BIOACTIVE COMPOUNDS IN THE AQUATIC ENVIRONMENT: STUDIES ON THE MODE OF UPTAKE OF DDE BY THE AQUATIC MIDGE, *CHIRONOMUS TENTANS* (DIPTERA: CHIRONOMIDAE)

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The mode of uptake of DDE-^{14}C by *Chironomus tentans* larvae was investigated. There was no difference in the amount of DDE-^{14}C accumulated by live and dead fourth instar larvae as a function of exposure time. Dead and live larvae were also exposed to an aqueous and substrate source of DDE-^{14}C contamination and again no differences were found in the amount of DDE-^{14}C accumulated indicating passive accumulation. Cuticle surface area and DDE-^{14}C uptake relationships were found to have a high degree of correlation. The amount of DDE-^{14}C concentrated by the larvae was increased by manipulation of water hardness. Calcium and magnesium ion concentrations in the water were increased and a subsequent increase in DDE-^{14}C accumulation by the larvae resulted. An adsorption-diffusion mechanism is proposed to account for the mode of uptake and biological concentration capabilities of the midge.

The occurrence of *p,p'*-DDE [2,2-*bis*(*p*-chlorophenyl)-1,1-dichloroethylene] in surface water, benthic sediments, and fishes is well documented by established pesticide monitoring programs (Weaver *et al.* 1965, Henderson *et al.* 1969). Organochlorine compounds are accumulated by aquatic organisms under field conditions by a trophic level transfer mechanism and direct adsorption from the aqueous environment (Woodwell *et al.* 1967, Hunt and Bischoff 1960). Macek and Korn (1970) found that brook trout, *Salvelinus fontinalis* (Mitchill), accumulated ten times more DDT from food intake than directly from water. Conversely, many investigators calculate that aquatic invertebrates accumulate organochlorine compounds as a result of direct adsorption from water (Johnson *et al.* 1971, Wilkes and Weiss 1971, Derr and Zabik 1972). However, quantitative data concerning and exemplifying the mode of uptake and factors affecting accumulation of bioactive materials by aquatic invertebrates are not available.

The purpose of this study was threefold: (1) to investigate the accumulation of *p,p'*-DDE from aqueous solution and contaminated substrate by fourth instar midge larvae, *Chironomus tentans,* as a function of exposure time and to differentiate between possible physical and biological modes of DDE sorption by comparing the DDE accumula-

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tion of live and dead insect larvae, (2) to investigate the role of the larval cuticle in DDE accumulation, and (3) to determine the effect of water hardness on the uptake relationships and accumulation of DDE.

Methods and materials

Significance of physical and biological modes of uptake. Five dead and five live fourth instar *C. tentans* larvae (20.0 ± 2.0 mm total length) were placed on each side of a glass partition in a three-liter glass aquarium. One side of the partition contained the substrate described by Derr and Zabik (1972) (chicken feed and paper towel homogenate) and the other contained no substrate.

Uniformly ring-labeled C^{14} *p,p'*-DDE with a specific activity of 2.4 mCi/mM was delivered in ethanol to four liters of diluent water to give approximately 2.76×10^5 dpm per 100 ml of test water. A 100-ml aliquot of contaminated water was then added to each side of the partition. The substrate-water system was allowed to equilibrate for two hours before the experiment was initiated.

Dead larvae were obtained by refrigeration at 4°C for two hours. Care was taken to prevent larval freezing so that ice crystal formation and cell lysis would not occur. They were then allowed to come to room temperature before introduction into the test aquaria.

After exposure to the DDE-^{14}C-contaminated water and substrate both the live and dead larvae were dried, weighed, and subjected to digestion in one ml of 1M hyamine-hydroxide for two days, a scintillation fluid was then added (4g BBOT/liter toluene), and the samples were counted on a Nuclear Chicago Mark 1 scintillation counter. Each larval sample was counted three times with quenching corrected by automatic external standardization. Exposure periods were 1, 2, 4, and 8 hours, and each treatment was replicated three times. One additional larval culture with no DDE-^{14}C was maintained in the prescribed substrate with the addition of a *Chorella* sp. algal population to facilitate the understanding of whether the *C. tentans* larvae would adequately construct tubes and feed in an eight-hour time period.

