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Identifying body residues of HCBP associated with 10-d mortality and partial life cycle effects in the midge, *Chironomus riparius*

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Abstract

The relationship between the body residue of 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP) and its effects, including 10-d mortality and chronic sublethal effects on the midge, *Chironomus riparius*, are examined in a partial life cycle assessment. The alga, *Chlorella vulgaris*, was loaded with ¹⁴C-labeled HCBP and fed to midges as the method for delivery of the toxicant. In a 10-d bioassay, median lethal body residue (LR₅₀) was 0.57 (95% CI: 0.49–0.66) mmol/kg. In the partial life cycle test, midges were fed a mixture of ¹²C- and ¹⁴C-HCBP-laden algae and exposed in four separate tests to assess the different developmental stages representing 2nd to 3rd instar, 2nd to 4th, 2nd to pupa, and 2nd to adult stages. A variety of sublethal endpoints were monitored, including developmental time within a stadium, body concentration at the end of each stadium, body weight, and fecundity (the number of ova) for the female pupae and adults. Overall, midge body concentrations of HCBP increased with increasing exposure concentration. Body weight was not significantly affected by HCBP except during the 4th instar. Body residue also increased with each successive stadium. Developmental time increased significantly with increasing body concentration in 2nd to 4th, 2nd to pupa, and 2nd to adult tests, while there was no statistical significance in developmental time for the 2nd to 3rd instar test. The number of ova decreased significantly in adults with increasing body concentration of HCBP, with an average of 345 ova in controls, 289 ova at 0.028 mmol/kg of HCBP, and 258 ova at 0.250 mmol/kg. These data, which relate chronic endpoints to body residues, suggest that sublethal endpoints in invertebrates are useful for defining sublethal hazards of PCBs. These data also suggest that ecological consequences may result from relatively low body burdens of PCBs. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Body residues; HCBP; Life cycle test; Midges; Chronic endpoints

1. Introduction

Bioassays should describe the effects associated with environmental contaminants to be useful in

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understanding the ecological risks associated with contaminants. The bioassays used to estimate the hazard of environmental contaminants in aquatic systems assume that the environmental concentration is proportional to the concentration at the target site. The assumption consists of two components: first, the contaminant concentration in environmental media is proportional to body residues and; second, the body residue is proportional to the concentration at the site of action, which governs the intensity of the toxic effect.

The first assumption does not always hold true particularly for sediment bioassays (Kane Driscoll et al., 1997; Kukkonen and Landrum, 1998). That is, environmental concentrations do not always correlate with body residues due to the diversity of interactions between organisms and the environmental media, the biology of organisms, and physicochemical factors of the environment. For instance, three different benthic organisms, *Chironomus riparius*, *Lumbriculus variegatus*, *Diporeia* spp. exposed to the sediments spiked with pyrene, benzo(a)pyrene, and trans-chlordane, exhibited significantly different levels of bioaccumulation with *C. riparius* showing lowest bioaccumulation and *Diporeia* spp. the highest (Harkey et al., 1994). This was attributed to differences in feeding selectivity, lipid content, and physiological/metabolic variation between organisms. Similar results were obtained when a series of invertebrates with varying feeding habits were exposed to sediment contaminated with chlordane. In this case, surface-feeders or surface-dwellers exhibited higher bioaccumulation than suspension feeders by a factor of three (Wilcock et al., 1993). Finally, PCP concentrations in zebra mussels varied by a factor of 177, when zebra mussels were exposed to PCP at different pH and temperature combinations (Fisher et al., 1999a). These examples clearly demonstrate that the way organisms interact with the environment or characteristics of the environment can cause significant changes in exposure and subsequent bioaccumulation.

The second assumption, i.e., the proportionality of the whole body concentration to the target site concentration, is harder to test due to shifts in modes of action for single compounds with different exposure conditions, multiple modes of action

for contaminants, difficulties in finding the target site and measuring the contaminant concentration at target sites. Some contaminants have more than one mode of action, thus rendering it difficult to choose a representative target concentration. For instance, DDT is known to be a neurotoxin, affecting membrane permeability to critical ions (Matsumura, 1985). Recently, DDT along with its major metabolite DDE was also found to be endocrine disruptors (Kelce et al., 1995). Also, modes of action have been found to change with increasing dose. For instance, the mode of action for lipophilic organophosphorus insecticides was found to change with increasing body residues in guppies from specific acetylcholine esterase inhibition to narcosis (Ohayo-Mitoko and Deneer, 1993). Similarly, PCP can act as an oxidative phosphorylation uncoupler for one species and a narcotic for another (Landrum and Dupis, 1990). Above all, it is hard to measure the precise amount of the toxicant reacting with an endogenous target molecule. Therefore, the whole body residue remains an easier and more reliable way of referencing the dose with the continued assumption that total accumulation or, in some cases, normalized concentration, e.g., to lipids, reflects the concentration at the target site.

There exists a rich body of bioaccumulation data that establishes the degree of contamination existing in many organisms. However, if contaminant levels are not related to biological effects, no use can be made of the data base other than to establish that exposure occurred. Bioaccumulation data representing body residues may be a better means of assessing the hazard of real world exposures, if the toxicological significance can be correlated to the bioaccumulation data. McCarty and Mackay (1993) proposed the use of body residues instead of environmental concentrations in assessing environmental hazard since accumulated dose integrates the uncertainties in bioavailability, assimilation and metabolism of the contaminants. Relating toxicological effects to body residues will permit interpretation of biological significance of bioaccumulation data resulting in improved hazard evaluation performed by governmental organizations such as US EPA and US Army Corps of Engineers (USACE) (Bridges et al., 1996; US ACE, 1996).

This work presents methods for 10-d mortality and partial-life cycle bioassays for the midges, *C. riparius*. These methods are used to define the toxicity response spectrum for 2,2',4,4',5,5'-hexachlorobiphenyl using body residues instead of environmental concentrations. Even though body residues have been successfully used to describe the mortality from the environmental contaminants (McCarty et al., 1992; Pawlisz and Peters, 1993), there are little data relating body residues to their specific chronic effects in invertebrates that are commonly used as sediment biomonitoring organisms (US EPA, 1994). Therefore, the methods used in this study will help develop a better understanding of the ecological consequences of low-level environmental contamination.

2. Materials and methods

2.1. Chemicals

¹⁴C-2,2',4,4',5,5'-hexachlorobiphenyl (HCBP) was purchased from Sigma Chemical Co. (St. Louis, MO) and ¹²C-HCBP was obtained from Chem Service (West Chester, PA). HCBP was chosen because of its availability in radiolabeled form and environmental significance. Both ¹²C- and ¹⁴C-labeled compounds were used in combination to lower the experimental cost. The specific activity was then recalculated to include isotopic dilution. The radiopurity of HCBP was determined using thin layer chromatography (TLC) on silica gel plates in a solvent system of hexane:benzene 80:20 and was >98% pure. Total contaminants were quantified using liquid scintillation counting (LSC, Beckman 6000 LSC, Fullerton, CA) in 5 ml of scintillation cocktail (1000 ml 1,4-dioxane, 100 g naphthalene, 5 g 2,5-diphenoloxazole (PPO)).

