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Lethal Body Residue of Chlorophenols and Mixtures of Chlorophenols in Benthic Organisms

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Abstract. The lethal body residue (LBR) of a few chlorophenol congeners were measured in the oligochaete worm *Lumbriculus variegatus*, and the LBR of pentachlorophenol was measured also in a midge, *Chironomus riparius* larvae. LBR is defined as the concentration of the compound in the organism, on molar basis, to cause death, and the LBR_{50} is defined as the calculated LBR value to cause a 50% mortality in population after a given time. Groups of 30 or 40 organisms were exposed to different chlorophenol concentrations in artificial soft fresh water to achieve differential mortality. Exposure times were either 24 h or 48 h. In addition to exposures with individual congeners, mixtures of chlorophenols were also tested. After each exposure, the surviving organisms were collected and the body burden of chlorophenols was measured by gas chromatography with electron capture detection. The measured body burden was related to the percent mortality in the group. The trichlorophenols and pentachlorophenol have a 48-h LBR_{50} of 0.45–0.66 μmol/g wet weight in *L. variegatus*. The 48-h LBR_{50} of pentachlorophenol for *C. riparius* was 0.15 μmol/g wet weight, indicating a slight difference in the sensitivity of these two species. The 48-h LBR_{50} of 2,3,4,6-tetrachlorophenol is 0.91 μmol/g wet weight, and the value for 2,6-dichlorophenol is 1.2 μmol/g wet weight in *L. variegatus*. The 48-h LBR_{50}s of the chlorophenol mixtures ranged from 0.50 to 0.83 μmol/g wet weight, demonstrating an additive toxicity.

Evaluating the effect of contaminants on various levels of the aquatic food chain has traditionally used concentrations in the external environment. When mixtures of chemicals or multiple sources are involved and the exposure becomes complicated due to significant bioavailability limitations (such as exposures in sediments), then assessing effects based on the external environment may not be very predictable. Rather, there is a body of knowledge that is developing to evaluate the effect of chemicals based on the internal concentration in organisms. This is analogous to utilizing blood levels in mammals to predict drug effects and behavior. McCarty (1986) derived that the molar whole body concentration of nonpolar narcotic chemicals at the time of death, referred as lethal body burden or critical body residue (CBR), is constant. This concept is based on idea that residue levels at the cell membranes (where the toxic action of these chemicals takes place) are well correlated with the whole body concentrations. Some studies have demonstrated that CBRs of nonpolar narcotic chemicals are indeed fairly constant varying from 2 to 8 mmol kg^{-1} for acute toxicity to 0.2 to 0.8 mmol kg^{-1} for chronic responses in fish (Van Hoogen and Opperhuizen 1988; McCarty *et al.* 1992; McCarty and Mackay 1993; Sijm *et al.* 1993) and even in some invertebrates (Landrum *et al.* 1991, 1994; Pawlisz and Peters 1993a, 1993b; Kane Driscoll 1997a, 1997b; Fisher *et al.* 1999a). However, some results indicate that there might be some differences between species in susceptibility to nonpolar narcotic chemicals (van Wezel *et al.* 1995a).

The range of concentrations that produce effects vary with both mechanism of action and duration of exposure. Besides nonpolar narcosis, the CBR approach for relating mortality to the affective body burden has been applied to chemicals with different mechanisms of toxic action in different organisms: polar narcosis (van Wezel *et al.* 1995b; de Wolf *et al.* 1992; Urrestarazu Ramos *et al.* 1998); decoupling of oxidative phosphorylation by pentachlorophenol in *Diporeia* and *Mysis relicta* (Landrum and Dupuis 1990), in zebra mussel (Fisher *et al.* 1999b), and in earthworms (Fitzgerald *et al.* 1996, 1997) and by 2,3,5-trichlorophenol in a midge larvae (Ristola *et al.* 1999); neurotoxicity by tri-n-butyltin in amphipods (Meador *et al.* 1993); acetylcholinesterase inhibition by organophosphorus pesticides in fish (de Bruijn *et al.* 1991; Deneer *et al.* 1999); and effects of nitrobenzenes in fish (Deneer *et al.* 1987).

