

Toxicokinetics of DDE and 2-Chlorobiphenyl in *Chironomus tentans*

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Abstract. Uptake, biotransformation, and elimination rates for DDE and 2-chlorobiphenyl (2-CB) were examined using fourth instar midge larvae (*Chironomus tentans*) under a water-only exposure. A two-compartment model that included biotransformation described the kinetics for 2-CB and a one-compartment model was used for DDE. The uptake clearance coefficient (k_u) was $65.96 \pm 2.09 \text{ ml} \cdot \text{g}^{-1} \text{ midge} \cdot \text{h}^{-1}$ for 2-CB and $84.1 \pm 2.7 \text{ ml} \cdot \text{g}^{-1} \text{ midge} \cdot \text{h}^{-1}$ for DDE. DDE demonstrated no measurable elimination, while 2-CB showed elimination with faster elimination of parent compound ($k_{ep} = 0.100 \pm 0.008 \text{ h}^{-1}$) compared to the polar metabolites ($k_{em} = 0.073 \pm 0.016 \text{ h}^{-1}$). The biotransformation rate for 2-CB ($k_m = 0.031 \pm 0.005 \text{ h}^{-1}$) appeared to be time-dependent with faster rates at the beginning of the accumulation process slowing to a constant once the midge was loaded with chemical. Experimental results indicate a difference in the ability of *C. tentans* to eliminate 2-CB and its metabolites more quickly than DDE, and these differences were related to the bioconcentration potential of the compounds.

Biotransformation, or changes in the structure or nature of a compound caused by the enzymatic action of the organism, can effect the bioconcentration potential of a compound for organisms living in aquatic systems. Chemicals that are biotransformed will tend to have lower bioconcentration factors than predicted by their hydrophobicity (De Wolf *et al.* 1992; Van Loon *et al.* 1997; Driscoll *et al.* 1997). The biotransformation process often creates metabolites that are more polar than the parent compound, thereby allowing for faster elimination of the compound from the organism. Thus, the biotransformation potential of a compound can have a pronounced effect on its bioconcentration when the metabolites are eliminated at a different rate than the parent compound (Leversee *et al.* 1982; Spacie *et al.* 1983).

Chlorinated hydrocarbon compounds are generally persistent and bioaccumulated within the aquatic environment. However, the compounds have a range of physical and chemical properties. The compounds chosen for this study have widely varying properties and provide an indication of the range of behavior in the interaction of this class of compounds with the midge.

2-Chlorobiphenyl (2-CB) is a polychlorinated biphenyl congener that is moderately persistent in nature. PCBs have been used in electrical insulations, transformers, and hydraulic systems; as plasticizers; and in the production of paint until 1979, when they were banned from production and distribution (Cairns and Mount 1990). On the other hand, dichlorodiphenyldichloroethylene (DDE) is a decomposition product of the persistent organochlorine pesticide DDT, which was widely used until it was banned from production and distribution in the United States in 1972 (Solomons 1992). DDE concentration frequently exceeds that of the parent compound (DDT) and is more environmentally persistent than DDT (Gills *et al.* 1995; Hoke *et al.* 1997; Murdoc *et al.* 1997). Because of the persistence and toxic properties of organochlorine compounds in the environment, it is important to establish the bioconcentration potential of these compounds in aquatic organisms.

This study was conducted to determine the ability of *Chironomus tentans* to metabolize two chlorinated compounds, namely 2-CB and DDE, and examine how biotransformation influences bioconcentration potential. Specific objectives include: (1) determine toxicokinetic parameters such as the uptake clearance coefficient (k_u), elimination rate constant for parent compound (k_{ep}) and metabolites (k_{em}), biological half life ($t_{1/2}$), and bioconcentration factor (BCF) for each of the compounds by conducting short-term bioconcentration studies using a water-only exposure; and (2) develop toxicokinetic models that describe the uptake, elimination, and biotransformation of DDE and 2-CB in the midge.

Methods and Materials

Organism Culture

C. tentans were obtained from existing cultures present in the Environmental Toxicology Core Facility in the Department of Biological Sciences at Wichita State University and maintained in accordance with the standard operating procedures of the Environmental Protection Agency (EPA) for static cultures (US EPA 1993). Slight modifications to these procedures were necessary and included maintaining the midges in mixed-age brood cultures and replacing natural water with moderately hard reconstituted water. Midges were collected from the brood cultures and fourth instar larvae (head capsule width of 0.63–0.71 mm and body length of 1.0 cm or longer) were used in all tests (US EPA 1993).

