

Lethal Body Residues for Pentachlorophenol in Zebra Mussels (*Dreissena polymorpha*) under Varying Conditions of Temperature and pH

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Pentachlorophenol (PCP) toxicity was measured in the zebra mussel under varying conditions of pH (6.5, 7.5, or 8.5) and temperature (10, 17, or 25°C). Toxicity decreased significantly with increasing pH at all temperatures. At a given pH level, toxicity increased significantly with increasing temperature. PCP was most toxic at pH 6.5, 25°C and least toxic at pH 8.5, 10°C. Toxicokinetic parameters were determined at trace PCP concentrations under each combination of pH and temperature. Increasing temperature generally increased the PCP uptake clearance (k_u) although elimination rate constants (k_d) were unaffected. The effect of pH on toxicokinetic parameters was inconsistent but k_u tended to decrease as pH and ionization of PCP increased. Lethal body residues (LR_{50s}), estimated from kinetic parameters determined at trace PCP concentrations and the LC₅₀ values, varied by a factor of 122 as a function of environmental conditions while LC_{50s} varied by a factor of 381. LR_{50s} were also estimated from the measured PCP tissue concentrations and varied by a factor of 8 across conditions. Calculated LR_{50s} were always higher than measured LR_{50s}, determined under identical conditions, by at least a factor of five. However, when LR₅₀ values were recalculated using k_u values measured at the LC₂₅ concentration, the resulting adjusted LR_{50s} varied only by a factor of 2.5 across the range of conditions studied and were more consistent with measured LR₅₀ values. Thus, variance in the PCP concentration required to produce toxicity is reduced when LR_{50s} are used in place of LC_{50s}. Further, the method by which lethal residues (LR₅₀ values) are determined can significantly affect the results and their interpretation. © 1999 Academic Press

Key Words: critical body residue; zebra mussels; pentachlorophenol; toxicokinetics

INTRODUCTION

Pentachlorophenol (PCP) is an ionizable phenol with a pKa of 4.8. As the pH of the medium increases, the

ionization of PCP proceeds and is nearly complete at pH 8.5–9.0. As PCP is ionized, its properties as a toxicant also change. The un-ionized compound is relatively lipophilic with a log K_{ow} of 4.2 (Saarikoski and Viluksela, 1982). In this form, the potential for bioconcentration in living tissues is greatest and PCP is most toxic to aquatic species (Kobayashi *et al.*, 1979; Kobayashi and Kishino, 1980; Spehar *et al.*, 1985; Fisher and Wadleigh, 1986; Stehly and Hayton, 1990). However, as ionization takes place, PCP becomes significantly less toxic because the ionized form does not penetrate membranes as readily (Kobayashi *et al.*, 1979; Kobayashi and Kishino, 1980). Although the pentachlorophenate ion contributes to toxicity at alkaline pHs (Fox and Rao, 1978; Saarikoski and Viluksela, 1982), toxicity of PCP generally decreases with increasing pH.

Temperature also affects the toxicity of PCP (Crandall and Goodnight, 1959; Hodson and Blunt, 1981). In most cases, PCP toxicity to aquatic organisms increases with increasing temperature. The observed increases in toxicity are related to increased ventilation rates as temperature increases, which expose the organism to more toxicant. In addition, increases in PCP toxicity at elevated temperatures may be due to increased sensitivity of aquatic life to respiratory uncouplers (Fisher, 1986; Persoone *et al.*, 1989).

Because the toxicity of PCP changes significantly with environmental conditions, large errors in estimating the hazard of PCP are possible. However, the recently promulgated concept of the critical body residue (CBR) may reduce the necessity of measuring toxicity under diverse environmental conditions. Briefly, the CBR hypothesis states that the use of environmental toxicant concentrations to gauge hazard can be misleading because the environmental concentration necessary to cause effect varies with the route and duration of exposure, type of contaminated medium, and species used for testing (McCarty and Mackay, 1993). However, the toxicant dose required to produce an effect at the target site in an organism should not change dramatically with route of exposure or duration, provided that the toxicity mechanism does not change and that the injury does not

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accumulate over time. Indeed, for a large number of chemicals that act as membrane narcotics, the whole-body tissue concentration of chemical (CBR) necessary to cause 50% mortality by narcosis varies between 2 and 8 mmol/kg across a range of species (McCarty *et al.*, 1992; McCarty and Mackay, 1993). CBRs have also been estimated for nonnarcotic chemicals; for respiratory uncouplers such as PCP, an acute CBR is estimated at 0.11–0.30 mmol/kg (McCarty, 1990; van den Heuvel *et al.*, 1991) while the chronic CBR is estimated at 0.09 mmol/kg (Spehar *et al.*, 1985).

PCP was used as a model chemical for testing the hypothesis of the CBR in zebra mussels (*Dreissena polymorpha*), a recent invader of the Great Lakes (Herbert *et al.*, 1989; Roberts, 1990). In an effort to standardize the terminology and avoid uncertainty in interpreting what is meant by "critical body residue" or the more recent derivative, lethal body burden (LBB) (Sijm *et al.*, 1993), the term lethal body residue (LR₅₀) was chosen to denote the tissue concentration of toxicant needed to cause 50% mortality in the test population analogous to LD₅₀, the lethal dose to cause 50% mortality.