The use of CBR has been applied also to chronic endpoints, like the scope for growth in mussels (Widdows and Donkin 1989) and to changes in energy metabolism in some invertebrates (Penttinen and Kukkonen 1998, 2000). Additionally, this approach is used to study the compound interactions and mixture toxicity of polyaromatic hydrocarbons (PAHs) (McCarty and Mackay 1993; Landrum *et al.* 1989, 1991) and chlorobenzenes (van Wezel *et al.* 1996). Further more, there is already a

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first attempt to establish water and sediment quality criteria for nonpolar narcotic chemicals using critical body burdens (Di Toro *et al.* 2000; Di Toro and McGrath 2000).

Chlorophenols are toxic to aquatic life because of their relatively high lipophilicity and capability to either induce polar narcotic effects or interfere with intermediary metabolism by uncoupling oxidative phosphorylation (Coulston and Kolbye 1994). Toxicity depends on the quality of the compounds, such as the degree of chlorination, the isometric position of the chlorine atoms, and the molecular weight as well as the pH of the medium.

Various chemical products derived from chlorophenols have been used for over 35 years as fungicides, pesticides, and for some other agricultural, industrial, and commercial uses (Sterling and Arundel 1986). In addition, chlorophenols can be formed in combustion of organic material, in chlorobleaching of pulp, and even as by-products during the chlorination of drinking water. Even though the discharge of chlorophenols has decreased during the last decade, they are an important group of environmental pollutants. For example, in Finland, the production and use of chlorophenols has led to severe soil, water, and ground water contamination at many industrial sites, like sawmills (Valo *et al.* 1985; Lampi *et al.* 1992). Further, in water chlorophenols from different discharges or runoffs from contaminated sites sorb onto particles and eventually end up in sediments (Paasivirta *et al.* 1990; Kukkonen *et al.* 1996; Lyytikäinen *et al.* 2001).

Benthic organisms that are in direct contact with sediments, like oligochaete worms or larval stages of midges, are exposed to sediment-associated chlorophenols via both porewater and food intake. In this kind of exposure situation the CBR approach is useful, but besides pentachlorophenol and 2,4,5-trichlorophenol (Landrum and Dupuis 1990; Penttinen and Kukkonen 1998; Ristola *et al.* 1999) there are no CBR data for benthic organisms, which are exposed the most to the sediment-associated chlorophenols. Furthermore, to use this approach in risk assessment, it should work also for mixtures of chlorophenols, and that data is not available for benthic organisms.

The objectives of this study were (1) to measure the lethal body residue of few individual chlorophenols in an oligochaete worm *Lumbriculus variegatus*; (2) to measure the lethal body residue of mixtures of chlorophenols in *L. variegatus*; and (3) to compare the lethal body residue of pentachlorophenol in *L. variegatus* and in a midge larvae, *Chironomus riparius*.

Materials and Methods

Organisms

The cultures of oligochaete worms, *L. variegatus* (Müller), and midges, *C. riparius* (L.), were reared in the Aquatic Ecology and Ecotoxicology Laboratory at the University of Joensuu under conditions previously described (Penttinen *et al.* 1996). The *L. variegatus* used in the experiments were taken from the culture aquaria. The chironomids originated from the egg batches from the culture and were grown in 2-L beakers with cellulose as a substrate and fed three times a week with fish food flakes (TetraMin) until they reached fourth instar. The wet weight of *L. variegatus* and *C. riparius* was 7 ± 3 mg and 3.5 ± 0.7 mg, respectively.