During each experimental time period larvae from this aquarium were dissected to ascertain the presence of green algal cells in the gut which would indicate that feeding had occurred.

Functional role of larval cuticle in DDE uptake. To better understand the significance of adsorption of DDE by the midge larvae, another series of experiments was conducted to facilitate the understanding of the role of the larval cuticle in the sorption mechanism.

Five live fourth instar larvae (21 ± 3 mm total length, 11.1 ± 3.9 mg wet weight) were placed in 100-mm diameter crystallizing dishes with 100 ml of diluent water containing approximately 1.7×10^5 dpm of uniformly labeled DDE-^{14}C. Larvae were exposed for 1, 2, 4, and 8 hours. The sample (five larvae per sample) of exposed larvae was subjected

to a cuticle wash or dip into one ml of redistilled hexane for five seconds. The larvae were digested and residue data procured as described earlier. The hexane wash residue was quantitated by adding the scintillation fluor to the wash and radiometric analysis was performed. Each treatment was replicated three times in a completely randomized design.

Relationship of cuticle surface area to DDE uptake. The diffusion equation (1) (Davson and Danielli 1952) describes the importance of the area of cuticle exposed to the diffusing material to the amount of adsorption that takes place. Thus, an experiment was conducted to investigate the surface area to DDE accumulation relationship to further develop the importance of the role of cuticular uptake of DDE by larval *C. tentans.*

$$s = C_o V\ (1\text{-}e^{-Pat/v}) \qquad (1)$$

where s = amount of material diffused into insect body
t = time after application of material
V = volume of the insect
A = area of the cuticle which is exposed to the diffusing material
P = permeability constant (cm/sec) at a certain temperature (°C)
C_o = initial concentration of material applied on cuticle

Chironomus tentans larvae were randomly selected from stock culture tanks which had a distribution of first through fourth instar larvae. Ten of these larvae were then placed in a 100-mm diameter crystallizing dish containing approximately 4.26×10^6 dpm of DDE-^{14} in 100 ml of water and exposed for 1, 2, 4, and 8 hours to the experimental DDE concentration. The insects were removed at the prescribed time and each one was subjected to a total length and width measurement. Total lengths were taken from the head to the extreme posterior gill and width measurements were taken at the fifth abdominal segment. The individual insect was then digested in 1.0 ml of 1 M methanolic hyamine-hydroxide for two days, a scintillation fluid was added, and the sample was counted. There were three replicates of each treatment and the design was completely randomized. Surface areas were calculated by treating the larvae as a cylinder and then using the equation.

$$S = 2\pi rh + 2r^2 \qquad (2)$$

where S = surface area (mm^2) of midge larvae
r = one-half the width (mm) at the fifth abdominal segment of the larvae
h = total length (mm) of the midge larvae

Effect of water hardness on DDE uptake. To further develop the significance of accumulation of DDE by adsorption to the larval cuticle another experiment was con-

ducted to investigate the effect of water chemistry on larval uptake of DDE-^{14}C. It was thought that if adsorption is related to the cuticular properties of the insect then the chemistry of the aquatic environment to which the midge is exposed should have an observable effect on the accumulation of DDE. Water hardness was chosen as the water chemistry parameter that would be altered to investigate this hypothesis. Two experimental synthetic waters were prepared, one of which, here denoted as "soft" and following that described by Cairns (1969), served as the control. The other synthetic water, denoted as "hard" paralleled that of the soft except that the magnesium and calcium ion concentrations were increased twofold (Table I).

Table I. *Molar Concentration and Chemical Properties of Synthetic Dilution Waters Used for Exposure of Fourth Instar* Chironomus Tentans *Larvae to DDE*^{14}*C*

Chemical	Molar concentration	
	Soft	Hard
KCL	2.68×10^{-4}	2.68×10^{-4}
$NaHCO_3$	1.63×10^{-4}	1.63×10^{-4}
$MgSO_4 \cdot 7H_2O$	1.62×10^{-4}	3.89×10^{-4}
$Ca(NO_3)_2 \cdot 4H_2O$	1.69×10^{-4}	1.71×10^{-4}
$CaCO_3$	1.00×10^{-4}	2.25×10^{-4}
K_2HPO_4	1.14×10^{-5}	1.14×10^{-5}
$FeCL_3$	2.60×10^{-6}	2.70×10^{-6}
Conductivity (μmhos/cm^2)	1.71×10^2	3.61×10^2
pH	7.81	7.65
Hardness as ppm $CaCO_3$	60.0	136.2

