2 days; mortality was assessed daily. After 10 days had elapsed, all organisms were collected for analysis with liquid scintillation counting (LSC). Worms were placed directly into 20-ml scintillation vials to which 5 ml of ^{14}C-scintillation cocktail (dioxane/naphthalene/diphenyloxazole, 1000:100:5) were added. Previous studies in our laboratory have shown that the extraction efficiency for PCBs was greater than 95% using this method. Vials were counted in a Beckman 6000 model LSC using automatic quench control. The instrument was recalibrated prior to each use with a ^{14}C-standard purchased from Beckman. Vials were counted for 10 min or until the standard deviation was less than 5%. Counting efficiency for worms varied between 80 and 96%.

2.5. Chronic mortality and sublethal effects

Tests were initiated by removing ten worms, 7–10 mg each, from culture, blotting worms dry and weighing to determine initial biomass. Thereafter, worms were placed in 500-ml of HSRW in 1-l glass beakers. Five grams of shredded brown paper towel were added to each beaker as substrate. All beakers were held in an environmental chamber at 21 ± 2°C. Five concentrations of each contaminant were tested in triplicate along with an unspiked control. Test duration was 35 days. Water was changed completely and new spiked algae were added to each replicate at 7-day intervals. The endpoints monitored in this test were total biomass, reproduction based on total number of individuals present and mortality.

Mortality was assessed on a daily basis. Dead organisms were removed, blotted dry, weighed and placed in a 20-ml glass scintillation vial containing 10 ml of scintillation cocktail. Vials were allowed to sit for 24 h to permit solvent extraction of radioactivity from the worms. Thereafter, total radioactivity was determined using LSC. On day 35, the experiment was terminated. All beakers were broken down; the total biomass per beaker was again determined and the contaminant concentration in each organism, living and dead, was measured using LSC. Body residues were calculated from adjusted SA values.

2.6. Toxicokinetic studies

Toxicokinetic studies were conducted to provide a mechanistic evaluation of failure to find toxicity in 10-day studies. Because the reference point in these studies is internal tissue concentration, the route of exposure can be altered from exposure to contaminated algae to a waterborne exposure without compromising the validity of results (McCarty and Mackay, 1993).

Uptake clearance rates were measured by exposing triplicate groups of ten worms to trace levels (0.0044–0.015 μg l^{-1}) of each ^{14}C-contaminant in 500 ml HSRW at 21°C. Worms were withdrawn at 0.5, 1, 1.5, 2, 3, 4 and 5 h and prepared for LSC. Water samples (2.0 ml) were taken initially and after 5 h.

Elimination rates were measured by exposing worms to trace contaminant levels as described above and then moving them to clean water in triplicate flowthrough systems consisting of glass crystallizing dishes (75 × 150 mm). Each dish was continuously flushed with uncontaminated HSRW using 15-l glass carboys as reservoirs of HSRW. The crystallizing dishes contained an overflow tube that was positioned to maintain a constant volume of 500 ml; flow rates were approximately 5 ml min^{-1}. Worms were removed at 6, 12, 24, 36 and 54 h and prepared for LSC.

2.7. Lipid analysis

Fifteen worms were analyzed per test for whole body lipids. Tissues from each individual were homogenized in 3 ml chloroform/methanol 2:1. A 0.5-ml sample of each homogenate was analyzed for lipids using the colorimetric method of van Handel (1985). A wet to dry weight conversion was performed by weighing individual worms and then drying in an oven to a constant dry weight.

2.8. Metabolism of PCBs

The ability of worms to metabolize PCBs was assessed by exposing triplicate groups of ten worms to MCBP and DCBP, the contaminants most likely to be metabolized. After 7 days of exposure, the worms were collected, and extracted