Chemicals

Two ^{14}C -labeled chlorinated compounds, 2-chlorobiphenyl and DDE, were used during this project. Both chemicals were purchased from Sigma Chemical Company (St. Louis, MO) and found to be $\geq 95\%$ purity as determined by thin layer chromatography (TLC) on normal phase silica gel plates using hexane:benzene (80:20) as the developing solvent. ^{14}C 2-CB has a formula weight of $188.7 \text{ g} \cdot \text{mole}^{-1}$, and specific activity of $12.1 \text{ mCi} \cdot \text{mmole}^{-1}$; ^{14}C DDE has a formula weight of $318.3 \text{ g} \cdot \text{mole}^{-1}$, and specific activity of $12.7 \text{ mCi} \cdot \text{mmole}^{-1}$.

Uptake Experiments

Uptake experiments were performed under static conditions at 20°C in 600-ml beakers. At the beginning of an uptake study, a predetermined amount of ^{14}C -labeled compound, dissolved in acetone, was added to each beaker. The acetone was allowed to evaporate prior to the addition of 500 ml of reconstituted moderately hard water (US EPA 1993). Test parameters of temperature, pH, dissolved oxygen, and conductivity were recorded for the water at the beginning and end of each experiment. Basic water chemistry parameters remained constant and within US EPA (1993) guidelines throughout all of the experiments. Temperature was maintained at $20^\circ\text{C} \pm 1^\circ\text{C}$, and dissolved oxygen levels were $>80\%$ of saturation in all tests. Conductivity remained between $330\text{--}360 \mu\text{S} \cdot \text{cm}^{-1}$ and pH ranged from 7.8–8.1.

Beakers were covered with aluminum foil to slow evaporation and then placed in a Sherer Dual-Jet Environmental Chamber maintained with a 16 light:8 dark photoperiod for 24 h to allow for equilibration between the compound and glassware. The following day, 25 midges were placed into each of 12 beakers per compound, and then the beakers were returned to the environmental chamber. No substrate was used in the uptake experiments to avoid possible sorption to sediment and to minimize microbial degradation of the test compounds. Sample times for both compounds were chosen from preliminary tests (sampling times: 0.5, 1, 2, 4, 6, and 8 h). At each sampling time, larvae were removed from the beakers (two replicate beakers were sampled at each time point), rinsed with distilled water, blotted dry, and weighed on a Sartorius H51 analytical balance (Westbury, NY) to the nearest 0.1 mg. Midges from each beaker were separated into two groups. The first group of midges were placed directly into vials containing 10 ml Scintisafe 50% scintillation cocktail, and then sonicated for 20 s using a Tekmar Sonic Disruptor (Model #TM501) for determination of total ^{14}C activity present in the midges at the allotted time period. The second group of midges was extracted using the procedure detailed in the biotransformation section. All midges remained in the cocktail for at least 24 h prior to counting to allow for decay of possible chemiluminescence and to achieve a constant level of disintegration per minute (DPM) in the samples. One-milliliter water samples were also taken from each beaker when its exposure group was analyzed. Water samples were placed directly into 10 ml of cocktail and then counted on a Packard liquid scintillation counter. 2-CB concentrations decreased slightly over the course of the uptake experiment (approximately 8%), but DDE concentrations did not decrease during the uptake phase. Average water concentrations for DDE were $0.0227 \pm 0.002 \text{ nmole} \cdot \text{ml}^{-1}$, and average 2-CB concentrations were $0.0269 \pm 0.002 \text{ nmole} \cdot \text{ml}^{-1}$. These concentrations are at least a factor of 50 below levels needed to cause a lethal response as determined from preliminary experiments.

All scintillation counting was performed using a Packard 1900 TR liquid scintillation counter (LSC) equipped with automatic quench control for 5 min per vial. Sample counts were corrected for background and quench using the external standards ratio method.