2.2. Organisms

C. riparius was reared in the Environmental Toxicology Laboratory at The Ohio State University according to the method of Estenik and Collins (1978). The midge larvae were initially

collected in 1974 from the Jackson Pike Water Treatment Plant in Columbus, OH and have been maintained in culture with periodic outbreeding to wild midges. Midges were chosen as test organisms because they are sediment dwelling, are easy to culture, are of ecological importance, and have a broad geographical distribution (US EPA, 1994).

2.3. Production of contaminated media

The midges were exposed to HCBP by feeding a green alga or trout chow previously dosed with HCBP. The green alga, *Chlorella vulgaris* is a round alga that does not have cilia or flagella for locomotion. The alga was chosen as contaminant medium because it settles quickly and was readily ingested by midges based on preliminary tests.

A pure stock of *C. vulgaris*, purchased from Carolina Biological Supply (Burlington, NC), was cultured in amended Bold's Basal medium (James, 1978). Cultures were kept in an environmental chamber at 20°C under fluorescent lights with photocycle of 14:10 light:dark cycle. Air was bubbled mildly through tubes to supply oxygen and to promote the suspension of algal cells.

The mixture of ¹²C- and ¹⁴C-HCBP dissolved in acetone at series of predetermined contaminant concentrations was put into a flask containing known mass of algae in minimal amount of algal medium. The algae were agitated over night by a rotary shaker. Algae were spiked with mixture of ¹²C- and ¹⁴C-HCBP dissolved in acetone at a series of predetermined contaminant concentrations. The final contaminant concentration of HCBP on algae was measured using LSC. The algal cell concentration used was 2 000 000 cells/ml. The number of algae was quantified on Coulter Counter[®] (Coulter Electronics Inc., Hialeah, FL). To ensure accurate spiking of algae, 1 ml algal solution was filtered through microfilter paper (0.5 µm, 25 mm; MSI, Westboro, MA) and dried overnight in an oven to obtain an accurate dry weight. Dry algal weight was estimated from the difference in filter paper weight before and after filtering the algae. The amount of contaminant needed to obtain series of predetermined concentrations was then calculated.

Commercial trout chow was also used as a contaminant delivery source when it became clear, in a pilot test, that midges did not grow successfully to the adult stage when fed only *C. vulgaris*. Trout chow was also spiked with HCBP at concentrations corresponding to those in the algae. Trout chow was sieved using no. 35 mesh sieve (500 μm). Because water became fouled rapidly due to high fat content, the trout chow was defatted by soaking in ether for 30 min with gentle swirling before spiking with HCBP. The ether was evaporated using a rotary evaporator (Büch Laboratory-Technik, Switzerland). HCBP was dissolved in acetone and trout chow was added to the acetone. After soaking for 30 min, acetone was evaporated using a rotary evaporator. The actual concentration was reconfirmed by LSC of 10 mg of spiked trout chow.

2.4. Water

Hard standard reference water (HSRW), pH 8.3–8.5, alkalinity 120 mg/l as CaCO_3 , and hardness 160 mg/l as CaCO_3 was used in all experiments (US EPA, 1975). Necessary salts were predissolved in smaller vessels and then added to deionized water to produce HSRW. The water was mixed over night before use to ensure required water quality parameters. The water quality parameters were confirmed according to the laboratory standard operation protocols.

2.5. 10-d mortality test

Mortality tests (10-d) used five exposure concentrations, 100, 500, 1000, 2000, 5000 mg HCBP/kg algae and a control. Three replicates of twenty 3rd instar midge larvae were used for each concentration placed in 1-l beakers with 500 ml HSRW and only spiked algae at 2 000 000 cells/ml. Unlike in the partial-life cycle test, no trout chow was used in the 10-d mortality test. The contaminated algae along with HSRW were renewed every two days to deliver a relatively constant concentration of contaminant to midges and to keep the acceptable level of oxygen. The beakers were covered with aluminum foil to prevent excessive water evaporation. Beakers were

kept at 20°C with a photoperiod of 14:10 h light:dark. Dead midges were removed daily, weighed, and the HCBP body residues were determined with LSC immediately. After 10 days, all organisms, alive or dead, were removed, weighed, and body residues were measured using LSC. The radioactivity attributable to undigested algae inside the gut was not subtracted from the total radioactivity because the chemical retained on food in the gut was found to be minimal (5–10% of total radioactivity) based on a test performed separately.

2.6. Partial-life cycle tests

As in the 10-d mortality test, five treatment groups and a control were used for the chronic tests. Trout chow, spiked at the concentrations corresponding to those used in algae, was also added to the test beakers to provide burrowing material and an additional food source. The whole exposure system was renewed every 2 days. Sublethal chronic tests were conducted over the partial midge life cycle in a series of separate assays. In each case, exposure began with mixed sex 2nd instar midges. This stage was the earliest that could endure handling stress. The first test ended when the midges reached 3rd instar (2nd to 3rd test). Subsequent chronic sublethal tests were begun with 2nd instar larvae and were concluded at 4th instar (2nd to 4th), the pupal stage (2nd to pupa-1), adult (2nd to adult-1), respectively. Twenty 2nd instar midges for 2nd to 3rd and 2nd to 4th tests were placed in twenty 50 ml beakers, while thirty midges were placed in thirty 50 ml beakers for the 2nd to pupae and the 2nd to adults. Midges were individually housed in a 50 ml beakers through out whole tests. The 2nd to pupa and the 2nd to adult tests were repeated to confirm the reproducibility of the results and entitled as 2nd to pupa-2 and 2nd to adult-2 tests, respectively. The contaminant concentrations in the algae and trout chow for the 2nd to pupa-2 test were 1, 3, 5, 7, and 10 mg/kg. For other tests, the HCBP concentrations in algae and trout chow were 1, 5, 10, 50, 100 mg/kg. The midges in the 2nd to pupa-1, and the 2nd to adult-1 and 2 were sexed and data were recorded separately for each

sex. The number of organisms necessary for the test was calculated according to Dean and Voss (1995) to assure statistical power of the data. Organisms were examined daily for mortality. For both sexes, weight at the end of each stadium (from the 2nd instar to a given developmental stage, e.g. adult) and developmental time within each stadium were assessed. In addition, the number of ova in ovaries was counted for each female pupa and adult except for the 2nd to pupa-2 test. Midges that died during exposure were weighed and body residue was measured by LSC as were midges that reached the desired stadium. The mortality that occurred prior to 2nd water change (4-d), i.e. ~4%, was considered attributable to handling stress and was not included in data analysis.

