BIOLOGICALLY ACTIVE COMPOUNDS IN THE AQUATIC ENVIRONMENT:

A STUDY OF TWO ENVIRONMENTAL CONTAMINANTS, DDE AND AROCLOR 1254, ON THE AQUATIC MIDGE, CHIRONOMUS TENTANS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
STEVEN KROGH DERR
1972

This is to certify that the

thesis entitled

BIOLOGICALLY ACTIVE COMPOUNDS IN THE AQUATIC ENVIRONMENT: A Study of Two Environmental Contaminants, DDE and Aroclor 1254, on the Aquatic Midge, Chironomus tentans.

presented by

Steven Krogh Derr

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Entomology

Major professor

Date October 27, 1972

O-7639

ABSTRACT

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The midge, Chironomus tentans, was exposed from egg through adult to varying concentrations (.07 to 2.2 parts per billion) of (1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene), DDE. The amount of DDE accumulated by the midge was determined by gas-liquid partition chromatography. The accumulation of DDE from aqueous solution by the midge as a function of DDE concentration and exposure time was determined and the distribution of the accumulated DDE residue was quantitated (pupa, exuvia, adult and egg mass). Field studies were conducted and the results obtained supported laboratory findings.

The DDE accumulation by the midge demonstrated a dose-dependent relationship, accumulating DDE exponentially with increased concentration at a given exposure time. At any given concentration of DDE in the water, accumulation increased with increased exposure time. On a part per million basis the midges concentrated DDE approximately 15,000 times over that level which was present in

the water. No metabolism of the parent p,p'-DDE occurred in the midge larvae.

The exuvia did not demonstrate a major route of residue elimination as only 1.4 to 4.9 percent of the pupal burden was lost via the exuvia. The process of egg deposition eliminated 11.6 to 30.9 percent of the adult female burden of DDE residue. The exposure of C. tentans to DDE resulted in a decrease in the number of second generation emerging adults.

The presence of the polychlorinated biphenyl, Aroclor 1254, did not affect the uptake of DDE either in static or continuous flow experiments. Pre-exposing the larvae to Aroclor 1254 and then exposure to DDE did not affect uptake of DDE either. Elimination dynamics were also studied and DDE was found to have a half-life of 6.8 days in the larvae.

The mode of uptake of DDE by larvae was investigated. There was no difference in the amount of DDE accumulated by live and dead fourth instar larvae. Dead and live larvae were also exposed to an aqueous and substrate source of DDE contamination and again no differences were found in the amount of DDE accumulated. Cuticle surface area and DDE uptake relationships were studied and found to have a high degree of correlation. The amount of DDE 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 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.

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The evaluation and applicability of capturing emergent chironomids as a possible mechanism of pesticide removal from sewage oxidation pond facilities is discussed.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Entomology

1972

ACKNOWLEDGEMENTS

I wish to express my appreciation to Dr. Matthew J. Zabik for his guidance and encouragement during the course of this study, and whose advice and kindness in areas other than research has helped me greatly. I also wish to express my gratitude to committee members Drs. Gordon E. Guyer, Ronald E. Monroe, Alan R. Putnam and Howard E. Johnson for their advice and interest in this study, all of who I enjoyed knowing.

Sincere appreciation goes to my wife Lynn for her constant interest, ideas, good humor and patience during my graduate program.

During this study I was supported by a National Defense Educational Act fellowship and funds partially derived from the National Institute of Health Grant No. CC-00246-03 and FD-00223-05.

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INTRODUCTION

The desire of man for an improved level of living has led to control and management of pest populations for the increased utilization of food and fiber crops. The recognition of the insecticidal properties of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) in the late 1930's and implementation into pest control programs (Frear, 1955) has led to development of other synthetic organic chemicals as tools that can be utilized in management programs. Since its discovery, 3.5×10^9 pounds of DDT has been globally distributed (Westlake and Gunther, 1966).

The beneficial contributions of DDT have been well recognized in malaria control and other insect borne diseases control programs and thus has resulted in a higher standard of well being in many economically depraved areas. However, environmentally undesirable effects soon became recognizable (Carson, 1962). The deleterious biological effects of this compound has resulted in curtailed use in the United States and restricted use in Michigan. The chlorinated hydrocarbon pesticides as a class have received particular attention because of their long persistence and adverse affects to some birds and aquatic ecosystems.