DDE-^{14}C in ethanol was added to the two stock synthetic waters so that a 75-ml aliquot of each contained approximately 6.75×10^4 dpm. This 75-ml aliquot was put into a 100-mm diameter crystallizing dish which served as the experimental test container. Aliquots of both DDE contaminated waters were extracted with portions of redistilled hexane and introduced into the liquid scintillation counter for confirmation that DDE was equally soluble in both. Three fourth instar larvae (20.2 ± 2.6 mm total length, and 11.3 ± 3.9 mg wet weight) were then placed in the experimental chambers. Exposure times were 1, 3, 9, and 18 hours and each sample was subjected to a cuticle wash with hexane and quantitation was achieved by the procedure described earlier. Each treatment was replicated three times.

Results

Physical and biological modes of uptake of DDE. Both live and dead larvae demonstrated a time related accumulation of DDE-^{14}C (Figure 1). Generally with live larvae there was a period of linear uptake (0-4 hr) followed by an equilibrium or plateau which was reached at 4-8 hours after initial exposure. A sample of three to four individual larvae taken at 1, 2, 4, and 8 hours from the *Chlorella* cultures were dissected and found to contain algal cells indicating that tube construction and feeding had occurred throughout the experimental time period.

This experiment suggests that adsorption is a major mechanism by which the accumulation of DDE by *C. tentans* larvae is accomplished, since no differences in uptake were attributed to contamination by the feeding process and thus the DDE body burden has its origin in the adsorptive process. Another factor that supports some mechanism of sorption is that there was no difference between live and dead larvae accumulation of DDE, suggesting that DDE uptake is passive and not an energy-requiring process; thus adsorption should be considered as a possible mode of uptake of environmental contaminants similar to DDE.

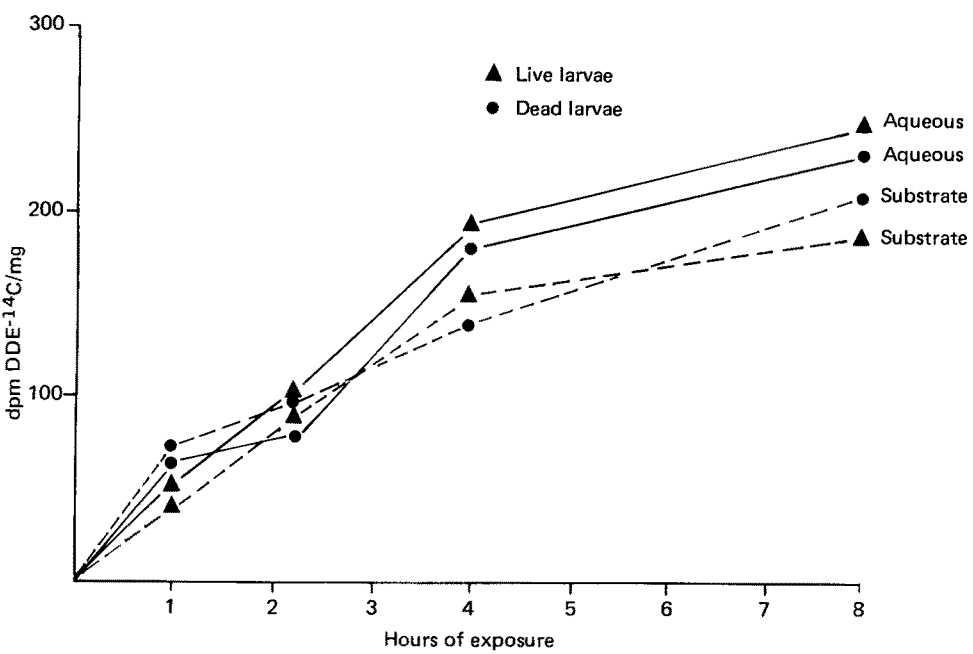

Fig. 1. Accumulation relationship with time between live and dead *Chironomus tentans* larvae exposed to an aqueous and substrate contamination of DDE-^{14}C for eight hours. Each point represents the mean of three cuticular samples with each sample consisting of five larvae.