2.7. Lipid analysis

Ten midges from each of 4th instar larvae, pupae, adult male and adult female midges were analyzed for the total lipid content. The method was a spectrophotometric assay modified from van Handel (1985). One midge was placed in a disposable culture tube (16 × 125 mm) and crushed with a glass rod in 0.5 ml of 1:2 chloroform:methanol solution. Test tubes were tightly sealed with aluminum foil and allowed to sit for 2 h to facilitate the extraction of lipids. Then, the tubes were placed in a heating block at 100°C and the solvent was allowed to evaporate. Subsequently, 0.2 ml of sulfuric acid were added and the sample was heated for 10 min at 100°C. Thereafter, 5 ml of vanillin–phosphoric acid reagent were added and the absorbance was read in a Spectrophotometer (Beckman Model DU-40, Fullerton, CA) at 525 nm. A standard curve was prepared with series of concentrations of 100% commercial vegetable oil. The actual midge lipid content was determined by comparison to the calibration curve.

2.8. HCBP analysis

The body residue was determined by LSC after weighing. Whole midges were individually transferred to scintillation vials, after which 0.5 ml of

tissue solubilizer (Packard Instrument Company, Inc. Meriden, CT) were added. After digestion, 5 ml of scintillation cocktail (1,4-dioxane (1000 ml), naphthalene (100 g) and 2,5-diphenoloxazole (PPO) (5 g)) were added and vials were allowed to stand overnight to minimize chemiluminescence. The samples were then counted by LSC for 5 min. The amount of HCBP accumulated was determined from the ^{14}C activity with recalculated specific activities adjusted for dilution with ^{12}C -HCBP. The data were corrected for quench using the external standards ratio method after subtracting background.

2.9. Data analysis

For 10-d mortality tests, median lethal residue (LR_{50}) values and LC_{50} values were calculated using Probit analysis (Finney, 1971). LR_{50} s were based on contaminant concentration in dead midges, while LC_{50} s were based on the contaminant concentration in algae. Confidence intervals (95%) were also reported.

For the partial life cycle test, data from each exposure concentration were pooled and subjected to the general linear model (GLM) analysis followed by Duncan's multiple range test on SAS[®] (SAS Institute Inc., 1992) to determine lowest observed effective residue (LOER) for each endpoint. The null hypothesis of no difference was rejected at P value of 0.05 or lower.

3. Results

3.1. Lipid analysis

The lipid content of midges increased with the developmental stage. The lipid content (standard deviation) of 4th instar larvae, pupae, male adults and female adults were 1.53 (0.24), 2.93 (0.41), 2.96 (0.65) and 3.53 (0.77)% of wet weight, respectively. Others found much lower lipid content in larval midges, i.e. 3.72% in dry weight (Harkey et al., 1994), and 0.6% in wet weight (Looser et al., 1998). Harkey et al.'s 3.72% in dry weight may be converted to 0.74% wet weight with assumption that 80% of midges' whole weight is water. The

differences in the lipid content between present study and the literature values may be attributable to the feed, the midges in our lab were fed high lipid trout chow, while Harkey et al. (1994) and Looser et al. (1998) fed midges Tetramin®. Female adults had more lipid than males but only by a factor of 1.2 as a percentage of wet weight. However, when expressed as total lipid mass in micrograms, female adults contained $126.02 \pm 52.03 \mu\text{g}$ with average body mass of $3.53 \pm 0.77 \text{ mg}$ which was more than twice the lipid content of male adults, $49.47 \pm 18.94 \mu\text{g}$, at an average body weight of $2.96 \pm 0.65 \text{ mg}$. The increasing lipid content was reflected in increasing body residues of HCBP with developmental stage of midges (Table 2).

3.2. 10-d mortality test

Dose-responsive mortality was found with increasing body residues (Table 1). At the end of 10-d, 11.8% mortality occurred at the lowest exposure concentration, 98.6 mg/kg, while 87.7%

mortality occurred at the highest exposure concentration, 5528.4 mg/kg. The LC_{50} for 10 days, calculated from the algal and trout chow concentrations, was 2.38 (95% C.I.; 1.05–5.35) mmol/kg. The LR_{50} based on midge body residue for 10 days of exposure was 0.57 (0.49–0.66) mmol/kg.

3.3. Partial-life cycle tests

Body residues increased with each successive stadium, which probably resulted from longer exposure time and higher lipid content (Tables 2, 6 and 7). Also, mean body residues of each exposure group increased with increasing exposure concentration. In the 2nd to 3rd instar test, mean body residues ranged from 0.0014 to 0.0063 mmol/kg, when midges turned to 3rd instar. In the 2nd to 4th instar test, mean body residues ranged from 0.001 to 0.086 mmol/kg in midges which had entered the 4th instar. Body residues of pupae significantly increased with increasing HCBP concentrations, ranging from 0.003 to 0.106 mmol/kg in females and 0.002 to 0.0922

Table 1
Exposure concentrations (S.D.), body residues (S.D.) and mortality in 10-d mortality tests

Nominal algal conc. (mg/kg)	Measured algal conc. (mg/kg)	Body residue (mmol/kg)	Total midges	Dead	Corrected mort. (%)
Control			61	2	
100	98.6 (14.9)	0.2 (0.05)	60	10	11.8
500	505.5 (77.8)	0.3 (0.12)	60	14	18.9
1000	889.79 (160.6)	0.74 (0.54)	60	35	55.9
2000	1910.5 (470.3)	1.12 (0.48)	57	45	77.7
5000	5528.4 (1056.2)	1.34 (0.26)	60	53	87.7

Table 2
Body residues (S.D.) of midges in mmol/kg at the end of different developmental stages

Nominal alga conc. (mg/kg)	2nd to 3rd	2nd to 4th	2nd to pupa		2nd to adult	
			Female	Male	Female	Male
1	0.0014 (0.0003)	0.001 (0.0005)	0.003 (0.002)	0.002 (0.001)	0.005 (0.001)	0.004 (0.001)
5	0.0017 (0.0003)	0.005 (0.001)	0.009 (0.002)	0.008 (0.004)	0.018 (0.007)	0.015 (0.006)
10	0.0027 (0.001)	0.008 (0.002)	0.016 (0.008)	0.009 (0.004)	0.028 (0.013)	0.030 (0.010)
50	0.0040 (0.001)	0.035 (0.018)	0.063 (0.025)	0.046 (0.016)	0.085 (0.039)	0.096 (0.068)
100	0.0063 (0.002)	0.086 (0.023)	0.106 (0.078)	0.092 (0.031)	0.250 (0.025)	0.195 (0.043)

Table 3
Developmental time (S.D.) in days from the beginning of 2nd instar to the start of each stadium*

Nominal alga conc. (mg/kg)	2nd to 3rd	2nd to 4th	2nd to pupa		2nd to adult	
			Female	Male	Female	Male
Control	5.1 (1.7) ^a	8.4 (0.6) ^a	20.6 (1.8) ^{ab}	15.4 (1.9) ^a	26.7 (2.0) ^{ab}	22.6 (2.0) ^a
1	5.7 (1.5) ^a	8.8 (0.7) ^b	22.1 (2.4) ^{ab}	16.7 (3.1) ^{ab}	26.6 (2.3) ^{ab}	22.3 (1.6) ^a
5	6.6 (2.5) ^b	8.8 (0.7) ^b	21.6 (1.6) ^{ab}	17.3 (3.1) ^{bc}	27.5 (0.7) ^a	24.0 (2.6) ^a
10	5.7 (1.9) ^a	8.6 (0.7) ^a	20.1 (2.8) ^a	14.8 (3.1) ^a	25.1 (2.7) ^{bc}	23.1 (2.0) ^a
50	6.4 (2.3) ^a	8.9 (0.7) ^b	21.4 (2.0) ^{ab}	15.5 (2.0) ^{ab}	27.2 (2.5) ^{ab}	23.4 (2.5) ^a
100	6.3 (2.9) ^a	9.0 (0.8) ^b	22.6 (3.2) ^b	17.7 (2.6) ^c	23.7 (1.2) ^c	22.6 (1.2) ^a

* Numbers with different superscripts within a stadium are statistically different.