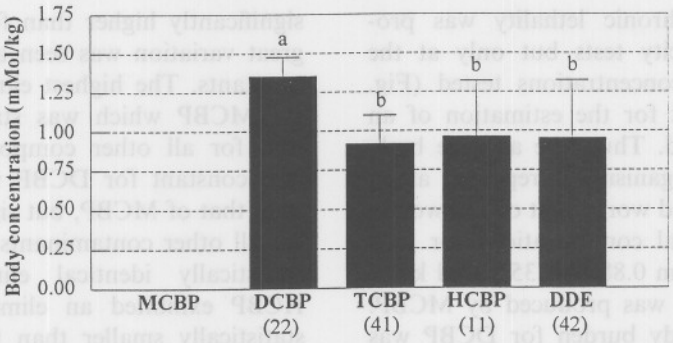

Fig. 1. Body residues (wet weights) of narcotics in dead organisms in 35-day exposures. Histograms with identical letters are not significantly different at $P < 0.05$. Numbers of animals showing lethality are given in parentheses beneath each histogram.

with chloroform/methanol, 9:1 three times. The extracts were combined, dried over anhydrous sodium sulfate and a 10–25-μl sample was spotted on a TLC plate, developed and screened for parent compound and metabolites as previously described.

2.9. Data analysis

The change in biomass was assessed as a percent increase or decrease in live animals from day 0 to day 35. Inhibition of reproduction was assessed as the percent change in the number of live animals present on day 35 relative to controls. For each endpoint, results among different contaminants were analyzed by ANOVA; means were separated using Tukey's HSD method (Poly Software International, 1996). Significant differences were determined at the $P < 0.05$ level.

Toxicokinetic data were analyzed using a two-compartment model:

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

where: C_a is the concentration of contaminant in animal (ng g^{-1}); C_w is the concentration of contaminant in water (ng ml^{-1}); k_u is the uptake clearance rate (ml g^{-1} h^{-1}); and k_d is the elimination rate constant (h^{-1}).

The model assumes that there is no biotransformation and that the contaminant concentration in water does not change significantly over the course of the uptake phase.

For TCBP, HCBP and DDE, k_d was slow enough that a simplification of the above model could be used:

$$\frac{dC_a}{dt} = k_u C_w \quad (2)$$

which integrates to:

$$C_a = k_u C_w t \quad (3)$$

The elimination data were fit to a first order decay model:

$$\ln C_a = \ln C_a^0 - k_d t \quad (4)$$

where, C_a^0 is the initial organism concentration (ng g^{-1}) at the beginning of the elimination experiment.

3. Results

3.1. Lethal body residues

Concentrations of contaminants used in 10-day toxicity assays were identical to those listed in Table 2 for chronic toxicity assays. However, these contaminant concentrations were not acutely toxic to *L. variegatus*. Thus, no data were

reported. However, chronic lethality was produced in 35-day toxicity tests but only at the highest contaminant concentrations tested (Fig. 1). Mortality sufficient for the estimation of an LD_{50} was not obtained. Thus, the average body burden of all dead organisms is reported along with the number of dead worms out of 150 worms exposed. Average lethal concentrations for four compounds ranged from 0.88 to 1.35 mmol kg^{-1} (Fig. 1). No mortality was produced by MCBP. The average lethal body burden for DCBP was significantly greater than for the other contaminants which produced mortality; however, the average lethal body burdens for the latter contaminants were not significantly different from one another.

3.2. Toxicokinetics

The failure to produce mortality in 10- and 35-day tests with MCBP prompted an investigation of the toxicokinetics of each contaminant (Table 1). The model assumptions were verified. The lack of metabolism was verified using TLC for MCBP and DCBP; only the parent compound was detected. Water samples taken at 0 and 5 h during the uptake phase verified that contaminant concentrations did not vary by more than 5–7%. Thus, the two compartment model could be used.

Uptake clearance rates were not significantly different for MCBP, DCBP and HCBP. The uptake clearance rates for TCBP and DDE were significantly higher than for DCBP. In contrast, great variation was seen among elimination rate constants. The highest elimination rate was seen for MCBP which was statistically greater than that for all other compounds. The elimination rate constant for DCBP was significantly lower than that of MCBP, but significantly greater than for all other contaminants. DDE and TCBP had statistically identical elimination rates while HCBP exhibited an elimination rate that was statistically smaller than for all other contaminants and not significantly different from zero.