Role of larval cuticle in DDE uptake. Uptake of DDE-^{14}C by the larvae demonstrated a time-dependent relationship as seen before, with a plateau being reached in eight hours (Figure 2). An exponential period of accumulation was observed up to four hours' exposure and resulted in a linear period of uptake. The hexane-washed cuticle appeared to reflect a modal relationship in that a peak in DDE contamination was reached after only two hours' exposure and then decreased to a level below that obtained after one hour's exposure. The peak in DDE concentrations obtained by the cuticle wash after two hours exposure exceeded that of the insect after eight hours subjection to DDE-^{14}C. Similar results were also obtained when dead larvae were subjected to a hexane wash.

The rate of accumulation and subsequent entry of DDE-^{14}C through the cuticle of *C. tentans* larvae deviated from the theoretical rate of diffusion given by the diffusion equation (1). No actual calculations of diffusion rates were attempted with the data obtained to demonstrate a deviation from the theoretical rate of diffusion. However, according to the diffusion equation, penetration should be proportional to the concentration of DDE-^{14}C and exponential to the penetration time. The larval cuticle did not demonstrate an exponential relationship with exposure time and thus did not adhere to the theoretical rate of diffusion. This same type of deviation from the expected theoretical diffusion was found with cuticle of the American cockroach (*Periplaneta americana*)

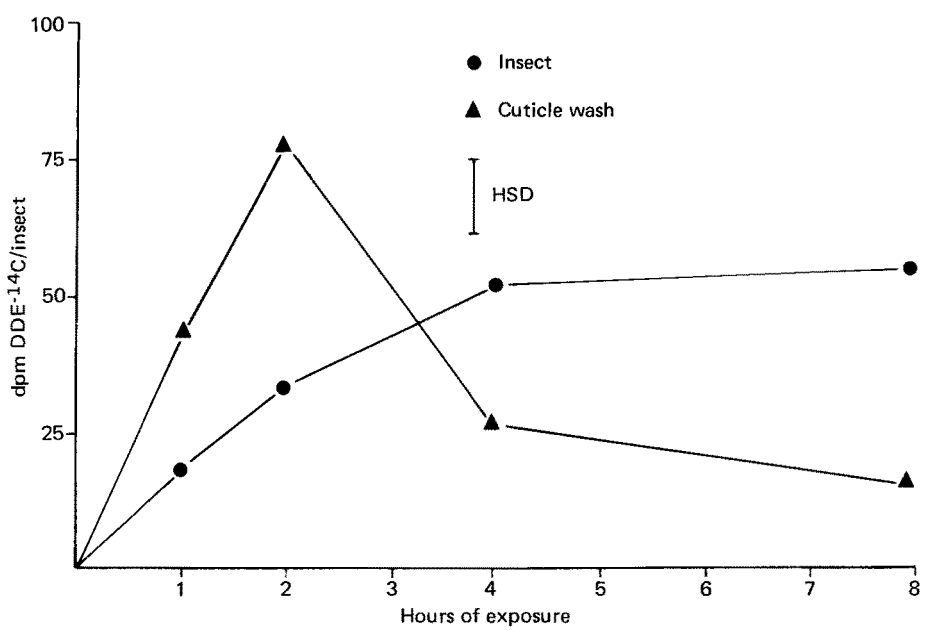

Fig. 2. Uptake of DDE-^{14}C by *Chironomus tentans* fourth instar larvae when subjected to a cuticle wash of hexane. Each point represents the mean of three replications. HSD-honest significant difference at the 5% level by Tukey's W procedure.

when malathion was applied (Matsumura 1963). Treherne (1957) has shown that the rate of penetration of non-electrolytes through the cuticle of *Schistocerca gregaria* is related to the solubility of the compound in water, but again some deviation from the theoretical was observed.