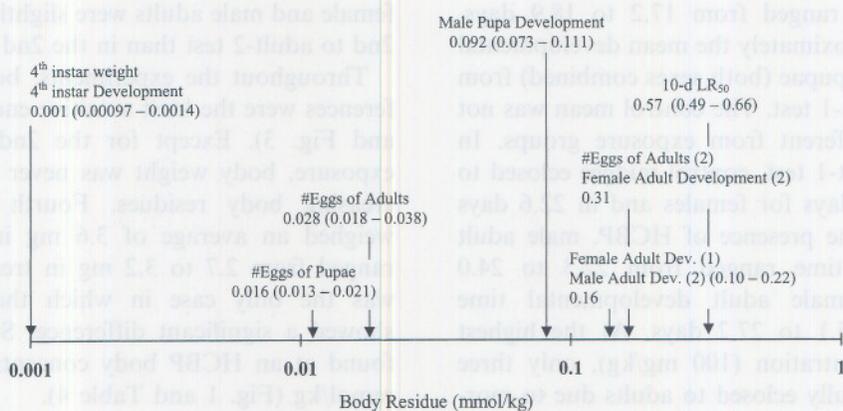


Fig. 1. Response spectrum of midges, *C. riparius*, to HCBP in relationship to body residues. Arrows represent the body residues at which significant effects are first detected. Body residue for female adult developmental time is estimated because of high pupal mortality. The range in the parenthesis is 95% confidence interval. The number 1 in the parenthesis indicates the 2nd to adult-1 test, while the number 2 indicates the 2nd to adult-2 test.

mmol/kg in males in the 2nd to pupa-1 test (Table 2). Body residues varied from 0.002 to 0.009 mmol/kg in the 2nd to pupa-2 test (Table 6). The same trend was also found in adults where body residues ranged from 0.004 to 0.195 mmol/kg in males and from 0.005 to 0.250 mmol/kg in females in the 2nd to adult-1 test (Table 2). In the adult-2 test, body residues varied from 0.003 to 0.306 mmol/kg in females and from 0.003 to 0.158 mmol/kg in males (Table 7). The higher body residue of female midges likely results from longer exposure times and higher fat content.

Mean developmental time increased with increasing body residues (Table 3). From 2nd to 3rd instar, the mean developmental time was 5.1 days in control group and varied from 5.7 to 6.4 days

among the treatments. The developmental time, thus, increased in a dose-responsive manner with increasing HCBP body residues, but was not statistically significant. When exposure took place from the 2nd larval instar to the beginning of the 4th, the midges turned to 4th instar in an average of 8.4 days without HCBP. However, when the midges were fed the algae contaminated with HCBP, mean developmental time ranged from 8.6 to 9.0 days depending on body residue. The developmental time during this exposure increased significantly at body residues of 0.001 mmol/kg compared to the control group (Fig. 1 and Table 3).

When the midges were analyzed by sex in the 2nd to pupa-1 and the 2nd to adult tests, the

developmental times of males were shorter than females'. In the 2nd to pupa-1 test, midges in the control group pupated in 20.6 and 15.4 days for females and males, respectively. In the treatment groups, however, pupal developmental time varied from 20.1 to 22.6 days in females and from 15.4 to 17.7 days in males depending on body residues. Male pupal developmental time increased significantly at a body residue of 0.09 mmol/kg (Fig. 1 and Table 3), while there was no statistical difference in female pupa developmental time compared to controls (Table 1 and Fig. 3). In the 2nd to pupa-2 test (Table 6), the developmental time ranged from 17.2 to 18.9 days, which was approximately the mean developmental time of control pupae (both sexes combined) from the 2nd to pupa-1 test. The control mean was not significantly different from exposure groups. In the 2nd to adult-1 test, control midges eclosed to adults in 26.7 days for females and in 22.6 days for males. In the presence of HCBP, male adult developmental time ranged from 22.3 to 24.0 days, while female adult developmental time ranged from 25.1 to 27.2 days. At the highest exposure concentration (100 mg/kg), only three midges successfully eclosed to adults due to mortalities and had a mean developmental time of 23.7 days. No statistical significance was found between body residue and developmental time for either male or female adults. However, this is somewhat misleading due to high mortality at the highest exposure concentration (100 mg/kg). Developmental time for female adults increased up to 4th exposure concentration (50 mg/kg), even though statistical significance was not found. Also, there is a large difference in body residues

(0.08 vs. 0.25 mmol/kg) between 50 and 100 mg/kg exposure concentrations. Therefore, biological differences are suspected to exist and LOER for female adult developmental time was estimated to be approximately 0.16 mmol/kg (Figs. 1 and 2). In the 2nd to adult-2 test, the developmental time of female adults ranged from 19.8 to 25 days, while the developmental time of male adults were from 15.3 to 24.6 days (Table 7). The female developmental time was statistically different from control at 0.306 mmol/kg body residue, while the significance in the male was found at 0.158 mmol/kg. The developmental time of both female and male adults were slightly shorter in the 2nd to adult-2 test than in the 2nd to adult-1 test.

Throughout the experiments, body weight differences were the least sensitive endpoint (Table 4 and Fig. 3). Except for the 2nd to 4th instar exposure, body weight was never affected by increasing body residues. Fourth instar midges weighed an average of 3.6 mg in controls and ranged from 2.7 to 3.2 mg in treatments, which was the only case in which the body weight showed a significant difference. Significance was found at an HCBP body concentration of 0.001 mmol/kg (Fig. 1 and Table 4).