Table 1
Toxicokinetic parameters for narcotics in *L. variegatus*

Contaminant	k_u (ml g^{-1} h^{-1})[a]	k_d (h^{-1})[a]	log K_{ow}
MCBP	125.6[1,2] (6.30)	0.22[1] (0.009)	4.53[b]
DCBP	105.4[1] (7.81)	0.03[2] (0.001)	5.33[b]
TCBP	135.6[2] (7.28)	0.005[3] (0.0009)	6.10[b]
HCBP	133.9[1,2] (7.08)	0.0013[4] (0.0009)	7.15[b]
DDE	137.4[2] (8.89)	0.005[3] (0.0016)	6.37[c]

Standard deviations are given in parentheses.
[a] For a given toxicokinetic parameter, numbers followed by the same superscript number are not significantly different at $P<0.05$.
[b] Gschwend and Schwarzenbach (1993).
[c] Montgomery (1993).

3.3. Sublethal endpoints

Body residues of all contaminants increased as exposure concentrations increased (Table 2). The contaminants with significantly higher elimination rates (MCBP and DCBP) required much higher exposure concentrations to achieve body residues that would result in significant changes in mortality, biomass and reproduction. The contribution of algae present in the organisms' gut at the time of sampling was estimated to contribute less than 5% to overall body residue and was, thus, ignored.

Reproduction, as assessed by organism number, and biomass as significantly reduced by all contaminants studied at body residues ranging from 0.34 to 0.56 mmol kg^{-1} (Table 2). The body residues of MCBP and HCBP needed to produce a significant effect were significantly lower than for DCBP and DDE (Fig. 2). Further, the effective body residues of MCBP, TCBP and HCBP were not significantly different from each other, nor were the effective body residues of DCBP and TCBP. The contaminant concentrations needed to produce significant sublethal effects were 1.6–2.5-fold lower than those necessary to produce mortality for the same contaminant. The exception was MCBP for which no mortality was obtained.

Significant depressions in biomass occurred at the same body concentrations as significant reductions in organism number relative to controls (Table 2, Fig. 2). At the highest concentrations of each contaminant, biomass on day 35 was reduced below starting levels. At the penultimate high concentration, biomass was reduced below

Table 2
Tissue residues and corresponding sublethal effects in *L. variegatus*

Contaminant	Algal exposure concentration (wet weight; mmol kg^{-1})	Mean body conc. (±SE) (mmol kg^{-1})	Mean biomass change (%)	Mean change in number (%)
DDE	0.0	0.0	+53.3 (2.5)	0.0
	0.4	0.029 (0.003)	+30.8 (8.7)	−0.21 (9.4)
	0.8	0.125 (0.000)	+46.1 (6.7)	−14.1 (5.4)
	1.6	0.249 (0.005)	+30.6 (23.1)	−13.6 (3.0)
	3.1	0.561 (0.021)	−37.7* (5.1)	−38.2* (2.5)
	6.3	1.038[a] (0.0)	−88.1* (12.2)	−94.2* (5.9)
MCBP	0.0	0.0	0.0+4.9 (1.4)	0.0
	1.1	0.024 (0.0015)	+0.4 (1.9)	−8.6 (5.0)
	2.1	0.043 (0.0014)	−2.2 (5.4)	+2.7 (8.6)
	4.2	0.090 (0.0034)	−5.7 (9.0)	+4.5 (2.5)
	8.4	0.176 (0.021)	−10.4 (5.9)	+9.0 (4.9)
	16.9	0.343 (0.061)	−23.1* (1.1)	−22.8* (0.53)
TCBP	0.0	0.0	+14.6 (6.1)	0.0
	0.5	0.048 (0.0007)	−1.1 (0.6)	−17.8 (4.5)
	1.0	0.073 (0.03)	−0.2 (4.5)	−26.3 (9.1)
	2.0	0.169 (0.1)	−5.7 (9.0)	−23.7 (3.8)
	4.1	0.386 (0.013)	−11.3* (2.3)	−28.1* (6.2)
	8.2	0.658[a] (0.0)	−75.1* (25.4)	−82.0* (18.2)
DCBP	0.0	0.0	+16.7 (4.5)	0.0
	2.2	0.126 (0.006)	−2.4 (6.7)	−38.6 (4.5)
	4.4	0.283 (0.016)	+2.2 (2.3)	−14.2 (5.3)
	8.9	0.532 (0.032)	−15.3* (3.5)	−38.1* (2.6)
	13.4	0.665 (0.025)	−9.6* (0.9)	−52.3* (0.0)
	17.9	1.340 (0.06)	−82.6* (7.0)	−84.5* (8.2)
HCBP	0.0	0.0	+20.5 (0.9)	0.0
	0.3	0.02 (0.001)	+16.4 (0.6)	−13.3 (4.1)
	0.6	0.04 (0.003)	+21.6 (5.8)	−19.3 (3.1)
	1.1	0.09 (0.005)	+26.2 (5.1)	−18.9 (3.8)
	2.2	0.18 (0.002)	+8.3 (10.8)	−19.6 (5.1)
	4.4	0.35 (0.016)	−2.7* (5.7)	−38.4* (3.2)