Chemicals

Chlorophenols used in this study (Table 1) were obtained as follows: 2,6-dichlorophenol (> 98%; 26-DCP), 2,4,5-trichlorophenol (> 99%; 245-TCP), and pentachlorophenol (> 99%; PCP) from Fluka AG (Buchs, Switzerland); 2,4,6-trichlorophenol (> 98%; 246-TCP) from Aldrich (Steinheim, Germany); and 2,3,4,6-tetrachlorophenol (> 99%; 2346-TeCP) from Riedel-de Haën (Seelze, Germany). The stock solutions of the chlorophenols were prepared in 99% ethanol (Primalco, Finland). Internal standard, 236-TCP (purity 99.9%), was produced by Labor Dr. Ehrenstorfer (Augsburg, Germany). Other chemicals used were of analytical grade produced by Merck (Darmstadt, Germany) or J. T. Baker (Deventer, The Netherlands), except iso-octane, which was of grade for organic trace analysis (Merck). Acetic anhydride was redistilled in the laboratory prior to use. The glassware was washed with acetone, ethanol (94%), and distilled water to avoid contamination.

Artificial fresh water was used in all experiments. It was made from Milli-Q-grade water by adding the following inorganic salts: $CaCl_2 \times 2H_2O$ 58.8 mg/L, $MgSO_4 \times 7H_2O$ 24.7 mg/L, KCl 1.1 mg/L, and $NaCO_3$ 13.0 mg/L (total Ca + Mg hardness 1.0 mmol/L). The water was buffered with phosphate buffer (0.5 mmol), and pH was adjusted to 6.5.

Experimental Setup

The *L. variegatus* and *C. riparius* were used in water-only exposures even though both species normally live in sediments. Artificial fresh water was dosed either with an individual chlorophenol congener or with a mixture of chlorophenols slowly by adding small amounts (< 0.01% v/v) of stock solution in 50% ethanol with stirring. The water sample was allowed to stir for an additional 30 min after dosing. Aliquots of 500 ml were placed into 600-ml beakers and 30 or 40 organisms were added. A water sample was taken for analysis to verify the concentration in the beginning of the exposure. A range of different concentrations of chlorophenols (Table 2) was used to produce no mortality, partial mortality, and 100% mortality. A control beaker with maximum the amount of carrier solvent and without chlorophenols was always included. The exposure was performed at 20 ± 1°C in the dark. After 24 or 48 h, the number of surviving organisms was recorded, and the survivors were placed on paper towel, weighed, and analyzed for body residue. A water sample was also taken at the end of the exposure for the chlorophenol analysis. The oxygen concentration and pH were monitored and O_2 saturation was always > 80% and pH was 6.5 ± 0.1 at the end of exposures.

Chemical Analysis

Chlorophenols and the internal standard in a water sample (1 ml) were acetylated with acetanhydride in 50 ml of 0.01 M K_2CO_3 and extracted with iso-octane. A small amount of ascorbic acid was added to prevent the oxidation of chlorophenols to quinones during the process. Iso-octane was dried over Na_2SO_4 before analyzing with a gas chromatograph (HP 5890) equipped with an automatic sampler (HP 7370) and two ^{63}Ni electron capture detectors. The columns used were HP-1 and PAS-1701 (Lyytikäinen *et al.* 1997).

After weighing, organisms were transferred to a test tube, acidified with 0.4 M H_2SO_4 and a mixture of hexane and acetone (1:1, v/v 5 ml) was added. Sonication (5 min) was used to facilitate extraction. After sonication the sample was centrifuged (10 min, 3,000 g), and the organic layer was collected. The extraction was performed twice, and the solvents were combined. The extract was transferred to a separation funnel containing 50 ml 0.1 M K_2CO_3 and ascorbic acid; the mixture was shaken 3 min and organic layer was discarded. The

Table 1. Physical-chemical characterization of model compounds (Mackay *et al.* 1995) and calculated % of nonionized form at the test pH (6.5)

Congener	Molecular Weight	log K_{ow}	pK_a	% Nonionized
26-DCP	163.00	2.9	6.8	66.6
245-TCP	197.45	3.7	6.9	71.5
246-TCP	197.45	3.7	6.1	28.5
2346-TeCP	231.89	4.4	5.4	7.4
PCP	266.34	5.1	4.7	1.6

The percentage of nonionized form is calculated from the Henderson-Hasselbach equation.