The number of ova per female pupa in the 2nd to pupa-1 test significantly decreased with increasing body residues of HCBP (Table 5 and Fig. 3). The mean number of ova per pupa was 323.2 in the control and ranged from 281.7 to 326.7 in treatment groups. The statistically significant reduction in the number of ova occurred at a body residue of 0.016 mmol/kg where an average of 292 ova were found (Figs. 1 and 3). The average number of ova from adult midges in the 2nd to

Table 6

Body residues, developmental time, body mass and fecundity of the midges in the first 2nd to pupa-2 test*

Nominal alga conc. (mg/kg)	Body residue (mmol/kg)	Developmental time (days)	Weight (mg)
Control		17.2 (2.4) ^a	3.5 (0.8) ^a
1	0.002 (0.001)	18.9 (2.5) ^a	3.0 (1.0) ^a
3	0.003 (0.002)	18.3 (4.6) ^a	3.2 (1.5) ^a
5	0.004 (0.001)	18.0 (2.0) ^a	3.7 (0.9) ^a
7	0.005 (0.002)	18.8 (2.7) ^a	3.5 (0.6) ^a
10	0.009 (0.004)	17.3 (4.6) ^a	3.3 (1.5) ^a

* The numbers with different superscripts in a stadium are statistically different.

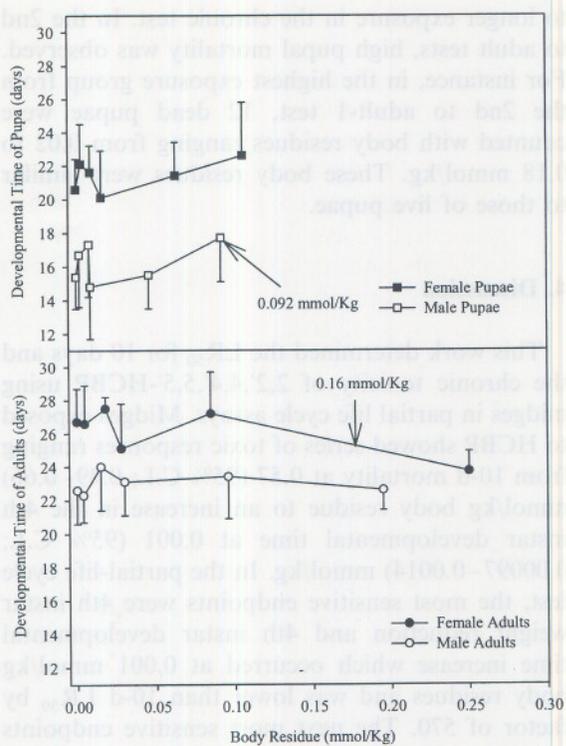


Fig. 2. Developmental time change related to body residue. Male pupa showed a significant developmental time increase at 0.92 mmol/kg body residue. Due to high mortality in female adults, only three midges in the early stage of the experiment survived. Because there was a big difference in body residues between the 4th and 5th exposure concentrations, it was assumed that developmental time would be, if there was no mortality, significantly increased and was estimated to be 0.16 mmol/kg. Arrows are placed to show LOER. Error bars represent the standard deviation.

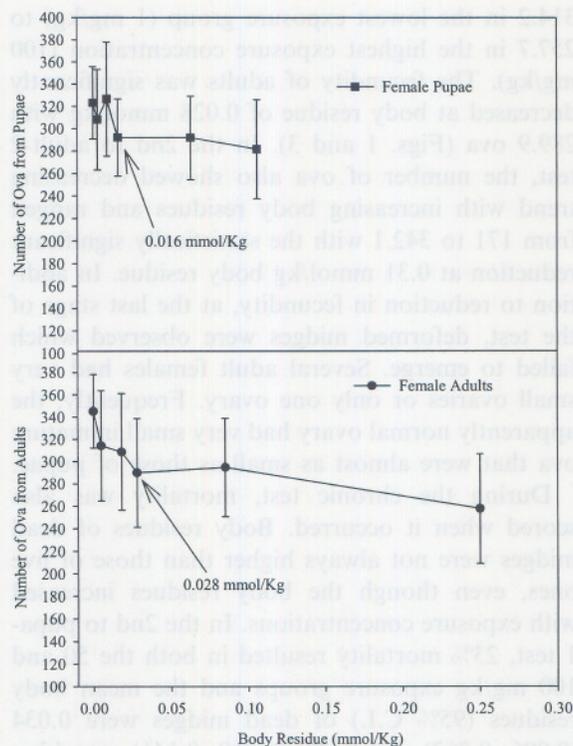


Fig. 3. The number of ova related to the body residue. The number of ova was drastically affected by increasing body residues in both pupa and adult. Arrows are placed to show the LOER. Error bars represent the standard deviation.

Table 4
Body mass of midges in mg (S.D.) in each developmental stadium*

Nominal alga conc. (mg/kg)	2nd to 3rd	2nd to 4th	2nd to pupa		2nd to adult	
			Female	Male	Female	Male
Control	2.1 (0.4) ^a	3.6 (0.7) ^a	5.6 (0.6) ^a	4.0 (0.5) ^a	2.5 (0.3) ^a	1.4 (0.2) ^a
1	1.8 (0.3) ^b	3.0 (0.5) ^{bc}	5.1 (0.9) ^a	3.9 (0.4) ^a	2.5 (0.3) ^a	1.4 (0.3) ^a
5	1.8 (0.3) ^{ab}	3.0 (0.6) ^b	5.2 (0.6) ^a	3.8 (0.4) ^a	2.6 (0.3) ^a	1.4 (0.2) ^a
10	1.8 (0.3) ^b	3.2 (0.6) ^b	5.7 (0.8) ^a	4.1 (0.6) ^a	2.3 (0.5) ^a	1.4 (0.2) ^a
50	1.7 (0.5) ^b	2.9 (0.5) ^c	5.5 (0.4) ^a	3.8 (0.5) ^a	2.3 (0.3) ^a	1.4 (0.2) ^a
100	1.9 (0.4) ^{ab}	2.7 (0.4) ^c	5.1 (0.8) ^a	4.2 (0.6) ^a	2.6 (0.2) ^a	1.5 (0.2) ^a

* The numbers with different superscripts in a stadium are statistically different.

314.2 in the lowest exposure group (1 mg/kg) to 257.7 in the highest exposure concentration (100 mg/kg). The fecundity of adults was significantly decreased at body residue of 0.028 mmol/kg with 289.9 ova (Figs. 1 and 3). In the 2nd to adult-2 test, the number of ova also showed decreasing trend with increasing body residues and ranged from 171 to 342.1 with the statistically significant reduction at 0.31 mmol/kg body residue. In addition to reduction in fecundity, at the last stage of the test, deformed midges were observed which failed to emerge. Several adult females had very small ovaries or only one ovary. Frequently, the apparently normal ovary had very small immature ova that were almost as small as those of pupae.

