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The effect of temperature on uptake rate constants, depuration rate constants, and bioconcentration factors (BCF) for six organochlorines in the aquatic insect, *Chironomus riparius*

McIntyre, Dennis Owen, Ph.D.

The Ohio State University, 1988
THE EFFECT OF TEMPERATURE ON UPTAKE RATE CONSTANTS, DEPURATION RATE CONSTANTS, AND BIOCONCENTRATION FACTORS (BCF) FOR SIX ORGANOCHLORINES IN THE AQUATIC INSECT, CHIRONOMUS RIPA RIIUS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

By

Dennis Owen McIntyre, B.S., M.S.

* * * * *

The Ohio State University

1988

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ACKNOWLEDGEMENTS

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I. Introduction

A. Bioaccumulation, an Overview

The focus of this review and study is the uptake and elimination of xenobiotic chemicals that accumulate in aquatic organisms at concentrations above that of the surrounding water. Bioaccumulation, as defined by Esser and Moser (1982), is the general phenomenon of an uneven distribution of chemicals between biota and other environmental compartments. The chemicals generally associated with bioaccumulation are hydrophobic in nature which have a strong affinity to the organic components of the environment. Two similar terms that pertain to bioaccumulation are bioconcentration and biomagnification. Bioconcentration is a special case of bioaccumulation in that it restricts the accumulation process to a partitioning of the chemical between an aquatic organism and the water it lives in, and excludes any dietary influence on uptake of the chemical. Biomagnification, which is more typically observed in terrestrial systems than aquatic systems, is that phenomenon where higher concentrations of a chemical are found at higher trophic levels.

Bioaccumulation of xenobiotic chemicals first gained prominence in the 1960's when organochlorine insecticides and methyl mercury were concentrated by fish and linked to such calamitous events as the death of 680,000 coho salmon fry in a Michigan hatchery (DDT) and
reproductive failure in fish-eating birds (DDT) (Spacie and Hamelink, 1985; Johnson and Ball, 1972). Since that time, the monitoring of residue levels in aquatic biota coupled with advances in analytical technology have demonstrated that many compounds enter aquatic ecosystems and concentrate in living tissue. For example, a paper presented at the 1984 national meeting for the Society of Environmental Toxicology and Chemistry, "Hazard Evaluation of Recently Identified Contaminants in Great Lakes Fish" (Passino, 1984), reported that nearly 500 different xenobiotic organic compounds were identified from a lake trout sample collected from Lake Michigan. The hazard associated with bioaccumulation is that when organisms are exposed to low levels of certain chemicals in the environment those chemicals may accumulate in the organisms and cause acute or chronic toxicity. Such was the case in the biomagnification of DDT in fish-eating birds and the bioconcentration of DDT in coho salmon fry cited above. Because it is pragmatically impossible to ascertain the chronic effects for the vast number of environmental pollutants to all organisms, bioaccumulation is used as a measure of potential hazard.

Parallel to monitoring of residue levels in the natural environment and its organisms are laboratory investigations that examine the interaction of compounds with aquatic biota. Although many test organisms ranging from algae to amphibians have been used (Geyer et al., 1984; Oliver, 1987; Govers et al., 1984; Miramand et al., 1981; Wilkes and Weiss, 1971; Solbakken et al., 1983), the predominance of these laboratory efforts have been with fish.

Major objectives of laboratory investigations are to evaluate the
acute and chronic toxicity and relative hazard of these compounds or predict their disposition in organisms, particularly their ability to bioaccumulate. Measurements of toxicity to aquatic organisms is traditionally reported in the units, $EC_{50}$ or $LC_{50}$, which are the concentrations of chemical that cause a toxic response or lethality in fifty percent of the test population. In bioaccumulation studies the unit used to measure the ability of a chemical to accumulate in an organism is the bioconcentration factor (BCF). The bioconcentration factor (BCF), is defined as a ratio: the concentration of chemical in the organism divided by the concentration of chemical in the water (Kenaga, 1972).

Two experimental approaches have been commonly used to obtain BCF values. In the plateau or steady-state approach, test organisms are exposed to a chemical dissolved in water which is held at a constant concentration. Exposure time is varied and body content is measured in groups of organisms over time until the concentration of the chemical in the organisms reaches a plateau or steady-state (see Figure 1 under Accumulation Model section) (Spacie and Hamelink, 1985). At steady-state the chemical concentrations in both the organism and water are measured to determine the BCF. The plateau level at steady-state and the time required to reach steady-state are highly variable and depend on the nature of the chemical, the test species, and conditions of the test. For example, steady-state concentrations in fish increase as the aqueous concentration of organochlorines is increased (Blanchard et al., 1977; Oliver and Nilmi, 1983). Also in fish, the exposure time needed to reach a steady-state concentration for the majority of
hydrophobic chemicals investigated by Macek et al. (1975) and Veith et al. (1979) took 21 to 32 days. Smaller organisms, such as the insect, Chironomus tentans, have reached steady-state for a series of pyrethroids within 24 hours (Muir et al., 1985). Other chemicals may take considerably longer, however, as demonstrated by DDE not reaching a steady-state concentration in Chironomus tentans after 30 days (Derr and Zabik, 1974).

The second approach to bioaccumulation measurements is an accelerated test that calculates BCF values of chemicals from their uptake rates and elimination rates. The initial uptake rate of a chemical is measured in groups of organisms exposed to an aqueous concentration of the chemical where the maximal exposure period is relatively short (for fish, <4 days). The elimination rate of the chemical is determined in a separate experiment where loss of chemical is measured from groups of organisms over time. The organisms used in the elimination rate experiment are initially exposed to the chemical to obtain a body burden and subsequently transferred to uncontaminated water where the loss of the compound from the body is measured, a process that is termed depuration. The data from these experiments are used to determine the uptake rate constant and depuration rate constant, the ratio of which is the BCF (see equation 5 in the Accumulation Model section). This technique for measuring BCF values is frequently called the dynamic method (Branson et al., 1975) and is valued for the economy of experimental time relative to the steady-state method. Branson et al. validated the accelerated method by comparing it to the steady-state method and obtaining similar
values.

B. The Test Organism, *Chironomus riparius*

This study investigates the uptake, loss and accumulation of six organochlorines in the larval stage of the midge, *C. riparius*. As a group, midges occupy a large biomass in the aquatic ecosystem and provide a significant role in community energy flow both as consumer and prey (Merrit and Cummins, 1984). The four larval instars of *C. riparius*, which are fully aquatic, inhabit the benthic regions of slower reaches of streams as well as standing water bodies. Under rearing conditions in the laboratory the maturation from egg to pupa takes about 3 weeks (Detra, 1984). Pupae rise to the surface of the water where eclosion to adult occurs. The adults are winged terrestrial forms that engage in a reproductive swarming behavior soon after eclosion. *C. riparius* larvae can occur in very large numbers (100,000/m²) in sediment heavily contaminated with organic matter (Muirhead-Thompson, 1971) and can routinely survive in moderately polluted natural water. Having a significant ecological role and occupying a niche that may contain many xenobiotic chemicals make *C. riparius* a appropriate test organism for an investigation of accumulation. Moreover, the information from the accumulative behavior of the test compounds would add to a growing toxicological database for the colony of *C. riparius* used in this study. Such data includes the acute toxicity of insecticides (Estenik, 1978; Shank, 1984), mixed-function oxidase activity (Estenik and Collins, 1979; Lucas, 1983), organophosphate toxicology (Detra and Collins, 1987), carboxylesterase activity, (McIntyre, 1984), chemical uptake relative
to physical/chemical properties (Lohner and Collins, 1987), and the
effect of pH on the acute toxicity and uptake of pesticides (Fisher,
1985; Fisher, 1985a; Fisher and Wadleigh, 1986; Fisher and Lohner,
1986). Using the midge in a study of accumulation of chemicals,
therefore, is not only environmentally significant but also is
important as a model aquatic invertebrate for understanding basic
toxicological principles in this kind of organism.

C. The Test Chemicals

The six organochlorines used in this investigation are
1,4-dichlorobenzene, 1,2,4-trichlorobenzene,
1,2,4,5-tetrachlorobenzene, hexachlorobenzene, lindane and DDE.
As a class of chemicals, organochlorines are environmentally
significant due primarily to their persistence in the environment and
affinity to biological tissue. Of the six compounds selected for this
study, four (DDE, lindane, 1,2,4,5-tetrachlorobenzene, and
hexachlorobenzene) have been placed on the U.S. Environmental
Protection Agency's Priority Pollutant List and two (DDE and lindane)
are suspected to be human carcinogens.

The uses and toxicological characteristics of the study compounds
are quite varied. For example, 1,4-dichlorobenzene is used as a moth
repellant, soil fumigant, pesticide, and an intermediate in the organic
chemistry industry (Verschueren, 1983), and has moderate acute toxicity
to fathead minnows (96 hr LC50 = 33.7 mg/L) and grass shrimp (96 hr
LC50 = 69 mg/L) (Curtis et al., 1979). Lindane, which is used
primarily as a scabicide and insecticide, has considerable acute
toxicity to aquatic organisms as demonstrated by the 24 hr LC50 of 3.6
ug/L to G. riparius (Estenik and Collins, 1979), 96 hr LC$_{50}$ of 4.5 ug/L to the stonefly, Pteronarcys californica (Sanders and Cope, 1968), and 96 hr LC$_{50}$'s of 27 to 131 ug/L to four species of fish (Macek and McAllister, 1970). Hexachlorobenzene and 1,2,4-trichlorobenzene are used predominantly in the chemical manufacturing industry: hexachlorobenzene in the manufacturing of pentachlorophenol, aromatic fluorocarbons, the herbicide DCPA (dimethyl tetrachloroterephthalate), and the pesticide PCNB (pentachloronitrobenzene); 1,2,4-trichlorobenzene as a solvent, lubricant, and as an intermediate in the manufacturing of dyes (Verschueren, 1983).

DDE, an impurity, a major metabolite, and an environmental conversion product of the insecticide DDT, typifies the environmental problems that are associated with organochlorines. DDE has been found in the tissues of freshwater and marine invertebrates, freshwater and marine fish, birds, aquatic mammals, wild terrestrial mammals, and domestic mammals (McEwen and Stephenson, 1979). DDE, as well as two other compounds used in this study, lindane and hexachlorobenzene, have also been reported in human milk (Mes and Davies, 1979). Laboratory experiments have shown that when freshwater plants and animals are exposed to DDE, the organisms can concentrate the level of DDE in their tissues 10,000 to 60,000 times more than the level that they were exposed to in the water (Metcalf et al., 1975).

D. Accumulation Model

The mathematical model used to describe the uptake/depuration dynamics of the six chlorinated hydrocarbons used in my experiments
treated the midge as a single compartment in an open system and is given by the equation:

\[
\frac{dC_a}{dt} = \text{uptake} - \text{loss} = ku \times C_w - kd \times C_a, \text{ where} \quad (1)
\]

\( C_a \) = concentration of the chemical in the animal (ng/mg)

\( C_w \) = concentration of the chemical in the water (ng/ml)

\( t \) = time (h)

\( ku \) = uptake rate constant (mlg\(^{-1}\)h\(^{-1}\))

\( kd \) = depuration rate constant (h\(^{-1}\)).

The rate constants, \( ku \) and \( kd \), are both assumed to be first-order rate processes, that is the rate of uptake or depuration depends only on the concentration of the chemical. However, the rate constants, \( ku \) and \( kd \), are independent of chemical concentration, therefore, they make them excellent measurements for comparisons between experiments conducted under different concentrations. Assuming, \( ku \), \( kd \), and \( C_w \) remain constant, equation 1 can be integrated to yield (Spacie and Hamelink, 1984),

\[
C_a = \frac{ku}{kd} \times C_w (1 - e^{-kd}) \quad (2)
\]

Graphically, equation 2 is depicted in Figure 1, showing the change in the concentration of the chemical in the animal with exposure time. The curve in Figure 1 actually represents the net accumulation of the chemical in the organism because it is the product of two processes that occur simultaneously, namely, uptake and elimination. During the initial stage of uptake, the accumulation curve is approximately linear because the amount of chemical the animal absorbs far exceeds the amount that is eliminated. As \( C_a \) becomes larger, the amount of internal chemical available for elimination increases and
Figure 1.

Expository uptake and accumulation of a xenobiotic compound by an aquatic organism; $Ca$, concentration of the chemical in the organism vs. exposure time.
plateau, 
\[ dCa/dt = 0 \]

\{ initial rate \}

Exposure time
more chemical is eliminated from the body, thereby causing a damping effect on the curve, i.e. departure from linearity. Net accumulation gradually decreases until eventually the rate of uptake equals the rate of elimination and a steady-state condition exists (plateau with slope of zero). At steady-state equation 1 can be expressed as,

\[
\frac{dC_a}{dt} = k_u \times C_w - k_d \times C_{ss},
\]

where \( C_{ss} = \) concentration of the chemical in the animal at steady-state. With \( \frac{dC}{dt} \) equal to zero at steady-state, rearrangement of equation 3 yields,

\[
C_{ss} = \left(\frac{k_u}{k_d}\right) \times C_w, \quad (4a)
\]

\[
C_{ss}/C_w = k_u/k_d \quad (4b)
\]

The previously described term, bioconcentration factor (BCF), is a measurement of the bioaccumulation potential of a chemical by an organism relative to aqueous concentration and is defined by,

\[
BCF = C_{ss}/C_w = k_u/k_d \quad (5)
\]

Therefore, by knowing the steady-state values, \( C_{ss} \) and \( C_w \), or the kinetic rate constants, \( k_u \) and \( k_d \), the bioaccumulation potential can be determined.

E. Bioaccumulation Research

A great deal of research in the bioaccumulative behavior of chemicals has been devoted to the development of correlative models that can predict the degree of chemical accumulation in an organism from a physical/chemical property of the chemical. Such models provide an inexpensive laboratory assessment or prediction of the bioaccumulation potential of a chemical, thereby circumventing the labor-intensive experimentation necessary to obtain experimental BCF
values for all chemicals (Davies and Dobbs, 1984). The models generally are a linear correlation of the log BCF of selected chemicals (ordinate) with the log of the physical/chemical measurement (abscissa).

An example of a correlative model relating BCF values to partition coefficients is that reported by Neely et al. (1974) who determined BCF values for eight chemicals using rainbow trout. A regression analysis was performed on the log of the partition coefficients (obtained from the literature) for each chemical with the log of the experimental BCF values for each chemical. Neely et al. found that the log of the BCF values were linearly correlated to the log of the partition coefficients, and reported the linear equation, log BCF = 0.542 log partition coefficient + 0.124, to have a correlation coefficient (r value) of 0.95. Such a highly correlated relationship inspired interest and research in the possibility of using physical/chemical characteristics to predict BCF values in fish. In fish, for example, BCF has been correlated with octanol/water partition coefficients ($K_{ow}$) (Neely et al., 1974; Lu and Metcalf, 1975; Veith et al., 1979), triolein/water partition coefficients (Chiou, 1985), water solubility (Lu and Metcalf, 1975; Chiou et al., 1977; Metcalf et al., 1973), molecular connectivity (Sabljic and Protic, 1982), and molecular weight (Kanazawa, 1982). Although the predominance of BCF correlative models are developed for fish, other test organisms have been used, such as algae (Geyer et al., 1984), mussels (Ernst, 1977; Geyer et al., 1982), and Daehnia (Govers et al., 1984).

However, in another study, the correlations of log BCF with log $K_{ow}$ for fish were nonlinear for chemicals with log partition
coefficients above 6 (Konemann and van Leeuwen, 1980; Spacie and Hamelink, 1982; Chiou, 1985). Mackay (1980) describes a possible explanation for the nonlinearity as a function of the higher molecular weight compounds (>290) being "less soluble" in octanol. Established correlations have been amended by adding a melting point term (Banerjee et al., 1980) and using the fugacity concept (Mackay, 1980).

The uptake rate constant, $k_u$, and the depuration rate constant, $k_d$, have also been correlated with water solubility and $K_{ow}$. Konemann and van Leeuwen (1980) found $k_u$ values in fish to show a parabolic relationship to $K_{ow}$ with the $k_u$ values decreasing above log $K_{ow}$ 5, whereas $k_d$ remained linear through log $K_{ow}$ 6. Spacie and Hamelink (1982) suggested that $k_d$ serves as a better parameter than $k_u$ in correlative models since a plot of log $k_d$ with log $K_{ow}$ has a steeper slope than log $k_u$ with log $K_{ow}$ (rate constant values taken from two fish investigations). In contrast, Lohner and Collins (1987), working with midges, found that the product of $k_u$ times the molecular weight of the compound is sensitive to changes in log $K_{ow}$ with a regression coefficient for log $k_u \times MW$ with log $K_{ow}$ similar to log $k_d$ with log $K_{ow}$ for the above fish data reported by Spacie and Hamelink.

In addition to correlative models, bioaccumulation research includes investigation of the variables that affect accumulation such as metabolism (Mayer, 1976; Southworth et al., 1981; Lech and Bend, 1980; Gerould et al., 1983), pH (Fisher and Wadleigh, 1986), food chain transfer (Reinert et al., 1972; Ribeyre et al., 1980), and body size (Matsumura, 1977). Esser and Moser (1982) listed species as a source of variation in the accumulation of chemicals by aquatic organisms.
Midges, for example, have been found to accumulate chemicals faster than fish (Muir et al., 1982). Accumulation variation exists within arthropods as it does in fish, as demonstrated by an order of magnitude difference in BCF values for the chemical, anthracene, between a scud (Landrum, 1982) and midge (Gerould et al., 1983).

The speed of accumulation of a toxic compound relates to attaining a threshold of toxicity and expressing toxic symptoms. In his book on selective toxicity, Albert (1973), gives three principles of selectivity, namely, comparative biochemistry, comparative cytology, and differential distribution. When rapid net accumulation overwhelms protective processes, the animal dies. Slower accumulation allows defensive processes to operate for degrading or eliminating the chemical illustrated by the "opportunity factor" of O'Brien (1967).

Certainly a great many variables affect the accumulation process of chemicals in aquatic organisms. Temperature, as a variable, may have the most profound influence on chemical-aquatic organism dynamics, since with the exception of a few aquatic mammals, all aquatic organisms are ectothermic.

F. Temperature

The effect of temperature on toxicity of chemicals to aquatic organisms has been extensively tested and reviewed by Cairns et al. (1975) and Mayer and Ellersieck (1986). It has been generally shown that acute toxicity of most chemicals is positively correlated with temperature. Notable exceptions to this relationship are DDD, DDT, methoxychlor, and some pyrethroids whose toxicity decline with increasing temperature (negative temperature correlation). Both Cairns
and Mayer and Ellersieck (1986) relate the temperature coefficient, $Q_{10}$, to the effects found with the positive correlation of temperature and toxicity. Mayer and Ellersieck go on to say that the $Q_{10}$ concept may not only apply to the rates of reaction at the site of action, but also to the uptake, metabolism and depuration rates. This statement is substantiated by Murphy and Murphy (1971) who found increased DDT uptake at a higher temperature to be related to oxygen uptake across the gill membrane in mosquito fish.

Laboratory efforts have shown temperature to influence the uptake, elimination, and steady-state concentrations of chemicals. The uptake and elimination of both organic and inorganic xenobiotic compounds have been demonstrated in the literature to increase with an increase in temperature (Reinert et al., 1974; Ribeyre and Boudou, 1972; Ribeyre et al., 1980; Boudou et al., 1979; Miramand et al., 1981; Graney et al., 1984; Gunkel, 1981; Niimi and Palazzo, 1985; Solbakken et al., 1984). No trend is especially evident with steady-state concentrations, however, where BCF values have been found to increase, decrease, or remain unaffected with increases in temperature (Fossato and Canzonier, 1976; Gerould et al., 1983; Harris et al., 1977; Veith et al., 1979; Powell and Fielder, 1983).