* For a given contaminant, numbers followed by an asterisk are significantly different from controls at $P<0.05$.
[a] Only one replicate had living animals at the end of the experiment.

that of controls for all contaminants except HCBP. At the lower body concentrations, biomass sometimes increased relative to starting values but was less than controls.

Reduction in organism numbers was referenced directly to controls since 30 organisms (ten per replicate) were used in all exposures and controls (Table 2). For four contaminants, there was a general trend of successive decreases in organism number as exposure concentration and body concentration increased. The exception was MCBP where small increases in organism number relative to controls were seen at intermediate body burdens. Reductions in organism number at the lowest contaminant level needed to produce a significant effect ranged from 22.8 to 38.4% of control values. As with changes in biomass, the body concentration of DCBP and DDE needed to significantly reduce organism number was statistically higher than those needed to produce a significant result with MCBP and HCBP (Fig. 2).

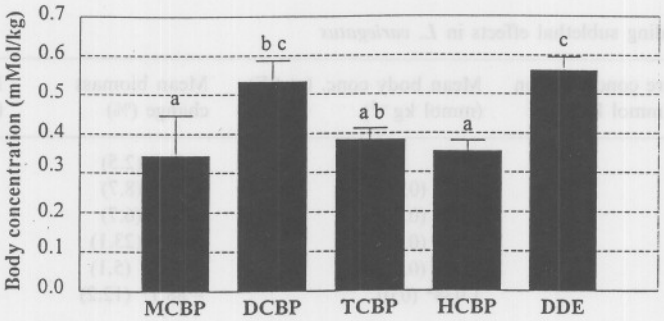

Fig. 2. Lowest body residues (wet weights) of narcotics at which significant differences ($P < 0.05$) in biomass and organism numbers relative to controls were detected. Histograms with identical letters are not significantly different.

Lipid levels did not vary significantly among organisms used in experiments. The average lipid level for worms used in these experiments was 8.8% (SE = 0.35) of dry weight.

4. Discussion

4.1. Chronic lethal body residues in *L. variegatus*

Toxicity experiments conducted over a 10-day time period did not result in toxicity. Thus, lethal narcotic potential in *L. variegatus* had to be investigated in 35-day exposures. Because this exposure period exceeded the reproductive span of the organism, the results must be considered chronic as opposed to acute.

Chronic lethal body residues for narcosis are reported to be in the range 0.2–0.8 mmol kg^{-1} for fish (McCarty and Mackay, 1993). Our results show that lethal residues for chronic PCB narcosis in *L. variegatus* varied between 0.88 and 1.35 mmol kg^{-1} (Fig. 1). While this range falls slightly above that identified by McCarty and Mackay (1993), it accords well with the limited lethal body residue data in invertebrates. In this case, most of the reported values are for acute narcosis. For instance, Nebeker and Puglisi (1974) reported that lethal residues for crustacea poisoned by Arochlor 1242 and 1254 mixtures were in the range 1.4–1.9 mmol kg^{-1}. Pawlisz and Peters (1993) demonstrated that the geometric mean of body burdens for narcosis for nine polar narcotics was 3.1 mmol kg^{-1}. In a study of a mixture of 11 PAHs in crustacea, Landrum et al. (1991) reported a lethal tissue concentration for 50% mortality of 6.1 mmol kg^{-1} as the molar sum of PAHs in the mixture. This is similar to the lethal concentrations of pyrene (6–9 mmol kg^{-1}; Landrum et al., 1994) and fluoranthrene (6.5 mmol kg^{-1}; Kane Driscoll et al., 1997a) to *Diporeia* spp. and for fluoranthrene in *Hyallela azteca* (3.6–5.6 mmol kg^{-1}; Kane Driscoll et al., 1997b). Finally, Kukkonen and Landrum (1994) found that a pyrene body burden of 1.6 mmol kg^{-1} was associated with 17% mortality in *L. variegatus*. Thus, body residues for acute narcosis in invertebrates appear to fall in the range 1.4–9.0 mmol kg^{-1}. Our chronic lethality residues fall below the acute lethal range as would be expected. A greater range for chronic mortality may be identified when contaminants with a greater range of lipophilicity are studied (Pawlisz and Peters, 1993).