Elimination Experiments

The contaminant loading for the elimination study was conducted at the same time as the uptake studies, and the methods for the loading phase of the elimination experiments were identical to those used in the uptake experiments (Lydy *et al.* 1994). Preliminary tests indicated an 8-h exposure was sufficiently long to ensure adequate accumulation of the compounds in the midges so that elimination experiments could be conducted. An elimination experiment was conducted by transferring dosed midge larvae from the 8-h exposure into a flow-through system maintained at a constant 17 L volume of reconstituted water in a 20-L aquarium with a flow rate of $1.0\text{--}1.2 \text{ L} \cdot \text{h}^{-1}$. Based on the analysis of water samples taken at each of the sampling times, this flushing rate was sufficient to keep radioactivity in the water at background levels during the course of the elimination experiments.

For each elimination experiment, two replicate samples of 20 midges each were taken at the time of transfer to uncontaminated water (time zero). Two replicate samples of 20 larvae each were taken at a total of 8 and 14 sampling times for DDE and 2-CB, respectively. Elimination experiments were conducted for a total of 64 h. At each sampling time, midge larvae were removed from the aquarium, blotted dry on a paper towel, and once again separated into two groups. The first group of midges was weighed and prepared for liquid scintillation counting as previously described. The second group of midges was then weighed and biotransformation determined as detailed in the following section.

Biotransformation

Midge larvae in both the uptake and elimination experiments were placed in a mortar with 3 ml of acetonitrile, then ground with a pestle to a fine residue. The extract was decanted into a 50-ml beaker. The midge residue remaining in the mortar was extracted twice more with acetonitrile, collecting extracts in the beaker after each extraction. The extract was dried over 10 g of anhydrous magnesium sulfate, which was then poured into a funnel lined with filter paper, and finally collected into a 10-ml graduated cylinder. The magnesium sulfate and filter paper were rinsed three times with 1 ml of acetonitrile and a measurement of the final extract recorded. One milliliter of the extract was then placed into a vial containing 10 ml of scintillation cocktail (midge extract concentration) and subsequently counted by LSC. The extract residue from the extraction process was air dried, weighed on the analytical balance, and placed in a vial containing 10 ml of scintillation cocktail (midge residue concentration). The extract and residue samples remained in scintillation cocktail for 24 h to aid in the extraction process and then counted for 5 min per vial by LSC. After calculating the concentration of ^{14}C 2-CB and ^{14}C DDE in the total midge, midge extract, and midge residue, the total accountability was determined by comparison of the midge extract and midge residue concentrations to the total midge concentrations (Lydy *et al.* 1994). The mass balance for 2-CB and DDE, measured as (midge extract + midge residue)/total, averaged 95.0% and 96.7%, respectively.

The remaining extract volume was reduced under a stream of nitrogen to 1 ml for TLC analysis. TLC analyses were conducted on silica-gel plates using an 80 hexane:20 benzene solvent system. The plate was then spotted with 10 μl of a 2-CB or DDE standard and each succeeding strip spotted with 25 μl of the concentrated midge extract ($\times 2$ replicates) for each sampling time. The spotted TLC plates were allowed to develop. The plate was then subdivided into equal increments, indicating the area on each strip identified by the standard as the parent compound, and all other increments located below the parent as polar metabolites. The increments were then cut from the plate and added to vials containing 10 ml of scintillation cocktail. The TLC samples remained in the scintillation cocktail for 24 h to aid in the extraction process prior to counting. The percentage of metabolites compared to parent compound was calculated from the TLC extract strips for each sampling time.

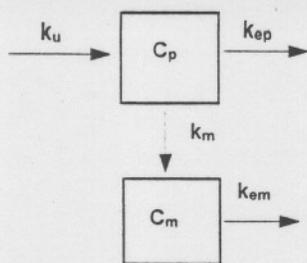


Fig. 1. Two compartment toxicokinetic model for 2-chlorobiphenyl in the midge (k_u = conditional uptake clearance coefficient; k_{ep} = parent compound elimination rate constant; k_m = metabolite formation constant; k_{em} = metabolite elimination rate constant; C_p = concentration of parent chemical in the animal; C_m = concentration of metabolite in the animal)

Data Analysis

DDE Toxicokinetics: For DDE, the midge weights declined slightly over the course of the uptake and elimination phases of the experiment. This was not observed for the 2-CB experiment. The decrease in weight has an impact on the ability to model the slow elimination of DDE and may affect the uptake rate as well. Thus, the concentrations were corrected for loss of weight, and this correction elevated the concentration in the midges. The weight change was found to fit a log linear form:

$$\ln W = \ln W_{(t=0)} - k_g t \quad (\text{Eq. 1})$$

where W = the weight of the midge (mg); k_g = the apparent loss rate constant (h^{-1}); and t = time (h). Thus, weight for the midges was corrected according to Equation 1, and the concentrations in the midge recalculated prior to modeling both uptake and elimination. This type of correction is only necessary when the rate of weight loss is comparable with or faster than the elimination rate. This is equivalent to but in the opposite direction of the impact of growth on the toxicokinetic processes.