Although seasonal variation of residue levels of both organic and inorganic compounds in natural populations of aquatic organisms may be due to a variety of factors acting in concert, temperature could be an important contributor. For example, environmental monitoring efforts have shown residue levels of cadmium in crustaceans and DDE and heptachlor in flounder to be highest in the winter (Zauke, 1982; Smith
and Cole, 1970). In a monthly monitoring program conducted over a year's time the highest residue levels of polychlorinated biphenyls (PCBs) and sDDT (DDT and all metabolites) in perch and roach were found in the spring, which gradually declined through summer and fall and rose again in winter (Edgren et al., 1981). In all three monitoring papers, the authors did not suggest temperature to be a factor in the seasonal variation of residue levels in the aquatic organisms, but rather an increased exposure during the peak periods due to introduced residues that were water-borne during higher stream flow conditions. The suggestion of introduced residues from meteorological events is a reasonable hypothesis, but another obvious possibility is a temperature effect. The point I make from this observation is that temperature, being such a basic variable in environmental study, is not very well understood with respect to its influence on the uptake, elimination and accumulation of residues in aquatic organisms in general. In addition, few systematic studies of the uptake and depuration of xenobiotic compounds have been conducted in invertebrates.

G. Premise For Study

The models that correlate physical/chemical properties with BCF have primarily been developed using a constant set of environmental conditions, such as hardness, alkalinity, organic carbon content, photoperiod, pH, and temperature. The validity of these models are limited if the effect of such environmental variables are not well understood. Fisher and Wadleigh (1986), for example, found the accumulation of pentachlorophenol (PCP) in G. riparius to be dependent on pH, with the midge accumulating significantly more PCP at lower pH's.
where more of the neutral form of the molecule was available for uptake. Therefore, if these models are going to be used to predict the accumulation potential of chemicals released into the environment, the effect of variations of the conditions in the environment should be understood. The effect of temperature on BCF values obtained in this study will add to the increasing data base of the effect of environmental variables. Using existing predictive models, such a data base can be used to better predict hazard to the environment, based on BCF values, for chemicals.

A classical concept in toxicology is the dose-response relationship. For aquatic organisms, the dose or internal concentration of most xenobiotic chemicals is obtained by partitioning processes with the surrounding water (Landrum and Giesy, 1985; Spacie and Hamelink, 1985). An assumption can be made that the internal concentration, \( C_a \), that causes a response in the organism may be predicted from the accumulation model, 

\[
C_a = \frac{C_u}{k_d} C_w x (1 - e^{-k_d t})
\]

This assumes the internal partitioning processes at the site of action are much faster than those between the organism and the water which is reasonable assumption for hydrophobic chemicals (Van Hoogen and Opperhuizen, 1988). According to the model, \( k_u \) would determine the speed of accumulation and \( k_u \) in combination with \( k_d \) would determine the maximum internal concentration. Therefore, any effect on \( k_u \) or \( k_d \) due to temperature can alter the time to reach an internal concentration that elicits a toxic response. Temperature has been shown to affect the \( k_u \) and \( k_d \) of anthracene in invertebrates (Landrum, 1982; Gerould et al., 1983). The study of the effect of temperature on the accumulation
dynamics of a class of chemicals, therefore, has not only environmental significance but relevance to understanding basic toxicological questions.

H. Objectives

The primary objective of this study is to determine the effect of temperature on the bioaccumulative behavior of six organochlorine compounds in *C. riparius* using the dynamic method of BCF determination.

By using the dynamic method, $k_u$, $k_d$, and BCF are obtainable. An objective of this study is to determine the effect of temperature on each of these accumulation parameters.

The experimental temperatures, 10°C, 16°C, 22°C, and 30°C, were chosen to represent a range of ambient water temperatures for a temperate climate.

The model, $\frac{dC_a}{dt} = k_u \times C_w - k_d \times C_a$, is used almost exclusively in the environmental toxicology literature to describe the accumulation dynamics of chemicals in aquatic organisms. This study examines how well the accumulation data generated from experiments determining the uptake and depuration of organochlorines in *C. riparius* fit the model.

The present study examines the effect temperature has on the acute toxicity of the test chemicals to the midge. The test chemicals used in the toxicity tests were restricted to those that expressed acute toxicity to the midge at concentrations below their aqueous solubility.

Finally, as a continuance of Lohner’s work with *C. riparius* that correlated log $k_u$ with the physical/chemical properties log $K_{ow}$ and log AS, an objective of this study is to determine if $k_d$ as well as BCF correlate with log $K_{ow}$ and log AS.
II. Materials and Methods

A. Chemicals

The six radiolabelled chlorinated hydrocarbons shown in Table 1 were used in the uptake and depuration experiments. The table lists each compound by chemical name, the abbreviation as it appears in the text, purity, and specific activity. Nonradiolabelled compounds used in preliminary studies and as standards in gas-liquid chromatography analyses are listed in Table 2. All radioactive chemicals, purchased from Pathfinder Laboratories, were $^{14}$C uniformly ring-labelled and checked for specific activity and chemical purity prior to experimental use.

Specific activity of a compound was obtained by determining its mass by gas-liquid chromatography and determining its radioactivity by liquid scintillation counting. Gas-liquid chromatography analyses were performed using a Varian Aerograph Model 1440, equipped with a $^{63}$Ni electron capture detector and a 183 cm by 2 mm (ID) glass column packed with 3% SE-30 on 100-120 mesh Gas Chrom Q. Column temperatures were as follows: diCB, 100°C; triCB, 120°C; tetraCB, 140°C; hexaCB, 172°C; DDE, 195°C; lindane, 170°C. The carrier gas flow was 25 ml/min N$_2$ and the chart speed, 0.2 in/min.

Analytical grade nonradiolabelled compounds were used under the above conditions to establish standard curves of peak height vs. mass from which the masses of at least triplicate samples of the
Table 1
Radiolabelled Chemicals Used in Experiments with Chironomus riparius

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Abbreviation</th>
<th>Specific activity^a</th>
<th>Purity^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-dichlorobenzene</td>
<td>dICB</td>
<td>165 dpm/ng</td>
<td>98%</td>
</tr>
<tr>
<td>1,2,4-trichlorobenzene</td>
<td>trICB</td>
<td>171 dpm/ng</td>
<td>98%</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>tetraCB</td>
<td>35 dpm/ng</td>
<td>98%</td>
</tr>
<tr>
<td>hexachlorobenzene</td>
<td>hexaCB</td>
<td>109 dpm/ng</td>
<td>99.6%</td>
</tr>
<tr>
<td>1,1-dichloro-2,2-bis(4-chlorophenyl)-ethylene</td>
<td>DDE</td>
<td>339 dpm/ng</td>
<td>98%</td>
</tr>
<tr>
<td>gamma-hexachlorocyclohexane</td>
<td>lindane</td>
<td>325 dpm/ng</td>
<td>98%</td>
</tr>
</tbody>
</table>

^a determined experimentally

^b provided by supplier; confirmed by GLC

Radioisotopes were interpolated and used to calculate an average value. Stock solutions of known concentrations of radioactive chemicals were then prepared. The stock solutions of radiolabelled trICB, tetraCB, hexaCB, and lindane were prepared in benzene:hexane (1:1). The stock solutions of radioactive DDE and dICB were made in benzene and toluene, respectively. Three 50 μL replicates from each of these stock solutions were directly added to vials containing scintillation cocktail and counted to determine radioactivity. The amount of radioactivity per unit mass is the specific activity of the compound.
Table 2

Nonradiolabelled Chemicals Used in Experiments with *Chironomus riparius*

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source</th>
<th>Purity (label)</th>
</tr>
</thead>
<tbody>
<tr>
<td>diCB</td>
<td>Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>triCB</td>
<td>Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>tetraCB</td>
<td>Aldrich</td>
<td>98%</td>
</tr>
<tr>
<td>hexaCB</td>
<td>Aldrich</td>
<td>97%</td>
</tr>
<tr>
<td>DDE</td>
<td>Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>lindane</td>
<td>Nutritional Biochemical Corporation</td>
<td>100%</td>
</tr>
</tbody>
</table>

and these experimentally obtained values are listed in Table 1. Chemical purity of the compounds was evaluated by inspecting gas-liquid chromatograms for the presence of extraneous peaks. There were no extraneous peaks found for any of the radiolabelled compounds compared to the chromatograms of analytical standards under the same conditions. The purity values given in Table 1 were provided by Pathfinder Laboratories.

All stock solutions were further diluted to known concentrations in acetone. These solutions, termed working solutions, were made to provide a desired aqueous concentration of the chemical when a 1 ml aliquot was added to 6 L water. Working solutions and stock solutions were stored in a freezer at -20°C.

Radiopurity of triCB and HCB also was analyzed by thin-layer
chromatography (TLC) using Analtech preadsorbent 0.25 mm silica gel plates type GF. Aliquots of 50 ul of working solution (1.5 ug triCB, 0.3 ug hexaCB) were spotted and developed with a hexane:acetone (9:1) mobile phase. Nonradiolabelled standards of sufficient mass to enable detection under UV light (254 nm) were coeluted to determine the location of the migrated radioisotope. After determining the $R_f$ value of the analytical standard, 1 cm zones of silica gel were scraped from the plates onto glassine paper and transferred into 6 ml polyethylene scintillation vials to which was added 4 ml of scintillation cocktail (1000 ml dioxane: 100 g naphthlene: 5 g PPO). The vials were stored in darkness for 48 hours before counting radioactivity on a Beckman LS 7000 liquid scintillation counter. All radioactivity in this work was quantified on this counter using the $^{14}$C window in program 4 and automatic quench. Calculations of disintegrations per minute (dpm) were made by subtracting a background count from the sample count and dividing by the counting efficiency (determined by counting a $^{14}$C-standard). Both compounds were found to be >95% radiopure. The same procedure for radiopurity analysis was attempted for diCB, but presumably due to the compound's volatility, no radioactivity was recovered from the TLC plate.

B. Test Organism

The test organism used in all experiments was Chironomus riparius Meigen (= C. thummi Klefer). A culture of these insects was maintained in the laboratory according to the procedure of Detra (1982). Third and fourth instar larvae were used in all experiments performed in this work, the instar being determined by length of the midge larva.
According to Lucas (1983), third and fourth instar range from 5 to 15 mm.

C. Uptake Experiments

Uptake and depuration experiments were conducted with all six organochlorines at four temperatures in an incubator: 10, 16, 22, and 30°C. An uptake experiment consisted of exposing midge larvae to a sublethal aqueous concentration of a compound and periodically removing samples of organisms to determine the amount of compound that was sorbed by the animals over time. Aqueous concentrations were determined to be sublethal based on preliminary toxicity experiments and observation of the test organisms' behavior during exposure periods. Abnormal behavior was not noted for any of the test chemicals during the uptake experiments. Uptake was considered to be the amount of chemical absorbed and adsorbed by the organism.

All uptake experiments were done as follows: An aqueous solution of a compound was made by the addition of a 1 ml aliquot of the working solution to 6 L of aged, aerated tapwater with a vortex created by a stirring bar in a 6 L Erlenmeyer flask (at least two 6 L containers of solution were made for each experiment). The solution was stirred for approximately ten minutes and then poured into 575 ml glass-stoppered bottles, leaving each bottle about 10 ml short of being completely filled. The glass-stoppered bottles served as exposure vessels for the midge larvae. The nearly filled bottles were stoppered and transferred to an environmental chamber (incubator) set at the appropriate experimental temperature and allowed to equilibrate overnight with the chamber conditions. Midge larvae in 50 ml beakers (5 larvae in 10 ml
water) were also equilibrated overnight at the experimental temperature of the environmental chamber. Determination of an uptake rate constant for one chemical consisted of at least three replicates of each exposure period and a minimum of six exposure periods, the latter determined by the uptake characteristics of each chemical.

An uptake experiment was begun by pouring the five acclimatized larvae into each glass-stoppered bottle, the addition of the larvae being staggered five minutes between replicates to allow time to do all of the procedure for each replicate. Typically, six sampling times, each replicated three times for a total of eighteen samples for each chemical temperature were taken in an eight-hour exposure test. At sampling times, the five larvae were removed from the bottle by pouring the contents through a nylon net which retained the larvae. The animals were rinsed with distilled water, blotted dry, and quickly weighed to the nearest 0.1 mg on a Mettler analytical balance. The weighed organisms were transferred to a 6 ml polyethylene liquid scintillation vial to which 4 ml of the dioxane cocktail (see chemicals section) was added. The organisms remained in the cocktail for at least two days prior to counting to allow for ample extraction of the compound into the cocktail.

The method of extraction from the intact animal by the scintillation cocktail was compared to the Mahin and Lofberg (1966) method which completely oxidizes the organisms and to a sample preparation method using tissue solubilizer (RPI TS-1). Three separate uptake experiments (each consisting of six sample periods with three replicates per sample period) were performed using radiolabelled DDE.
The samples from the three different uptake experiments were prepared by the three different sample preparation methods and the slopes of the three different experiments were compared by an analysis of covariance (ANCOVA). No difference ($P > 0.05$; $\alpha = 0.74$) in detecting the amount of compound sorbed per mass of organism was found between methods (data given in Appendix 1). Therefore, the whole body technique was used because it eliminated one step from the procedure.

Water samples were taken from each 6 L Erlenmeyer flask ("zero time") and from each 575 ml glass-stoppered bottle when its exposure group was analyzed to determine the aqueous concentration of the test compound. The 1 ml sample was placed in a 6 ml liquid scintillation vial containing 4 ml dioxane cocktail and counted as described above. The measured aqueous concentrations of the chemicals at each sampling time for all uptake experiments are given in Appendix 2. These values are given as the mean of the three replicates taken at each sampling time.

D. Depuration Experiments

Depuration experiments measured the elimination of the compound from the organism at four temperatures (10°C, 16°C, 22°C, and 30°C). Elimination can occur by desorption, diffusion, excretion, and biotransformation. Biotransformation was not considered to be a source of elimination for the chemicals used in this work since earlier work by Lohner and Collins (1987) showed no metabolism in four of these same compounds by $C. \textit{plerocerus}$ during similar exposure times.

A depuration experiment was conducted by transferring to uncontaminated water a number of larvae previously exposed to a
chemical and measuring the loss of the chemical from the animal over
time. Larvae were exposed to the radiolabelled chemical for a period
sufficient to ensure a significant body burden of the compound for the
depuration experiment. Approximately 150 larvae were poured into a 6 L
Erlenmeyer flask containing approximately 1.5 L aqueous solution and
the flask was transferred to the environmental chamber set at the
experimental temperature. After an overnight exposure the contents of
the 6 L flask were poured through a nylon net, the retained larvae
were rinsed with distilled water and transferred to a crystallizing
dish (75 mm x 150 mm diameter) held in the environmental chamber that
was continuously flushed with uncontaminated water that was at the
experimental temperature.

The flow through delivery mechanism was a gravity feed system.
Two 4 L brown bottles served as reservoirs for the uncontaminated water
which was delivered to the crystallizing dish via 3 mm (O.D.) glass
tubing. The crystallizing dish contained an overflow tube which was
positioned to maintain a constant 500 ml volume in the vessel. Flow
rates through the dish were maintained at approximately 0.5-1 l/h.
Based on the analysis of 4 to 5 water samples taken periodically from
each depuration experiment, this flushing rate was sufficient to keep
radioactivity in the water in the crystallizing dish at or below
background level during the course of a depuration experiment
(McIntyre, unpublished).

In a depuration experiment three replicate samples of five larvae
each were taken at the time of transfer to uncontaminated water (time
zero) and three replicate samples of five larvae each were taken in at
least seven sampling times after starting exposure to uncontaminated water. The sampling times, selected according to the chemical being tested, were based on preliminary experiments. Analysis for radioactive content was the same as for uptake experiments. Larvae were blotted dry, weighed, and placed in 6 ml scintillation vials to which was added 4 ml dioxane cocktail. Samples were held at least two days prior to counting on the liquid scintillation counter.

E. Determination of Accumulation Parameters

The data obtained from the uptake and depuration experiments were used to calculate the rate constants, $k_u$ and $k_d$, by a number of different methods. The subsections that follow address each method used in the determination of the rate constants and BCFS.

a. $k_d$, experimental

Depuration is the elimination of the compound from the body of midges held in uncontaminated water. In this situation, the uptake component of equation 1 is removed, reducing the model to,

$$\frac{dC_a}{dt} = -k_d \times C_a. \tag{6}$$

Equation 6 integrates and rearranges to,

$$\ln C_{a_t} = \ln C_0 - k_d t, \text{ where} \tag{7}$$

$C_0$ is the concentration of the chemical in the animal at the start of the depuration phase. A plot of the decline of $C_a$, on a logarithmic scale, against depuration time, on a linear scale, yields a straight line for a one compartment system, and a biphasic line for a two compartment system. The slope of the straight line in the one compartment system is the $k_d$.

The determination of $k_d$ for a two compartment system was done by
"feathering" the body residue data (Notari, 1980). A best-fit line for the beta phase was drawn first to determine the slope (-beta) and Y-intercept (B). Points from the beta line were subtracted from the experimental points and the differences replotted. The difference plot, termed the alpha phase, yielded the slope, (-alpha) and the Y-intercept (A) and together with beta and B were used to calculate \( k_{21} \) and \( k_d \). The following equations were used to calculate \( k_d \). An explanation of the individual rate constants and their derivation can be found in Notari (1980).

\[
A' = \frac{A}{C_0} \\
B' = \frac{B}{C_0} \\
k_{21} = A' \times \beta + B' \times \alpha \\
k_d = \frac{(\alpha \times \beta)}{k_{21}}
\]

b. half-life, \( t_{1/2} \)

The half-life of a chemical is the time it takes for one half of the chemical to be eliminated from the midge. Given that elimination is a first-order process, the \( t_{1/2} \) is a constant for that rate process. The depuration experiments measured the loss of Ca over time and is explained by equation 7. Equation 7 can be rearranged to give

\[
\ln(C_{a}/C_0) = -k_d t \tag{8}
\]

The \( t_{1/2} \) can be termed as \( C_a/C_0 \) when \( C_a \) is half of \( C_0 \), or incorporating into equation 8

\[
\ln0.5 = -k_d t, \text{ or} \tag{9} \\
t_{1/2} = \frac{0.693}{k_d} \tag{10}
\]

The time it takes to reach steady-state concentration, \( C_{ss} \), depends only on the \( t_{1/2} \) of the chemical. According to Notari (1980) the time
to reach 95% of theCss may be calculated by multiplying the t1/2 by 4. This calculation was used to estimate Css values for midges.

c. ku and kd, nonlinear regression

Both ku and kd were determined simultaneously from the data obtained in the uptake phase alone. A nonlinear regression analysis estimated the rate constant parameters by Marquardt iterative least squares (SAS, 1979). The nonlinear program used equation 2 as a function to simultaneously estimate ku and kd.

d. ku, slope

The slope of the linear region of the uptake curve was used in the determination of ku. This method, used by Lohner and Collins (1987), assumed the linear portion of the curve was in the initial rate phase where elimination of the chemical is quantitatively insignificant. Using the initial rates assumption, equation 1 reduces to,

\[ \frac{dCa}{dt} = ku \times Cw, \]  \hspace{1cm} \text{(11)}

dCa/dt is the slope of the initial rate. ku can be calculated from a rearrangement of equation 11, to,

\[ ku = \frac{\text{slope}}{Cw}. \]  \hspace{1cm} \text{(12)}

e. ku, initial rate

The uptake experiments, run in glass stoppered bottles, were conducted in a closed system. Thus, the mass of the chemical was conserved within the system. The distribution of the chemical in the system can then be described by a mass flux equation,

\[ A = Qa + Qw, \]  \hspace{1cm} \text{(13)}

A = the initial mass of the chemical in the system,

Qa = the mass of the chemical in the animal, and
Q_w = the mass of the chemical in the water.