MATERIALS AND METHODS

Chemicals

Pentachlorophenol (>99% pure) was obtained from Aldrich Chemical Co. (Milwaukee, WI) for use in toxicity tests. [¹⁴C] PCP (>98.0% pure, specific activity = 7.9 mCi/mmol) was purchased from Sigma Chemical Co. (St. Louis, MO). The purity of PCP was checked, prior to use, with thin-layer chromatography (Fisher, 1986).

Organisms

Adult zebra mussels (>20-mm shell length) were collected from Lake Erie near Kelly's Island by SCUBA divers. Mussels were transported to holding facilities in Columbus, wrapped in wet paper towels. Animals were held in culture for up to 6 months at densities of 2000 mussels per 200 liter of water at <10°C (Bruner *et al.*, 1994). Animals were fed a daily diet of Tetra Min fish food sufficient to maintain their condition as reflected by lipid content and ability to reform the byssus within 48 h after severing (Atanasoff, 1993). Zebra mussels were acclimated to laboratory conditions for at least 2 weeks prior to use in experiments.

Media

Soft standard reference water (SRW) (40–48 mg/liter as CaCO₃, total alkalinity = 30–35 mg/liter as CaCO₃), was adjusted to the appropriate pH (6.5, 7.5, or 8.5) using 1 M NaOH or 1 M HCl (USEPA, 1975). Because potassium salts are toxic to zebra mussels (Fisher *et al.*, 1991), 1.0 M Na₂HPO₄ was substituted for the recommended

K₂HPO₄ buffer. The water was held at 10, 17, or 25°C in environmental chambers set at the appropriate temperature. pH and temperature were measured prior to introduction of zebra mussels to test vessels and at the conclusion of toxicity tests. In no case did pH vary more than 0.2 pH units nor did temperature vary more than 0.1°C from the desired values during the course of the experiments.

Twenty-Four-Hour Toxicity Tests

The toxicity of PCP to zebra mussels was measured using a modified method of Waller *et al.* (1993). Briefly, adult zebra mussels were removed from culture by severing their byssal threads. Ten to 13 mussels were then placed on 9-cm-diameter glass petri dishes and allowed to secrete new byssal threads. If, after 48 h, new threads were not formed, the mussels were discarded.

Petri dishes, containing at least 10 mussels, were submerged in 2.5 liters of SRW, previously adjusted to the appropriate pH and temperature, in 4 liter glass jars. Mussels were allowed to acclimate to conditions for 12 h before exposure to PCP. Thereafter, PCP was added to each jar in a volume of 2.5 ml acetone (1 ml/liter) to give a range of five concentrations plus a control which received acetone only. Three replicates of each concentration were made. After 24 h of exposure, mortality was scored, the criterion for which was failure to respond to the touch of a blunt probe. Closed mussels were gently gaped open and a probe was inserted. If no adductor muscle activity was noted, the animals were considered dead. Tests were invalidated if control mortality exceeded 10%.

Three additional tests were conducted at two extreme sets of conditions (pH 6.5, 25°C; pH 8.5, 10°C) and one intermediate set of conditions (pH 7.5, 17°C). These tests were conducted in a manner identical to that described above except that trace [¹⁴C] PCP (3 µg/liter) was added to each vessel along with unlabeled PCP at the appropriate concentration. After mortality was scored, dead and live mussels were noted and total PCP in the tissue of each individual was measured using liquid scintillation counting (LSC) as described below. The specific activity of PCP was recalculated for each concentration to reflect the contributions of labeled and unlabeled PCP to total body burden.

Uptake Clearance and Elimination Rates

The methods of Bruner *et al.* (1994) were used to determine toxicokinetic parameters. Mussels were removed from culture 3 days prior to an experiment and allowed to attach individually to 2.5 × 7.5-cm glass slides. Attached mussels were allowed to acclimate to experimental conditions for 6 h prior to use. Mussels were then immersed individually in 500 ml SRW in 1-liter beakers spiked with trace levels of

[^{14}C] PCP (0.02 $\mu\text{mol/liter}$). A second set of uptake studies was performed at extreme conditions (pH 6.5, 25°C; pH 8.5, 10°C) and an intermediate set of conditions (pH 7.5, 17°C) in which PCP was present at a toxic LC_{25} concentration determined for each set of conditions. The later tests were conducted as previously described except that mussels were preconditioned by being exposed to the LC_{25} concentration of PCP for 6 h prior to initiation of kinetic tests. The behaviour of the mussels during the uptake phase was monitored every 30 min during exposure; if siphons were extended, the animals were considered to be filtering. One-milliliter water samples were withdrawn from each beaker initially and at the end of each exposure for LSC. Five mussels were sampled at 0.5, 1, 1.5, 2, 4, and 6 h and processed for LSC. Animals were blotted dry and weighed and tissue was extracted from each shell. Thereafter, the tissue of each individual was placed in 20-ml glass scintillation vials with 5 ml of cocktail (1000 ml dioxane, 500 g naphthalene, 5 g diphenyloxazole) for 24 h to extract >90% of PCP. Valves and tissue from each mussel were counted separately using a Beckman LS 6000IC scintillation counter (^{14}C efficiency >95%) with automatic quench control. To complete a mass balance in each exposure, beakers that contained the exposure water were rinsed with 20 ml of acetone and 1-ml sample of the acetone wash was taken for LSC.