Table 2. The concentration ranges of chlorophenols (μg/L) used in single exposures or in mixture exposure for *Lumbriculus variegatus* in water-only tests

Congener	Single Exposure	245-TCP + PCP	2346-TeCP + PCP	245-TCP + 246-TCP + PCP
26-DCP	3.000–16.400	—	—	—
245-TCP	650–1,690	420–810	—	350–500
246-TCP	788–3,160	—	—	440–600
2346-TeCP	130–980	—	130–550	—
PCP	72–360	45–130	28–75	25–70

phenols were acetylated by shaking with acetic anhydride (3 min) and the acetates were extracted with 10 ml of iso-octane. Iso-octane was dried over Na_2SO_4 before analyzing with gas chromatography (GC) installed with two electron-capture detectors. The GC procedure, instrumentation, and conditions are described in Lyytikäinen *et al.* (1997).

Recoveries for the water samples in the beginning of the exposure were from 92% to 109% of the nominal concentration for each of the chlorophenol congener tested. Recoveries for the worm extraction procedure were determined to be (mean [SD], n = 4) 129.8% (4.1), 116.7% (0.5), 107.5% (0.7), 93.3 (5.1), and 80.5 (6.6) for 26-DCP, 245-TCP, 246-TCP, 2346-TeCP, and PCP, respectively. The measured body residue results were not corrected for recovery. The limit of detection for chlorophenols was 20–130 ng g^{-1} ww depending on the compound.

Calculations

The LC_{50} and LBR_{50} values were calculated by the generalized linear model described by Kerr and Meador (1996). For the calculations of LC_{50} values, the measured concentrations (μg/L) in the beginning of the exposure were used and for the calculations of LBR_{50} values the measured body residues (μmol/g wet weight organisms) of surviving organisms were used.

Results

In the controls, more than 93% of organisms survived in every exposure. The 24-h and 48-h LC_{50} values of the chlorophenols to *L. variegatus* and 24-h and 48-h LC_{50} values of PCP to *C. riparius* are presented in Table 3. After 48-h exposure, the concentration was 85.3% (± 1.2), 79.6% (± 5.9), 89.8% (± 6.6), 86.0% (± 8.3), and 54.6% (± 7.1) of the starting concentration for 26-DCP, 245-TCP, 246-TCP, 234,6-TeCP, and PCP, respectively. The PCP is 80 (24 h) to 140 (48 h) times more toxic to *L. variegatus* than 26-DCP when comparing LC_{50} values on a molar basis. The toxicity of the other congeners are intermediate to these two compounds. These results were expected as these conditions are similar to those reported for some other aquatic organisms (Smith *et al.* 1994; Devillers and Chambon 1986; Saarikoski and Viluksela 1981). Based on the LC_{50} values, it seems that *C. riparius* is a substantially more tolerant species than *L. variegatus* for PCP toxicity (Table 3).

There was a very sharp dose-response curve for these compounds (*e.g.*, Figure 1). The shape of this curve is very different from those for nonpolar narcotics (van Wezel *et al.* 1995c) where the partial effect range is much larger. LBR_{50} values were calculated from the data set for the different chlorophenols, and there was no great difference between congeners on molar basis. The LBR_{50} values for *L. variegatus* varied from 1.72 μmol/g for 26-DCP (24 h) to 0.45 μmol/g for PCP (48 h) (Table 4). When comparing the two species based on calculated LBR_{50} values, it appears that *C. riparius* is more sensitive to the PCP than *L. variegatus*, exactly the opposite of the result implied by the LC_{50} values.

When using mixtures of chlorophenols, the water concentration of each component was so low (Table 2) that individual chlorophenol alone could not cause high mortality observed, but the combined effect was expected. The dose-effect curve for the mixtures was similar to the one single congener exposures. The LBR_{50} values for the mixtures varied between 0.5 μmol/g and 1.2 μmol/g (Table 5), which is exactly the same range where the individual chlorophenol congeners caused the mortality.