The dynamics of the flux of the chemical between the water and the animal is described by the model equation,

\[
dQ_a/dt = k_{um} x Q_a - k_d x Q_a, \text{ where}
\]

\[
k_{um} = \text{the system uptake rate constant (h}^{-1})
\]

Substituting for Q_w, equation 14 converts to,

\[
dQ_a/dt = k_{um} (A - Q_a) - k_d x Q_a.
\]

After rearrangement and integration equation 15 yields,

\[
Q_a = \left[ k_{um} x A (1 - e^{-k_{um} + k_d} t)}\right]/k_{um} + k_d.
\]

Using the initial rates assumption k_d can be removed from equation 16,

\[
Q_a = \left[ k_{um} x A (1 - e^{-k_{um} x t)}\right]/k_{um}.
\]

Factoring equation 17 yields,

\[
Q_a = A (1 - e^{-k_{um} x t}).
\]

After taking the natural log of both sides and rearrangement, equation 18 can be used to calculate k_{um}.

\[
k_{um} = -[ln(1 - Q_a/A)]/t
\]

Conversion of k_{um} to k_x was done by multiplying k_{um} by the volume of water in the system (565ml) and dividing by the mass of the five larvae (g). The conversion to k_x gave the units, mlg^{-1}h^{-1}, which is consistant and comparable to other methods in this work and to the literature. The rate constant, k_x, is actually the clearance rate constant for the water.

f. k_x, using the experimental k_d

k_x values were calculated using an equation that utilized a k_d value from a depuration experiment (see "a") and Ca; C_w, and t from an uptake experiment. These values were used in a rearrangement of
equation 2 to solve for $ku$.

$$ku = \frac{(Ca \times kd)}{Cw(1 - e^{-\alpha \times t})}$$  \hspace{1cm} (20)

g. $ku$, BCF $\times$ kd

A rearrangement of equation 5,

$$ku = kd \times BCF,$$ \hspace{1cm} (21)
gives a calculable solution to $ku$ if the accumulation process has reached a steady-state condition. As the only compound to reach steady-state in these experiments, dICB was the only chemical for which this method of $ku$ determination was used.

h. BCF

BCFs were calculated by $ku/kd$ (equation 5) for all chemicals. The steady-state determination of BCF, $C_{ss}/C_w$, was used solely for dICB.

F. Toxicity Tests

Acute toxicity tests were conducted with dICB, tICB, tetaCB, hexaCB, and lindane. DDE was not tested, because previous exposure in other experiments at or near the maximum aqueous solubility of DDE did not produce any symptoms of toxicity to the midge.

Toxicity tests were performed at both 10°C (in an environmental chamber) and at room temperature (21 ± 1°C) for each chemical using fourth instar G. riparius larvae in 575 ml glass-stoppered bottles. Each toxicity test had two replicates per concentration and ten larvae per replicate. On the day of exposure, the chemicals were delivered to the water at experimental temperature in 0.50 ml aliquots of fresh acetone stock solutions. Acetone controls were run with each set of toxicity tests. After mixing, larvae which had been equilibrated for eight hours to the temperature of the experiment were added to the
bottles.

A toxic response was determined by a failure to complete three consecutive "figure 8" movements after squeezing the posterior segment of the larvae as described by Estenik (1978). Toxicity measurements were determined at 24 hours for diCB, triCB, and lindane, and at 42 hours for tetraCB and hexaCB.

The concentration of chemical that caused a toxic response in 50% of the test organisms (EC$_{50}$ value) was determined for each test where sufficient toxicity was observed. Estimates of EC$_{50}$ values were obtained using the computer program (TOXDAT) cited in Peltier and Weber (1985). This program estimates an EC$_{50}$ using one of three methods: binomial, moving average or probit analysis. The method selected is based on the shape of the concentrations-effects curve and the number of concentrations with partial toxic responses (toxic response greater than 0% but less than 100%). The probit and moving average methods both estimate the EC$_{50}$ with 95% confidence limits.

G. Data Analysis

To test differences between ku values, kd values, and EC$_{50}$ values across temperatures and among chemicals pairwise t-tests at alpha = 0.05 were performed.

ANCOVA was performed on kd values and BCF values across temperature data for diCB, triCB, tetraCB, and lindane to determine if the effect of temperature was different among the chemicals (SAS, 1987).

A conservative estimation of the 95% confidence intervals (CI) was determined for the BCF values used in Figures 17, 18, and 19. The
upper bounds of the 95% CI for the BCF values were determined by dividing the upper bound of the 95% CI of the $k_u$ values ($k_u + 95\% \text{ CI}$) by the lower bound of the 95% CI of the $k_d$ values ($k_d - 95\% \text{ CI}$). Conversely, the lower bound of the 95% CI for the BCF values were determined by dividing the lower bound of the 95% CI of the $k_u$ values ($k_u - 95\% \text{ CI}$) by the upper bound of the 95% CI of the $k_d$ values ($k_d + 95\% \text{ CI}$).
III. Results

A. Depuration

Five compounds (diCB, triCB, tetraCB, hexaCB, and DDE) exhibited one compartment elimination from the midge at all four temperatures (10°C, 16°C, 22°C, and 30°C). A representation of one compartment elimination of diCB (Figure 2) shows four linear plots of ln Ca against depuration time at each experimental temperature. Lindane, the only nonaromatic compound in the group that I studied, did not exhibit one compartment elimination. The plot of ln Ca against depuration time for lindane yielded a biphasic line for all four temperatures. Figure 3 illustrates the two compartment elimination of lindane from the midge at 16°C and 22°C.

The kd values and their 95% confidence intervals determined in depuration experiments (experimental kd) and calculated from the uptake data (nonlin kd) for all six organochlorines at 10°C, 16°C, 22°C, and 30°C are listed in Table 4. Values of experimental (expt‘l) kd were not obtainable for hexaCB and DDE at 10°C and 16°C. No loss of either chemical was measured over the course of the depuration experiments which ranged from 66 h for DDE at 16°C and 125 h for DDE at 10°C, to 100 h for hexaCB at 10°C and 16°C. The plot, ln Ca against depuration time, yielded a slope of 0 for both chemicals at both temperatures, indicating a negligible kd for the time period tested. Regression analysis of ln Ca with depuration time for diCB, triCB, and tetraCB at
Figure 2.

One compartment elimination of diCB from midge larvae at 10°C, 16°C, 22°C, and 30°C; ln Ca, ng diCB/g midge vs. time, h; each point is the average of 3 replicates; value in parenthesis is the standard error of the regression line.

10°C: ln Ca = -0.073 (± 0.002) t + 6.47 $r^2 = 0.98$
16°C: ln Ca = -0.111 (± 0.007) t + 5.70 $r^2 = 0.92$
22°C: ln Ca = -0.318 (± 0.016) t + 5.77 $r^2 = 0.96$
30°C: ln Ca = -0.416 (± 0.009) t + 6.02 $r^2 = 0.98$

Table 3

Concentration of diCB in the Midge (Ca) at Each Sampling Time of Experiments for Figure 2

<table>
<thead>
<tr>
<th>Time, h</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.641 ± 0.064</td>
<td>0.337 ± 0.062</td>
<td>0.322 ± 0.056</td>
<td>0.384 ± 0.016</td>
</tr>
<tr>
<td>0.25</td>
<td>0.600 ± 0.023</td>
<td>0.281 ± 0.044</td>
<td>0.144 ± 0.020</td>
<td>0.263 ± 0.014</td>
</tr>
<tr>
<td>0.75</td>
<td>0.544 ± 0.032</td>
<td>0.195 ± 0.044</td>
<td>0.057 ± 0.005</td>
<td>0.118 ± 0.005</td>
</tr>
<tr>
<td>1</td>
<td>0.425 ± 0.026</td>
<td>0.160 ± 0.029</td>
<td>0.044 ± 0.007</td>
<td>0.014 ± 0.002</td>
</tr>
<tr>
<td>2.75</td>
<td>0.404 ± 0.021</td>
<td>0.154 ± 0.012</td>
<td>0.026 ± 0.004</td>
<td>0.004 ± 0.0003</td>
</tr>
<tr>
<td>3</td>
<td>0.309 ± 0.039</td>
<td>0.100 ± 0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.75</td>
<td>0.221 ± 0.022</td>
<td>0.046 ± 0.019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.103 ± 0.008</td>
<td>0.024 ± 0.004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ca, ng diCB/g midge (log scale)
Figure 3.

Two compartment elimination of lindane from midge larvae at 16°C and 22°C. Ca. ng lindane/g midge vs. time, h; each point is the average of 3 replicates; bars represent one standard deviation of the mean.
Ca, ng lindane/g midge (log scale)
10°C, 16°C, 22°C, and 30°C, and hexaCB and DDE at 22°C and 30°C yielded significant slopes (different than zero) as determined by the f-test at alpha = 0.05. The negative values of these significant slopes are the expt’l kd values which are given in Table 4.

Table 4
Depuration Rate Constants (kd) for Six Organochlorines with Chironomus riparius at Four Temperatures

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Method</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>dICB</td>
<td>expt’l</td>
<td>73 ± 4</td>
<td>111 ± 14</td>
<td>318 ± 34</td>
<td>461 ± 19</td>
</tr>
<tr>
<td>dICB</td>
<td>nonlin</td>
<td>237 ± 67</td>
<td>743 ± 97</td>
<td>1520 ± 770</td>
<td>2840 ± 700</td>
</tr>
<tr>
<td>triCB</td>
<td>expt’l</td>
<td>18 ± 3</td>
<td>27 ± 3</td>
<td>50 ± 3</td>
<td>86 ± 6e</td>
</tr>
<tr>
<td>triCB</td>
<td>nonlin</td>
<td>28 ± 178</td>
<td>273 ± 246</td>
<td>333 ± 248</td>
<td>453 ± 189</td>
</tr>
<tr>
<td>lindane</td>
<td>expt’l</td>
<td>23</td>
<td>24</td>
<td>59</td>
<td>96</td>
</tr>
<tr>
<td>lindane</td>
<td>nonlin</td>
<td>277 ± 233</td>
<td>349 ± 341</td>
<td>441 ± 444</td>
<td>482 ± 445</td>
</tr>
<tr>
<td>tetraCB</td>
<td>expt’l</td>
<td>27 ± 8</td>
<td>50 ± 6</td>
<td>66 ± 4</td>
<td>94 ± 7e</td>
</tr>
<tr>
<td>tetraCB</td>
<td>nonlin</td>
<td>224 ± 143</td>
<td>191 ± 75</td>
<td>288 ± 140</td>
<td>148 ± 70</td>
</tr>
<tr>
<td>hexaCB</td>
<td>expt’l</td>
<td>...d</td>
<td>...d</td>
<td>2.8 ± 3f</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>hexaCB</td>
<td>nonlin</td>
<td>117 ± 92</td>
<td>87 ± 41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDE</td>
<td>expt’l</td>
<td>...d</td>
<td>...d</td>
<td>1.6 ± 1f</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>DDE</td>
<td>nonlin</td>
<td>49 ± 255</td>
<td>0.5 ± 4</td>
<td>3 ± 8</td>
<td>19 ± 31</td>
</tr>
</tbody>
</table>

a all values in rows are significantly different; all values in columns are significantly different except as noted by c, e, and f (t-test: P < 0.05)

b expt’l method of kd determination used data from depuration experiments deriving kd from lnC = lnCo - ktd

c no confidence intervals were obtained due to inability to statistically treat "feathered" data

nonlin method of kd determination used data from uptake experiments in a nonlinear regression analysis
d no loss of chemical from the animal; slope of curve was zero
e not significantly different as determined by t-test ($P < 0.05$)
f not significantly different as determined by t-test ($P < 0.05$)

Statistical comparisons between $k_d$ values were restricted to the
expt'l $k_d$ values for diCB, triCB, tetraCB, hexaCB, and DDE. Statistical
treatment of the expt'l $k_d$ values for lindane was excluded because the expt'l $k_d$ values were obtained by "feathering" the data and a calculation of their variation was not determined. Statistical comparisons of the calculated nonlin $k_d$ values were not performed because, as discussed later, the uptake data which was used to determine the nonlin $k_d$ values did not fit the model (see Accumulation Model in the Discussion). Therefore, statistical treatment of the nonlin $k_d$ values was not meaningful.

For diCB, triCB, tetraCB, hexaCB, and DDE, temperature had a
significant effect on expt'l $k_d$ ($P > 0.05$) (Rows, Table 4). Statistical
comparisons of expt'l $k_d$ values between chemicals at a given temperature also resulted in significantly different $k_d$ values between all chemicals ($P > 0.05$) with the exception of triCB ($0.008 h^{-1}$) and tetraCB ($0.094 h^{-1}$) at 30°C, and hexaCB ($0.0028 h^{-1}$) and DDE ($0.0016 h^{-1}$) at 22°C ($P < 0.05$) (Columns, Table 4).

The chemical with the highest water solubility of the test
compounds, diCB, had the fastest depuration rate at each temperature as determined by expt'l $k_d$. In contrast, the chemicals with the lowest water solubility, hexaCB and DDE, had the slowest depuration rate at all temperatures.
Figure 4 illustrates the effect of temperature on the experimental kd's for the test chemicals (the 95% confidence intervals for the data points on Figure 4 are given in Table 4). The slopes of log kd versus temperature for the four chemicals that had measurable experimental kd values at all four experimental temperatures, namely diCB, triCB, tetraCB, and lindane, were found not to be significantly different (ANCOVA: P < 0.05). All four slopes were significant (P > 0.05), indicating an increase in temperature (in the experimental range) had a significant and similar effect of increasing the experimental kd values for these four compounds. The same ANCOVA also determined that the experimental kd values for diCB, triCB, tetraCB, and lindane, were significantly different (P > 0.05) from each other across temperature and chemical.

DDE and hexaCB, having experimental kd values for only 22°C and 30°C, were not included in the ANCOVA. The experimental kd values for these two compounds, however, generally showed a more sensitive response to temperature. The 4.3-fold increase in experimental kd values between 22°C and 30°C for hexaCB was greater than any increase between neighboring temperatures for any other chemical. The increase in the experimental kd value for DDE over the same temperature interval was a 2.5-fold increase, greater than any increase demonstrated by diCB, triCB, tetraCB, or lindane, with the exception of diCB between 16°C and 22°C which had exhibited a 2.9-fold increase in experimental kd.

The half-lives (t1/2) and the time it takes to reach 95% of the steady-state concentration (t95Css) for the six compounds at each temperature are given in Table 5. Since the values of t1/2 and the t95Css were determined by factoring kd with a constant, the
Figure 4.

The effect of temperature on depuration rate constants (expt'1 kd) for six organochlorines: log kd, h\(^{-1}\) vs. temperature, °C; each point is the average of 3 replicates (95% confidence limits given in Table 4).
Figure 4

A graph showing the relationship between kd h⁻¹ (log scale) and Temp., °C for various compounds:
- diCB
- lindane
- tetra CB
- tri CB
- hexa CB
- DDE

The graph is on a log scale for kd h⁻¹ and a linear scale for Temp., °C.
relative statistical variation for each value was the same as it was for \( k_d \). Therefore, significantly different values in Table 5 are identical to that of Table 4. The response of the \( t_{1/2} \)’s and \( t_{95\%SS} \)’s to temperature was reverse to that of \( k_d \), because \( t_{1/2} \) is an inverse function of \( k_d \). The chemical with the highest \( k_d \) (diCB at 30°C, \( k_d = 0.461 \text{ h}^{-1} \)) had the lowest \( t_{1/2} \) (1.5 h) and \( t_{95\%SS} \) (6 h), whereas the chemical with the lowest \( k_d \) (DDE at 22°C, \( k_d = 0.0016 \text{ h}^{-1} \)) had the highest \( t_{1/2} \) (433 h) and 95% SS (1733 h).

Table 5

The Half-Lives and the Time Required to Reach 95% of the Steady-State Concentrations for Six Organochlorines with Chironomus riparius at Four Temperatures

<table>
<thead>
<tr>
<th></th>
<th>10°C (t( t_{1/2} ) (95%\text{SS} ))</th>
<th>16°C (t( t_{95%\text{SS}} ))</th>
<th>22°C (t( t_{95%\text{SS}} ))</th>
<th>30°C (t( t_{95%\text{SS}} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>diCB</td>
<td>10 38</td>
<td>6 25</td>
<td>2 9</td>
<td>1.5 6</td>
</tr>
<tr>
<td>triCB</td>
<td>39 154</td>
<td>26 103</td>
<td>14 55</td>
<td>8(^b) 32(^b)</td>
</tr>
<tr>
<td>lindane</td>
<td>30 121</td>
<td>29 116</td>
<td>12 47</td>
<td>7 29</td>
</tr>
<tr>
<td>tetraCB</td>
<td>26 103</td>
<td>14 55</td>
<td>11 43</td>
<td>7(^b) 29(^b)</td>
</tr>
<tr>
<td>hexaCB</td>
<td>... ...(^c)</td>
<td>... ...(^c)</td>
<td>248(^d) 990(^d)</td>
<td>58 231</td>
</tr>
<tr>
<td>DDE</td>
<td>... ...(^c)</td>
<td>... ...(^c)</td>
<td>433(^d) 1733(^d)</td>
<td>173 693</td>
</tr>
</tbody>
</table>

\(^a\) significant differences derived from Table 4

\(^b\) not significantly different as determined by t-test (\( P < 0.05 \))

\(^c\) not able to calculate because no loss of chemical was measured

\(^d\) not significantly different as determined by t-test (\( P < 0.05 \))
B. Uptake

The accumulation dynamics of the six organochlorines varied greatly. The extremes of this variability are illustrated in the uptake curves of diCB and DDE at 22°C (Figure 5). The accumulation of diCB by midge larvae in the 22°C uptake test reached steady-state after only 5 hours, whereas DDE accumulation at 22°C was still linear after 24 hours. Figure 6 gives the accumulation plots (Ca vs. exposure time) of the remaining four organochlorines at 22°C showing intermediate degrees of accumulation dynamics.

In the Materials and Methods section, five different methods of calculating ku are presented. If accumulation data fit the mathematical model from which the equations to calculate ku are derived, and if all assumptions are met for the respective calculation method, all five different calculation methods should result in similar ku values. An assumption for both the slope method (equation 12, Materials and Methods) and the initial rate method (equation 19) is that only data from the linear portion of the accumulation curve may be used to calculate ku. For four of the test chemicals (diCB, triCB, tetraCB, and lindane), sufficient data was not obtained in this study from the linear portion of their accumulation curves (Figures 5 and 6) to calculate ku values from either the slope or initial rate method. Equation 20 \((ku = \frac{(C_a \times kd)}{C_w(1-e^{-kd})})\) does not require data to be in the linear portion of the curve, thereby allowing ku values to be determined for each chemical at all four temperatures. These ku values and their 95% confidence intervals are given in Table 6.
Figure 5.