No biotransformation was noted for DDE during the uptake or elimination phases of the experiment; therefore, conditional uptake clearance coefficients (k_u) were calculated by fitting the corrected concentration data to a simple linear model (SAS 1991):

$$C_a = k_u \cdot C_w \cdot t \quad (\text{Eq. 2})$$

where C_a = concentration of chemical in the animal ($\text{nmole} \cdot \text{g}^{-1}$); k_u = conditional uptake clearance coefficient ($\text{ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$); and C_w = concentration of chemical in the water ($\text{nmole} \cdot \text{ml}^{-1}$). This model assumes that elimination is not important during the uptake phase, that the flux of contaminant into the organism is much greater than the flux out, and that the concentration in the water remains constant. There was no measurable change in DDE water concentrations over the course of the uptake phase.

The elimination rate for DDE was fit independently as a first-order rate process according to the equation:

$$\ln C_a = \ln C_a^{(t=0)} - k_{ep} t \quad (\text{Eq. 3})$$

where k_{ep} = parent compound elimination rate constant (h^{-1}).

2-CB Toxicokinetics: For 2-CB, the modeling effort was necessarily more complex because of biotransformation (Figure 1), but no correction for weight loss was required. The data for both the parent compound and metabolite were fit simultaneously. The model assumes

that biotransformation is first order with respect to the concentration of the parent compound. In addition, the model was set up so that the uptake and elimination phases could be modeled simultaneously. The data were fit by performing an iterative least squares fit to the following differential equations using the fourth order Runge-Kutta approach in the software package, Scientist (MicroMath, Salt Lake City, UT).

$$\frac{dC_{\text{tot}}}{dt} = (k_u C_w) - (k_{ep} C_p) - (k_{em} C_m) \quad (\text{Eq. 4})$$

$$\frac{dC_p}{dt} = (k_u C_w) - (k_m C_p) - (k_{ep} C_p) \quad (\text{Eq. 5})$$

$$\frac{dC_m}{dt} = (k_m C_p) - (k_{em} C_m) \quad (\text{Eq. 6})$$

where C_{tot} = concentration of total amount of compound on a molar basis ($\text{nmole} \cdot \text{g}^{-1}$); C_p = concentration of parent chemical in the animal ($\text{nmole} \cdot \text{g}^{-1}$); C_m = concentration of metabolite in the animal ($\text{nmole} \cdot \text{g}^{-1}$); k_{ep} = parent compound elimination rate constant (h^{-1}); k_m = metabolite formation constant (h^{-1}); and k_{em} = metabolite elimination rate constant (h^{-1}).

An initial rates procedure was used to obtain the initial estimate for the uptake clearance coefficient (Landrum *et al.* 1992). Initial rates estimates were calculated from the slope of the tangent line in the initial linear part of the uptake curve according to Equation 2. This model also assumes that the water concentration of the contaminant will remain constant (within 10% of the original concentration) throughout the uptake portion of the experiment (Landrum *et al.* 1992). Contaminant concentrations decreased only slightly over the course of the uptake experiments for 2-CB (averaging 8%). The average measured values were used as the water concentration for modeling purposes.

Bioconcentration factors (BCF) were estimated from the kinetics using the following equations:

$$\text{DDE:} \quad \text{BCF} = \frac{C_p}{C_w} = \frac{k_u}{k_{ep}} \quad (\text{Eq. 7})$$

This model can be used because biotransformation is not measurable and does not contribute to the loss of the contaminant.

$$\text{2-CB:} \quad \text{BCF} = \frac{C_p}{C_w} = \frac{k_u}{(k_{ep} + k_m)} \quad (\text{Eq. 8})$$

In the case of 2-CB, to properly evaluate a BCF for the parent compound, the total loss of parent compound must be accounted for. Thus, not only must the elimination rate of the parent compound be considered but also the loss rate via biotransformation in order to determine the steady state BCF for a metabolized compound.