Discussion

In this study, the concept of lethal body residue (LBR) was applied and the lethal body residue 50% value (LBR_{50}) was calculated. LBR_{50} value defines the body residue at which 50%

Table 3. Acute toxicity of chlorophenols (LC_{50}) to *Lumbriculus variegatus* and PCP to *Chironomus riparius* in water-only exposures

Congener	Time (h)	LC_{50} (μg/L)	95% Confidence Limits	LC_{50} (μmol/L)	95% Confidence Limits
26-DCP	24	16,127	15,172–17,667	98.9	93.1–108.4
	48	12,171	11,703–12,670	74.7	71.8–77.7
245-TCP	24	1,160	1,127–1,191	5.88	5.71–6.03
	48	865	854–876	4.38	4.33–4.44
246-TCP	24	2,614	2,636–2,690	13.2	12.8–13.6
	48	1,356	1,333–1,380	6.87	6.75–6.99
2346-TeCP	24	822	777–918	3.54	3.35–3.96
	48	690	691–716	2.98	2.89–3.09
PCP	24	328	309–354	1.23	1.16–1.33
	48	143	139–147	0.54	0.52–0.55
PCP*	24	1,192	389–1,993	4.48	1.46–7.48
	48	898	795–1,131	3.37	2.99–4.25

* Results for *C. riparius*.

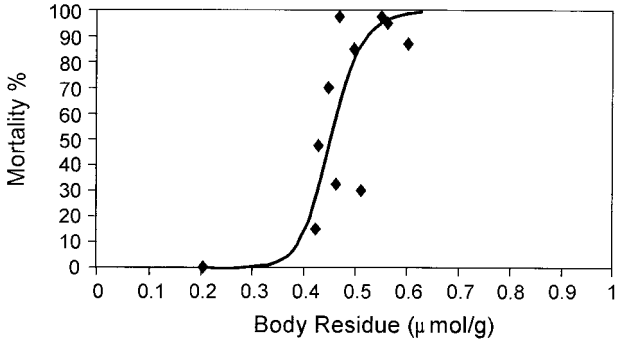

Fig. 1. Body burden–mortality relationship for *L. variegatus* exposed to pentachlorophenol for 48 h. Each dot represents the response of a group of 30 organisms. The shown body residues are analyzed in surviving organisms

Table 4. Lethal body residues (LBR_{50}) of chlorophenols in *Lumbriculus variegatus* and LBR_{50} of PCP in *Chironomus riparius*

Congener	Time (h)	LBR_{50} (μmol/g)	95% Confidence Limits
26-DCP	24	1.72	1.68–1.76
	48	1.24	1.22–1.27
245-TCP	24	0.63	0.60–0.66
	48	0.54	0.53–0.55
246-TCP	24	0.81	0.80–0.83
	48	0.66	0.64–0.67
2346-TeCP	24	0.95	0.87–1.15
	48	0.91	0.85–0.96
PCP	24	0.70	0.67–0.74
	48	0.45	0.44–0.47
PCP*	24	0.22	0.20–0.24
	48	0.15	0.14–0.16

* Results for *C. riparius*.

Table 5. Lethal body burdens (LBR_{50}) of mixtures of chlorophenols in *Lumbriculus variegatus*

Mixture	Time (h)	LBR_{50} (μmol/g)	95% Confidence Limits
245-TCP + PCP	24	0.58	0.56–0.62
2346-TeCP + PCP	24	1.20	1.15–1.26
	48	0.83	0.82–0.84
245-TCP + 246-TCP + PCP	24	0.66	0.59–0.72
	48	0.50	0.41–0.59

of the test population died at the fixed exposure durations of 24 h or 48 h and gives a 95% percent confidence limits for the estimate. With the same method, it is possible to calculate for example the LBR_{90} or LBR_{20} values. To compare the species or to conduct environmental risk assessment, we need this type of predetermined effect level approach. In some of the earlier studies, the tissue concentrations at which organisms die were analyzed and results given in the form of average value or range of concentrations, referred as lethal body burden (LBB; for example, van Wezel *et al.* 1995b; Landrum *et al.* 1991). In the studies where LBB estimates are based on the calculations from the LC_{50} and toxicokinetics, we may say that the modeled body residue value represents a level where 50% of population shows a response (McCarty *et al.* 1993; Sijm *et al.* 1993). In many cases the results obtained from the two approaches are very similar, but there is a need to clarify the terminology behind these different measurements and concepts.