Accumulation curves of diCB and DDE by midge larvae at 22°C; Ca, ng chemical/mg midge vs. exposure time, h; each point is an average of 3 replicates; error bars represent one standard deviation of mean.
Figure 6.

Accumulation curves of triCB, tetraCB, hexaCB, and lindane by midge larvae at 22°C; Ca, ng chemical/mg midge vs. exposure time, h; each point is an average of 3 replicates; error bars represent one standard deviation of the mean.
Ca, ng chemical/mg midge

Exposure time, h

- tetraCB
- tri CB
- hexa CB
- lindane
Table 6

Uptake Rate Constants (ku) for Six Organochlorines with Chironomus riparius at Four Temperatures\(^a\)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>diCB</td>
<td>57 ± 11</td>
<td>119 ± 33</td>
<td>127 ± 48</td>
<td>169 ± 47</td>
</tr>
<tr>
<td>triCB</td>
<td>60 ± 11</td>
<td>139 ± 28</td>
<td>216 ± 42</td>
<td>185 ± 30</td>
</tr>
<tr>
<td>lindane</td>
<td>38 ± 9</td>
<td>56 ± 17</td>
<td>66 ± 19</td>
<td>76 ± 21</td>
</tr>
<tr>
<td>tetraCB</td>
<td>95 ± 21</td>
<td>172 ± 27</td>
<td>245 ± 37</td>
<td>199 ± 31</td>
</tr>
<tr>
<td>hexaCB</td>
<td>NA(^b)</td>
<td>NA(^b)</td>
<td>311 ± 31</td>
<td>237 ± 26</td>
</tr>
<tr>
<td>DDE</td>
<td>NA(^b)</td>
<td>NA(^b)</td>
<td>150 ± 13</td>
<td>220 ± 12</td>
</tr>
</tbody>
</table>

\(^a\) Equation 20 of Methods section was used to calculate ku values.

\(^b\) Equation 20 determination not applicable due to lack of expt'l kd value.

Only one of the six test chemicals, diCB, reached steady-state during the uptake exposure period. Therefore, it is the only compound for which ku can be calculated from values of BCF and kd (equation 21). Table 7 lists the determination of ku with BCF and kd for diCB along with the slope and initial rate calculations of ku for hexaCB and DDE. The ku values for hexaCB and DDE could be determined by the slope and initial rate methods because sufficient accumulation data were obtained in the linear portion of their curves. An example of their accumulation curves are illustrated in Figures 5 and 6.
Table 7
Uptake Rate Constants (ku) for Six Organochlorines with *Chironomus riparius* at Four Temperatures

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Methoda</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>diCB</td>
<td>BCFxkd</td>
<td>19</td>
<td>27</td>
<td>56</td>
<td>71</td>
</tr>
<tr>
<td>hexaCB</td>
<td>slope</td>
<td>58 ± 8</td>
<td>94 ± 57</td>
<td>318 ± 69</td>
<td>220 ± 59</td>
</tr>
<tr>
<td></td>
<td>initial</td>
<td>58 ± 8</td>
<td>155 ± 43</td>
<td>333 ± 30</td>
<td>238 ± 34</td>
</tr>
<tr>
<td>DDE</td>
<td>slope</td>
<td>53</td>
<td>65 ± 8</td>
<td>136 ± 11</td>
<td>178 ± 21</td>
</tr>
<tr>
<td>DDE</td>
<td>initial</td>
<td>53 ± 13</td>
<td>72 ± 4</td>
<td>147 ± 16</td>
<td>216 ± 13</td>
</tr>
</tbody>
</table>

a Methods of ku determination are referenced to equations listed in the methods section that were used to calculate the rate constants.

BCFxkd, equation 21
slope, equation 11
initial, equation 19

b Statistical treatment not applicable.

As previously stated, if the accumulation data fit the model and if all assumptions for the respective calculation method are met, the different calculation methods should result in similar ku values. A comparison of the ku values determined by the different calculation methods and given in Tables 6 and 7 showed inconsistent results. The ku values for hexaCB and DDE determined by equation 20 (Table 6), the slope method (Table 7), and the initial rate method (Table 7) all demonstrated similar results. The equation 20 determinations of ku (Table 6) for diCB, however, did not compare similarly to the BCF x kd determinations (Table 7) for diCB. At all four temperatures the equation 20 estimate of ku was markedly higher than the BCF x kd estimate, which ranged from a 2.3-fold difference at 22°C to a 4.4-fold
difference at 16°C. Because neither the BCF x kd calculation nor the equation 20 calculation violated any assumptions the difference in ku values was due to the accumulation data not fitting the model equation.

Equation 20, \( ku = \frac{(Ca \times kd)}{Cw(1-e^{-kd\cdot t})} \), can be used to determine ku values for each sampling time of an uptake exposure test using expt’l kd values (Table 4) from a depuration test and experimental values of Cw, Ca, and t from an uptake test. (The ku values determined by equation 20 that are listed in Table 6 represent a mean value for all 6 sampling times, n = 18.) Figures 7 to 12 illustrate plots of ku values determined by equation 20, for each sampling time, against exposure time for each compound at the four experimental temperatures. The compounds diCB, triCB, lindane, and tetraCB demonstrated a rapid decrease in ku values over the early exposure times (up to 2 hours). The rate of decrease in ku diminished in the later exposure times for these compounds, leveling off to nearly a constant ku. A similar pattern of ku values decreasing with exposure time was found for hexaCB at 30°C (Figure 11). This pattern was not evident, however, in hexaCB at 22°C (Figure 11) or DDE at 22°C and 30°C (Figure 12) (note: equation 20 could only be applied to hexaCB and DDE at 22°C and 30°C since expt’l kd values were negligible at 10°C and 16°C).

Since hexaCB and DDE were not eliminated from the midge larvae at 10°C and 16°C after at least 60 hours, the initial rate stage (the linear section of the accumulation curve where elimination of the chemical is insignificant) is no longer an assumption, but documented. Therefore, the initial rate method, which does not consider elimination, can be used to calculate ku for each sampling time
(analogous to equation 20 calculations of other chemicals that were used in Figures 7 to 12) for plotting against exposure time. Figures 13 and 14 illustrate these plots for hexaCB and DDE at 10°C and 16°C, showing that the ku values for hexaCB at 16°C and DDE at 10°C decreased with time while the ku values for hexaCB at 10°C and DDE at 16°C did not.

The pattern of a higher ku decreasing rapidly to a constant lower ku, as found in the plots of ku with time for diCB, triCB, lindane, and tetraCB, indicate the data from the uptake tests for these compounds do not fit the accumulation model (see discussion). The pattern of ku values decreasing over exposure time suggested that possibly two processes contribute to the accumulation of these compounds by midge larvae. The first process accounted for a rapid accumulation of the compound by the midge and is described by the rate constant, $ku_{fast}$. An accurate determination of $ku_{fast}$ cannot be obtained from the data using the model, $dCa/dt = kuCw - kdCa$, since only one ku is described by this model. A best estimate of $ku_{fast}$ can be obtained from the ku value of the first sampling time in the uptake exposure test. The second process accounted for a slower accumulation of the chemical by the midge and is described by the rate constant, $ku_{slow}$. The $ku_{slow}$ values were estimated by averaging the ku values which formed a plateau in the final 2 to 4 sampling times of an uptake exposure test. In Figures 7 to 10, $ku_{fast}$ is depicted by the highest ku value at the first sampling time, and $ku_{slow}$ is the average of the lower ku values in the
The effect of exposure time and temperature on uptake rate constants, $k_u$, for diCB determined by equation 20*; $k_u$, mlg$^{-1}$h$^{-1}$ vs. exposure time, h; each point is an average of 3 replicates.

* $k_u = \frac{(C_a \times kd)}{C_w(1 - e^{-k_{ad}})}$

Table 8

Uptake Rate Constants ($k_u$) for diCB at Each Sampling Time of Experiments for Figure 7

<table>
<thead>
<tr>
<th>Sampling time, h</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>109 ± 5</td>
<td>255 ± 28</td>
<td>314 ± 14</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>82 ± 17</td>
<td>162 ± 38</td>
<td>236 ± 8</td>
<td>260 ± 27</td>
</tr>
<tr>
<td>1</td>
<td>61 ± 14</td>
<td>122 ± 3</td>
<td>143 ± 16</td>
<td>182 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>57 ± 1</td>
<td>104 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.25</td>
<td>95 ± 23</td>
<td></td>
<td>98 ± 4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>49 ± 11</td>
<td>62 ± 6</td>
<td>80 ± 4</td>
<td>87 ± 6</td>
</tr>
<tr>
<td>4</td>
<td>37 ± 8</td>
<td>54 ± 2</td>
<td>73 ± 6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>66 ± 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.25</td>
<td></td>
<td>64 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>37 ± 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>42 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.25</td>
<td>25 ± 3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 8.
The effect of exposure time and temperature on uptake rate constants, $k_u$, for triCB as determined by equation 20*; $k_u$, mg$^{-1}$h$^{-1}$ vs. exposure time, h; each point is an average of 3 replicates.

\[ *k_u = \frac{(Ca \times kd)}{Cw(1 - e^{-kt})} \]

Table 9
Uptake Rate Constants ($k_u$) for triCB at Each Sampling Time of Experiments for Figure 8

<table>
<thead>
<tr>
<th>Sampling time, h</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>106 ± 5</td>
<td>233 ± 11</td>
<td>332 ± 35</td>
<td>270 ± 39</td>
</tr>
<tr>
<td>0.5</td>
<td>81 ± 6</td>
<td>199 ± 42</td>
<td>304 ± 28</td>
<td>226 ± 46</td>
</tr>
<tr>
<td>1</td>
<td>64 ± 15</td>
<td>151 ± 12</td>
<td>271 ± 99</td>
<td>210 ± 52</td>
</tr>
<tr>
<td>2</td>
<td>44 ± 6</td>
<td>136 ± 14</td>
<td>208 ± 41</td>
<td>221 ± 34</td>
</tr>
<tr>
<td>2.25</td>
<td></td>
<td></td>
<td>208 ± 41</td>
<td>221 ± 34</td>
</tr>
<tr>
<td>4.25</td>
<td></td>
<td></td>
<td>159 ± 8</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>41 ± 8</td>
<td>109 ± 13</td>
<td>170 ± 25</td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>40 ± 7</td>
<td>80 ± 2</td>
<td>110 ± 5</td>
<td>111 ± 14</td>
</tr>
<tr>
<td>10.8</td>
<td></td>
<td>66 ± 7</td>
<td>114 ± 1</td>
<td></td>
</tr>
<tr>
<td>11.25</td>
<td>46 ± 11</td>
<td></td>
<td></td>
<td>99 ± 12</td>
</tr>
<tr>
<td>13.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 9.

The effect of exposure time and temperature on uptake rate constants, $k_u$, for lindane as determined by equation 20*; $k_u$, mlg$^{-1}$h$^{-1}$ vs. exposure time, h; each point is an average of 3 replicates.

$* k_u = \frac{(Ca \times kd)}{Cw(1 - e^{-k_u t})}$

Table 10
Uptake Rate Constants ($k_u$) for Lindane at Each Sampling Time of Experiments for Figure 9

<table>
<thead>
<tr>
<th>Sampling time, h</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>69 ± 11</td>
<td>118 ± 7</td>
<td>134 ± 21</td>
<td>155 ± 38</td>
</tr>
<tr>
<td>0.5</td>
<td>54 ± 10</td>
<td>93 ± 4</td>
<td>109 ± 10</td>
<td>108 ± 8</td>
</tr>
<tr>
<td>1</td>
<td>47 ± 5</td>
<td>69 ± 6</td>
<td>85 ± 3</td>
<td>98 ± 6</td>
</tr>
<tr>
<td>2</td>
<td>36 ± 2</td>
<td>42 ± 1</td>
<td>50 ± 6</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>4.5</td>
<td>22 ± 1</td>
<td>27 ± 2</td>
<td>30 ± 4</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>8.5</td>
<td>18 ± 1</td>
<td>22 ± 1</td>
<td>26 ± 1</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>33 ± 1</td>
</tr>
<tr>
<td>10.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>27 ± 1</td>
<td></td>
</tr>
<tr>
<td>12.25</td>
<td>19 ± 2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 10.

The effect of exposure time and temperature on uptake rate constants, $k_u$, for tetraCB as determined equation 20*: $k_u, \text{ml}^{-1}\text{h}^{-1}$ vs. exposure time, h; each point is an average of 3 replicates.

* $k_u = \frac{(C_a \times k_d)}{C_w(1 - e^{-k_d \times t})}$

Table 11

Uptake Rate Constants ($k_u$) for tetraCB at Each Sampling Time of Experiments for Figure 10

<table>
<thead>
<tr>
<th>Sampling time, h</th>
<th>10°C</th>
<th>15°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>177 ± 10</td>
<td>259 ± 25</td>
<td>361 ± 40</td>
<td>279 ± 21</td>
</tr>
<tr>
<td>0.5</td>
<td>111 ± 13</td>
<td>204 ± 26</td>
<td>295 ± 63</td>
<td>244 ± 72</td>
</tr>
<tr>
<td>1</td>
<td>99 ± 22</td>
<td>176 ± 23</td>
<td>210 ± 25</td>
<td>198 ± 31</td>
</tr>
<tr>
<td>2</td>
<td>67 ± 6</td>
<td>155 ± 18</td>
<td>247 ± 16</td>
<td>201 ± 33</td>
</tr>
<tr>
<td>4.5</td>
<td>130 ± 6</td>
<td>188 ± 39</td>
<td>138 ± 25</td>
<td></td>
</tr>
<tr>
<td>4.75</td>
<td>67 ± 16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>51 ± 6</td>
<td>105 ± 3</td>
<td>166 ± 22</td>
<td>133 ± 14</td>
</tr>
</tbody>
</table>
Figure 11.

The effect of exposure time and temperature on uptake rate constants, \( k_u \), for hexaCB as determined by equation 20*; \( k_u, \text{mg}^{-1} \text{h}^{-1} \) vs. exposure time, \( h \); each point is an average of 3 replicates.

\[
* k_u = \frac{(C_a \times k_d)}{C_w(1 - e^{-k_d t})}
\]

Table 12

Uptake Rate Constants (\( k_u \)) for hexaCB at Each Sampling Time of Experiments for Figure 11

<table>
<thead>
<tr>
<th>Sampling time, h</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td></td>
<td>301 ± 77</td>
</tr>
<tr>
<td>1</td>
<td>305 ± 39</td>
<td>255 ± 42</td>
</tr>
<tr>
<td>1.25</td>
<td></td>
<td>208 ± 29</td>
</tr>
<tr>
<td>1.5</td>
<td>341 ± 42</td>
<td>194 ± 46</td>
</tr>
<tr>
<td>2</td>
<td>357 ± 59</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.75</td>
<td>341 ± 70</td>
<td></td>
</tr>
<tr>
<td>6.5</td>
<td>266 ± 64</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>253 ± 50</td>
<td></td>
</tr>
<tr>
<td>11.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The effect of exposure time and temperature on uptake rate constants, $k_u$, for DDE as determined by equation 20*; $k_u$, ml g$^{-1}$ h$^{-1}$ vs. exposure time, h; each point is an average of 3 replicates.

* $k_u = \frac{(Ca \times kd)}{Cw(1 - e^{-k_d*t})}$

Table 13

<table>
<thead>
<tr>
<th>Sampling time, h</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>150 ± 54</td>
<td>242 ± 36</td>
</tr>
<tr>
<td>3</td>
<td>149 ± 33</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>145 ± 22</td>
<td>162 ± 10</td>
</tr>
<tr>
<td>6</td>
<td>144 ± 24</td>
<td>261 ± 37</td>
</tr>
<tr>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>148 ± 9</td>
<td>181 ± 10</td>
</tr>
<tr>
<td>10.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>162 ± 25</td>
<td>235 ± 31</td>
</tr>
<tr>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>239 ± 37</td>
<td></td>
</tr>
</tbody>
</table>
Figure 13.

The effect of exposure time and temperature on uptake rate constants, $k_u$, for hexaCB as determined by mass balance (equation 19 in methods section); $k_u$, mlg$^{-1}$h$^{-1}$ vs. exposure time, h; each point is an average of 3 replicates.

Table 14

Uptake Rate Constants (ku) for hexaCB at Each Sampling Time of Experiments for Figure 13

<table>
<thead>
<tr>
<th>Sampling time, h</th>
<th>10°C</th>
<th>16°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>81 ± 15</td>
<td>206 ± 34</td>
</tr>
<tr>
<td>1</td>
<td>58 ± 8</td>
<td>203 ± 51</td>
</tr>
<tr>
<td>2</td>
<td>59 ± 12</td>
<td>247 ± 127</td>
</tr>
<tr>
<td>2.5</td>
<td>52 ± 14</td>
<td>133 ± 52</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>94 ± 10</td>
</tr>
<tr>
<td>3.5</td>
<td></td>
<td>104 ± 34</td>
</tr>
<tr>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>48 ± 4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.25</td>
<td>55 ± 8</td>
<td></td>
</tr>
</tbody>
</table>
Figure 13: Plot showing the relationship between exposure time (h) and a variable measured in ml g⁻¹ h⁻¹ for two temperatures: 16° and 10°. The graph indicates a decrease in the variable with increasing exposure time.
Figure 14.

The effect of exposure time and temperature on uptake rate constants, \( k_u \), for DDE as determined by mass balance (equation 19 in methods section); \( k_u, \text{mlg}^{-1}\text{h}^{-1} \) vs. exposure time, h; each point is an average of 3 replicates.

Table 15

Uptake Rate Constants (\( k_u \)) for DDE at Each Sampling Time of Experiments for Figure 14

<table>
<thead>
<tr>
<th>Sampling time, h</th>
<th>10°C</th>
<th>16°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53 ± 5</td>
<td>81 ± 8</td>
</tr>
<tr>
<td>1.5</td>
<td>41 ± 5</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>3</td>
<td>34 ± 9</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>24 ± 6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>71 ± 6</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>28 ± 4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td></td>
<td>70 ± 1</td>
</tr>
<tr>
<td>12.5</td>
<td></td>
<td>71 ± 12</td>
</tr>
<tr>
<td>24.5</td>
<td></td>
<td>73 ± 16</td>
</tr>
</tbody>
</table>
"leveled off" region of the line. The two uptake rate constants, $k_{u_{fast}}$ and $k_{u_{slow}}$, were only calculated for diCB, triCB, lindane, and tetraCB because only for these four chemicals was the two process accumulation by the midge clearly demonstrated at all four temperatures (Table 16). Two processes were not evident for the $k_u$ values of hexaCB and DDE at all four temperatures.

It was the occurrence of two processes with different uptake rates that caused the difference between the $k_u$ values of diCB as determined by the $BCF \times kd$ method and equation 20 (Tables 6 and 7). The $BCF \times kd$ determination calculated $k_u$ from accumulation data at steady-state where the $k_u$ estimate was not time-dependent and involved both uptake processes. The equation 20 calculation of $k_u$ given in Table 6, however, estimated the $k_u$ of diCB by averaging $k_u$ values from each of the six sampling periods. The result was an average $k_u$ value which was weighted by the fast uptake process in early sampling periods.