Similarly, the biological half-life was also computed differently for each compound:

$$\text{DDE:} \quad t_{1/2} = (0.693) \cdot (k_{ep})^{-1} \quad (\text{Eq. 9})$$

$$\text{2-CB:} \quad t_{1/2} = (0.693) \cdot (k_{ep} + k_m)^{-1} \quad (\text{Eq. 10})$$

Results and Discussion

A two-compartment model was developed that described the kinetics for 2-CB (Figure 1). The correlation coefficient for the fitted equation was high for the fit to the total 2-CB equivalents (C_{tot} , $r^2 = 0.909$) and for the parent compound (C_p , $r^2 = 0.978$). However, the fit for the metabolites was not as good (C_m ,

$r^2 = 0.632$); this was primarily due to a poor fit to the metabolism during the uptake phase (Figure 2). The overall model fit was $r^2 = 0.938$ and showed that the model adequately characterized the uptake and elimination of 2-CB. Even though midges were not fed throughout the 2-CB tests, there was no appreciable decrease in midge weights; therefore, no weight loss correction was needed for this experiment.

On the other hand, a one-compartment model was used to determine the kinetics for DDE in the absence of biotransformation. The correlation coefficient for the fitted uptake equation was also high ($r^2 = 0.933$, Figure 3). The nonmeasurable elimination rate and the constant water concentration both confirm the assumptions for using a simple linear uptake model (Equation 2). The midge weights did drop slightly during the uptake phase (2.4%) for DDE. There was a greater drop in weight during the elimination phase (18%). This decrease in weight followed a simple log linear decline over time (Equation 1, $r^2 = 0.30$, $p = 0.0015$), and the concentration in the organism was corrected as follows prior to modeling the DDE data. The apparent first-order loss rate constant (k_g) was $0.003 \pm 0.0008 \text{ h}^{-1}$. DDE data were corrected for this first-order loss term that amounted to a 2.4% change in concentration during the uptake phase and an 18% change in concentration during the elimination phase. Application of this correction factor for DDE did not greatly influence the uptake data, however it did flatten out the elimination. Prior to correction, the midges appeared to be accumulating compound in the absence of exposure. This is the result of faster weight loss than loss of contaminant.

A summary of the toxicokinetic model parameters for DDE and 2-CB is provided in Table 1. The uptake clearances (k_u) of both compounds by *C. tentans* (84.1 ± 2.7 and $65.96 \pm 2.09 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, respectively, for DDE and 2-CB) were similar to those values found by other researchers for DDE and chlorinated biphenyls in several invertebrate species (Landrum 1988; Evans and Landrum 1989; McIntyre 1989). For example, Evans and Landrum (1989) performed toxicokinetic studies on DDE and hexachlorobiphenyl (HCB) in the amphipod *Diporeia* sp. and the mysid shrimp *Mysis relicta* and found uptake clearances for *Diporeia* sp. of 79.2 and $53.5 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, whereas *M. relicta* values were 46.0 and $57.5 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ for DDE and HCB, respectively. In a separate study, Landrum (1988) examined the toxicokinetics of biphenyl and tetrachlorobiphenyl (TCB) in *Diporeia* sp. and reported uptake clearances of 95.4 ± 28.2 and $134.9 \pm 13.9 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, respectively. Finally, McIntyre (1989) exposed *Chironomus riparius* to DDE and found an uptake clearance of $78.0 \pm 4.0 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$. Toxicokinetic factors, such as uptake clearances, may vary among species (Leversee et al. 1982; Eadie et al. 1983) and, within a species, may be modified by such factors as size, lipid content, feeding regime, and season (Landrum et al. 1991). Change in the uptake clearances for compounds of differing log K_{ow} s have been observed for some classes of compounds, e.g., *Diporeia* sp. accumulation of PAHs where the uptake increases with increasing log K_{ow} (Landrum 1988). However, a response to changes in hydrophobicity does not always occur. *Diporeia* sp. did not show a significant change in uptake clearance with log K_{ow} for PCB congeners (Landrum et al. 1998). Thus, the observation that 2-CB and DDE have similar uptake clearances is not particularly surprising. Furthermore, the finding of similar uptake clearances among organisms of similar size is