A possible explanation for this deviation in diffusion and absorption of DDE from the theoretical rate by midge larvae is that the DDE is in some way altering the cuticular properties of the insect and thus the cuticle is not functioning in the same manner throughout the experimental period of exposure. The process of DDE uptake is adsorption onto the cuticle and subsequent diffusion or incorporation into the body with time. As the exposure time increases the cuticle is altered causing a decrease in the amount of residue absorbed with a slower rate of diffusion into the body. This explanation appears to describe the two relationships depicted in Figure 2: (1) the apparent plateau reached by the insect after only six to eight hours exposure to DDE-^{14}C, and (2) the modal relationship of cuticular adsorption of DDE over time.

Relationship of cuticle surface area to DDE uptake. When concentrations of DDE (dpm) in the larvae at the prescribed exposure times were plotted against their corresponding surface areas (mm^2) and fitted with a least squares line, significant positive relationships were established (Figures 3-6). The correlation coefficients (r) at each exposure period of 1, 2, 4, and 8 hours were 0.91, 0.94, 0.91 and 0.87, respectively, and indicated a very

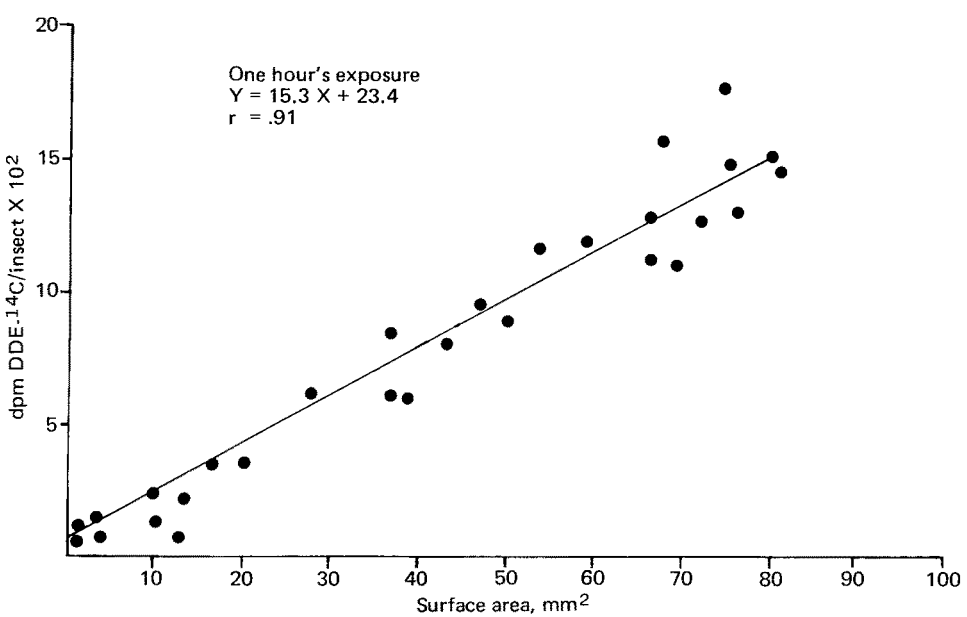

Fig. 3. Relationship of surface area to uptake of DDE-^{14}C by *Chironomus tentans* larvae after one hour's exposure.