Table 16

<table>
<thead>
<tr>
<th>Uptake Rate Constants, mlg$^{-1}$h$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>fast</td>
</tr>
<tr>
<td>diCB</td>
</tr>
<tr>
<td>triCB</td>
</tr>
<tr>
<td>lindane</td>
</tr>
<tr>
<td>tetraCB</td>
</tr>
</tbody>
</table>

$^a$ estimated from Figure 7
The three methods (equation 20, slope, and initial rate) used to calculate $k_u$ for DDE and hexaCB produced generally similar $k_u$ values (Tables 6 and 7). For DDE and hexaCB the equation 20 method was considered the most accurate estimation of $k_u$ at 22°C and 30°C, because this method takes into account the depuration that was measured in the depuration experiments at these temperatures. The initial rate method of calculating $k_u$ was chosen as the representative $k_u$ values for DDE and hexaCB at 10°C and 16°C.

The $k_u^{\text{slow}}$ values for diCB, triCB, tetraCB, and lindane; the $k_u$ values determined by equation 20 for DDE and hexaCB at 22°C and 30°C; and the $k_u$ values determined by the initial rate equation for DDE and hexaCB at 10°C and 16°C are the $k_u$ values that are considered most representative and are used in the following figures, statistical comparisons, calculation of BCF values, and in the discussion. Briefly, $k_u^{\text{slow}}$ was considered representative for diCB, triCB, tetraCB, and lindane because for diCB $k_u^{\text{slow}}$ approximated the steady-state determination of $k_u$ (BCF x kd). Table 17 lists these $k_u$ values. (A more detailed explanation of the $k_u$ values is given in the discussion.)

At each experimental temperature pairwise comparisons of the most representative $k_u$ values were made for all six chemicals listed in Table 17. Tables 18 and 19 list the $k_u$ comparisons and their statistical significance at alpha = 0.05. Pairwise comparisons were also performed on the $k_u$ values of a given chemical at each temperature. For this analysis the t-tests were performed only on the $k_u$ values of adjacent experimental temperatures. Table 20 lists the comparisons of $k_u$ values between adjacent temperatures that were found
to be significant at alpha = 0.05.

Table 17
Representative ku values for the Six Test Chemicals at Four Temperatures

<table>
<thead>
<tr>
<th>Chemical</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>diCB</td>
<td>35 ± 5.5</td>
<td>48 ± 3.9</td>
<td>65 ± 6.4</td>
<td>80 ± 6.2</td>
</tr>
<tr>
<td>triCB</td>
<td>43 ± 8.2</td>
<td>71 ± 5.7</td>
<td>112 ± 3.4</td>
<td>105 ± 14</td>
</tr>
<tr>
<td>lindane</td>
<td>20 ± 1.0</td>
<td>25 ± 1.3</td>
<td>28 ± 2.5</td>
<td>35 ± 3.0</td>
</tr>
<tr>
<td>tetraCB</td>
<td>59 ± 11</td>
<td>117 ± 4.9</td>
<td>177 ± 31</td>
<td>136 ± 20</td>
</tr>
<tr>
<td>hexaCB</td>
<td>58 ± 15</td>
<td>155 ± 87</td>
<td>311 ± 62</td>
<td>237 ± 51</td>
</tr>
<tr>
<td>DDE</td>
<td>53 ± 5.0</td>
<td>72 ± 8.7</td>
<td>150 ± 27</td>
<td>220 ± 44</td>
</tr>
</tbody>
</table>

Table 18
Pairwise Comparisons of ku Values of all Chemicals for the Temperatures 10°C and 16°C

<table>
<thead>
<tr>
<th>10°C</th>
<th>diCB</th>
<th>triCB</th>
<th>lindane</th>
<th>tetraCB</th>
<th>hexaCB</th>
<th>DDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>diCB</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
</tr>
<tr>
<td>triCB</td>
<td>S</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>lindane</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>tetraCB</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>hexaCB</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DDE</td>
<td>S</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>16°C</th>
<th>diCB</th>
<th>triCB</th>
<th>lindane</th>
<th>tetraCB</th>
<th>hexaCB</th>
<th>DDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>triCB</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>lindane</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>tetraCB</td>
<td>S</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>hexaCB</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DDE</td>
<td>S</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a^ \text{S = significant difference, P > 0.05; NS = no significant difference}

\(^b^ \text{symbols above the diagonal line are 10°C comparisons; symbols below the diagonal line are 16°C comparisons}\)
Table 19

Pairwise Comparisons of ku Values of All Chemicals for the Temperatures, 22°C and 30°C\textsuperscript{a,b}

\begin{tabular}{cccccc}
& \textit{diCB} & \textit{triCB} & \textit{lindane} & \textit{tetraCB} & \textit{hexaCB} & \textit{DDE} \\
\textit{diCB} & S & S & S & S & S & S \\
\textit{triCB} & NS & S & S & S & NS & \\
\textit{lindane} & S & S & S & S & S & S \\
\textit{tetraCB} & S & NS & S & NS & NS & NS \\
\textit{hexaCB} & S & S & S & NS & S & NS \\
\textit{DDE} & S & S & S & NS & NS & NS \\
\end{tabular}

\textsuperscript{a} S = significant difference, P > 0.05; NS = no significant difference

\textsuperscript{b} symbols above the diagonal line are 22°C comparisons; symbols below the diagonal line are 30°C comparisons

Table 20

Pairwise Comparisons of ku Values of a Chemical between Adjacent Experimental Temperatures\textsuperscript{a}

\begin{tabular}{cccc}
& \textit{10°C/16°C} & \textit{16°C/22°C} & \textit{22°C/30°C} \\
\textit{diCB} & NS & S & NS \\
\textit{triCB} & S & S & NS \\
\textit{lindane} & S & NS & NS \\
\textit{tetraCB} & S & NS & NS \\
\textit{hexaCB} & NS & NS & NS \\
\textit{DDE} & NS & S & NS \\
\end{tabular}

\textsuperscript{a} S = significant difference, P > 0.05; NS = no significant difference
The results of all the pairwise comparisons of ku values at a given temperature (Tables 18 and 19) show 37 significant differences out of a possible 60. This was a considerably smaller portion of significant differences than was found for all possible comparisons of kd values at a given temperature, which only found 2 comparisons not significantly different. Of the 23 comparisons that were not significantly different, two reasons accounted for 17. The first was that at 10°C ku values for diCB, triCB, tetraCB, hexaCB, and DDE were closely grouped in the range, 35 to 59 mlg⁻¹h⁻¹ (Figure 15). Lindane with a ku value of 20 mlg⁻¹h⁻¹ was found to be significantly different from all other ku values at 10°C. The range of ku values widened as temperature increased. The second reason contributing to the number of insignificant differences was the large standard deviations associated with hexaCB. This point is illustrated at 16°C where the difference in ku values between hexaCB and lindane was greater than 6 times and found to be statistically insignificant.

The ku values response to temperature was not as significant as the kd values response. All comparisons of kd values between adjacent temperature were significant. The ku values, however, were only found to be significantly different in 1/3 of all comparisons between adjacent temperatures (Table 20). The ku values for all test chemicals increased from 10°C to 22°C (Figure 15) with 1/2 of the ku values between adjacent temperatures significantly different over this range (Table 20). An increase in ku relative to an increase in temperature was not, however, found between 22°C to 30°C where no significant differences were determined between ku values.
C. BCF

The steady-state calculation ($C_{ss}/C_w$) for the determination of the bioconcentration factor (BCF) was done only for diCB. Figure 16 shows the uptake curves for diCB at 10°C, 16°C, 22°C, and 30°C. The asymptotic $C_{ss}$ values of 600 ng/g and 620 ng/g were experimentally reached at 22°C and 30°C. The $C_{ss}$ values of 960 ng/g and 900 ng/g were estimated for 10°C and 16°C by an eye-fit extension of the curve. The last sampling times for the 30°C (5.25 h) and 22°C (9 h) uptake tests for diCB were similar to the estimated times of 6 h (30°C) and 9 h (22°C) to reach 95% $C_{ss}$ values based on the expt'1 kd values (Table 5), reinforcing the asymptotic values interpolated from the curves for these two temperatures. The estimated time to reach 95% $C_{ss}$ (Table 5) for 10°C (38 h) and 16°C (25 h) exceeded the last sampling times for the uptake tests at 10°C and 16°C. The extrapolated asymptotic $C_{ss}$ values at 10°C and 16°C are thus less certain than the $C_{ss}$ values at 22°C and 30°C.

The kinetic calculation for BCF, $k_u/k_d$, (equation 5 in the methods section) was made for all chemicals at each temperature (except for hexaCB and DDE at 10°C and 16°C, for which expt'1 kd values were not determined). The kinetic determinations of BCF (BCF$_k$) and the steady-state determinations of BCF (BCF$_{ss}$) are listed in Table 21. The kd values used in the determinations of all BCF$_k$ values were the expt'1 kd values.
Figure 15.

The effect of temperature on \( ku \) for d1CB, t1CB, tetaCB, lindane, hexaCB, and DDE; \( ku, \text{ ml}g^{-1}h^{-1} \) vs. temperature, \( ^\circC \); each point represents 3 replicates; standard deviation of each data point is given in Table 17.
Figure 16.

Uptake and accumulation of diCB by midge larvae at 10°C, 16°C, 22°C, and 30°C; Ca, ng diCB/g midge vs. exposure time, h; each point is an average of 3 replicates.
Table 21

The Bioconcentration Factors, BCF, for Six Organochlorines with *Chironomus riparius* at Experimental Temperatures

<table>
<thead>
<tr>
<th></th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>dICB</td>
<td>270</td>
<td>242</td>
<td>185</td>
<td>159</td>
</tr>
<tr>
<td>dICB</td>
<td>479</td>
<td>432</td>
<td>204</td>
<td>171</td>
</tr>
<tr>
<td>triCB</td>
<td>2389</td>
<td>2630</td>
<td>2240</td>
<td>1221</td>
</tr>
<tr>
<td>lindane</td>
<td>870</td>
<td>958</td>
<td>475</td>
<td>365</td>
</tr>
<tr>
<td>tetraCB</td>
<td>2185</td>
<td>2388</td>
<td>2723</td>
<td>1447</td>
</tr>
<tr>
<td>hexaCB</td>
<td>...</td>
<td>...</td>
<td>111000</td>
<td>19833</td>
</tr>
<tr>
<td>DDE</td>
<td>...</td>
<td>...</td>
<td>93750</td>
<td>55000</td>
</tr>
</tbody>
</table>

* BCF<sub>ss</sub> values

An ANCOVA of log BCF (kinetic determination) with temperature for dICB, triCB, lindane, and tetraCB found the slopes for these four chemicals not significantly different (*P* < 0.05) (Figure 17). The ANCOVA also determined the common slope for the four compounds to be negative and statistically significant (*P* > 0.05) indicating temperature had a negative effect on the BCF values for dICB, triCB, lindane, and tetraCB. A comparison of the four y-intercepts by ANCOVA determined the four chemicals to be significantly different from each other with the exception of triCB and tetraCB which were not significantly different. The comparisons of the y-intercepts indicate the BCF values for dICB and for lindane were different from the BCF...
values of the other chemicals and the BCF values for triCB and tetraCB were only different from the BCF values of diCB and lindane.

HexaCB and DDE were not included in the ANCOVA because each chemical had only two BCF values. A cursory comparison of the BCF values for these two compounds can be done, however, by an examination of the conservative confidence intervals illustrated on Figure 17. For the BCF values of hexaCB, the confidence bounds at 22°C and 30°C do not overlap indicating the BCF at the higher temperature to be significantly lower. The confidence bounds for the BCF values of DDE at 22°C and 30°C do overlap indicating the decrease in BCF at the higher temperature may not be significant.

D. Correlation of Accumulation Parameters with Aqueous Solubility and n-Octanol/Water Partition Coefficient

The BCF's for diCB, triCB, lindane, tetraCB, hexaCB, and DDE increased according to the hydrophobic/lipophilic character of the chemical, i.e. the BCF's increased as the aqueous solubility of the chemical decreased. This relationship is illustrated in a plot of $\log_{10} BCF$ against $\log_{10} AS$ (aqueous solubility) (Figure 18) which spans a range of five orders of magnitude of solubilities and has a correlation of $r^2 = 0.88$. The BCF values used in Figure 18 were BCF$_k$ values determined at 22°C, and the aqueous solubilities were the average of literature values (Table 22) determined at 20°C to 25°C. (All of the following correlations use accumulation parameters determined at 22°C and physical/chemical values from Table 22.) Conversely, the bioconcentration of these chemicals were found to increase relative to an increase in the lipophilicity of the chemicals.
Figure 17.

The effect of temperature on the bioconcentration factors, BCF, for the six organochlorines; $\log_{10} \text{BCF vs. temperature, } ^\circ\text{C}$; each point represents 3 replicates; errors represent 95% confidence intervals.

Note: Both $\text{BCF}_k$ and $\text{BCF}_{ss}$ are plotted for diCB. All others are $\text{BCF}_k$. 
FIGURE # 17
Figure 19 shows this correlation with a plot of $\log_{10} BCF$ against $\log_{10} K_{ow}$ (n-octanol/water partition coefficient) which has a slope of near unity (1.06) and an $r^2$ value of 0.96.

Table 22

<table>
<thead>
<tr>
<th>chemical</th>
<th>$\text{AS(M)}^{ab}$</th>
<th>ref$^c$</th>
<th>$\log_{10} K_{ow}^{a}$</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>dICB</td>
<td>$5.1 \times 10^{-4}$</td>
<td>1,2,3</td>
<td>3.44</td>
<td>1,2,3,5,6</td>
</tr>
<tr>
<td>trICB</td>
<td>$1.5 \times 10^{-4}$</td>
<td>5</td>
<td>4.02</td>
<td>5</td>
</tr>
<tr>
<td>lindane</td>
<td>$2.9 \times 10^{-5}$</td>
<td>4,7,8</td>
<td>3.50</td>
<td>4,8,9</td>
</tr>
<tr>
<td>tetraCB</td>
<td>$1.9 \times 10^{-6}$</td>
<td>5</td>
<td>4.61</td>
<td>5,10</td>
</tr>
<tr>
<td>hexaCB</td>
<td>$2.2 \times 10^{-8}$</td>
<td>1,5,6,7</td>
<td>5.93</td>
<td>1,5,6,10,11</td>
</tr>
<tr>
<td>DDE</td>
<td>$4.6 \times 10^{-9}$</td>
<td>7</td>
<td>5.69</td>
<td>12</td>
</tr>
</tbody>
</table>

$^a$ value represents a mean of reference values

$^b$ solubility determined between 20°C and 25°C


The uptake rate constants used in correlations with aqueous solubility and $K_{ow}$ were the representative $k_u$ values listed in Table 17. Regression analysis of $k_u$ values determined at 22°C with literature $\text{AS}$ values showed an inverse relationship between uptake of the chemical and its solubility and yielded an $r^2$ value of 0.50. Multiplying the $k_u$ values by the molecular weight of the compound improved this correlation as reflected by its $r^2$ value of 0.62.
(Figure 20). A further improvement was found correlating ku x MW with log$_{10}$ $K_{OW}$ with an increased $r^2$ value of 0.83 (Figure 21). This plot demonstrated a trend of increasing ku's relative to an increase in $K_{OW}$ or lipophilicity.

Depuration demonstrated an inverse relationship to uptake to the effect of the hydrophobic/lipophilic nature of the chemical. Depuration generally increased relative to an increase in solubility and to a decrease in lipophilicity ($K_{OW}$). Figures 22 and 23 illustrate the inverse relationship of $k_d$ to $k_u$ by a positive slope for $k_d$ x MW against log$_{10}$ AS ($k_u$ against AS was negative) and a negative slope for $k_d$ x MW against log$_{10}$ $K_{OW}$ ($k_u$ was positive). Multiplying the $k_d$ values by the molecular weight of the chemical increased the correlation in these plots, similar to effect of this factor on uptake correlations, as demonstrated by an increase in $r^2$ from 0.50 to 0.58 ($k_d$ x MW vs. log$_{10}$ AS), and from 0.44 to 0.59 ($k_d$ x MW vs. log$_{10}$ $K_{OW}$). Still, neither are very strong correlations.

E. Effect of Temperature on the Acute Toxicity of Test Chemicals to the Midge

Acute toxicity tests were conducted at 10°C and room temperature (21 ± 1°C) for all six test chemicals using the midge, C. riparius, as the test organism (DDE was conducted only at room temperature). Two of the chemicals, hexaCB and DDE, were not found to be acutely toxic to the midge over a 48-hour exposure near their solubilities (3.5 µg/L, hexaCB; 1 µg/L, DDE). The remaining four chemicals demonstrated acute toxicity to the midge and their EC$_{50}$ values are given in Table 23.
Figure 18.

Correlation of $\log_{10}$ bioconcentration factor with $\log_{10}$ aqueous solubility in midge larvae for six organochlorines at 22°C; $\log_{10}$ BCF vs. $\log_{10}$ AS, mol/liter; each point represents 3 replicates; bars represent 95% confidence limits.

$$\log_{10} \text{BCF} = -0.52 \log_{10} \text{AS} + 0.74 \quad r^2 = 0.88$$
FIGURE # 18
Figure 19.

Correlation of $\log_{10}$ bioconcentration factor with $\log_{10}$ n-octanol/water partition coefficient in midge larvae for six organochlorines at 22°C: $\log_{10} \text{BCF} \text{ vs. } \log_{10} K_{ow}$; each point represents 3 replicates; bars represent 95% confidence limits.

$\log_{10} \text{BCF} = 1.06 \log_{10} K_{ow} - 1.12 \quad r^2 = 0.96$
Figure 20.

Correlation of the product of uptake rate constants and molecular weight with $\log_{10}$ aqueous solubility for six organochlorines in midge larvae at 22°C; $ku \times MW$ vs. $\log_{10}$ AS, mol/liter; each point represents 3 replicates; bars represent 95% confidence limits.

$ku \times MW = -11,483 \log_{10} AS - 29,616 \quad r^2 = 0.62$

Note: without DDE coordinates; $ku \times MW = -17,805 \log_{10} AS - 56,698 \quad r^2 = 0.88$
FIGURE # 20
Figure 21.

Correlation of the product of uptake rate constants and molecular weight with $\log_{10} n$-octanol/water partition coefficients for six organochlorines in midge larvae at 22°C; $ku \times MW$ vs. $\log_{10} K_{ow}$: each point represents 3 replicates; bars represent 95% confidence limits.

$ku \times MW = 25,265 \log_{10} K_{ow} - 79,920 \quad r^2 = 0.83$

Note: without DDE coordinates; $ku \times MW = 32,394 \log_{10} K_{ow} - 106,261 \quad r^2 = 0.98$
FIGURE # 21
Correlation of the product of depuration rate constants and molecular weight with \( \log_{10} \) aqueous solubility for six organochlorines in midge larvae at 22°C; \( kd \times MW \) vs. \( \log_{10} AS \), mol/liter; each point represents 3 replicates; bars represent 95% confidence limits.

\[ kd \times MW = 63 \log_{10} AS + 50 \quad r^2 = 0.58 \]
Log$_{10}$ Aqueous Solubility, mol/liter

Figure # 22
Figure 23.