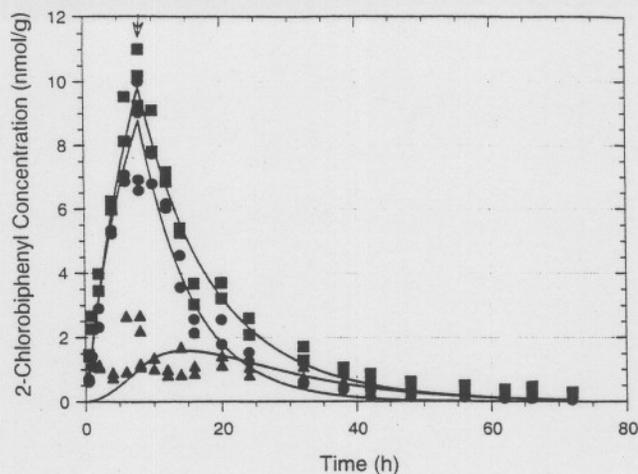


Fig. 2. Amount of 2-chlorobiphenyl ($\text{nmole} \cdot \text{g}^{-1}$) in the midge *Chironomus tentans*. Symbols indicate experimentally determined values ■ = total chemical, ● = parent compound, and ▲ = polar metabolites; the lines connect the corresponding model predicted points. The arrow represents the end of the uptake phase and the beginning of the elimination phase

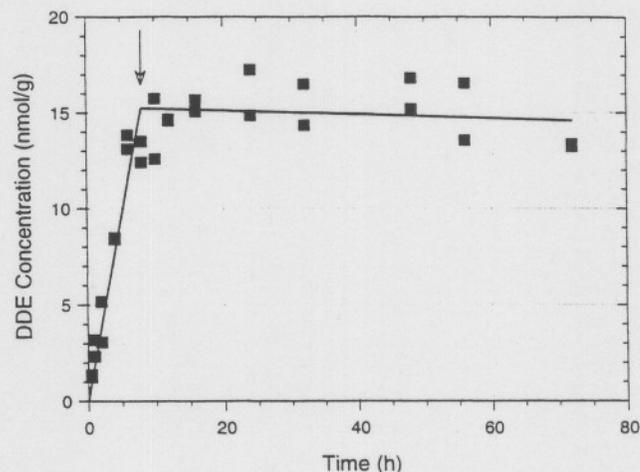


Fig. 3. Amount of DDE ($\text{nmole} \cdot \text{g}^{-1}$) in the midge *Chironomus tentans*. Symbols indicate experimentally determined values, ■ = total chemical. The lines represent the corresponding model predicted points. The arrow represents the end of the uptake phase and the beginning of the elimination phase

not surprising since respiration rates and surface area to volume ratios that can strongly influence uptake from water should be similar.

Even though the uptake clearances were similar in the present study, the elimination rate constants (k_{ep}) for the parent compounds differed greatly (Table 1 and Figures 2 and 3). DDE demonstrated no measurable elimination during the experiments. Other studies have also shown slow elimination rates for DDE in freshwater benthic invertebrates (Evans and Landrum 1989; McIntyre 1989). For example, Evans and Landrum (1989) found that *Diporeia* sp. and *M. relicta* are both capable of slowly eliminating DDE (0.0010 and 0.0005 h^{-1} , respectively) with these elimination rates decreasing even further, to

Table 1. Toxicokinetic model parameters (\pm SD) estimated for DDE and 2-chlorobiphenyl (2-CB)

Parameter	DDE	2-CB
k_u (ml · g midge ⁻¹ · h ⁻¹)	84.1 \pm 2.7	65.96 \pm 2.09
k_{ep} (h ⁻¹)	NA	0.100 \pm 0.008
k_{em} (h ⁻¹)	NA	0.073 \pm 0.016
k_m (h ⁻¹)	NA	0.031 \pm 0.005
Calculated BCF ^a	NA	504
$t_{1/2}$ (days) ^b	NA	5.7

* NA = not applicable

^a BCF = $(k_u) \cdot (k_{ep} + k_m)^{-1}$ for 2-CB

^b $t_{1/2} = (0.693) \cdot (k_{ep} + k_m)^{-1}$ for 2-CB

nondetectable levels, during certain months of the year. Elimination rate constants were not even obtainable for DDE at 10 and 16°C for *C. riparius* and were slow ($k_{ep} = 0.0016$ h⁻¹) at 22°C in a study by McIntyre (1989).