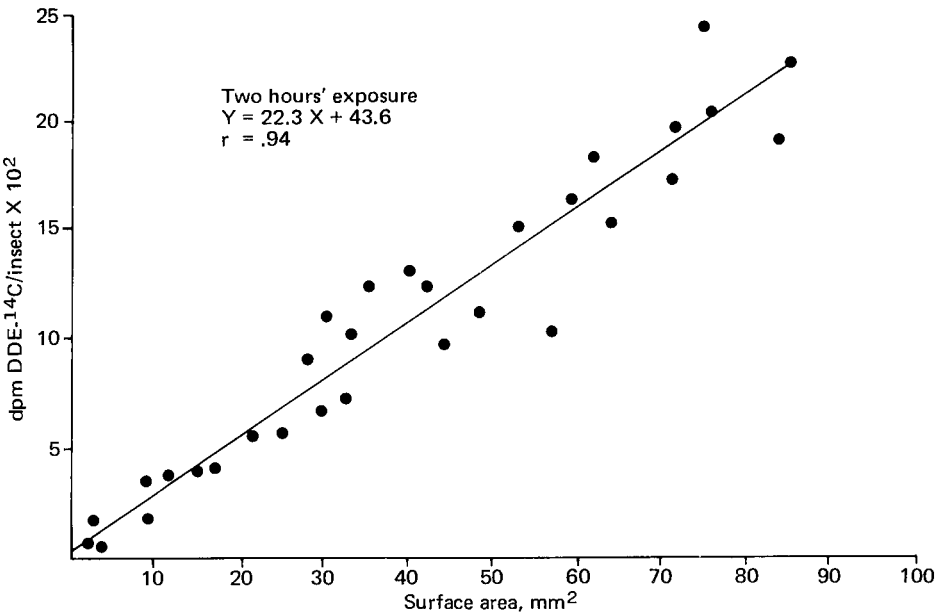

Fig. 4. Relationship of surface area to uptake of DDE-^{14}C by *Chironomus tentans* after two hours' exposure.

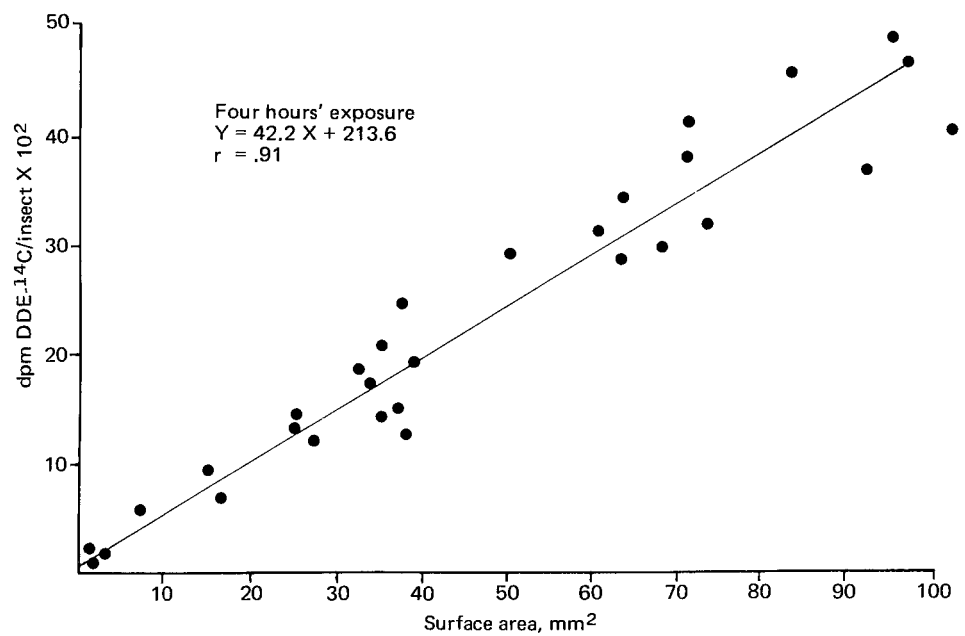

Fig. 5. Relationship of surface area uptake of DDE-^{14}C by *Chironomus tentans* larvae after four hours' exposure.

close fit and highly significant relationship. Thus, it appears that there is a direct proportional relationship between the surface area of the larvae and the amount of DDE-^{14}C accumulated. The surface area of the cuticle must be a very significant factor when one considers the mode of uptake of compounds favoring adsorption. This experiment clearly reflects that cuticular adsorption of DDE is a major route of DDE accumulation by *C. tentans* larvae.

Effect of water hardness on DDE uptake. Larvae exposed to the synthetic dilution water containing a higher concentration of calcium and magnesium ions significantly accumulated almost two times as much DDE after 18 hours as the control (Figure 7). The cuticle in both types of water behaved as in previous studies with a peak in adsorption being reached in the early periods of exposure with a gradual decrease over time. However, the cuticle exposed to hard water adsorbed almost three times more DDE than that exposed to soft water after one hour of exposure.