Elimination rates were determined by exposing mussels to PCP for 6 h as previously described and then placing exposed mussels in clean water which was renewed daily. Mussels were removed for analysis at 12, 24, 48, 72, 96, 144, and 168 h. Five mussels were collected at each sampling time and analyzed as previously described for PCP. Animals were fed during the elimination period.

Lipid Analysis

Whole-body lipid were measured by blotting 20 mussels dry and weighing eviscerated tissue. Tissues from 20 mussels were homogenized individually in glass test tubes in 6 ml of chloroform/methanol, 2:1. Tubes were sealed and refrigerated overnight. Subsequently, 0.5 ml of each homogenate was analyzed for total lipids using the methods of van Handel (1985). Lipid levels in zebra mussels varied from 10.8 to 13.5% of dry weight and were not significantly different among treatment groups.

Data Analysis

Uptake clearance constants (k_1), which describe the fractional change in the contaminant concentration in the water over time, were calculated for PCP under each set of exposure conditions. Contaminant partitioning between water

and organism is assumed such that:

$$k_1 = [-\ln(1 - Q_a/A)]/t - k_d Q_a,$$

where Q_a = quantity of PCP in animal (μg), k_1 = conditional uptake rate constant (h^{-1}), k_d = conditional elimination rate (h^{-1}), A = amount of contaminant in the system at T_0 (μg), and t = time (h).

It is also assumed that the amount of PCP in the system does not change and that no biotransformation of PCP takes place. Only nominal sorption of PCP to glassware occurred (<2% of total) and was, thus, not incorporated into the mass balance model. The uptake rate constant (k_1) is conditional and must be converted to the system independent clearance rate (k_u) by the following equation:

$$k_u = k_1 \times (\text{volume of water/wet mass of tissue}).$$

Elimination rate constants were determined independently from a first-order elimination model.

$$\ln C_a = \ln C_{a0} - k_d t.$$

where C_a = concentration of PCP in organism (ng/g) and C_{a0} = contaminant concentration in organism at start of elimination experiment (ng/g). The elimination rate constant is obtained from the slope of the regression line between $\ln C_a$ vs t .

Bioconcentration factors (BCFs) were calculated from the ratio of the two kinetic rate constants:

$$\text{BCF} = \frac{k_u}{k_d}.$$

Statistical differences in log-transformed k_u and BCF values were analyzed by a general linear analysis of variance (SAS, 1982) and means were separated using least-squares means test (SAS, 1982).

Significance was determined at $P < 0.05$. Elimination rate constants were tested for homogeneity of slopes and significant slopes were separated using nonoverlapping 95% confidence intervals. Individuals with uptake clearance rates < 10 $\text{ml g}^{-1} \text{h}^{-1}$ or from systems with mass balance < 70% were excluded from data analysis.

Toxicity data were analyzed using probit analysis (Litchfield and Wilcoxon, 1949). LC_{50} values were considered significantly different when 95% confidence limits did not overlap. Three types of LR_{50} values were determined: (1) **Measured** LR_{50} values were estimated from body burdens of animals used in toxicity tests with probit analysis. Measured LR_{50} values that were determined under two extreme sets of conditions (pH 6.5, 25°C and pH 8.5, 10°C) and one set of intermediate conditions (pH 7.5, 17°C) were corrected for

percentage ionization at the internal pH of the zebra mussel using the Henderson-Hasselbalch equation. (2) **Calculated** LR_{50} values were determined from the equation (Sijm *et al.*, 1993)

$$LR_{50} = k_u/k_d \times LC_{50} (1 - e^{-k_d t}).$$

(3) Finally, **adjusted** LR_{50} s were calculated from the above equation using the k_u determined at the LC_{25} concentration of PCP.

RESULTS

The dramatic effects of pH and temperature on the acute toxicity of PCP to the zebra mussel are summarized in Table 1. At a given pH, there was a significant increase in the toxicity of PCP as temperature increased from 10 to 25°C. Likewise, decreasing pH significantly increased toxicity within each temperature. As expected, the mussels exhibited the highest susceptibility to PCP under conditions of the lowest pH coupled with the highest temperature (pH 6.5, 25°C) while the lowest toxicity was observed at the highest pH coupled with the lowest temperature (pH 8.5, 10°C). The toxicity of PCP varied 381-fold between these two extreme sets of conditions.

Tissue Residues

Radiolabeled PCP was added in trace amounts to unlabeled PCP and toxicity was again assessed at the two extreme sets of conditions and one intermediate set of conditions. Measured median lethal residues (LR_{50}) were estimated from the tissue residues in poisoned animals, both dead and alive, following 24 h of exposure (Table 2). In contrast to the LC_{50} values, which varied by a factor of 381 between the extremes, the measured LR_{50} s, determined

from the tissue residues of PCP in each mussel, varied only by a factor of 8 (Table 3).