During the chronic test, mortality was also scored when it occurred. Body residues of dead midges were not always higher than those of live ones, even though the body residues increased with exposure concentrations. In the 2nd to pupa-1 test, 23% mortality resulted in both the 50 and 100 mg/kg exposure groups and the mean body residues (95% C.I.) of dead midges were 0.034 (0.006–0.062) and 0.080 (0.019–0.141) mmol/kg, respectively. This was lower than the 10-d LR₅₀ by 16.8 and 7.1 times, respectively. In the 2nd to pupa-2 test, 0 to 16% mortality occurred but was not dose responsive. When these residues were compared to the body residue of midges showing the similar mortality from 10-d mortality test, body residues are still lower by one to six times in the chronic study. This mortality response at decreased body residues was somewhat expected due

to longer exposure in the chronic test. In the 2nd to adult tests, high pupal mortality was observed. For instance, in the highest exposure group from the 2nd to adult-1 test, 12 dead pupae were counted with body residues ranging from 0.02 to 0.18 mmol/kg. These body residues were similar to those of live pupae.

4. Discussion

This work determined the LR₅₀ for 10 days and the chronic toxicity of 2,2',4,4',5,5'-HCBP using midges in partial life cycle assays. Midges exposed to HCBP showed series of toxic responses ranging from 10-d mortality at 0.57 (95% C.I.; 0.49–0.66) mmol/kg body residue to an increase in the 4th instar developmental time at 0.001 (95% C.I.; 0.00097–0.0014) mmol/kg. In the partial-life cycle test, the most sensitive endpoints were 4th instar weight reduction and 4th instar developmental time increase which occurred at 0.001 mmol/kg body residues and was lower than 10-d LR₅₀ by factor of 570. The next most sensitive endpoints were fecundity of pupae and adults as represented by the counts of ova, followed by male pupa developmental time increase. The least sensitive endpoint in the partial-life cycle test was the female adult developmental time increase, which occurred at body residues that were a factor of 1.9 lower than the 10-d LR₅₀. This study strongly suggests that the toxicity test based on short-term mortality is not sufficient to protect environmental integrity.

Table 5
Fecundity (S.D.) shown in the number of ova in each of pupa and adult female*

Nominal alga conc. (mg/kg)	2nd to pupa		2nd to adult	
	Body residue (mmol/kg)	The number of ova	Body residue (mmol/kg)	The number of ova
Control		323.2 (32.5) ^a		345.1 (33.0) ^a
1	0.003 (0.002)	308.2 (45.0) ^{ab}	0.005 (0.001)	314.2 (49.5) ^{ab}
5	0.009 (0.002)	326.7 (51.2) ^a	0.018 (0.007)	308.7 (52.2) ^{ab}
10	0.016 (0.008)	292 (34.5) ^b	0.028 (0.013)	289.9 (48.7) ^{bc}
50	0.063 (0.025)	291.7 (37.3) ^b	0.085 (0.039)	294.6 (44.1) ^{bc}
100	0.106 (0.078)	281.7 (44.2) ^b	0.250 (0.025)	257.7 (48.9) ^c

* The numbers with different superscripts are statistically different.

There have been numerous publications measuring bioaccumulation based on the assumption that the bioaccumulated contaminants exert adverse effects on organisms. However, there are very few studies relating levels of bioaccumulation to the resulting biological effects. The current research will help bridge the difficulties in understanding the relationship between bioaccumulation of 2,2',4,4',5,5'-HCBP and its effects.

4.1. 10-d mortality test

The body residues from live organisms, when the test was terminated, were similar to those of dead midges suggesting that the genetic variability in sensitivity to HCBP exists among individual organisms within population as expected. This result is consistent with the finding that live earthworm body residues were similar or slightly lower than those of dead earthworms exposed to pentachlorophenol (Fitzgerald et al., 1996). The LR_{50} value (0.57 mmol/kg) for 10-d mortality for the midges was lower than acute mortality body residues proposed by McCarty and Mackay (1993) (2–8 mmol/kg) for nonpolar narcotics, or measured using fish (2.02–8 mmol/kg) (Sijm et al., 1993). Given that toxicity is the function of exposure, exposure duration, and potency of the toxin (Hong et al., 1988), the lower value is somewhat expected because the mortality began occurring after 6 days, while the data of McCarty and Mackay (1993), Sijm et al. (1993) were based on the 96-h mortality tests. Besides the difference in exposure duration, this difference may be also attributable to the differences in genetic susceptibility to HCBP and differences in lipid content of each organism. The lipid content of fathead minnows used by McCarty et al. (1992) and of fathead minnows and guppies used by Sijm et al. (1991) reportedly ranged from 3 to 8% of wet weight, while lipid content of 4th instar and pupal stage midges, which are aquatic life stages, were determined to be around 1.53 (\pm S.D.; 0.24) and 2.93 (\pm S.D.; 0.41)% of wet weight. This lower lipid content would translate into lower storage capacity for the organism. Thus, less total compound is required to reach toxic concentration at the receptor. On a lipid normalized basis, the

respective mortality response would be \sim 36–145 mmol/kg lipid for fish and 53.6 for chironomids, thus the effects concentrations are similar. It is important to consider lipid content for nonpolar narcosis because storage lipid removes the contaminant from the site of action, the membrane must be filled before sufficient concentration would be attained at the site of action. Therefore, organisms of differential lipid content would be expected to exhibit differential responses on a whole body basis, but similar responses on a lipid normalized basis for nonpolar narcosis. Such lipid normalization will also be important for other endpoints, since storage lipid will remove contaminant from other sites of actions as well until filled to the critical level.

4.2. Partial-life cycle tests

HCBP produced a variety of adverse effects on midges at much lower concentrations than that required for mortality (Fig. 1). For instance, the ova count reduction in adult female required lower body residues than 10-d LR_{50} by factor of 20 and the adult female developmental time increase required lower residues than 10-d LR_{50} by a factor of 3.6 in the 2nd to adult-1 test. In the presence of a toxicant like HCBP, the organisms have to allocate their energy to cope with the stresses that otherwise can be used to support growth and reproduction (Gibbs et al., 1996). Therefore, slower development and fecundity reduction are likely due to energy required to cope with the stress caused by the presence of xenobiotics. In the present study, developmental time increases in male pupae in the 2nd to pupa-1, female adults in the 2nd to adult-1, and female and male adults in the 2nd to adult-2 test were observed (Fig. 2 and Table 7). However, no significant developmental time changes were observed in the 2nd to pupa-2 test, while a statistically significant increase in developmental time for male pupae was observed in the 2nd to pupa test-1. The absence of adverse effects in the 2nd to pupa-2 test is likely due to the low body residues attained. These residues reached only 10% of those for pupae from the 2nd to pupa-1 test. This example shows that critical body

Table 7
Body residues, developmental time, and body mass of the midges in the repeated 2nd to adult-2 test*