Biotransformation potential was examined for DDE during both uptake and elimination phases in the present study; however, no biotransformation of DDE was noted for *C. tentans* throughout any of the experiments. This result was not totally unexpected, since DDE is very stable and considered a terminal product for many organisms (Addison and Willis 1978). Biotransformation of DDE can occur in some insects, birds, and mammals with a conversion of DDE to 2,2-bis(p-chlorophenyl)-1-chloroethylene (DDMU) via a reductive dechlorination step. Even though the present study did not measure enzyme activity, it appears as though *C. tentans* lacks the enzymes necessary to initiate this biotransformation.

On the other hand, the elimination of 2-CB was very rapid and had contributions from both the biotransformation process and the elimination of parent compound. The elimination rates of parent compound appear to dominate the total elimination with a larger rate constant for elimination of parent compound ($k_{ep} = 0.100 \pm 0.008$ h⁻¹) compared to the rate constant for biotransformation ($k_m = 0.031 \pm 0.005$ h⁻¹). So that approximately three times as much parent compound is eliminated directly compared to the amount biotransformed. Further, the elimination rate constant for metabolites ($k_{em} = 0.073 \pm 0.016$ h⁻¹) is also smaller than that of the parent compound, indicating that metabolites are more slowly eliminated. Other studies have also shown a similar trend with faster elimination of parent compound versus metabolites. For example, polar metabolites were eliminated more slowly than parent compound by *C. riparius* for anthracene (Gerould *et al.* 1983) and for p-nitroanisole for the sea urchin *Strongylocentrotus purpuratus* (Landrum and Crosby 1981).

The model developed for this study, which has a fixed biotransformation rate ($k_m = 0.031 \pm 0.005$ h⁻¹), underestimates the observed formation of metabolites during the first 8 h, but accurately follows the data for the remainder of the experiment (Figure 2). Thus, it appears as though the rate of biotransformation is faster during the first 8 h when the amount of metabolite is substantially greater than the modeled values, and then slows during the remainder of the study when the model tracks the metabolite concentration. We can speculate on two possible reasons for this observation. When organisms are initially accumulating contaminants, the distribution of the contaminant is not uniform throughout the various tissues, and even the relative distribution among tissues, based on their

capacity, will be out of equilibrium. Thus, greater proportions of contaminants can be found in the membrane and aqueous phases of cells and not yet in fat storage (Gardner *et al.* 1990). While this disequilibrium occurs, a greater portion of the total body residue may be available to the metabolic enzymes than will occur later when a substantial portion of the body residue is locked up in storage tissue. Second, it may be that early in the exposures there may be an abundance of co-factors to allow for rapid biotransformation and that depletion of the co-factors results over time with the constant biotransformation demand. The model assumes that the rate of metabolism is first order with respect to the concentration of the parent compound and only allowed for a single biotransformation rate constant. To incorporate the observed changing pattern of biotransformation would require a substantially more detailed set of measurements to permit development of a more complex model. The actual enzymes required for the biotransformation of 2-CB were not investigated, however, possible reactions that could have converted 2-CB for polar metabolites include reductive dechlorination, epoxidation, hydroxylation, and formation of glutathione conjugates (West *et al.* 1997).

Finally, BCF and biological $t_{1/2}$ values for 2-CB were 504 and 5.7 days, respectively. The BCF value obtained in the present study is similar to those found by Leverage *et al.* (1982) who reported a BCF of 200 for benzo(a)pyrene which is actively biotransformed by *C. riparius*. In a separate study, Gerould *et al.* (1983) calculated a BCF of 915 for the same species of midge exposed to anthracene. The relatively small BCF and short biological $t_{1/2}$ found for 2-CB was primarily due to the midge's ability to eliminate parent compound and not due to elevated elimination of polar metabolites. The elimination of parent compound dominated the total elimination, with approximately three times as much parent compound eliminated compared to the amount being biotransformed. On the other hand, BCF and biological $t_{1/2}$ values could not be determined for DDE, since k_{ep} could not be measured. Therefore, the results of this study indicate a difference in the ability of *C. tentans* to eliminate 2-CB and its metabolites more quickly than DDE; however, these differences are only partially due to differences in biotransformation potential of the chemicals.

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