The raw data from the measured LR_{50} determinations provide additional insight into the processes leading to intoxication (Table 2). At pH 8.5, 10°C, dead mussels contained three- to five fold more PCP in their tissues than did live mussels. However, at pH 6.5, 25°C, live and dead mussels contained roughly equal amounts of PCP after 24 h of exposure. At the intermediate set of conditions (pH 7.5, 17°C) dead mussels had higher tissue residues than live ones, but the difference was not as pronounced as the tissue residue data at pH 8.5, 10°C.

In the mussels held at pH 6.5, 25°C, there was clear evidence of a dose-response based on tissue concentrations of PCP (Table 2). That is, as the exposure concentration of PCP in water increased, there were concomitant increases in the tissue concentration of PCP and mortality. The data obtained at pH 8.5, 10°C and pH 7.5, 17°C did not exhibit the expected dose-response as strongly. Further, there was an interesting anomaly in the data obtained at pH 8.5, 10°C: mortality dropped from 55 to 20% as the PCP concentration in water increased from 31.5 to 39.4 µmol/liter. The average tissue concentration in the mussels also decreased from 128.0 to 87.0 µmol/kg as the exposure concentration went from 31.5 to 39.4 µmol/liter (Table 2). Thus, there is evidence that lower mortality occurred when the animals absorbed a lower dose of PCP, even under conditions that do not generate a robust dose-response based on aqueous concentrations.

The measured LR_{50} tissue concentrations were adjusted for ionization that occurs at the internal pH of the zebra mussel, pH 7.5 (O'Donnell *et al.*, 1995), and compared to predicted levels of PCP (Table 3). The actual amounts of unionized PCP causing 50% mortality varied by a factor of 8 between exposure conditions with a lower amount of PCP resulting in 50% mortality at pH 6.5, 25°C. The bulk of the tissue concentration (>99.8%), under all exposure regimes, was contributed by the phenate form of PCP.

Predicted tissue concentrations of both PCP and Phenate were always higher, by at least a factor of three, than observed tissue concentrations. However, the predicted concentrations of unionized PCP were similar (Table 3).

TABLE 1
Toxicity of PCP to *Dreissena polymorpha* as a Function of pH and Temperature

pH	Temperature (°C)	LC_{50}^a	(µmol/liter)
6.5	10	3.5 ^c	(1.9-6.2)
	17	0.8 ^{ab}	(0.5-1.3)
	25	0.09 ^a	(0.06-0.16)
7.5	10	11.6 ^c	(10.3-13.0)
	17	2.3 ^{bc}	(1.3-4.1)
	25	0.2 ^a	(0.12-0.42)
8.5	10	34.3 ^f	(31.4-37.3)
	17	7.8 ^d	(6.5-9.6)
	25	1.2 ^b	(0.83-1.80)

Note. LC_{50} values with nonoverlapping 95% confidence limits are significantly different.

Toxicokinetic Measurements

To partition out the relative influences of pH and temperature, toxicokinetic measurements were made for each combination of pH and temperature studied (Table 4). Uptake clearance rates were significantly affected by changing temperature. In general, k_u values decreased as temperature was lowered from 25 to 10°C. However, k_u values at the median temperature, 17°C, were higher than the values obtained at 25°C at pHs 8.5 and 6.5 while the k_u at 17°C, pH 7.5 was lower than that obtained at 25°C, pH 7.5. The effect of pH

TABLE 2
PCP Levels in Living and Dead Mussels Exposed under Extremes of Environmental Conditions

Exposure conditions	Aqueous exposure conc. (μmol/liter)	% Mortality	Ave. PCP residue in all mussels (μmol/kg)	Ave. PCP residue in dead mussels (μmol/kg)	Aver. PCP residue in live mussels (μmol/kg)
PH 8.5, 10°C	23.6	23	95.0	237.0	47.0
	27.6	35	112.0	200.0	60.0
	31.5	55	128.0	174.0	65.0
	39.4	20	87.0	180.0	63.0
	43.3	65	135.0	170.0	68.0
pH 7.5, 17°C	0.9	30	27.4	28.9	27.4
	1.9	45	36.5	49.6	26.1
	9.9	70	56.7	71.8	28.5
	19.8	95	117.8	121.8	58.0
pH 6.5, 25°C	0.0098	10	0.40	0.39	0.39
	0.0200	15	0.75	0.71	0.75
	0.0390	20	1.30	1.10	1.40
	0.0980	30	3.80	3.70	3.90
	0.1960	50	13.70	12.10	14.60

Note. LD₅₀ + 95% C.L. = 14.4 μmol/kg, 8.8–32.8 (pH 6.5, 25°C); 36.8 μmol/kg, 30.4–45.1 (pH 7.5, 17°C); 121.9 μmol/kg, 116.9–128.8 (pH 8.5, 10°C).

upon the uptake clearance rate was highly variable. The k_u values tended to be higher at pH 6.5, the pH at which PCP was least ionized. However, a k_u of 2023 liters kg⁻¹ day⁻¹ was measured at pH 7.5, 25°C which was higher than two k_u values obtained at pH 6.5 at 25 and 10°C. It appears that interactions between temperature and pH were occurring to thwart a straightforward interpretation of the results.