Nominal alga conc. (mg/kg)	Body residue (mmol/kg)		Developmental time (days)		Weight (mg)		Fecundity (no. of ova)
	Female	Male	Female	Male	Female	Male	
Control			22.4 (1.3) ^a	19.1 (5.0) ^{ac}	2.8 (0.2) ^a	1.6 (0.2) ^a	340.4 (57.5) ^a
1	0.003 (0.001)	0.003 (0.002)	19.8 (2.1) ^{ab}	15.3 (3.3) ^b	2.6 (0.5) ^a	1.4 (0.3) ^a	342.1 (66.4) ^a
5	0.016 (0.007)	0.010 (0.005)	20.7 (1.4) ^a	18.4 (5.0) ^{abc}	2.8 (0.4) ^a	1.4 (0.2) ^a	315.4 (53.9) ^a
10	0.024 (0.006)	0.019 (0.008)	20.2 (1.8) ^a	16.8 (4.9) ^{abc}	2.5 (0.3) ^a	1.8 (0.6) ^a	329.9 (42.8) ^a
50	0.147 (0.048)	0.095 (0.052)	22.3 (2.7) ^{ab}	21.7 (3.9) ^{cd}	2.5 (0.5) ^a	1.5 (0.3) ^a	294.2 (46.3) ^a
100	0.306	0.158 (0.110)	25.0 ^c	24.6 (4.6) ^d	2.2 ^a	1.7 (0.5) ^a	171 ^b

* The numbers with different superscripts in a stadium are statistically different.

residues must be attained to achieve a given effect and consistent with the result of Fisher et al. (1999b). Further, developmental time responses were the most inconsistent of the sublethal responses. Both reductions and increases in total developmental time were observed (Fig. 2). The developmental time decreased and then increased at lower levels of body residue for all stages (Fig. 2) up to almost 0.03 mmol/kg. Subsequently, the pupa developmental time again increased as body residue increased with a significant increase for male pupae at 0.09 mmol/kg. The observed drop in developmental time response at low body residues may be an expression of hormesis or physiological compensation which occurs at low-levels of contamination (Bengtsson, 1979; Laughlin et al., 1981; Calabrese, 1999). Hormesis is described as transient overcorrections by control mechanisms to inhibitory challenges (Laughlin et al., 1981). Bengtsson (1979) reported that the growth of minnows with body residues of PCB Clophen A50 from 0.4 to 31 $\mu\text{g/g}$ (~ 0.0012 – $0.095 \mu\text{mol/g}$) wet weight significantly increased compared to that of minnows in the control group. The author hinted that thyroid hormone-like activity of PCB might have increased in the growth of minnows. It is difficult to determine whether the decrease in the developmental time in midges with low level of HCBP is due to hormesis. However, the trend was consistent across the both sexes and all developmental stages, suggesting that the midges growth might be boosted due to low level of HCBP contamination.

The weight of organisms has been reported to decrease with increasing body residues of contaminants. The metabolic rates of benthic organisms like *C. riparius*, oligochaetes (*L. variegatus*), bivalves (*Sphaerium corneum*) increased in the presence of 2,4,5-trichlorophenol (Penttinen et al., 1996). In fact, the length of *C. riparius* larvae has also been shown to decrease with exposure to contaminants, e.g. nickel in 30-d growth test (Powlesland and George, 1986). However, in the present study, no significant weight reduction except for the 4th instar larvae was found while significant developmental time increases occurred (Fig. 2). This may be explained by the insect-specific physiology. The onset of metamorphosis to pupae in most insects is generally associated with attaining a certain body mass (Denlinger and Zdarek, 1994). When midges are exposed to HCBP, they likely take longer time to reach the critical body weight due to energy allocation for coping with HCBP-induced stress. Overall, the weight difference of the specific developmental stage was not a very good endpoint for *C. riparius* in the partial life cycle test in assessing chronic effects of HCBP.

Another problem in using body weight as an endpoint is that pupae lose weight until they eclose to adults. As pupation starts, midges stop eating and go through the reconstruction process in which larval structures are destroyed and adult structures are elaborated (Gullan and Cranston, 1994). Midges have to depend on the reserved energy sources during this period. Moreover,

midges continue to purge their guts in the early pupal stage, which further leads to body weight loss. Therefore, early stage pupae are usually heavier than later ones. The body weight during the pupal stage varies depending on the degree of pupal maturity and the representative body weight for the pupal stage is difficult to determine. Thus, pupal weight as an endpoint is likely to be problematic.

PCBs are known to have adverse effects on the reproduction in aquatic organisms. For example, Aroclor 1254 decreased the reproductive capacity of a meiobenthic copepod, *Microarthridion littorale* (DiPinto et al., 1993). Unfortunately, the copepod body residues associated with this effect were not reported. Nor did they perform a congener-specific analysis. In another study, strong correlation ($r = 0.701$, $P = 0.0002$) between egg mortality and total PCB concentrations in the eggs was found for Lake Trout (Mac et al., 1993). The above results match well with our study, even though the confirmation of relationship between body residues and their effect could not be made, because experimental designs were different. In both the 2nd to adult-1 and -2 tests, the number of ova significantly decreased with increasing body residue of HCBP in female midges and both abnormal ovary development and oocyte development were observed. However, LOER for fecundity from the 2nd to adult-2 test was higher than that from the 2nd to adult-1 test by a factor of 10. This discrepancy may be attributed to the low statistical power in the 2nd to adult-2 test due to the lower number of females that resulted by random selection of experimental organisms. Because there is no way of discerning sex of the 2nd instar midges, the number of each sex cannot be determined at the initiation of the test.

Given that many midges were observed with abnormal ovaries, one ovary, or abnormally small ova in ovarioles in the high exposure concentration, it is strongly suggestive that HCBP blocks either normal ovariole formation or yolk deposition in ova. This response may be either specific to HCBP, or due to chronic stress that interferes with ovary development at high chronic exposures.

4.3. Experimental difficulties

The measurement of HCBP body residues in *C. riparius* was fraught with difficulties. The timing of recovering dead midges was critical for determining an accurate weight. Upon dying, midges start absorbing water and disintegrating, which made it difficult at times to measure an exact weight of dead organisms. To avoid this problem, Penttinen et al. (1996) used live organisms to measure body residues which were similar or just slightly lower than dead organisms. However, when the live organisms are used to obtain the body residues, it is hard to determine which midges are on the brink of death. Thus sampling can be performed only once at the end of experiment or must be made from separate replicate exposures in which the measurement of body residues is separated from effect assessment. In the current study, midges were sampled only when they reached specified developmental states. Thus, sampling only at the end of experiment was not possible and measures in dead organisms had to be taken when they occurred.

Distinguishing each larval stadium is also somewhat difficult because individual size can be variable depending on the food or water intake. Therefore, some error can be associated with determining developmental stadium prior to pupation. However, there is no problem in distinguishing pupae or adults because pupae or adults are morphologically different from the previous stadia. Thus, measuring body residue and developmental time at these stages should be the easiest to perform and give the most reliable data.

Collecting adult midges is also difficult. Adult midges were collected using two techniques, e.g., using forceps and with an aspirator as was depicted in a USEPA document (US EPA, 1994). In the present research, forceps were mainly used, because an aspirator was somewhat bulky to use in 50 ml exposure chambers. Difficulties in recovering flying midges with forceps resulted in the loss of ~5% of adult midges.