Correlation of the product of depuration rate constants and molecular weight with $\log_{10}$ n-octanol/water partition coefficient for six organochlorines in midge larvae at 22°C: $kd \times MW$ vs. $\log_{10} K_{ow}$; each point represents 3 replicates; bars represent 95% confidence limits.

$$kd \times MW = -12 \log_{10} K_{ow} + 70 \quad r^2 = 0.59$$
Table 23
Acute Toxicity of dlCB, trICB, tetraCB, and lindane to Chironomus riparius at 10°C and Room Temperature

<table>
<thead>
<tr>
<th>Chemical</th>
<th>EC50 ± 95% Confidence Limits, ug/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10°C</td>
</tr>
<tr>
<td>dlCBa</td>
<td>1,574 ± 170°C</td>
</tr>
<tr>
<td>trICBa</td>
<td>1,136 ± 103°C</td>
</tr>
<tr>
<td>tetraCbb</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>lindanea</td>
<td>2.7 ± 0.7</td>
</tr>
</tbody>
</table>

a 24-hour exposure  
b 48-hour exposure  
c Values are significantly different from all others in table

The effect of temperature on the acute toxicity was varied among the chemicals. Two of the chemicals in Table 23, dlCB and trICB, were more acutely toxic at 10°C than at room temperature. One chemical, tetraCB, was found to be acutely toxic at room temperature (EC50 = 343 ug/L) but not at 10°C where no toxicity was observed in the highest test concentration of 500 ug/L. The remaining chemical, lindane, had somewhat similar values at the two temperatures with a slightly higher EC50 value at 10°C (2.7 ug/L) than at room temperature (2.0 ug/L).

A comparison of the EC50 values among chemicals found the insecticide, lindane, to be two orders of magnitude more toxic than any of the chlorinated benzenes. For the chlorinated benzenes, the acute toxicity to the midge increased as the number of chlorines on the benzene ring and lipophilicity of the chemical increased (Table 23).
IV. Discussion

A. The Accumulation Model, Assumptions and Limitations

The transfer of chemical between water and midge larvae may be expressed mathematically by rate constants. The rate constants are proportionality constants quantifying the transfer rate for the transferrable concentration of a chemical from one compartment to another. Transfer rate constants have both a toxicological and environmental significance in that they describe the dynamics of xenobiotic chemical flux between the organism and the water and, using the midge as a model for benthic arthropods, between a faunal community and water. It is important, therefore, to estimate as accurately as possible the rate constants involved in these transfer processes.

Before a discussion of the accumulation parameters I obtained can be made with, for example, comparisons to literature accumulation parameters or the effect of temperature, one must decide on what set of transfer rate constants will be used. A number of methods and calculations were used to determine the rate constants, $k_u$ and $k_d$, according to the model, $\frac{dC_a}{dt} = k_u \times C_w - k_d \times C_a$. The following section details the explanations, assumptions, and limitations of the rate constants that were chosen for discussion and used in determining the effect of temperature, BCF calculations, and correlational analyses.

The depuration rate constant, $k_d$, was determined by two different methods using independent data. The expt'1 $k_d$, determined from actual
depuration experiments (data), was found to have much lower values and less statistical error than the nonlinear, which used data from the uptake test and was calculated by nonlinear regression analysis (Table 4). The difference in \( k_d \) values between the two methods can be explained by the configuration of the accumulation curve (data from an uptake test) relative to the monoexponential function, \( C_a = \frac{k_u}{k_d}C_w \times (1 - e^{-k_d t}) \), describing the curve. Figures 7 to 10 illustrated that the accumulation process was not adequately described by the monoexponential model, \( \frac{dC_a}{dt} = k_u \times C_w - k_d \times C_a \), due to the systematic decline in \( k_u \) values during shorter exposure times for diCB, triCB, lindane, and tetraCB. Indeed, if the data fit the above model, \( k_u \) would be independent of exposure time. Therefore, there is another process that enhanced the damping of the curve and caused steady-state to be reached sooner. In contrast, the \( k_u \) values of DDE and hexaCB did not, in general systematically decline during shorter exposure times (Figures 11 to 14). Both these compounds (DDE and hexaCB) exhibited accumulation curves that were basically still in the initial rate stage (Figures 5 and 6) and therefore no "enhanced damping" could be detected.

The monoexponential function, \( C_a = \frac{(k_u/k_d)C_w \times (1 - e^{-k_d t})}{1} \), can be separated into a term that defines the steady-state concentration in the animal, \( (k_u/k_d)C_w \), and an exponential term, \( (1 - e^{-k_d t}) \), that describes the nonlinearity of the curve. As exposure time increases the exponential term ranges from 0 \( (t = 0) \) to 1 \( (t = \infty) \). The impact a larger \( k_d \) value will have on the function, \( C_a = \frac{(k_u/k_d)C_w \times (1 - e^{-k_d t})}{1} \), is a shortening of the time it takes the exponential term to
reach 1 and thereby a shortening of the time to reach steady-state. 
The enhanced damping of the curve, therefore, can be accounted for in 
the nonlinear regression method by a larger kd value. The kd values 
that will be used in the discussion are those obtained from the 
depuration data (expt'l kd).

The uptake rate constant, ku, was derived by a number of different 
calculation methods, but all used data from the same uptake tests. Of 
the two calculation methods for ku that needed a kd value, 
k = BCF x 
kd (equation 21) and 
k = (Ca x kd)/CW(1 - e-kdt) (equation 20). The 
needed kd values were obtained from depuration experiments (expt'l kd). 
None of the ku determinations, therefore, were derived from totally 
independent data as was the situation for kd (expt'l kd's were 
determined from depuration data and nonlin kd's were determined from 
uptake data). All of the values for ku obtained by the various 
calculation methods were similar with the exception of the values 
obtained for dicB (Table 6). The ku values for dicB calculated by BCF 
x kd were substantially lower than the ku values for dicB calculated by 
the other four methods (Table 6). Assuming the kd values and the 
experimental value of BCF obtained at steady-state for dicB were 
accurate, the ku values calculated by BCF x kd are considered an 
accurate estimate of ku.

The reason the remaining ku values calculated by the other 
methods are so different from the ku values calculated by BCF x kd is 
that the other calculations average the ku values over the course of an 
uptake test. Evidence for significant change of ku values during an 
experiment is illustrated in Figures 7 to 10, which show a rapid
decline in ku values between 0.25 h and 2 h. From Figures 7 to 10 two
uptake rate constants, ku.fast and ku.slow, were determined for diCB,
triCB, lindane, and tetraCB. The ku.slow values (10°C, 35 mg⁻¹h⁻¹;
16°C, 48 mg⁻¹h⁻¹; 22°C, 65 mg⁻¹h⁻¹; 30°C, 80 mg⁻¹h⁻¹) for diCB
approached the ku values obtained from steady-state BCF's (BCF x kd)
(10°C, 19 mg⁻¹h⁻¹; 16°C, 27 mg⁻¹h⁻¹; 22°C, 56 mg⁻¹h⁻¹; 30°C, 71
mg⁻¹h⁻¹). With the above cross-check of ku values showing that ku.slow
of diCB as being accurate, the ku.slow values for diCB, triCB, lindane,
and tetraCB were accepted as more reliable.

ku.slow was the rate constant that described the predominance of
the accumulation transfer process, because ku.slow/kd was approximately
equal to Css/Cw for diCB, and is the ku value for diCB, triCB, tetraCB,
and lindane used in the BCF calculations, correlations and discussion.
The ku values determined by equation 20 for hexaCB and DDE at 22°C and
30°C, and the ku values determined by the initial rate calculation for
hexaCB and DDE at 10°C and 16°C are the uptake rate constants used in
the BCF calculations and discussion.

The amount of chemical accumulated in the animal was determined
by a ¹⁴C total count which would include parent chemical as well as any
metabolite if biotransformation had occurred. Proposing that two
uptake rate processes rather than one are exhibited assumes no
significant metabolism of the parent chemical occurred during the
uptake and depuration experiments. Metabolism of the parent chemical
can account for an enhanced damping of the curve and a diminishing ku
with time as was found for diCB, triCB, lindane, and tetraCB (Figures
7 to 10). A biotransformation rate constant could be incorporated in
the exponential term of the function as well as the steady-state term and could also account for a departure from the \( \frac{dC_a}{dt} = ku \times C_w - kd \times C_a \) model similar to what was found in the present study.

I have no direct evidence that metabolism did not occur in the depuration experiments for diCB, triCB, lindane, and tetraCB. A monophasic elimination was found for diCB, triCB, and tetraCB in depuration experiments, whereas lindane exhibited a biphasic curve. Metabolites, according to Glesy and Landrum (1985), will likely be eliminated at different rates than parent compounds thereby resulting in a multiphasic elimination curve. If this indeed were true, the monophasic elimination of diCB, triCB, and tetraCB would be evidence against metabolism, but according to Notari (1980) two simultaneous first-order losses (in this example, loss of parent chemical and loss of metabolite) from one compartment (midge) can have different rate constants and still exhibit a monophasic in Ca vs. time plot.

The assumption that no significant metabolism had occurred and therefore two uptake rate processes exist was also based on work done by Lohner (1984). Lohner performed uptake experiments using the same culture of *C. riparius* and three of the same four chemicals, 1,4-diCB, lindane, and 1,2,4,5-tetraCB (Lohner used 1,3,5-triCB, this work 1,2,4-triCB) that displayed diminishing \( ku \)'s with time in my experiments. Lohner used gas-liquid chromatography to quantify organic solvent extracts of acidified midge homogenates (40 midges/extract). He found no evidence of metabolites based on the absence of unidentified peaks on the gas-liquid chromatograms after midges had been exposed to the chemicals for two to three hours (this assumes any metabolite is
extracted by the solvent system, and is resolved and detected by the GC). According to the rapid decline in ku values (Figures 7 to 10) between 0.25 h and 2 h, if metabolism was a significant reaction, an extractable quantity of metabolite should be present in the midge after 2 h of exposure.

In summary, the rate constants $k_{u\text{slow}}$ and $k_{u\text{fast}}$ were proposed, because the accumulation dynamics of the six test compounds in the midge did not fit the mathematical model, $dC_a/dt = ku x C_w - kd x C_a$. In order to build a more accurate model, additional compartments must be sampled (pers. comm., Yowming Wang).

B. Depuration

The five aromatic compounds all showed well defined, monophasic, one compartment elimination from the midge. Giam et al. (1980) found one compartment elimination of hexaCB by killifish as did Konemann and van Leeuwen (1980) for dICB and hexaCB in guppies. The latter authors, however, reported biphasic elimination plots for 4 other chlorobenzenes in the same paper.

I found lindane to exhibit a biphasic two compartment elimination from C. riparius (Figure 3). Yamato et al. (1983) found lindane to be eliminated biphasically from guppies and monophasically from short-necked clams.

Data on elimination of chlorobenzene, lindane or DDE from chironomid larvae were not found in the literature. Gerould et al. (1983) and Leversee et al. (1982), however, reported triphasic elimination for anthracene and benzo(a)pyrene in C. riparius and Muir et al. (1982) found two compartment elimination for herbicides,
terbutryn and fluridone, in C. tentans. Gerould et al. (1983) and Leversee et al. (1982) determined their kd values by depurating the chemicals from midge larvae in the presence of a paper substrate. They found that midges depurated faster in the presence of the paper substrate than when depurated in water alone. The authors hypothesized that the increased activity associated with the presence of the substrate increased the rate of depuration over that when no substrate was present. An experiment was performed in this study that examined the effect of the presence of substrate versus food versus water alone on the depuration of DDE from midge larvae (see Appendix 4). Another set of experiments was performed in this study examining the effect of binary mixtures on the uptake and depuration of two chemicals by midge larvae. These data are given in Appendix 3.

Elimination of the test compounds by the midge ranged from slower to faster when compared to the elimination of the same compounds by other species. Konemann and van Leeuwen (1980) found guppies eliminated diCB slower than the midge, reporting a kd at 21°C of 0.042 h⁻¹ (this work, 0.318 h⁻¹ at 22°C) while Galassi et al. (1982) reported a similar kd of 0.11 h⁻¹ (this work, 0.073 h⁻¹ at 10°C) for diCB in rainbow trout alevins (young fish) at 10°C. Galassi and Calamari (1983) found an elimination rate similar to the midge for triCB in hatching rainbow trout at 10°C with a kd of 0.036 h⁻¹ (midge kd at 10°C, 0.018 h⁻¹). Niimi and Palazzo (1985) measured the elimination of hexaCB from rainbow trout at three different temperatures and found the kd's to be about an order of magnitude slower than the midge. Giam et al. (1980), however, found killifish eliminated hexaCB about 25
times faster than the midge. A depuration experiment conducted by Ernst (1977) reported a $kd$ of 0.0313 h$^{-1}$ for lindane in the mussel, *Mytilus edulis*, similar to the $kd$ of 0.059 h$^{-1}$ found in this work for lindane in the midge at 22°C.

Elimination of all the chemicals, as reflected by the $kd$ values, increased as temperature increased (Figure 4). The compounds diCB, triCB, tetraCB, lindane, and DDE generally followed a $Q_{10}$ coefficient of 2 in that $kd$ approximately doubled with a 10°C increase in temperature (Figure 4). According to Kelister and Buck (1974) simple chemical reactions generally follow the Van’t Hoff rule of $Q_{10} = 2$, but biological processes can vary widely. Murphy and Murphy (1971) did find, however, both respiration ($O_2$ uptake) and DDT uptake in mosquito fish followed the Van’t Hoff rule.

An exception to the $Q_{10}$ coefficient of 2 was found in the depuration of hexaCB whose $kd$ values increased 5-fold over an 8°C increase in temperature (only $kd$ values that could be determined were at 22°C and 30°C). Niimi (1985) reported a response to temperature with hexaCB in rainbow trout similar to that of diCB, triCB, tetraCB, lindane, and DDE in the midge. Niimi found the $kd$ values to increase progressively from 4°C to 12°C to 18°C and that they generally doubled over a 10°C increase in temperature. Gerould et al. (1983) reported the depuration of anthracene from *C. riparius* was not as dependent on temperature by showing insignificant differences in $kd$ values between 16°C, 25°C, and 30°C. Landrum (1982) investigated the depuration of anthracene from the scud, *Pontoporeia hovii*, at 4°C, 7°C, and 10°C and found a 2.5-fold increase in $kd$ from 4°C to 7°C and then a decrease at
Landrum suggested that 7°C was an optimum temperature for *P. hovii* since it inhabits the deep benthic region of Lake Michigan and has been found to reproduce optimally in the laboratory at 7°C. The kd values' varying response to an increase in temperature for the poikilothermic organisms reviewed above suggests that biological processes may influence the elimination of xenobiotic compounds. Other depuration studies, such as vanadium in shrimp (Miramand *et al.*, 1981) and phenanthrene in mussels (Solbakken *et al.*, 1983) have shown elimination increased with increased temperature.

Midge larvae contain only 1.6% lipid (Shank, 1984) whereas rainbow trout contain about 8% (Oliver, 1987) and guppies 5.4% (Konemann and van Leeuwen, 1980). Even with this low lipid level the midge, as has been shown with fish, can function as an organic phase in a partitioning process of a chemical with water. The more water soluble a compound is, the faster it will transfer from the organic phase to the aqueous phase. Conversely, the more lipid soluble (higher *K*<sub>ow</sub>) a compound is, the slower it will transfer from the organic phase to the aqueous phase.

The relationship described above was found to exist with the organochlorines used in this study with the midge. The transfer from the organic phase (midge) to the aqueous phase, as determined by the kd value, was found to increase as the water solubility of the chemical increased. This relationship is illustrated in the kd x MW vs. log AS and kd x MW vs. log *K*<sub>ow</sub> correlations in Figures 22 and 23.

Multiplying the rate constant by the molecular weight of the compound increased the correlations with AS and *K*<sub>ow</sub>. This
transformation was done by Lohner and Collins (1987) to enhance the correlation of the uptake rate constant, ku, with log AS. Since diffusion of a chemical is inversely related to its molecular weight (Starr and Taggart, 1981), the increased correlation due to the transformation by multiplying by MW seems reasonable. It has also been shown that for compounds with K_{OW}'s above 6 or have a molecular weight greater than 290, the transfer processes between organism and water and octanol and water are impeded (Konemann and van Leeuwen, 1980; Zitko, 1976; Mackay, 1980).

A correlation of log kd with log K_{OW} for 6 chlorobenzenes in guppies (Konemann and van Leeuwen, 1980) was found to be similar to the same correlation in the midge. Konemann and van Leeuwen reported a linear regression equation, log kd = 1.44 - 0.42 log K_{OW}, for this correlation which had an r^2 of 0.98. Linear regression of the same correlation for the data in this work yielded the equation, log kd = 1.85 - 0.76 log K_{OW} (r^2 = 0.85). Spacie and Hamelink (1982) combined the data from Konemann and van Leeuwen with the fish data from Neely et al. (1974) and performed the same regression analysis yielding the equation, log kd = 1.47 - 0.41 log K_{OW} (r^2 = 0.90). A comparison of the slopes in the regression equations between the midge (-0.76) and fish (-0.41) reflects that the midge kd's increased faster with decreased lipid solubility. This difference may be a function of the lower lipid content in the midge relative to fish.

C. Uptake

The uptake of organochlorines by the midge was faster, compared to other species. The midge at 22°C sorbed diCB 17 times more than
guppies at 21°C (Konemann and van Leeuwen, 1980), as determined by the ku values (midge, 65 mlg\(^{-1}\)h\(^{-1}\); guppies, 3.75 mlg\(^{-1}\)h\(^{-1}\) [gram units of guppies are a wet weight conversion from a lipid weight]). Galassi et al., (1983) measuring the uptake of diCB in rainbow trout alevins at 10°C and Neely et al., (1974) measuring diCB uptake in rainbow trout muscle also reported lower ku values (Galassi, 7.8 mlg\(^{-1}\)h\(^{-1}\); Neely, 5.7 mlg\(^{-1}\)h\(^{-1}\)) than was found for the midge (35 mlg\(^{-1}\)h\(^{-1}\) at 10°C). Ernst (1977) reported a ku value of 3.13 mlg\(^{-1}\)h\(^{-1}\) for lindane uptake in mussels, approximately one-ninth the ku for lindane in the midge (28 mlg\(^{-1}\)h\(^{-1}\)). Neely et al., (1974) and Konemann and van Leeuwen (1980) found similar ku values of 19 and 21 mlg\(^{-1}\)h\(^{-1}\) for hexaCB in rainbow trout muscle and guppies, respectively. These ku values are approximately one-fifteenth the ku value (311 mlg\(^{-1}\)h\(^{-1}\)) found in the midge at 22°C. The faster uptake by the midge was also reported from an investigation by Muir et al., (1982) comparing the accumulation dynamics of two herbicides between midges and rainbow trout. The midge, *G. tentans*, was found to have ku values 28 and 3 times higher than the rainbow trout for fluridone and terbutryn, respectively. ku values obtained for other hydrophobic compounds in arthropods compare favorably to the present study. Gerould et al., (1983) and Leversee et al., (1982) reported ku values of 155 and 214 mlg\(^{-1}\)h\(^{-1}\) for anthracene and benzo(a)pyrene in *G. riparius* and Landrum (1982) reported ku values of 125 to 200 mlg\(^{-1}\)h\(^{-1}\) for anthracene in *P. hoyi*.

The ku values reported by Lohner and Collins (1987) for diCB, tetraCB, lindane, and DDE in *G. riparius* were reported in liter x 10\(^{-4}\) midge\(^{-1}\)h\(^{-1}\) units. Assuming a midge larva weighs 5 mg those ku units
can be converted to $\text{mlg}^{-1}\text{h}^{-1}$ (the form of data in this work). Table 24 lists the $k_u$ values obtained in the present study at 22°C and the converted $k_u$ values obtained at room temperature by Lohner and Collins.