A second set of k_u values was determined under extreme conditions and one set of intermediate conditions at the LC₂₅ concentration of PCP for each set of conditions (Table 4). The uptake clearance rates determined at concen-

trations at which PCP would be expected to exert some toxicity were significantly lower than k_u values measured at trace concentrations for the corresponding set of pH and temperature conditions.

Elimination rates were not as variable as conditions changed. k_d values were not significantly altered by changing pH or temperature, although the k_d values measured at

TABLE 4

Toxicokinetic Parameters and Calculated Lethal Body Residues (LR_{50s}) for Zebra Mussels Exposed to PCP under Varying Conditions of pH and Temperature

pH	T (°C)	k_u (liter kg ⁻¹ day ⁻¹)	k_d (day ⁻¹)	BCF	Calculated LR ₅₀ (μmol/kg)
8.5	25	1015 ^a (57.8)	0.96 ^a (0.26)	1057	782
	17	1221 ^f (8.4)	0.91 ^a (0.18)	1342	6253
	10	369 ^e (26.4)	0.86 ^a (0.20)	429	8489
	10*	0.33 (0.12)			
7.5	25	2023 ^d (67.2)	1.34 ^a (0.38)	1510	245
	17	1001 ^{abefg} (201.6)	1.18 ^a (0.23)	848	1351
	17*	5.4 (0.78)			
6.5	10	631 ^c (39.6)	1.03 ^a (0.34)	613	4569
	25	1524 ^b (9.12)	1.56 ^a (0.39)	977	69
10°C	25*	66.3 (4.01)			
	17	2133 ^{abcdfg} (108.0)	1.56 ^a (0.23)	1369	863
	10	1313 ^a (12.5)	1.10 ^a (0.21)	1194	2787

Note. Standard errors are given in parentheses. Toxicokinetic parameters followed by the same letter are not significantly different.

*Uptake studies were performed at the LC₂₅ concentration of PCP.

TABLE 3
Observed and Predicted LR₅₀ Values of PCP and Phenate Ion under Extreme Conditions of pH and Temperature

Conditions	Chemical form	Chemical concentration zebra mussels (μmol/kg)	
		Observed	Predicted ^a
pH 6.5, 25°C	PCP + phenate	14.5	69.0
	PCP only ^b	0.03	1.1
pH 7.5, 17°C	PCP + phenate	36.8	1351.0
	PCP only	0.07	2.7
pH 8.5, 10°C	PCP + phenate	121.9	8489.0
	PCP only	0.24	1.7

^aPredicted concentrations were determined from the equation $C_a = k_u C_w / k_d (1 - e^{-k_d t})$, where C_w is the aqueous concentration of PCP produced 50% mortality in LC₅₀ assays.

^bConcentrations of un-ionized PCP were determined from the Henderson-Hasselbalch equation.

pH 8.5 were consistently lower than those obtained at the other two pH levels.

Changes in BCF tended to mirror the changes in k_u , the parameter which drives BCF more strongly for this compound and organism (Table 4). Thus, BCF values were generally higher at higher temperatures at least at pHs 7.5 and 8.5. At pH 6.5, there was no pronounced temperature effect. The effect of pH on BCF was highly variable, with a consistent result achieved only at 10°C, where there was an increase in BCF with a decrease in pH.

Median Lethal Residues

Calculated LR₅₀ values (using trace PCP concentrations) were consistently higher with each decrease in temperature (Table 4). The LR₅₀ values calculated at pH 8.5 were most consistent and varied by a factor of 10.9 as temperature decreased from 25 to 10°C. At pH 6.5, the temperature effect was most variable; calculated LR₅₀ values differed by a factor of 40 across the range of temperatures tested at a fixed pH.

The effect of pH on calculated LR₅₀s was very consistent with theory (Table 4). As pH decreased, the calculated LR₅₀ dropped with successive decreases in pH. Thus, the lowest calculated LR₅₀s were found at pH 6.5, the pH at which PCP is least ionized and most toxic. Despite the consistent impact of both pH and temperature on calculated LR₅₀ values, these LR₅₀s varied by a factor of 122 from the lowest LR₅₀ (pH 6.5, 25°C) to the highest LR₅₀ (pH 8.5, 10°C) when kinetics were measured using trace concentrations of PCP.

Comparison of Different Methods for Estimating Body Residues

When aqueous concentrations of PCP, needed to cause intoxication, were used to describe the toxic potential of PCP, the resulting LC₅₀s proved to be the most variable estimate of toxicity (Table 5). When tissue residues were measured in toxicity assays, measured LR₅₀ values were determined. There was significantly less variability among measured LR₅₀s across the range of conditions tested than for LC₅₀ values. Indeed, measured LR₅₀ values varied only by a factor of 8 across conditions. Calculated LR₅₀s, determined from LC₅₀s in conjunction with toxicokinetic parameters at trace concentrations of PCP, were more consistent than LC₅₀ values but still ranged from 69 to 8489 µmol/kg as a function of environmental conditions, a range of 122-fold. The calculated LR₅₀s were consistently higher than the measured LR₅₀ values determined under identical conditions. However, when LR₅₀s were recalculated using k_u values measured under LC₂₅ concentrations of PCP (adjusted LR₅₀, Table 5), these values varied only by a factor of 2.5 across the range of conditions studied. In addition,