The relationship between acute and chronic lethal residues may change as additional contaminants are studied. For the invertebrate *H. azteca*, the estimated chronic median lethal residue (LR_{50}) for fluoranthrene is 1.1 mmol kg^{-1} (Kane Driscoll and Landrum, 1997) while the acute value for 10-day exposures was 3.6–5.6 mmol kg^{-1} (Kane Driscoll et al., 1997b). Thus, at least for *H. azteca*, the chronic lethal body residue is approximately five-fold lower than the acute value. In fish, a ten-fold difference between acute and chronic body residues is anticipated (McCarty and Mackay, 1993). The apparent reduction in range between acute and chronic lethal residues in invertebrates may relate to exposure durations (4 days for fish;

and 10 days for amphipods) and the relative proportions of the total life span each exposure duration represents for each organism. Time to mortality, thus, appears to be an issue which must be explored in order to standardize interpretation of lethal residues.

Despite reasonable agreement between our data and the limited published body residue data, some caveats are in order. First, there is not yet consensus about what constitutes a 'lethal body residue' as defined by McCarty and Mackay (1993). In order to be comparable among species, the measurement should be referenced to a predetermined effect level such as the LC_{50}. A lethal body residue thus defined would measure the body residue at which 50% of the test population died. Some tissue-based residues, such as the lethal body burden (LBB) a derivative of the critical body residue hypothesis explicitly bases calculations on the LC_{50}, uptake and elimination rate constants, thus ensuring that the tissue based interpretation of hazard is referenced to the 50% population level of response (Sijm et al., 1993). Other authors simply record the average tissue concentration at which organisms die (Landrum et al., 1991; Pawlisz and Peters, 1993). If the internal concentration needed to cause effect is constant, all members of a population should be similarly affected at a particular body residue, varying only with individual differences in genetic susceptibility. We obtained mortality only at the highest exposure concentrations tested (Fig. 1, Table 2), thus we could only report average lethal body residues. However, the number of organisms showing this response (mortality) is provided with each histogram to indicate variability in effect level with each contaminant.

The lack of mortality in some exposures appears to relate to an inability to maintain a lethal body burden. The average body concentration data (Table 2) show that body residues were simply not high enough to cause mortality, assuming the legitimacy of the 0.88–1.35 mmol kg^{-1} range, until the organisms were exposed to the highest concentrations. Moreover, an elimination rate that was significantly higher for MCBP than for all other contaminants, made it impossible to achieve a lethal body residue for that contaminant. The relatively fast elimination rate of DCBP made it difficult to achieve a toxic body residue. However, at very high exposure concentrations, lethality did occur. It was necessary for both MCBP and DCBP to use exposure concentrations that were substantially higher than for the other contaminants (Table 2).

The exposures in our experiments were designed to result in high body residues in as short a time as possible. We did not use aqueous exposures because the water solubility of the contaminants was so low, that effective body burdens could not have been achieved on a time scale relevant to the organism. Indeed for contaminants such as HCBP which is extremely water insoluble, lethal body burdens could not have been achieved using water-only exposures. Thus, we selected a solid phase medium with a high organic carbon content which would permit concentration of the contaminant on the particle approximately five orders of magnitude above the maximum aqueous concentration. Moreover, we chose algal particles rather than sediment to deliver the contaminants to *L. variegatus* even though *L. variegatus* will readily consume contaminated sediment because the assimilation efficiencies of the contaminants selected are generally much lower when the contaminants are delivered on sediment than on algal particles (Bruner et al., 1994). Since the worms voraciously consumed algae, the algal route of exposure appeared to be the most efficacious. It is likely that contaminant desorbed from the algal cells and secondary uptake from water took place (Stange and Swackhammer, 1994). However, a benefit of tissue-based measurements is that it explicitly integrates all routes of exposure; it is, thus, not important to separate exposure routes experimentally.