Table 24

A Comparison of $k_u$ Values for diCB, tetraCB, lindane, and DDE in *Chironomus riparius*

<table>
<thead>
<tr>
<th>chemical</th>
<th>present study$^a$</th>
<th>Lohner and Collins$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>diCB</td>
<td>65</td>
<td>109</td>
</tr>
<tr>
<td>lindane</td>
<td>28</td>
<td>76</td>
</tr>
<tr>
<td>tetraCB</td>
<td>177</td>
<td>214</td>
</tr>
<tr>
<td>DDE</td>
<td>150</td>
<td>390</td>
</tr>
</tbody>
</table>

$^a$ values obtained at 22°C  
$^b$ values obtained at room temperature

The $k_u$ values obtained in the present study were all lower than the values found by Lohner and Collins. The reason for the lower values for diCB, tetraCB, and lindane was due to the different method of calculating $k_u$ values. Lohner and Collins used shorter exposure periods, calculated $k_u$ by the slope method, and forced the beginning of the curve through the origin. The result was a $k_u$ value that included the fast uptake process as well as the slow uptake process. The $k_u$ values of the present study primarily describe the slow uptake process by the midge, resulting in lower $k_u$ values. The
difference between DDE values cannot be explained with the present information.

The uptake rate constants generally did not increase with temperature as fast as the depuration rate constants. For example, the ku values of dieldrin and lindane increased 2.3 times and 1.7 times from 10°C to 30°C, whereas the kd values increased 6.3 times and 4.2 times, respectively over the same temperature range. The ku values of DDE increased only 1.5 times from 22°C to 30°C compared to a 2.5 times increase in kd. The ku values for triCB and tetraCB exhibited similar increases to kd between 10°C and 22°C, but then declined at 30°C, whereas kd still increased. The differences in the magnitude of response to temperature between ku and kd will be discussed further in the BCF section.

My determination that an increase in temperature from 22°C to 30°C caused no increase in ku for triCB, tetraCB, and hexaCB was not found by Landrum (1982) for anthracene in P. hovii. However, Gerould et al., (1983) did find no basic change in ku from 25°C (155 mg⁻¹h⁻¹) to 30°C (152 mg⁻¹h⁻¹) for anthracene in C. riparius. Reasons for this phenomenon at this point are purely speculative but may pertain to the wax layer of the epicuticle. The wax layer is a mixture of C_{25}-C_{31} hydrocarbons and esters of even-numbered fatty acids and alcohols (C_{24}-C_{34}) (Locke, 1974). Lockey (1976) states that the wax layer is characterized by weak molecular interactions comparable in intensity to van der Waals interactions. The increased temperature may provide sufficient energy to destroy the integrity of this layer and thereby affect its’ role in the partitioning process and its’ permeability.
Higher temperatures may also cause adverse physiological effects on the whole animal and resulting in a decrease in uptake.

An alternative hypothesis for explaining the lack of increase in ku from 22°C to 30°C is relative to a decrease in the affinity of the chemicals with the wax layer at the higher temperature. Recent investigations (Jones and Lee, 1985; Antunes-Madeira and Madeira, 1986; Antunes-Madeira and Madeira, 1984) have demonstrated a decrease in the partitioning of hydrophobic compounds (lindane, DDT, and parathion) into synthetic lipid bilayers, liposomes, and native membranes with an increase in temperature. The increase in temperature from 22°C to 30°C may be a range where a decrease in the partitioning process eclipsed or superceded the increase due to the effect of the Q\textsubscript{10} coefficient (includes chemical reaction rates as well as biological), resulting in a ku value that did not increase or decrease.

The correlations of the product \( k_u \times MW \) with log AS and \( k_u \times MW \) with log \( K_{ow} \) for this study were not as highly correlated as was found by Lohner and Collins. Lohner and Collins reported a higher \( r^2 \) (0.97) for \( k_u \times MW \) with log AS than their correlation of \( k_u \times MW \) with log \( K_{ow} \) (\( r^2 = 0.93 \)). The present study found \( k_u \times MW \) correlated with log \( K_{ow} \) (\( r^2 = 0.83 \)) to have the higher \( r^2 \) value (\( k_u \times MW \) with log AS, \( r^2 = 0.62 \)). DDE was the outlier in these relationships (Figures 20 and 21) as evidenced by the increased \( r^2 \) values (0.88, log AS: 0.98, log \( K_{ow} \)) obtained when both correlations were analyzed without the DDE coordinates. The DDE ku values were repeatable in separate replicated experiments with both radiolabelled DDE and nonradiolabelled DDE. DDE was the only biphenyl structure used in these experiments. Perhaps a
structural or steric hinderence due to the biphenyl configuration caused the decreased ku of DDE relative to the other compounds.

D. BCF

BCF data in the literature for the six organochlorines used in this study are predominantly for fish. The BCF values for the midge were about the same or higher compared to literature BCF values for fish and other species. Table 25 lists some literature BCF values for the test compounds, the test organisms, the temperature the literature value was obtained at (if given), and the midge BCF obtained at a comparable temperature in this study.

The negative temperature correlation of BCF with temperature (Figure 17) that was found for organochlorines in the midge has also been reported by other investigators. Harris et al., (1977) found petroleum hydrocarbon BCF's to be negatively correlated to temperature in copepods. Fucik and Neff (1977) reported naphthalene BCF's to have a negative correlation with temperature in two species of clams and Collier et al., (1978) found naphthalene to be at much higher levels in various tissues of coho salmon at 4°C than at 10°C. The BCF was unaffected by temperature in investigations with anthracene in C. riparius (Gerould et al., 1983) and with hydrocarbons in M. edulis (Fossato and Canzonier, 1976). In contrast, BCF has also exhibited a positive correlation to temperature with DDT in rainbow trout (Reinert et al., 1974) and with arochlor 1254 in fathead minnows, rainbow trout, and green sunfish (Veith et al., 1979).

It has been suggested by Davies and Dobbs (1984) that the reason BCF increases with temperature is that the uptake rate of the chemical
increases more than the elimination rate when temperature increases. The present study, for the most part, demonstrated the reverse occurs.

Table 25

Comparison of BCF Values for Six Organochlorines with
Chironomus riparius to Literature BCF Values

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Species</th>
<th>BCF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Temp (°C)</th>
<th>Midge BCF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>dICB</td>
<td>guppy</td>
<td>97</td>
<td>21</td>
<td>185</td>
<td>1</td>
</tr>
<tr>
<td>dICB</td>
<td>alevin</td>
<td>60</td>
<td>10</td>
<td>270</td>
<td>2</td>
</tr>
<tr>
<td>dICB</td>
<td>trout muscle</td>
<td>215</td>
<td>12</td>
<td>270</td>
<td>3</td>
</tr>
<tr>
<td>triCB</td>
<td>hatched trout</td>
<td>349</td>
<td>10</td>
<td>2389</td>
<td>4</td>
</tr>
<tr>
<td>triCB</td>
<td>fathead</td>
<td>2100</td>
<td>15</td>
<td>2630</td>
<td>5</td>
</tr>
<tr>
<td>triCB</td>
<td>sunfish</td>
<td>2300</td>
<td>15</td>
<td>2630</td>
<td>5</td>
</tr>
<tr>
<td>triCB</td>
<td>trout</td>
<td>890</td>
<td>15</td>
<td>2630</td>
<td>5</td>
</tr>
<tr>
<td>triCB</td>
<td>Chlorella</td>
<td>250</td>
<td>NG</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>lindane</td>
<td>M. edulis</td>
<td>100</td>
<td>NG</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>lindane</td>
<td>killifish</td>
<td>375</td>
<td>NG</td>
<td></td>
<td>8</td>
</tr>
<tr>
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<td>guppy</td>
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<td>clam</td>
<td>121</td>
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<tr>
<td>lindane</td>
<td>fathead</td>
<td>180</td>
<td>25</td>
<td>475</td>
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<tr>
<td>lindane</td>
<td>Chlorella</td>
<td>240</td>
<td>NG</td>
<td></td>
<td>6</td>
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<tr>
<td>tetraCB</td>
<td>trout</td>
<td>5300 to 13000</td>
<td>15</td>
<td>2388</td>
<td>10</td>
</tr>
<tr>
<td>hexaCB</td>
<td>trout muscle</td>
<td>7880</td>
<td>12</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>hexaCB</td>
<td>Chlorella</td>
<td>24000</td>
<td>NG</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>hexaCB</td>
<td>guppy</td>
<td>15660</td>
<td>21</td>
<td>111000</td>
<td>1</td>
</tr>
<tr>
<td>hexaCB</td>
<td>fathead</td>
<td>16200</td>
<td>15</td>
<td></td>
<td>5</td>
</tr>
<tr>
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<td>sunfish</td>
<td>21900</td>
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<td>5</td>
</tr>
<tr>
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<td>fathead</td>
<td>18500</td>
<td>25</td>
<td>111000</td>
<td>5</td>
</tr>
<tr>
<td>DDE</td>
<td>fathead</td>
<td>51000</td>
<td>25</td>
<td>93750</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>a</sup> BCF based on weight wet

<sup>b</sup> Temperature of midge BCF closest to literature temperature; all data by McIntyre

NG not given

ref: 'Konemann and van Leeuwen, 1980; 2Galassi et al., 1982; 3Neely et
for organochlorines in the midge. With the exception of trichlorobenzene (trichloro) and tetrachloro from 10°C to 22°C, the $k_d$ values all increased proportionately more than the $k_u$ values when temperature increased. Since BCF was calculated as the ratio, $k_u/k_d$, the resultant quotient would decrease with temperature due to a proportionately larger denominator ($k_d$) at higher temperatures. It should be noted that the sharpest declines in BCF with temperature were for trichloro, tetrachloro, and hexachlorobenzene (hexachloro) from 22°C to 30°C which was due primarily to the lower $k_u$ values determined at 30°C.

A number of hypotheses can be given for the negative correlation of BCF with temperature. Heisig-Gunkel and Gunkel (1982) found that the fat content in *Daphnia* had decreasing importance in atrazine accumulation as temperature increased. This finding supports a lowered partitioning capability of the chemical by the midge at higher temperatures as an explanation of the negative correlation. Such a hypothesis is also supported by the negative temperature correlations reported for partition coefficients (synthetic lipid bilayer, liposomes, native membranes) with hydrophobic compounds (as discussed in the $k_u$ section). The synthetic bilipid, diolphosphatidylycholine, partition coefficient for lindane, for example, decreased about 44% from 10°C to 30°C (Jones and Lee, 1985), which compares favorably to the 58% decline in BCF for lindane over the same temperature range found in this work. The chemicals, dichloro, trichloro, and tetrachloro similarly showed decreases in BCF values of 41%, 49%, and 33%, respectively. The negative...
relationship of BCF with temperature for organochlorines in *C. riparius* (Figure 17) was consistent with the negative temperature correlation reported for partition coefficients with the hydrophobic compounds, DDT, lindane, and parathion (Jones and Lee, 1985; Antunes-Madeira and Madeira, 1986; Antunes-Madeira and Madeira, 1984).

In a study by Opperhuizen et al. (1988) that used a series of chlorinated benzenes very similar to this study (1,3-dichlorobenzene, 1,3,5-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene) the effect of temperature on $K_{OW}$ and BCF values in guppies was reported. They found that all the chlorinated benzenes had a negative temperature correlation for $K_{OW}$ similar to the negative temperature correlation for lindane, DDT, and parathion using synthetic and native membrane/water partition coefficients (mentioned in the previous paragraph). The BCF values of the fish, however, were found to have positive temperature correlations for all five chlorinated benzenes. The authors discussed that the differences in the thermodynamic properties were due to an endothermic enthalpy change in the movement of the solute from the water to the fish and an exothermic enthalpy change in the movement from water to octanol. They hypothesized that the differences in enthalpy changes were caused by the different structure between fish lipids and octanol. If this hypothesis is true it should also be true for midges because the lipid structure of midges should be much closer to the structure of fish lipids than the structure of octanol.

Adsorption to a tissue such as the midge cuticle is another hypothesis that supports increased accumulation at decreased
temperatures. McEwen and Stephenson (1979) explain pesticide adsorption as an exothermic process, stating that when energy is added as heat the bonds adsorbing the chemical are broken and desorption occurs. Atrazine, diuron, and lindane have exhibited increased adsorption to various sediments at lower temperatures (McGlamery and Slife, 1976; Corwin and Farmer, 1980; Mills and Biggar, 1969). Whether this temperature-dependent phenomenon occurs with insect cuticles has not been shown. Wilkes and Weiss (1971) report, however, that a large portion of DDT accumulation in dragonfly nymphs is due to adsorption and Leversee et al., (1982) found 10% of the benzo(a)pyrene accumulation in C. riparius associated with exoskeleton.

BCF's were much more closely correlated to the physical/chemical properties of the test compounds than were ku or kd values. The higher $r^2$ values for the log BCF vs. log AS plot (Figure 18, $r^2 = 0.88$) and the log BCF vs. log $K_{ow}$ plot (Figure 19, $r^2 = 0.96$) correlations demonstrate this point. The fact that BCF and the physical/chemical values, $K_{ow}$ and AS, are thermodynamic properties, may contribute to this increased correlation over the kinetic constants, kd and ku.

A review paper on the prediction of BCF in fish by Davies and Dobbs (1984) lists a number of linear equations for these correlations that were collected from experimental investigations. The slopes of the equations correlating log BCF to log AS ranged from -0.32 to -0.85 of which the log AS coefficient from the present study falls in the middle (-0.52). The range of log $K_{ow}$ coefficients given in the review was 0.46 to 0.94. The 1.06 log $K_{ow}$ coefficient for the midge data was just outside this range, but as stated by Chiou (1985) who reported a
slope of 0.96 for a log (triolein/water) partition coefficient correlation with fish, the fact that the slope is nearly one and the intercept is small supports the postulate that lipid content is mainly responsible for accumulation of organic chemicals. The log $K_{ow}$ was the better predictor for all the accumulation parameters examined in this work as determined by the higher $r^2$ values. This finding is supported by Mackay et al. (1980) who suggested $K_{ow}$ is better than AS in correlating the partitioning behavior of organic solutes from water into biota since it is a more direct measurement of the activity coefficient of the solute in water.

E. Toxicity

The most acutely toxic of the test chemicals to the midge by far was the insecticide, lindane (Table 23). The EC$_{50}$ at 22°C for lindane was 2.0 ug/L which was similar to the EC$_{50}$ of 3.6 ug/L found by Estenik (1978) who used the same culture of C. riparius ten years earlier. Lindane was almost 200 times more toxic than tetraCB (EC$_{50}$ at 22°C, 343 ug/L), the most toxic (according to the aqueous concentration) of the three chlorinated benzenes that expressed acute toxicity to the midge. Lindane, an axonal poison, has a different mode of action than the chlorobenzenes, which have a general narcotic property (Van Hoogen and Opperhuizen, 1988). According to the EC$_{50}$ values the toxicity of the three chlorinated benzenes increased as the degree of chlorination increased.

The effect of temperature on the acute toxicity was varied when the chemicals were tested at 10°C and 21°C (Table 23). Two chemicals, dicCB and tricCB, were found to be more toxic at the lower temperature.
while tetraCB was toxic at 21°C but not at 10°C, and lindane had statistically the same acute toxicity at both temperatures.

An estimate of the amount of chemical accumulated in the midge (Ca) that caused a toxic response to 50% of the test population can be made using the equation, $Ca = \frac{\langle ku / kd \rangle Cw}{1 - e^{-kd t}}$ (see Accumulation Model in the Introduction, equation 2) ($Cw = EC_{50}$; $t$ = exposure time of the toxicity). A comparison of the toxicity between temperatures can then be made relative to the toxicokinetic estimate, Ca. Table 26 lists these estimates for diCB, triCB, tetraCB, and lindane at both temperatures along with the EC$_{50}$ values.

Table 26
Toxicokinetic Estimates for the Amount of diCB, triCB, tetraCB, and lindane Accumulated in the Midge (Ca) During the Exposure Time of Toxicity Tests and Their EC$_{50}$ Values

<table>
<thead>
<tr>
<th></th>
<th>10°C Ca</th>
<th>EC$_{50}$</th>
<th>21°C Ca</th>
<th>EC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>diCB</td>
<td>5.1 umol/g</td>
<td>1.6 mg/L</td>
<td>3.6 umol/g</td>
<td>2.5 mg/L</td>
</tr>
<tr>
<td>triCB</td>
<td>5.3 umol/g</td>
<td>1.1 mg/L</td>
<td>12.6 umol/g</td>
<td>1.5 mg/L</td>
</tr>
<tr>
<td>tetraCB</td>
<td>...</td>
<td>&gt;0.5 mg/L</td>
<td>3.4 umol/g</td>
<td>0.3 mg/L</td>
</tr>
<tr>
<td>lindane</td>
<td>3.4 nmol/g</td>
<td>2.7 ug/L</td>
<td>2.5 nmol/g</td>
<td>2.0 ug/L</td>
</tr>
</tbody>
</table>

*a units are in umol chemical/g midge*

The toxicokinetic estimates of diCB accumulation agreed with the toxicity data in that the amount of chemical that accumulated over a 24-hour exposure at 10°C was equal to or greater than the amount accumulated at 21°C. This was reflected in the lower EC$_{50}$ at 10°C. The Ca's for triCB, however, at 10°C (5.3 umol/g) was less than half
that at 21°C (12.6 umol/g), whereas greater toxicity was observed at 10°C. The acute toxicity of lindane was not significantly different between 10°C and 21°C, however, the Ca estimate showed a slightly greater accumulation at 10°C.

The acute toxicity to diCB and tetraCB at 21°C as determined by EC₅₀ values was found to be 2.5 mg/L and 0.3 mg/L, respectively. However, when the estimated Ca's of diCB and tetraCB that caused a toxic response in 50% of the test population are compared the values were similar (diCB, 3.6 umol/g; tetraCB, 3.4 umol/g). This agreed with Hoogen and Opperhuizen (1988) who reported the lethal dose of trichlorobenzene, tetrachlorobenzene, and pentachlorobenzene in fish to be between 2.0 and 2.5 umol/g fish indicating that the chlorobenzenes may have relatively equal toxicity to aquatic organisms such as fish and midges but accumulation properties of each chemical may cause different sensitivities to exposure concentrations.

F. Environmental Significance

The environmental significance of the effect of temperature on the accumulation of chemicals may best be addressed by two hypothetical scenarios describing the exposure of contaminants in the aquatic environment.

The first scenario is an oil spill that is discharged into a river. Such an event introduces to the river a large number of hydrophobic chemicals at very high environmental concentrations. The spill enters the river and is swept downstream as a plume exposing aquatic organisms to the contaminants until the plume flows past. (Albeit some of the contaminants will partition into the sediment
Increasing the exposure period at lower concentrations.) With water sampling analyses and flow rate information the length of the plume and the length of time a section of the river will be exposed to the plume can be determined. Given that the plume takes ten hours to pass a cross-section of the river, and given that the plume contains tetraCB as one of the contaminants at a concentration of 0.5 mg/L, a comparison can be made (using simplified conditions) of the estimated accumulation of tetraCB by a population of midges in February when the river is 10°C relative to June when the river is 22°C. Again using equation 2, $C_a = \left(\frac{k_u}{k_d}\right)C_w \times (1 - e^{-kt})$, and the toxicokinetic rate constants obtained at each temperature, estimates of tetraCB accumulation in the midges in February and June can be made. In February the midges would accumulate an estimated 1 umol tetraCB/g midge after ten hours and in June the estimated accumulation is 3 umol/g after the same exposure period. The effect of temperature, therefore, is a three-fold increase in body burden in June over February which may be a differential critical to survivorship of the population.