The discontinued use of DDT, however, has not completely solved the environmental problem of DDT contamination. Due to the metabolic degradative pathway of DDT in biological systems another

problem has arisen, that being DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene).

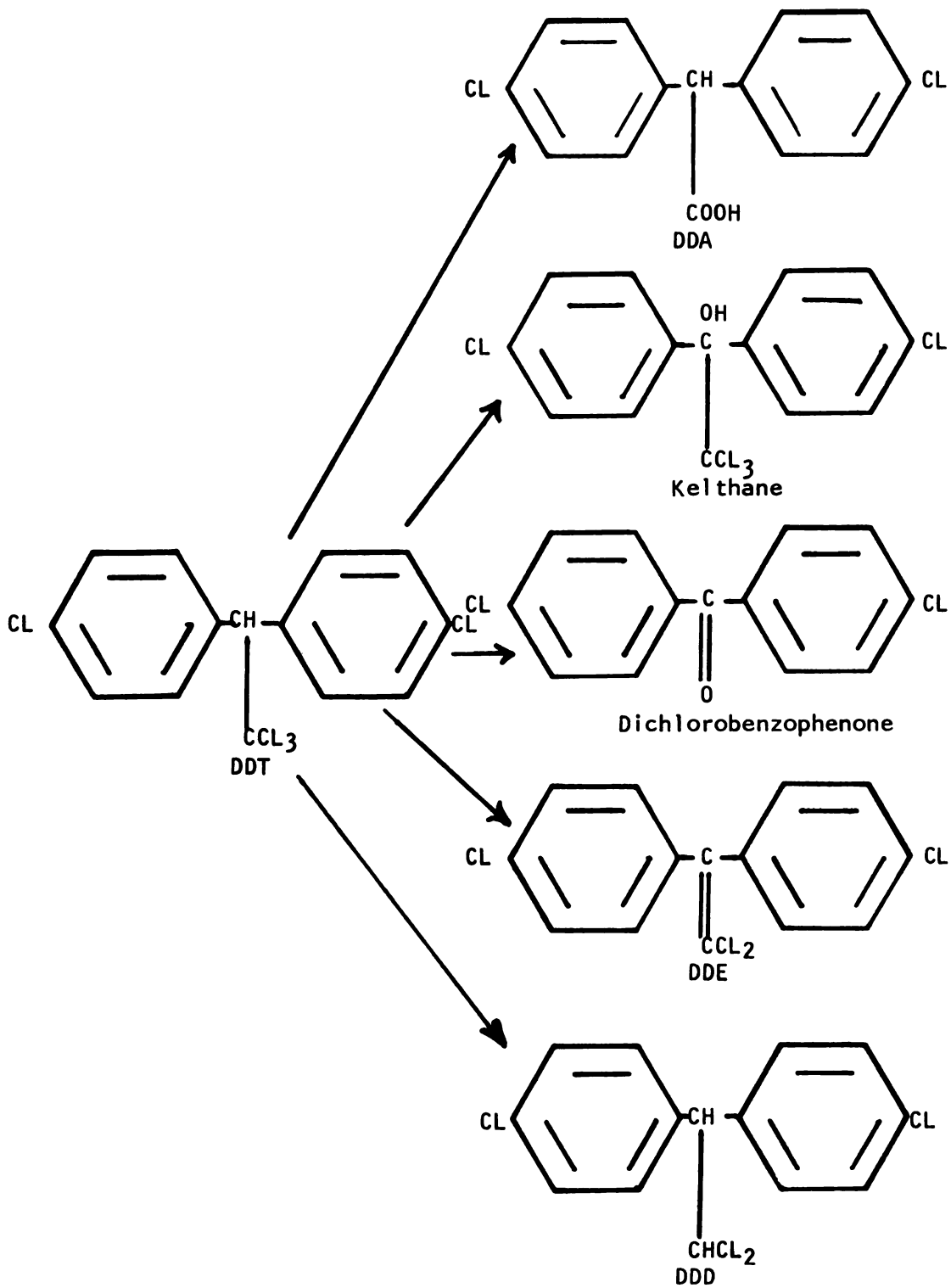
The major detoxification process of DDT in mammalian systems is reductive dechlorination of DDT to DDE (O'Brien, 1967) (Figure 1). DDE is also the form in which parent DDT is stored in body tissues due to its high liposolubility and low water solubility.