4.2. Sublethal effects in *L. variegatus*

Sublethal effects in *L. variegatus* in response to narcotics have been well documented and range from reductions in biomass and reproduction (Nebeker et al., 1989) to behavioral changes (Keilty et al., 1988; Kukkonen and Landrum, 1994), to alterations in physiology (Penttinen et al., 1996). As with body residues and mortality,

there is very little information tying these sublethal effects to body residues reported in the literature.

Nebeker et al. (1989) found that *L. variegatus* exposed to hexachlorobenzene with a body burden of 1.06 mmol kg^{-1} showed a non-significant reduction number of 33.7% relative to untreated controls in a 49-day study. This effect level and body burden are very consistent with the results of our study in which body burdens for chronic effects varied from 0.34 to 0.56 mmol kg^{-1} and the mean organism number was reduced by 22.8–34.4% relative to controls (Table 2). The primary difference between the two studies is the use of more exposure concentrations and replicates permitting the finding of statistical significance in our case.

No significant effects were seen in a whole life cycle exposure of *Chironomus tentans* and *L. variegatus* to 2,3,7,8-tetrachlorodi-benzo-*p*-dioxin (TCDD) delivered on algal cells even at body residues as high as 9533 ng g^{-1} (West et al., 1997). The authors attributed the lack of biological effect to the fact that invertebrates do not possess the aryl hydrocarbon receptor and consequently cannot respond to toxicity exerted by that mode of action. TCDD, like PCBs, could potentially act as narcotics. However, even the highest body residue attained in the study (9533 ng g^{-1}) would yield only a body residue of 29.7 μmol kg^{-1} which is an order of magnitude below the sublethal body residue and two orders of magnitude below the lethal range. No narcotic effects would, thus, be anticipated.

In a study of *Artemia* spp. exposed to PCBs on algal cells, Wang et al. (1998) found that significant reductions in growth occurred at body burdens of 0.08–0.9 mmol kg^{-1} (dry weight). The use of dry weight measurements makes it difficult to compare to our data directly. However, the reduction of *Artemia* growth appears to be occurring at a body burden that is similar to our measurements.

Despite the lack of comparable data, the range of tissue concentrations needed to produce sublethal effects was remarkably consistent, varying between 0.34 and 0.56 mmol kg^{-1} across the range of contaminants tested. The level of effect produced at those tissue residue levels was also consistent: average organism numbers were reduced by 22.8–38.4% relative to controls and average biomass was reduced by 2.7–37.7% (Table 2).

The body residues needed to significantly impact organism number were identical to those needed to cause significant depression in biomass. Clearly, a reduction in total biomass could be due to a reduction in organism number or a reduction in the mass of each organism. *L. variegatus* reproduces by budding off new individuals. It is not possible to distinguish parental from filial stock once reproduction has occurred. Nor is it possible to differentiate newly produced organisms, which are smaller, from organisms present at the beginning of the test but which failed to gain weight. As a result, it is not possible to separate measurements of biomass and reproduction. Thus, measurement of biomass and reproduction overlap but track different types of reproductive stress, and are therefore, both used as effects assessments.

The occurrence of sublethal narcosis at significantly lower body residues than are needed to produce death suggest that contaminants such as PCBs and DDE may be having important ecological effects on invertebrate populations well before lethality occurs. Failure to reproduce or a lengthier reproductive period may suppress population levels and/or result in the production of offspring which are themselves impaired. Further, subtle changes in behavior, such as substrate avoidance (Kukkonen and Landrum, 1994) or altered escape reflexes (Drewes, 1997) may increase exposure to predators and indirectly reduce population numbers. Both phenomena merit further scrutiny as hazard assessment becomes more refined.

The data support the critical body residue hypothesis for both lethal and sublethal effects. Our data extends the utility of this approach to an invertebrate which is commonly used in bioaccumulation tests for regulatory purposes (USEPA, 1992). Moreover, the consistency of the tissue residues producing both lethal and sublethal effects may permit interpretation of effects in other organisms for which body residue data is available.

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