There are some data in literature where the LBBs of various chlorophenol congeners have actually been measured in fish (Kobayashi *et al.* 1979; Hattula *et al.* 1981; Kobayashi and Kishino 1980; Spehar *et al.* 1985; van Wezel *et al.* 1995b; Hickie *et al.* 1995) and in some cases LBB was estimated from toxicity and bioaccumulation data (Saarikoski and Viluksela 1981; McCarty *et al.* 1992, 1993). The range of reported LBB values of chlorophenols for fish vary from 0.08 to 1.8 μmol g^{-1}, which might indicate differences in species tolerance to chlorophenols as well as variation in analytical procedures. Unfortunately, the recoveries or detection limits are not reported in every paper to facilitate the comparison. However, there is the trend that the LBB values for mono- and dichlorinated phenols are from 0.8 to 1.8 μmol g^{-1} and for higher chlorinated phenols from 0.2 to 1.0 μmol g^{-1}.

For invertebrates, the existing CBR data of chlorophenols is limited. Landrum and Dupuis (1990) measured the 96-h LBR_{50} of PCP in an amphipod, *Pontoporeia hoyi*, and in a mysid shrimp, *Mysis relicta*, to be 0.91 μmol g^{-1} and 4.4 μmol g^{-1}, respectively. It is interesting to notice a quite large different in

LBR_{50} of PCP in these two organisms. In *P. hoyi* the effect concentration for PCP is on the upper end of what can be expected for uncoupler of oxidative phosphorylation, but for *M. relicta*, LBR_{50} value is similar to that for narcotic compound (McCarty and Mackay 1993).

The LBB of PCP in the freshwater clam *Pisidium amnicum* was in the range of 0.15 to 0.27 μmol g^{-1} in a long-term exposure to two different concentrations (100 and 300 μg/L) of PCP at two different temperatures (5 and 19°C) at pH 6.5 (Heinonen *et al.* 2001). The time needed for toxic response was greatly affected by temperature, and the mean survival times were 5–15 times longer at 5°C than at 19°C. Despite the differences in survival times, the LBBs between the temperatures were the same.

LBB of PCP was relatively constant when measured in three different earthworms exposed to different PCP concentrations in different soil types and temperatures. The values ranged from 0.33 to 1.59 μmol g^{-1} and one was 2.65 μmol g^{-1} in worms exposed to soil with a very high PCP concentration (Fitzgerald *et al.* 1996, 1997). Kaila and Saarikoski (1977) performed an 8-day LD_{50} test with a crawfish, *Astacus fluviatilis*. The 8-day LD_{50} values for PCP and 2,3,6-TCB were 0.098 and 0.192 μmol g^{-1}, respectively. Ristola *et al.* (1999) measured the LBR_{50} value of 2,4,5-TCP to be 0.113 μmol g^{-1} for a midge larvae (*Chironomus riparius*) in a 10-day sediment test.

There is an interesting difference between the responses of the two species exposed to PCP. When considering the calculated LC_{50} values (Table 3), the midge larvae, *C. riparius*, seem to be more tolerant of PCP than *L. variegatus*. However, the LBR_{50} data (Table 4), suggest that *C. riparius* is more sensitive than *L. variegatus*. This difference between the species comes most likely from the metabolism of PCP. *C. riparius* is capable of metabolizing PCP and eliminating the metabolites to the water (Verrengia Guerrero *et al.* 2002). Thus, the concentration of the parent compound measured by GC is rather low. On the other hand, the metabolism of PCP by *L. variegatus* is very limited (Verrengia Guerrero *et al.* 2002).

Data in this paper (Table 4) provides the information on the short-term LBR_{50} values of chlorophenols in invertebrates that is measured at the same temperature and pH. The literature reviewed, however, shows quite a variation in published LBR_{50} and LBB values. The variation is much less than the variation in LC_{50} values (see Table 3) but large enough to be considered. The ability to metabolize chlorophenols may explain some of the difference between species. One other factor to consider is the lipid content of the organism, which may affect considerably the accumulated body burden (Landrum and Fisher 1998). Unfortunately, in this study the lipid content of the organisms was not measured. Published lipid content of *L. variegatus* from the same population varies from 5.2% to 7.7% of dry weight (Leppänen and Kukkonen 1998a, 1998b, 2000). The lipid content of *C. riparius* varies in the literature from 3.7% to 9.5% of dry weight (Choi *et al.* 2001; Hwang *et al.* 2001; Harkey *et al.* 1994). Based on these published values, it is not possible here to connect the measured difference in body residues to the different lipid content of the organisms.