Further due to the high liposolubility of DDE it can be readily partitioned into fat containing biological material in the organism and thus pose a problem in elimination dynamics.

The ubiquitous occurrence of DDE in surface waters is demonstrated by the national pesticides monitoring program (Litchenberg, et. al., 1970) and Risebrough's (1969) conclusion that p,p'-DDE may be the most abundant of the synthetic pollutants in the environment. Biological activity of DDE has been demonstrated in birds (Heath, et. al., 1970) and thus far thought as the compound responsible for the impairment of egg viability in a number of bird species.

DDE is comparatively resistant to degradation by the detoxification process usually employed by vertebrates, to microbial action, and to non-biological breakdown in the environment. Its half-life would appear to be greater than ten years (Risebrough, et. al., 1969). A model ecosystem for the precise evaluation of pesticide biodegradibility has recently been developed and a situation was constructed resembling that of Lake Michigan (Metcalf, et. al., 1971b). When p,p'-DDE was the starting material applied to this system little further metabolism occurred and storage in the animals of the ecosystem occurred at levels substantially higher than those

Figure 1. Degradative metabolic routes of p,p'-DDT.



Routes of DDT Metabolism (O'Brien, 1967)

Figure 1

of DDT. It is evident from this experiment that DDE is responsible for a major portion of the adverse environmental effects of concentration and storage in animal tissues occurring after the use of DDT.

Due to the widespread occurrence of DDE in surface waters and its biological activity this compound could be eliciting a detrimental response to aquatic insect populations, and thus affecting other components of the aquatic system.

Another area of recent concern in the aquatic environment is the appearance of synthetic industrial materials referred to as PCB's (polychlorinated biphenyls) in biological material.

PCB is similar to DDT (and metabolites) both in physical and biological properties and thus poses the same problem as DDT (and metabolites) in the aquatic environment. The occurrence of PCB and DDE together in the aquatic environment might pose a significant problem in the utilization and management of our aquatic resources.

The phenomenon of biological concentration (magnification) of these industrial pollutants and chlorinated hydrocarbon pesticides has been well studied in the field environment. However, in order to assess the significance of the biological concentration of contaminants in the aquatic environment it is necessary to have an understanding of the dynamics of pesticide uptake within each trophic level.

The importance of the ecological and biological characteristics of an aquatic insect must be considered as to their role in pesticide transfer. The midge, Chironomus tentans, was chosen as the test organism for this study based on the following factors; 1) C. tentans larvae are a major food source for many of the benthos

feeding fish, 2) the pupae due to their ascending planktonic nature are readily available to free swimming predators, 3) the midge occupies a substrate that is high in organic matter which would favor partitioning of non-polar pesticides, and chlorinated industrial pollutants, 4) C. tentans has a short developmental time in nature (egg to adult, 28-35 days) and thus a rapid turnover rate of the midge would be present, and 5) the insect can occupy a significant numerical proportion of a benthic community.

Since much remains to be known about pesticide dynamics within each trophic level and the mechanics of such dynamics could be applied to future aquatic contaminants, a study was proposed. Thus the purpose of this study is to increase the knowledge and understanding of the role of benthic insects in their relationship to biologically active compounds in the aquatic environment by accomplishing the following objectives:

1. Determination of the rate of uptake and concentration factor of DDE from egg to adult.
2. Determination of distribution of DDE burden accumulated during larval development (i.e., pupal exuvia, egg deposition).
3. Influence of the presence of PCB (1254) on the uptake of DDE from egg to adult.
4. Determination of the rate of elimination of DDE expressed as half-life within the larvae.
5. Determination of the mode of uptake of DDE during larval development.

6. To assess the significance of metabolism of p,p'-DDE by the aquatic insect.
7. Determination of the influence that DDE has on the reproductive process of the aquatic insect.
8. Confirmation of laboratory results by conducting field experiments.