TABLE 5
Comparison of PCP Toxicity as a Function of Environmental Concentration (LC₅₀, µmol/liter), Measured Lethal Issue Residue (LR₅₀, µmol/kg), Calculated Lethal Body Residue and Adjusted Lethal Body Residue

pH	Temperature (°C)	LC ₅₀	Measured LR ₅₀ ^a	Calculated LR ₅₀ ^b	Adjusted LR ₅₀ ^c
6.5	25	0.09 (0.06-0.16)	14.5 (8.8-32.8)	69	3.02
7.5	17	2.30 (1.3-4.1)	36.8 (30.4-45.1)	1351	7.29
8.5	10	34.30 (31.4-37.3)	121.9 (116.9-128.8)	8489	7.60

Note. Ninety-five percent confidence limits are presented in parentheses.

^aMeasured LR₅₀s were determined from tissue concentrations of PCP.

^bCalculated LR₅₀ values were estimated from LC₅₀ values and toxicokinetic parameters measured at trace PCP concentrations.

^cAdjusted LR₅₀s were calculated using the k_u value determined at the LC₂₅ concentration of PCP for each set of conditions.

adjusted LR₅₀s were much closer to measured LR₅₀ values than were LR₅₀s calculated from kinetics at trace concentrations of PCP (Table 5).

DISCUSSION

Effects of Environmental Conditions on Toxicity

The toxicity of PCP to the zebra mussel was altered significantly by changing environmental conditions. Significant increases in toxicity resulted from increasing pH (Table 1), consistent with other studies. For instance, the LC₅₀ of PCP to goldfish declined by a factor of 307 when pH increased from 5.5 to 10 (Kobayashi and Kishino, 1980). The negative relationship between pH and PCP toxicity has been explained in terms of PCP ionization. That is, as pH increases, so too does the degree of ionization of PCP, resulting in decreased absorption and toxicity. Several investigators have found that toxicity of PCP at different pHs was correlated with change in the apparent partition coefficient which had been corrected for ionization at different pHs (Kobayashi and Kishino, 1980; Saarikoski and Viluk-sela, 1982; Fisher and Wadleigh, 1986; Stehly and Hayton, 1990). This suggests that the degree of ionization drives accumulation and, thus, determines toxicity. However, these current kinetic data do not indicate a consistent influence of pH upon uptake (Table 4) and it is clear that while much of the pH-induced variability in toxicity data can be explained in this manner, other factors are operating as well. Stehly and Hayton (1990) stated that the LC₅₀ vs pH curve for PCP is displaced toward higher pHs in comparison to ionization vs pH curves for PCP. If ionization were the only

factor controlling PCP toxicity, the two curves should be superimposable. One reason for the displacement is the contribution of the ionized or phenate form of PCP to toxicity. Although the phenate ion is polar, it is apparently absorbed and appears to contribute significantly to toxicity in some organisms (Saarikoski and Viluksela, 1982; Spehar *et al.*, 1985). Konneman and Musch (1981) argued that the toxicity of ionized and un-ionized forms of phenols is essentially additive. The tissue residue data (Table 2), taken when [^{14}C] PCP was added as a tracer in toxicity tests, suggest that the phenate ion is absorbed but that its contribution to toxicity is not additive. At pH 8.5, there was little dose-response evident in tissue concentrations although differential mortality, sufficient to calculate an LR_{50} , was obtained at pH 8.5 in the combined dead and live mussel samples. Curiously, dead mussels took up approximately three times as much PCP as live mussels while at pH 6.5 roughly equal amounts were accumulated by live and dead mussels in a dose-responsive manner. One interpretation of this finding is that at pH 8.5, the ionized form constitutes a large proportion of the tissue residue and that this form is less toxic than the un-ionized form, thus obscuring the expected dose-response. At pH 8.5, the rates of uptake and distribution of the ionized and un-ionized forms may vary substantially. This coupled with possible differences in the mode of action for the two forms of PCP (Saarikoski and Viluksela, 1982), makes it harder to tie a tissues concentration of contaminant with a biological effect at pH 8.5.

Increasing temperature was also found to increase PCP toxicity significantly (Table 1). This is partly explained by a generally higher uptake clearance of PCP at elevated temperatures (Table 4) and is consistent with other studies on zebra mussels. For instance, Fisher *et al.* (1993) found that an increase in temperature from 4 to 23°C resulted in a 10-fold increase in oxygen consumption in zebra mussels and a doubling in the uptake clearance rates of benzo [*a*]pyrene (BaP) and hexachlorobiphenyl (HCBP) from water. However, BCFs for BaP and HCBP actually declined as temperature increased because elimination increased proportionally more than uptake as a function of increasing temperature. In this study, BCFs decreased as a function of decreasing temperature at pHs 8.5 and 7.5, while at pH 6.5, BCFs tended to increase with decreasing temperature. This finding suggests that the pH effects on PCP ionization override the changes in exposure due to altered temperature at pH 6.5. In other words, pH and temperature are interacting.