It appears that calcium and magnesium ion concentrations may alter the integrity of the cuticle, thus altering its adsorptive and permeability properties, and causing a difference in the resultant accumulation of DDE. Studies on the stonefly, *Pteronarcys californica,* have shown that the surface lipid composition of the adult and naiad differ in that a larger percentage of hydrocarbons, wax esters, free fatty acids, and sterols are found on the adult, while the naiad has more triglycerides (Armold *et al.* 1969). Such findings would indicate that any alteration in the surface lipid composition would affect the

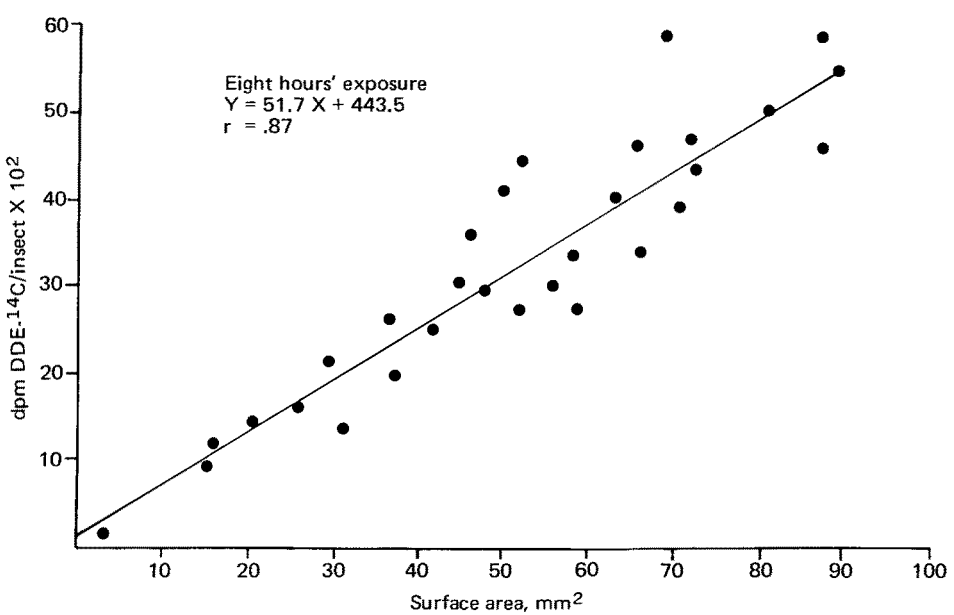

Fig. 6. Relationship of surface area to uptake of DDE-^{14}C by *Chironomus tentans* larvae after eight hours' exposure.

adsorption of nonpolar compounds. The water hardness experiment supports the concept that accumulation of DDE is significantly related to the cuticular adsorption process.

Discussion

Wallace and Brady (1971) found relatively low levels of dieldrin in the hellgramite, *Corydalis cornuta*, from a highly contaminated stream in South Carolina. Gut analysis revealed that this animal preyed on other aquatic insects, including Trichoptera and Simuliidae larvae. With the reported increase in concentration of chlorinated hydrocarbons up the food chain (Naqvi and Ferguson 1968, Macek and Korn 1970), it would be expected that *C. cornuta* would have higher pesticide levels than its prey. Instead the *Simulium* and hydropsychid larvae that represented the probable prey had 12 to 70 times more dieldrin than the hellgramite. Wallace and Brady (1971) then attributed this difference to two factors: Either *C. cornuta* does not store dieldrin or has some efficient mechanism for eliminating it. Another possible explanation can be offered, that is, that the cuticular properties of Simuliidae and Hydropsychidae are different from those of *Corydalis,* and this adsorption of dieldrin is favored more with Simuliidae and Hydropsychidae. The cuticles of the prey species are more membranous and less chitinized, which would allow for greater diffusion and adsorption of dieldrin (Matsumura 1963).