REVIEW OF LITERATURE

Distribution of DDE

The distribution and accumulation of p,p'-DDE residues by various trophic level components of the aquatic system is well documented in the field situation. In the marine system seals and porpoises from the Canadian Atlantic coast have been analyzed and found to contain 1.2 to 5.9 parts per million (ppm) in seal fat and 1.1 to 12.8 ppm in porpoise fat (Holden, 1967). The Northern Anchovy (Engraulis mordax) from the San Francisco Bay area had a total body burden of .21 to 10.20 ppm while the English Sole (Parophyrs vetulus) from the same area contained .14 ppm p,p'-DDE (Risebrough, 1969). The common surf-zone Sand Crab (Emerita analoga) was collected from various locations along the California coast and analyzed for total organo-chlorine pesticides. The analysis demonstrated a range of .024 ppm to 6.9 ppm wet weight p,p'-DDE in the sand crab (Burnett, 1971).

Distribution and accumulation of p,p'-DDE in fresh water systems has also been well documented in field environments. One of the most comprehensive studies on stream systems was accomplished by Grzenda (1965). His work demonstrated that chlorinated hydrocarbon pesticides can be distributed among various components of a lotic system. DDE was distributed among water, substrate, bottom fauna

and fish with the fish having .2 to 1.6 ppm DDE. In a number of comprehensive pesticide monitoring surveys both in the soil and water environments and estuaries and wildlife, p,p'-DDE was present (Spencer, 1967; Lyman, et. al., 1968; Wiersma, et. al., 1971).

Bird populations have been recently monitored for pesticide accumulation and p,p'-DDE has been found in a number of samples. Falconiform birds from Montana have a whole muscle wet weight burden of 10.6 to 14.0 ppm p,p'-DDE (Seidensticker, 1970).

Moribound Bald Eagles (Haliaeetus leucocephalus) from Florida and Connecticut were found to contain high levels of p,p'-DDE. Eagles from Florida demonstrated 28.9, 73.8 and 27.1 ppm wet weight in carcass, liver and brain, respectively. The eagles from Connecticut possessed 263.0, 170.0 and 88.6 ppm wet weight in carcass, liver and brain, respectively, (Reichel, et. al., 1969). Double-crested Cormorants (Phalacrocorax auritus) collected from the Muscongus Bay in Maine averaged 1.5 ppm for brain tissue, 6.5 ppm in gonads, 3.5 ppm in hearts and 6.5 ppm in eggs. No reproductive failure was noted in this cormorant population (Kury, 1969). Great Blue Heron (Ardea herodias) eggs from western Oregon were analyzed and found to contain 3.3 to 4.5 ppm wet weight p,p'-DDE. This egg burden was found not to be sufficient to elicit reproductive defects (Henny, 1971).

Trophic Level Transfer of Contaminants

The phenomena of biological concentration of pesticides and other chemical pollutants have received particular attention, as small concentrations of these contaminants have been shown to have

deleterious effects (Stickel, 1968). A concentration of 1.0 to 3.0 parts per trillion (ppt) DDT in Lake Michigan water has resulted in accumulation of DDT residues in several species of fish surpassing the Food and Drug Administration's temporary tolerance level for human consumption of 5 ppm (Reinert, 1970).

Field and laboratory studies have suggested that organochlorine compounds concentrate via a food chain mechanism, as well as accumulating by direct sorption from the water (Hunt and Bischoff, 1960; Woodwell, et. al., 1967).

There have been many studies demonstrating pesticide accumulation by fish under laboratory conditions using contaminated food pellets as a source of pesticide input (Buhler, et. al., 1969; Grzenda, et. al., 1970; Macek, et. al., 1970). Chadwick and Brocksen (1969) used dieldrin-contaminated midge larvae (Chironomidae) and tubificid worms (Tubifex sp.) as a source of pesticide introduction to sculpins. However, no data were presented as to the rate of uptake and accumulation of dieldrin by the midge larvae. Meeks (1968) described the accumulation of CL^{36}-DDT by bloodworms in a freshwater marsh. He found that the midges accumulated up to 5.2 ppm (dry weight) when a concentration of 3.0 parts per billion (ppb) was in the surface water.