Environmental pH is one factor that affects the accumulation and toxicity of chlorophenols. There is negative correlation between pH and toxicity of chlorophenols (Saarikoski and Viluksela 1981; Fisher *et al.* 1999b). This has been explained by ionization of chlorophenols. In Table 1 it is calculated the proportion of nonionized form at the pH 6.5 used in this set of exposures. If two trichlorinated congeners are compared, it can be seen that 245-TCP has a higher pK_a than 246-TCP and thus at pH 6.5 a higher portion of 245-TCP is in nonionized form. Because of this 245-TCP is more toxic than 246-TCP when comparing the LC_{50} values. However, if the toxicity of these two congeners is corrected for ionization 246-TCP becomes more toxic. This might indicate that also the nonionized form accumulates and contributes to toxicity of the congener (Saarikoski and Viluksela 1982; Spehar *et al.* 1985). Fisher *et al.* (1999b) showed the LBR_{50} of PCP in zebra mussel, *Dreissena polymorpha*, depended on the exposure pH. The measured LBR_{50}s varied over a range of 0.015 to 0.12 μmol g^{-1}, the lowest being for the pH 6.5 and the highest for pH 8.5. They discussed that this difference at different pHs is at least partly due to the fact that ionized and nonionized forms may have different mode of toxic actions. This is an important point when considering the use of critical body residue approach. However, results in this study indicates that the different chlorophenol congeners, except maybe 26-DCP, have the same mode of toxic action at pH 6.5.

In this study, only the surviving organisms were analyzed, because after death *L. variegatus* starts to swell and decompose rather quickly. The dead worms are very fragile and hard to handle and weight. The swelling also affects the wet-weight measurements and thus the results. The comparison of the measured body residues in dead and alive organisms was done in one case (PCP + 235-TCP exposure). It revealed that the dead organisms had on average 23% and 8% lower body residue of PCP and 235-TCP, respectively, than the live organisms. A similar observation was done by Penttinen and Kukkonen (1998). This difference can be partly due to the swelling and decomposition of the organisms and partly due to the difference of individual tolerance, which has been shown for fish in the case of nonpolar narcotic compounds (van Wezel *et al.* 1995c). Similarly, dead *P. hoyi* had on average a slightly lower carbaryl body burden than the estimated LBR_{50}, but there was no statistical difference between the values (Landrum and Dupuis 1990). On the other hand, Fisher *et al.* (1999b) reported that dead zebra mussels had higher PCP body residues than the live organisms at pH 8.5, but there was no difference at pH 6.5. Spehar *et al*. (1985) reported that dead fathead minnows had a PCP body residue about 0.28 μmol g^{-1}, and live minnows were below 0.13 μmol g^{-1}. Furthermore, Fitzgerald *et al.* (1996) reported similar or lower body residues of PCP in surviving earthworms than in dead ones. In this study, the use of surviving worms seemed to give reliable body residue estimates. Because the measured values were from a group of organisms, the figures represent more a response of a population, not just an individual.

The results of mixture toxicity of chlorophenols in this study generally support the hypothesis that the LBR_{50} value on molar basis is the same for the molecules having the same mode of toxic action. The LBR_{50} value was similar no matter whether the molar concentration resulted from a single chemical or by a group of chemicals (Table 5). This conclusion is in agreement with the results reported for the narcotic chemicals (Landrum *et al.* 1991; van Wezel *et al.* 1996). The CBR approach seems to work within chemicals having the same mode of toxic action. The next step would be to evaluate CBRs of mixtures of

chemicals having a different modes of toxic action and determine whether any type of general risk assessment procedure can be established, as suggested by van Wezel *et al.* (1996).

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