Changing temperature has profound effects on the physiology of organisms, which may account for elevated toxicity of PCP at higher temperatures. For instance, the toxicity of PCP to midges increased significantly at higher temperatures but the concentration of PCP in the midges at higher temperatures was statistically indistinguishable from body burdens at lower temperatures (Fisher, 1986). At higher

temperatures, organisms experience higher respiratory demands and are consequently more sensitive to respiratory uncouplers. In this study, the data suggest that both pH and temperature interact to produce greater sensitivity under certain conditions. The very low LC_{50} obtained at pH 6.5, 25°C, for instance, no doubt reflects the fact that the mussels were at the lower end of their pH tolerance and the upper end of their temperature preference (Nichols, 1993). This assertion is substantiated by the tissue residue data (Table 2) and the LR_{50} values corrected for ionization (Table 3). The former data indicate that a tissue residue of 13.7 $\mu\text{mol/kg}$ was associated with 50% mortality under the most stressful set of conditions (pH 6.5, 25°C). However, tissue residues of 128.0 $\mu\text{mol/kg}$ were required to produce 55% mortality under the least stressful set of exposure conditions (pH 8.5, 10°C) (Table 2).

Evaluation of Lethal Body Residues

The lethal body residues calculated from kinetics at trace concentrations of PCP varied from 69 to 8489 $\mu\text{mol/kg}$ (Table 4). The values varied by a factor of 122 from the highest LR_{50} (pH 8.5, 10°C) to the lowest (pH 6.5, 25°C) in contrast to LC_{50} values which varied 381-fold across the same range of conditions. Thus, the use of calculated LR_{50} s to estimate toxic potential does reduce the variability caused by environmental conditions interacting with the physiological state of the organism. However, calculated LR_{50} s were still much more variable than measured LR_{50} values (Table 5). In addition, calculated LR_{50} s were consistently higher than measured LR_{50} values. The apparent overestimation of calculated LR_{50} s is thought to result from the use of different exposure times and PCP concentrations in the two types of experiments. Toxicokinetic parameters, from which calculated LR_{50} s were determined, were assessed during a 6-h exposure period to a nontoxic, trace concentration of PCP. In contrast, measured LR_{50} s were determined after 24 h of exposure to PCP concentrations sufficient to cause mortality. These differences in exposure conditions appear to account for variation in tissue concentrations needed to cause mortality.

The first important factor which was altered between exposures was filtering rate. Previous studies with zebra mussels, exposed to three molluscicides (copper sulfate, TFM 3-trifluoromethyl-4-nitrophenol and KCl), demonstrated that the filtering rate of the mussels was sharply reduced in the presence of these compounds. Moreover, the reduction in filtering rate was concentration-responsive with significantly greater inhibition occurring at higher exposure concentrations (Chen, 1993). Thus, it is likely that the filtering rate of mussels exposed to PCP in nonlethal toxicokinetic studies would be much higher than in the mussels exposed to lethal concentrations of PCP in measured LR_{50} studies. This assertion is supported by the

large differences in observed and predicted levels of PCP determined under extreme and intermediate conditions (Table 3). Predicted values, calculated from the uptake clearance rates, were always much higher than observed tissue residues. Thus, actual filtering rates in lethal exposures were probably significantly lower than those that occurred during toxicokinetic measurements.

These observations prompted remeasurement of uptake clearance rates for two sets of extreme conditions and one set of intermediate exposure conditions at the LC_{25} concentration for each set of conditions (Table 4). As expected, the uptake clearance rates were sharply reduced under each set of conditions when the mussels were exposed to toxic levels of PCP. When adjusted LR_{50} s were calculated using these k_u values, more consistent LR_{50} s, varying only by a factor of 2.5, were obtained (Table 5). Furthermore, the adjusted LR_{50} s were much closer to the tissue residue-based LR_{50} s, as would be expected if calculated values can serve as a surrogate for measured values.

The adjusted LR_{50} s, while more consistent with measured values than calculated LR_{50} s, appear to underestimate the actual tissue residues. This discrepancy may have arisen from decreases in elimination rate during LC_{25} toxicokinetic measurements. The latter were not remeasured. If elimination rates slowed substantially during the LC_{25} exposures, the resulting adjusted LR_{50} values would be substantially depressed. However, even a reduction in elimination rates by a factor of 10 would not yield LR_{50} s as high as the measured values. In addition, it is clear from measurements of elimination under trace exposure conditions that elimination rates are unaffected even under highly stressful conditions (Table 4). Thus, the reason for the apparent underestimation of adjusted LR_{50} s remains obscure.

The contrast between calculated and adjusted LR_{50} s underscores one caveat in the use of LR_{50} s (or similar parameters) in interpretation of hazard. Clearly, in the case of zebra mussels, the biology of the animal was an important determinant of exposure. Other investigators have found that toxicokinetic parameters determined under sublethal exposure conditions could accurately predict the response at lethal conditions (van Eck *et al.*, 1997). However, these investigators used fish as their experimental animal in aqueous exposures. Fish have a limited ability to control exposure as conditions change. However, zebra mussels are known to alter filtering behavior, and consequently the uptake clearance rate, as a mechanism for avoiding intoxication (de Kock and Bowmer, 1993; Jenner and Janssen-Mommen, 1993). Such biological characteristics which may be peculiar to certain taxonomic groups must, therefore, be considered a source of variability in determining and interpreting lethal body residues.