Population Effects

The majority of effort towards the understanding of biologically active compounds and aquatic insects has been concerned with population disturbances and acute toxicity determinations.

Aquatic insect population effects ellicited by pesticide contamination have been shown by a number of authors. When DDT was applied to a Utah pond for mosquito control those organisms most commonly affected were Odonata, larger Coleoptera, particularly Dysticidae, Ephemeroptera and a genus of Corixidae. Various Crustacean and Corixidae appeared to be the most tolerant (Warniek, et. al., 1966). The effects of DDT larviciding on a Labrador stream are also noted when bottom invertebrates were sharply reduced as a result of the DDT. Caddisfly larvae were most affected and drifting insect larvae increased exponentially during each DDT application (Hatfield, 1969). Hitchcock (1965) has shown a reduction in total number of insects following a one pound per acre application of DDT for forest insect control in a Connecticut stream. The Ephemeroptera appeared to be the most affected while the Megaloptera appeared relatively unaffected. There was some indication that the DDT had affected adult emergence. Repopulation was also studied and recovery was noted qualitatively after two years. Similar results were also obtained in a Maine stream when 1.2 pounds per acre of DDT were used for Spruce Budworm control (Dimond, 1967). There was a 50% reduction in Trichoptera and Ephemeroptera and a 60% loss in Simuliidae and Chironomidae.

All normally present taxa were repopulated in two to three years. Delays in recovery were largely a result of slower repopulation of stoneflies and caddisflies. Mayflies recovered more quickly with the Chironomidae reinvading the fastest. Gypsy moth control in Pennsylvania has led to consequential effects of DDT on lotic insect population (Hoffman and Drooz, 1953). Mayflies and caddisflies were

severely affected with repopulation occurring three years later. The Chironomidae as in other studies was the first group to reinvade the denuded stream. Family level differences of repopulation were noted in a Montana stream when one gallon per acre of DDT was used for Spruce Budworm control (Hastings, et. al., 1961). The Trichopteran family Leptoceridae were found in the faunal composition four years after spraying for budworm control. The orders Ephemeroptera and Plecoptera completely recovered to pre-spray numbers after two years. Similar studies with like results have been obtained by other workers in the area of pesticides and aquatic insect population changes (Hoffman and Surber, 1945, 1949).

There has been a limited amount of effort directed towards evaluation of insecticides other than DDT on aquatic insect population. Methoxychlor as a simuliidae larvicide was shown to have minimal toxic effects to other stream insects as compared to a DDT application (Burdick, et. al., 1968). Also Methoxychlor did not pose a residue problem as did DDT. Situations affecting aquatic insect populations can arise from the utilization of insecticides for processes other than direct application for insect control. A woolen mill that used dieldrin as a moth-proofing agent released .2 ppm into the plant effluent which in turn emptied into a small South Carolina stream. The number of species of aquatic invertebrates downstream had been greatly reduced. Areas of the contaminated stream were completely denuded except for a few genera of Chironomidae (Wallace and Brady, 1971). Aldrin was investigated as to its effect on the invertebrates of an Illinois stream following application to 23,000 acres for Japanese beetle control (Moye and

Luckman, 1964). Of the four taxa studied only the Elmidae appeared unaffected by the treatment. Species of Ephemeroptera were severely reduced and the two remaining taxa, Trichoptera and Chironomidae, increased the summer following the treatment.

There is a paucity of knowledge dealing with the effects of organophosphate insecticides on aquatic insect populations. Much of our understanding of the behavior and significance of organophosphates in the aquatic environment has been derived from studies utilizing fish as the test organism (Parkhurst and Johnson, 1955; Henderson and Pickering, 1957; Darsie and Corriden, 1959; Henderson, et. al., 1960; Lewallen and Wilder, 1962; Pickering, et. al., 1962). There is one comprehensive study however that deals with malathion and its effects on benthic populations (Kennedy and Walsh, 1970). This study illustrated that the Chironomidae was the dominant family of aquatic insects and formed approximately 70% of the total benthos. This group showed a significant reduction in numbers in both high and low (.022 ppm and .002 ppm) treated ponds. Baetid mayflies formed about 24% of the benthos and were also definitely reduced in both high and low treated ponds.