Once a plume has passed over a cross-section of a river, temperature can have a significant impact on the $t_{1/2}$ of the chemicals in the organisms that inhabit the cross-section. For example, if a population of midges accumulated 20 ug hexaCB/g midge it would take approximately 52 days to eliminate 97% of the body burden (to approximately 0.6 ug/L) at 22°C. If, however, the temperature was raised to 30°C the loss of 97% of the hexaCB would only take 12 days. Temperature, therefore, is an important factor in the ability to predict the persistence of contaminants in a population of midges which
subsequently has relevance to fish populations that feed on the midges and ultimately man which feed on the fish.

The second example that illustrates the significance of temperature on the accumulation of chemicals in aquatic organisms is a situation where organisms are continuously exposed to low concentrations of hydrophobic organic contaminants (as opposed to temporary exposures to high levels as discussed above). Under a constant exposure to accumulative compounds a steady-state condition of chemical accumulation in aquatic organisms may be obtained. For certain hydrophobic chemicals temperature, as determined in this study, can have a significant effect on the magnitude of chemical accumulation. Hazard prediction of these compounds would be enhanced if there was information on increased accumulation at certain temperatures. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substance Control Act (TSCA), the Environmental Protection Agency (EPA) regulates the use of agricultural and industrial chemicals. One of the criteria that determines the regulation of these chemicals is the extent to which they bioaccumulate which is estimated from laboratory BCF determinations. To better estimate the potential hazard of a chemical an effort should be made to obtain BCF values at the temperature where accumulation is the highest.

Additionally, the significance of the negative correlation between BCF and temperature may explain some of the seasonal variation found with residue levels in aquatic biota. The hydrophobic compounds that exhibit a negative temperature coefficient would accumulate to higher levels in the colder months.
The negative temperature coefficient for BCF is also consistent with the negative temperature coefficient for toxicity of the hydrophobic compounds, DDT, methoxychlor, and pyrethroids to aquatic organisms. The target site for these insecticides is thought to be axonal membranes which are composed of mostly lipids. A decrease in temperature would then increase the partitioning of the compound into the animal, and once inside the animal increase the partitioning to the axonal membrane.

Conclusions

1. The accumulation dynamics of the six test compounds in the midge did not fit the mathematical model, \( \frac{dC_a}{dt} = k_u C_w - k_d C_a \). In order to build a more accurate model, additional compartments must be sampled (pers. comm., Yowming Wang).

2. Both \( k_u \) and \( k_d \) generally increase with increases in temperature from 10°C to 30°C. Generally \( k_d \) was more sensitive to temperature than \( k_u \).

3. BCF generally declined with an increase in temperature. Kinetically, this was due to \( k_d \) increasing faster than \( k_u \) to an increase in temperature (conclusion 3). Thermodynamically, this was due to the hydrophobic compounds not partitioning into the midge as much at the higher temperatures.

4. Midge \( k_u \)'s were found to be higher than fish \( k_u \)'s in the literature.

5. Midge BCF values were generally higher than literature BCF values for other species.

6. The correlations relating \( k_u \), \( k_d \), or BCF with \( K_{ow} \) were better than the correlations with AS.
7. BCF with $K_{ow}$ had the highest correlation.
REFERENCES


OECD. 1981. OECD guidelines for testing of chemicals, section 107, p. 3.


Ribeyre, F. and A. Boudou. 1982. Study of the dynamics of the accumulation of two mercury compounds - HgCl\textsubscript{2} and CH\textsubscript{3}HgCl - by Chlorella vulgaris: effect of temperature and pH factor of the environment. Intern. J. Environ. Stud. 20:35-40.


APPENDIX A

ACCUMULATION OF $^{14}$C-DDE IN CHIRONOMUS RIPARIUS
ANALYZED BY THREE DIFFERENT METHODS
Table 27

Accumulation of $^{14}$C-DDE, Ca, in *Chironomus riparius* Analyzed by Three Different Sample Preparation Methods

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Dioxane$^b$</th>
<th>TS$^c$</th>
<th>M &amp; L$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0.043 ± 0.009</td>
<td>0.039 ± 0.009</td>
<td>0.039 ± 0.002</td>
</tr>
<tr>
<td>3</td>
<td>0.076 ± 0.015</td>
<td>0.076 ± 0.010</td>
<td>0.064 ± 0.009</td>
</tr>
<tr>
<td>6</td>
<td>0.185 ± 0.021</td>
<td>0.173 ± 0.038</td>
<td>0.153 ± 0.023</td>
</tr>
<tr>
<td>9</td>
<td>0.260 ± 0.069</td>
<td>0.309 ± 0.021</td>
<td>0.300 ± 0.050</td>
</tr>
<tr>
<td>12</td>
<td>0.435 ± 0.092</td>
<td>0.438 ± 0.010</td>
<td>0.518 ± 0.104</td>
</tr>
<tr>
<td>24</td>
<td>1.288 ± 0.171</td>
<td>0.904 ± 0.189</td>
<td>1.046 ± 0.114</td>
</tr>
</tbody>
</table>

$^a$ each Ca value is a mean of three replicates

$^b$ extraction by dioxane cocktail method

$^c$ TS = tissue solubilizer method

$^d$ M & L = Mahin and Lofberg oxidation method
APPENDIX B

AQUEOUS CONCENTRATIONS OF TEST CHEMICALS IN THE UPTAKE EXPOSURE TESTS
Table 28

Aqueous Concentrations (C<sub>W</sub>) of dICB in the Uptake Exposure Tests

<table>
<thead>
<tr>
<th>Sampling Time (h)</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>3.42 (0.34)</td>
<td>3.30 (0.07)</td>
<td>3.89 (0.05)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>3.35 (0.45)</td>
<td>3.65 (0.12)</td>
<td>2.78 (0.65)</td>
<td>3.94 (0.07)</td>
</tr>
<tr>
<td>1</td>
<td>3.53 (0.39)</td>
<td>3.80 (0.14)</td>
<td>3.32 (0.09)</td>
<td>3.86 (0.10)</td>
</tr>
<tr>
<td>2</td>
<td>3.61 (0.33)</td>
<td>3.17 (0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.25</td>
<td></td>
<td>3.57 (0.25)</td>
<td></td>
<td>3.96 (0.02)</td>
</tr>
<tr>
<td>3</td>
<td>3.51 (0.25)</td>
<td>3.05 (0.78)</td>
<td></td>
<td>3.90 (0.10)</td>
</tr>
<tr>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3.67 (0.34)</td>
<td>3.84 (0.10)</td>
<td>3.84 (0.13)</td>
<td></td>
</tr>
<tr>
<td>5.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3.68 (0.29)</td>
<td>3.18 (0.11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>3.13 (0.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.25</td>
<td></td>
<td>3.77 (0.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.25</td>
<td>3.71 (0.33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x&lt;sub&gt;overall&lt;/sub&gt;</td>
<td>3.55 (0.11)</td>
<td>3.71 (0.06)</td>
<td>3.25 (0.10)</td>
<td>3.91 (0.03)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Each C<sub>W</sub> value for each sampling time represents a mean of three water samples taken from each of three replicate exposure bottles.
Table 29
Aqueous Concentrations (C<sub>w</sub>) of triCB in the Uptake Exposure Tests

<table>
<thead>
<tr>
<th>Sampling Time (h)</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>4.27 (0.09)</td>
<td>3.48 (0.06)</td>
<td>1.96 (0.10)</td>
<td>2.86 (0.14)</td>
</tr>
<tr>
<td>0.5</td>
<td>4.30 (0.02)</td>
<td>3.08 (0.17)</td>
<td>1.92 (0.21)</td>
<td>3.23 (0.09)</td>
</tr>
<tr>
<td>1</td>
<td>4.31 (0.02)</td>
<td>3.01 (0.29)</td>
<td>4.04 (0.57)</td>
<td>3.40 (0.17)</td>
</tr>
<tr>
<td>2</td>
<td>3.02 (0.77)</td>
<td>2.51 (0.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.25</td>
<td></td>
<td></td>
<td>3.42 (0.11)</td>
<td>3.31 (0.38)</td>
</tr>
<tr>
<td>4.25</td>
<td></td>
<td></td>
<td></td>
<td>3.05 (0.06)</td>
</tr>
<tr>
<td>4.5</td>
<td>3.48 (0.14)</td>
<td>1.99 (0.06)</td>
<td>3.32 (0.09)</td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>3.81 (0.03)</td>
<td>2.40 (0.23)</td>
<td>3.00 (0.01)</td>
<td>3.12 (0.11)</td>
</tr>
<tr>
<td>( \bar{x} ) overall</td>
<td>3.98 (0.09)</td>
<td>2.74 (0.14)</td>
<td>2.99 (0.18)</td>
<td>3.26 (0.09)</td>
</tr>
</tbody>
</table>

*a Each C<sub>w</sub> value for each sampling time represents a mean of three water samples taken from each of three replicate exposure bottles.
Table 30

Aqueous Concentrations ($C_w$) of tetraCB in the Uptake Exposure Tests

<table>
<thead>
<tr>
<th>Sampling Time (h)</th>
<th>$10^\circ$C</th>
<th>$16^\circ$C</th>
<th>$22^\circ$C</th>
<th>$30^\circ$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>3.66 (0.26)</td>
<td>4.58 (0.12)</td>
<td>3.41 (0.09)</td>
<td>3.24 (0.73)</td>
</tr>
<tr>
<td>0.5</td>
<td>4.01 (0.10)</td>
<td>4.15 (0.10)</td>
<td>3.45 (0.08)</td>
<td>2.43 (0.11)</td>
</tr>
<tr>
<td>1</td>
<td>4.84 (0.10)</td>
<td>4.56 (0.19)</td>
<td>4.08 (0.40)</td>
<td>3.06 (0.08)</td>
</tr>
<tr>
<td>2</td>
<td>4.37 (0.25)</td>
<td>4.14 (0.08)</td>
<td>4.19 (0.19)</td>
<td>3.22 (0.07)</td>
</tr>
<tr>
<td>4.5</td>
<td></td>
<td>4.13 (0.24)</td>
<td>3.60 (0.08)</td>
<td>3.80 (0.09)</td>
</tr>
<tr>
<td>4.75</td>
<td>4.40 (0.21)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>4.04 (0.11)</td>
<td>4.37 (0.16)</td>
<td>3.32 (0.09)</td>
<td>3.93 (0.16)</td>
</tr>
<tr>
<td>$\bar{C}_w$ overall</td>
<td>4.27 (0.11)</td>
<td>4.43 (0.07)</td>
<td>3.68 (0.11)</td>
<td>3.26 (0.13)</td>
</tr>
</tbody>
</table>

Each $C_w$ value for each sampling time represents a mean of three water samples taken from each of three replicate exposure bottles.
Table 31
Aqueous Concentrations (Cw) of hexaCB in the Uptake Exposure Tests

<table>
<thead>
<tr>
<th>Sampling Time (h)</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.60 (0.07)</td>
<td>0.76 (0.26)</td>
<td></td>
<td>0.66 (0.01)</td>
</tr>
<tr>
<td>1</td>
<td>0.64 (0.11)</td>
<td></td>
<td>0.73 (0.01)</td>
<td>0.75 (0.03)</td>
</tr>
<tr>
<td>1.25</td>
<td>0.83 (0.12)</td>
<td></td>
<td></td>
<td>0.76 (0.06)</td>
</tr>
<tr>
<td>1.5</td>
<td>0.67 (0.03)</td>
<td>0.55 (0.04)</td>
<td>0.67 (0.08)</td>
<td>0.75 (0.04)</td>
</tr>
<tr>
<td>2</td>
<td>0.66 (0.09)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.72 (0.08)</td>
<td>0.96 (0.15)</td>
<td>0.58 (0.03)</td>
<td>0.05 (0.07)</td>
</tr>
<tr>
<td>4.5</td>
<td>0.86 (0.03)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.75</td>
<td>0.84 (0.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>0.78 (0.07)</td>
<td></td>
<td>0.54 (0.03)</td>
<td></td>
</tr>
<tr>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.75 (0.04)</td>
<td>0.76 (0.07)</td>
<td>0.62 (0.02)</td>
<td>0.69 (0.03)</td>
</tr>
</tbody>
</table>

\( \bar{X} \) overall

Each Cw value for each sampling time represents a mean of three water samples taken from each of three replicate exposure bottles.
Table 32
Aqueous Concentrations (Cw) of lindane in the Uptake Exposure Tests

<table>
<thead>
<tr>
<th>Sampling Time (h)</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.49 (0.04)</td>
<td>0.44 (0.01)</td>
<td>0.47 (0.01)</td>
<td>0.46 (0.01)</td>
</tr>
<tr>
<td>0.5</td>
<td>0.59 (0.01)</td>
<td>0.49 (0.01)</td>
<td>0.47 (0.01)</td>
<td>0.48 (0.01)</td>
</tr>
<tr>
<td>0.75</td>
<td></td>
<td></td>
<td>0.49 (0.01)</td>
<td>0.48 (0.01)</td>
</tr>
<tr>
<td>1</td>
<td>0.49 (0.01)</td>
<td>0.48 (0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.48 (0.01)</td>
<td>0.48 (0.02)</td>
<td>0.44 (0.03)</td>
<td>0.45 (0.02)</td>
</tr>
<tr>
<td>4.5</td>
<td>0.48 (0.01)</td>
<td>0.49 (0.01)</td>
<td>0.49 (0.01)</td>
<td>0.45 (0.01)</td>
</tr>
<tr>
<td>8.5</td>
<td>0.50 (0.01)</td>
<td>0.50 (0.01)</td>
<td>0.51 (0.01)</td>
<td>0.50 (0.02)</td>
</tr>
<tr>
<td>( \bar{x}_{\text{overall}} )</td>
<td>0.49 (0.01)</td>
<td>0.48 (0.01)</td>
<td>0.48 (0.01)</td>
<td>0.47 (0.01)</td>
</tr>
</tbody>
</table>

\(^a\) Each Cw value for each sampling time represents a mean of three water samples taken from each of three replicate exposure bottles.
<table>
<thead>
<tr>
<th>Sampling Time (h)</th>
<th>10°C</th>
<th>16°C</th>
<th>22°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.27 (0.01)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>0.30 (0.02)</td>
<td>0.31 (0.02)</td>
<td>0.28 (0.02)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.28 (0.03)</td>
<td>0.30 (0.03)</td>
<td></td>
<td>0.36 (0.02)</td>
</tr>
<tr>
<td>4.5</td>
<td>0.30 (0.04)</td>
<td></td>
<td></td>
<td>0.36 (0.02)</td>
</tr>
<tr>
<td>5</td>
<td>0.36 (0.05)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>0.29 (0.01)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.30 (0.05)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>0.34 (0.01)</td>
<td></td>
<td></td>
<td>0.27 (0.01)</td>
</tr>
<tr>
<td>9</td>
<td>0.32 (0.02)</td>
<td>0.30 (0.03)</td>
<td>0.28 (0.01)</td>
<td>0.28 (0.01)</td>
</tr>
<tr>
<td>10.5</td>
<td>0.30 (0.03)</td>
<td>0.27 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.30 (0.02)</td>
<td>0.25 (0.02)</td>
<td>0.23 (0.01)</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.26 (0.01)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \bar{X}_{\text{overall}} )</td>
<td>0.30 (0.01)</td>
<td>0.30 (0.01)</td>
<td>0.28 (0.01)</td>
<td>0.28 (0.01)</td>
</tr>
</tbody>
</table>

\( a \) Each \( C_w \) value for each sampling time represents a mean of three water samples taken from each of three replicate exposure bottles.
APPENDIX C

UPTAKE AND DEPURATION OF A TEST CHEMICAL WHEN EXPOSED TO MIDGE LARVAE IN A BINARY SOLUTION
The effect on the uptake and depuration of a test chemical by midge larvae when more than one chemical is present in solution was examined. In uptake and depuration experiments, similar to those described in the methods section, ku and kd values were determined for DDE in the presence diCB and without the presence of diCB. Table 33 gives the kd and ku values for DDE in the presence and absence of diCB.

Table 34

Uptake and Depuration Rate Constants of DDE in *Chironomus riparius* in the Presence and Without the Presence of diCB\(^{a,b}\)

<table>
<thead>
<tr>
<th></th>
<th>With diCB</th>
<th>Without diCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>ku, mlg(^{-1})h(^{-1})</td>
<td>142</td>
<td>147</td>
</tr>
<tr>
<td>kd, h(^{-1})</td>
<td>0.006</td>
<td>0.003</td>
</tr>
</tbody>
</table>

\(^{a}\) diCB was present at 5 ug/l (nominally) in uptake and depuration experiments

\(^{b}\) experimental temperature, 22°C

\(^{c}\) ku values determined by mass balance equation

The reciprocal experiments were performed with diCB uptake and depuration examined in the presence and absence of DDE. Table 34 gives the kd and ku values for diCB in the presence and absence of DDE.
### Table 35
Uptake and Depuration Rate Constants of diCB in *Chironomus riparius* in the Presence and Without the Presence of DDE$^{a,b}$

<table>
<thead>
<tr>
<th></th>
<th>With DDE</th>
<th>Without DDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_u^c$, $\text{mg}^{-1}\text{h}^{-1}$</td>
<td>93</td>
<td>109</td>
</tr>
<tr>
<td>$k_d^d$, $\text{h}^{-1}$</td>
<td>0.301</td>
<td>0.360</td>
</tr>
<tr>
<td>$k_d^e$, $\text{h}^{-1}$</td>
<td>0.450</td>
<td>0.400</td>
</tr>
<tr>
<td>BCF$^f$</td>
<td>310</td>
<td>303</td>
</tr>
</tbody>
</table>

$^a$ DDE was present at 1 $\mu$g/L (nominally) in uptake and depuration experiments; diCB nominal exposure concentration in uptake experiment was 1 $\mu$g/L.

$^b$ Experimental temperature, room temperature.

$^c$ $k_u$ values determined by multiplying $k_d$ by BCF.

$^d$ Depuration experiment consisted of loading midges with diCB only before depurating in presence and absence of DDE.

$^e$ Depuration experiment consisted of loading midges with diCB and DDE before depurating in presence and absence of DDE.

$^f$ BCF values were determined from a mean of four steady-state Ca's divided by a mean of four measured concentrations of diCB in the exposure water.
APPENDIX D

A COMPARISON OF DEPURATION RATES OF DDE FROM MIDGE LARVAE IN THE PRESENCE OF GLASS WOOL, FOOD, AND NEITHER FOOD OF GLASS WOOL
Two separate experiments were conducted each examining the effect of glass wool, food, and water alone on the depuration of DDE by midge larvae. For both experiments, the depuration procedure was similar to that described in the methods section except that midge larvae were depurated in three separate vessels each containing a different treatment (glass wool, food, water alone). The slopes of the ln(Ca vs. time) plot were analyzed by ANCOVA for each experiment. For both experiments, it was found that the kd's for glass wool and water alone were not significantly different from each other, but both were significantly different (P < 0.01) than the kd for food. Table 35 lists these data.

Table 35

Depuration Rate Constants of DDE in Midge Larvae in the Presence of Glass Wool, Food, and Without Glass Wool and Food

<table>
<thead>
<tr>
<th></th>
<th>Glass Wool</th>
<th>Food&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Water Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>kd&lt;sup&gt;b&lt;/sup&gt;, h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.0069</td>
<td>0.0176</td>
<td>0.0035</td>
</tr>
<tr>
<td>kd&lt;sup&gt;c&lt;/sup&gt;, h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.0021</td>
<td>0.0072</td>
<td>0.0016</td>
</tr>
</tbody>
</table>

<sup>a</sup> food, purina trout chow

<sup>b</sup> experiment performed June 5/9, 1988; experimental temperature, 22°C

<sup>c</sup> experiment performed June 19/23, 1988; experimental temperature, 22°C