A second factor which varied between the LR_{50} estimates was the time of exposure. Mortality was assessed after 24 h of exposure while toxicokinetic experiments lasted for only

6 h. Since toxicity is a function of both exposure concentration and time (Hong *et al.*, 1988), it is reasonable that a greater effect would be observed at a lower concentration of PCP in the measured LR_{50} tests. Although mortality determined in LC_{50} measurements constitutes part of the calculated LR_{50} value and LC_{50} s were measured under conditions identical to those used for measured LR_{50} s, this was apparently insufficient to negate the overestimate of body burdens from toxicokinetic parameters.

A third factor which may have varied between exposure types is an interaction between PCP ionization and the internal pH of the zebra mussel. Once PCP is absorbed from water into the zebra mussels, the ratio of ionized PCP to un-ionized PCP should be determined by the internal pH of the organism and should remain unchanged even as external conditions are altered, at least as long as the animal is able to maintain homeostasis. The whole-body intracellular pH of the zebra mussel has been calculated from chemical shifts in inorganic phosphate resonance using nuclear magnetic resonance spectroscopy (O'Donnell *et al.*, 1995) and has been determined to be in the range 7.5–7.9 for healthy zebra mussels. However, if the mussels are exposed to a molluscicide or are deprived of oxygen, the intracellular pH of the mussels begins to decline; intracellular pHs in severely poisoned mussels have been measured in the range 5.9–6.3 (O'Donnell *et al.*, 1995). If a similar decline in intracellular pH is experienced during PCP exposure, as is likely, the decline in intracellular pH would exacerbate the effect of PCP by increasing the proportion of the more toxic un-ionized form in a positive feedback cycle. That is, as the animal begins to experience toxicity, the internal pH would begin to drop. The pH drop would increase the amount of un-ionized PCP in the mussels' tissue, causing further intoxication and decline in pH. These interactions between chemical ionization and the physiological state of the organism probably explain both the apparent increase in the sensitivity of PCP at lower pHs (Tables 1–5) and the discrepancies in toxic body burdens determined by measuring LR_{50} s versus calculating LR_{50} s. Normalization of lethal body residues to exposure concentration and time may assist in reducing variability between calculated and measured LR_{50} values. However, it is also clear that the method used to determine LR_{50} s must take the biology of the test organism into account if the utility of LR_{50} s in hazard assessment is to be maximized.

Calculated LR_{50} s were lowest at pH 6.5, where the values varied by a factor of 40 as temperature decreased from 25 to 10°C (Table 4). The temperature effect on calculated LR_{50} s became less variable with each successive increase in pH. At pH 8.5, the calculated LR_{50} s varied by a factor of 10.9 across temperatures. The authors believe that the lower calculated LR_{50} s, found at the lowest pH, are due to the greater concentration of the more toxic form of PCP, coupled with the fact that at pH 6.5, the mussels were

under considerable osmotic stress, which can disrupt homeostatic control of internal pH, giving rise to LR₅₀s that were generally lower than at other pH levels. When the mussels were held under conditions that were closer to their optimum, less variability in LR₅₀s was noted.

Lethal Residues in Other Organisms

The measured LR₅₀s determined in this study encompassed a range of 14.5 to 121.9 µmol/kg (Table 5), which is on the low end of the range of acute lethal body residues reported for PCP intoxication in other organisms. LR₅₀ values ranging from 570 to 1640 µmol/kg were reported for goldfish exposed to PCP under pHs which varied from 5.5 to 10.0 (Kobayashi and Koshino, 1980). Measurements of a 24-h LR₅₀ for PCP in *Diporeia* spp. of 920 µmol/kg and in *Mysis relicta* of 3787 µmol/kg (Landrum and Dupuis, 1990) were reported at pH 8.0. Thus, the reported measured LR₅₀s for PCP span a range from approximately 14.5 to 3787 µmol/kg among the species tested across a range of pHs from 6.5 to 9.0. Zebra mussels appear to be the most sensitive species tested, while *M. relicta* was least sensitive, having LR₅₀ values that were always at least an order of magnitude higher than those for zebra mussels.

The higher lethal residues found for *M. relicta* and goldfish at higher pHs suggest that PCP may be acting more like a nonpolar narcotic for those organism rather than by the specific mode of action of oxidative uncoupler. The spread in reported LR₅₀s in diverse species may herald a shift in the apparent mode of action from respiratory uncoupler to nonpolar narcotic as pH increases. Further work must be done to establish whether measured differences in LR₅₀s reflect differences in toxicodynamics or mode of action of the two forms of PCP or whether it reflects inherent variability in species or population-level sensitivity.

CONCLUSIONS

The data obtained in this study are generally consistent with the hypothesis of the critical body residue in that there is less variability in the tissue concentrations of poisoned animals than in the environmental concentrations necessary to produce the effects. However, the current data suggest that when lethal body residues are measured in animals under highly stressful conditions, there will be significant interactions between animal physiology and the amount of contaminant needed to cause intoxication. The utility of LR₅₀ in hazard assessment will be improved as the relationship between tissue residues and effects is determined for additional species, particularly those used as biomonitors. Additionally, standardization of the methodology is critical to providing a context in which the biology of the animal can be related to toxic potential.

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