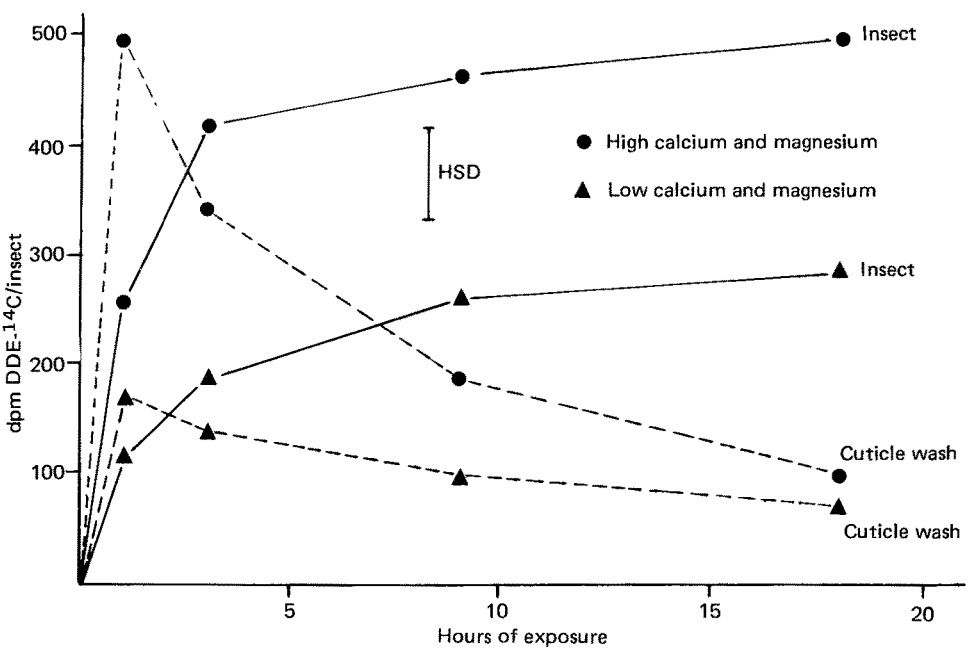

Fig. 7. Effect of exposing *Chironomus tentans* fourth instar larvae to two different synthetic waters varying in calcium and magnesium concentrations on uptake of DDE-^{14}C with time. Each point represents the mean of three replications, HSD-honest significant difference at 5% level by Tukey's W procedure.

This adsorption and diffusion concept would support the findings of Keith (1966), Peterle (1966), and Chadwick and Brocksen (1966) that aquatic organisms may not necessarily show pesticide concentrations relative to their position in the food chain.

The proposal submitted by Hamelink *et al.* (1971), that exchange equilibria determine the degree to which chlorinated hydrocarbons are biologically magnified, can also be incorporated into the adsorption-diffusion mechanism. The exchange equilibirum depends on the differences in solubility of the pesticide in water and fats. If the assumption is met that DDE, DDT, dieldrin, toxaphene, and lindane have similar solubilities in fats, then differences in the degree of biological concentration of the compounds observed in the aquatic organisms would be due to differences in their solubility in water. Conversely, if we assume that there is no difference in their solubility in water, then the degree to which they exhibit biological concentration is dependent upon their affinity or solubility in fats. The lipid layer of the epicuticle of an aquatic immature insect could then play a significant role in biological magnification based on the particular pesticide's solubility in this lipid layer.

The functional role of the epicuticle also explains some of the differences in the amounts of DDE accumulated by the invertebrate component (primarily ostracods, mites, dragonfly naiads, and some *Hyallella azteca* and *Chaoborus* sp.) of the Hamelink *et al.* (1971) study. When all the invertebrate DDT residues were pooled and plotted against the corresponding water concentration to which they were exposed, an accumulation factor of 10,000 resulted, which is comparable to the concentration factor obtained with DDE and *C. tentans.* However, when broken down into their respective taxonomic groups, the mites contained about 20 percent more DDT residue than the ostracods, while the odonates contained about half as much as ostracods. Thus the epicuticular lipid layer could indeed have different properties in each invertebrate group and therefore exhibit different accumulation rates of DDT (Armold *et al.* 1969).

Hamelink *et al.* (1971) also suggest that the degree of biological magnification and persistence of DDT in the lentic ecosystems appear to be promoted by oligotrophic conditions and retarded by eutrophic conditions, and that the primary factor controlling these relationships appears to be the concentration of free or unbound DDT in the water. Another possible explanation for this relationship is the observation in this study that the inorganic ion concentration (calcium and magnesium) affects the adsorption-diffusion mechanism and thus a change in the accumulation of residue is observed. Oligotrophic lakes are characteristically higher in alkalinity and free inorganic ions, whereas the inorganic ions in an eutrophic situation are usually tied up in sediment (Reid 1961). This factor aids in the further understanding of the biological concentration differences between eutrophic and oligotrophic ecosystems.

Acknowledgments

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