Additional experimental difficulties occurred with counting ova in pupae. Pupal ova, especially early stage pupal ova, are small and almost transparent which makes them very difficult to count

accurately. Because fecundity can be measured more easily by counting ova from adult midges, counting pupal ova is redundant and not statistically more sensitive. However, since yolk deposition occurs throughout the pupal stage, the number of ova in pupa can be different from that of adult if yolk deposition or yolk production itself is blocked by the presence of HCBP. Counting ova in pupa therefore may still be important for determining the extra damage to normal ova formation. In the current study, a number of adult female midges were found to have very small ova that are almost the same size of those in pupae, which is likely a reflection of altered yolk deposition by the contaminant.

4.4. Advantages and disadvantages in the use of body residues to assess the hazard

The hypothesis of constant body residue for the mortality has been challenged (Legierse et al., 1999; Yu et al., 1999). Yu et al. (1999) reported that the internal lethal median concentration (ILC_{50}) of series of halobenzenes in fish and juvenile crabs decreased with increasing natural logarithm of exposure time in a linear manner. In the graphical presentation, the authors showed that the relationship between ILC_{50} and $\ln LT_{50}$ is inversely linear. Legierse et al. (1999) showed that the lethal body residues of organophosphorus compounds in pond snails and the guppy decreased with increasing exposure time and the decreasing trend could not be modeled using critical body residue model. Also, the LR_{50} values of other PCB congeners and linear alkylbenzenesulfonate in the midges were also found to decrease with increasing exposure time (unpublished data). Therefore, it will be critical to determine the relationship between exposure time and body residue to produce mortality, e.g. Yu et al. (1999). Moreover, the exposure time question is likely less important for the chronic endpoints, since steady state is more likely established in the long-term test and the toxicity value approaches the lethal threshold concentration (Sprague, 1969).

Bioassays should be sensitive and discriminatory in order to be used in the hazard assessment. It has been reported that body residues for a toxic

response are not discriminatory (Pawlisz and Peters, 1993). Pawlisz and Peters (1993) found that *Daphnia magna* showed a wide ranges of responses ranging from no response to mortality at a given body residue and the body residues for *D. magna* showing the same response varied by two orders of magnitude, when *D. magna* were exposed to nine narcotic organic chemicals. If this is universal, body residues may not be useful in referencing toxicity. However, the present study finds that using body residues were both sensitive and discriminatory. While individuals in a population may respond at different levels, the median response concentration for a population should be as useful as other median measures.

Another problem using body residues may be associated with the presence of metabolites. Metabolites are usually included in total chemical amounts when radiolabeled chemicals are used in laboratory tests, but in environmental samples metabolites are usually disregarded, thereby overestimating the critical body residue unless the metabolites contribute equally to the effect. Thus, the extent of metabolism must be determined, and the toxicity of metabolites must be assessed. This may be resolved by performing two toxicity tests in parallel, i.e. with and without a mixed function oxidase suppressant, such as piperonyl butoxide. However, when differentially potent metabolites coexist, it may be difficult to determine contribution of each metabolite to the apparent toxicity unless studies with specific metabolites are performed. In a similar study, no metabolites were found in a 10-d mortality test with 4,4'-dichlorobiphenyl (DCBP) (unpublished data). Therefore, although biotransformation was not measured, it was assumed that midges did not metabolize HCBP which has higher level of chlorination than DCBP and is usually less readily biotransformed.

Finally, the potency of compounds may be different in different species, creating variation in interpreting the significance of body residues across species. Normalization to the storage capacity of the organism, e.g. lipid content, is expected to correct for much of this variability between species for non-polar narcotics. However, a limitation to the body residue approach is our current inability to measure and assess the contri-

bution of genetic variation — both intra- and inter-specific — to the production of toxicological effects.

Despite some difficulties and limitations, using internal body concentration has distinct advantages over using environmental concentrations as a toxicological index. In referencing toxicity to body residues, bioaccumulation data can be explained more meaningfully. Bioaccumulation data themselves have little meaning beyond confirmation of exposure and bioavailability. Making a connection between accumulated dose and toxicological effects will permit better interpretation of the hazard associated with complex exposure such as occurs with multiple exposure routes or from matrices such as sediment where bioavailability and exposure routes are not readily predicted.

Interpretation of field data will also improve. It is not easy to interpret the adverse effect of a toxic agent in the field using LC_{50} or EC_{50} values that were developed in a lab, because a series of extrapolations are required to estimate the adverse effect in the field and each step has uncertainties (Suter et al., 1985). In particular for sediment bioassays, greater variation may result, because of varying interactions of different organisms with sediments and concomitant differences in bioavailability (Harkey et al., 1994). These problems are largely alleviated by measuring body residues and knowing the relationship between accumulated dose and toxicological effect.

Problems with multiple exposure routes can also be solved by using body residues. With multiple exposure routes, it is difficult to determine the contribution of each route to the total exposure, since the bioavailability and contaminant concentration of each medium are different (Kukkonen and Landrum, 1995). Also, steady state between the concentration in the source compartment and that in an organism is not necessary with the tissue residue approach, because a toxicological effect is expressed at the threshold concentration in organisms for a given exposure duration (Sprague, 1969; Sijm et al., 1993).

4.5. Significance and application of findings

The current study has documented that HCBP can exert its toxicity including developmental time increases and fecundity reduction at levels which are significantly lower than for mortality. The reduction in fecundity and increase in developmental time in presence of HCBP may significantly impact midge populations. For instance, increased developmental time could lead to longer exposure to predators prior to reproduction, resulting in fewer adults during breeding cycles. Reductions in the growth of individuals and reductions in fecundity can cause population reductions and even extirpation (Klok and Roos, 1996). Also, in the experiment using a model *Daphnia* population to determine the effects of sublethal narcosis on population resistance, Hallam et al. (1993) showed that sublethal stress imposed by nonpolar narcotics to the individual organisms could lead to population extinction. Given that midges are important food source for fish, diminished midge populations due to contaminant exposures would adversely affect next trophic level. In a balanced ecosystem, the adverse effects on one level ultimately affects the whole ecosystem (Cole, 1994). Therefore, the partial life cycle test, in which fecundity reduction and developmental time increases are detectable, is a powerful and sensitive tool to assess the biological effects of the contaminants and to protect the organisms in the environment.

The causative relationships between body residues and their related effects will also help define the sublethal hazards of highly lipophilic compounds in the environment. By comparing LOER values developed in the current study to the body residue of non-polar narcotics from the organisms collected in the field, the hazard can be estimated. Because body residues are directly related to the effects, exposure estimation will not be necessary if measured residue levels are available for assessing risk, which should improve reliability by reducing uncertainties. Moreover, the method reported here can be successfully applied to other exposure routes, like sediment exposure, because problems such as different bioavailabilities, inconsistent feeding, or avoid-

ance behavior do not need to be accounted for when measuring body residues. Given that the benthic invertebrates are associated with sediments, ecological risk assessment, using biomonitoring in which severity of contamination in sediment is investigated using representative organisms, will be significantly improved by knowing the relationship between body residues and their effects.

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