Pesticide Toxicity Determination

Thus far laboratory studies involving pesticides and aquatic insects have been primarily directed towards acute toxicity determinations. These studies have just been "recently" conducted in relation to when the synthetic pesticides were first incorporated in pest control programs. The earliest work centered around the use of Daphnia as a test organism to evaluate the significance of the

pesticide in the aquatic environment (Anderson, 1945). The stonefly naiad (Plecoptera) has now been utilized as a representative test organism for acute toxicity studies. Acroneuria pacifica and Pteronarcys califoncica were exposed to various organic insecticides (Jensen and Gaufin, 1964a). The organochlorine insecticides used were DDT, aldrin, dieldrin and endrin. Organophosphates were represented by parathion, malathion, guthion, Dylox, Disyston and Bayer 29493. Endrin was the most toxic to both species with a 96 hour TL_m of .00039 ppm for A. pacifica and .0024 ppm for P. californica. DDT appeared to be the least toxic having a 96 hour TL_m of .32 ppm A. pacifica and 1.8 ppm for P. californica. The organophosphates were of intermediate toxicity between the extremes of endrin and DDT. With the insecticide parathion the resistance of P. californica was greater than that of A. pacifica.

Similar results were obtained in another study utilizing P. californica and two other different species of stoneflies (Sanders and Cope, 1968). Endrin and dieldrin were the most and DDT was the least toxic of the chlorinated hydrocarbon insecticides tested. Parathion was the most toxic of the organophosphates tested to P. californica while dursban was the most toxic to P. badia and C. subulosa naiads. Trichlorofon (Dipterex) was least toxic to all three species. There was a marked relationship between naiad size and susceptibility to the insecticides tested in both of the studies, with smaller naiads being more susceptible.

There is very little understanding and knowledge of the effects of pesticides on aquatic insect physiology. Long term chronic effects have often been neglected as a parameter of measuring the

significance of a pesticide in the aquatic environment. Five pesticides were assayed for their physiological impairment: DDT, parathion, malathion, Disyston and Dylox (Jensen and Gaufin, 1964b). Normal molting of A. pacifica and P. californica was inhibited at or near the four-day TL_m concentration of each compound tested. At concentrations less than the four-day TL_m molting was not altered until after 20 to 25 days of exposure.

Another study has shown the acute effects of organophosphate insecticides. In an attempt to evaluate the effectiveness of three organophosphates for nuisance mayfly and caddisfly control along the upper Mississippi River some toxicity values were generated (Carlson, 1966). The caddisfly larvae, Hydropsyche, was more susceptable to all three compounds tested (malathion, Co-Ral and Dylox) than was the mayfly Hexagenia.

Aquatic Insect Pesticide Resistance

The phenomenon of resistance and tolerance of insects to insecticides has received particular attention. However, this biological expression has only been recently recognized in aquatic insects other than the Culicidae. The mosquito, Culex pipiens, was one of the first insects (including terrestrial and aquatic) to demonstrate resistance to DDT (Mosna, 1947; Missiroli, 1947). Other studies in mosquito resistance to pesticides have followed this initial investigation (Deonier and Gilbert, 1950; Bowman, et. al., 1959; Abedi and Brown, 1960; Abedi, et. al., 1963). Resistance to DDT in other aquatic insects has now been shown. A mayfly population, Heptagenia hebe, in New Brunswick was exposed to DDT for Spruce

Budworm for eight consecutive years (Grant and Brown, 1967). When these naiads were tested against the same species taken from untreated streams they showed LC_{50} values three times higher than the control population. Populations surviving the air spray proved to be 12 to 40 times as DDT-resistant as the untreated population. The DDT resistant naiads of H. hebe detoxified DDT to p,p'-DDE 15 times faster than the control naiads. Another interesting result brought forward by this study was that the DDT-resistant naiads absorbed approximately twice as much DDT as did the control naiads. A follow-up study was conducted on a stonefly and mayfly population in a New Brunswick stream suspected of exhibiting DDT-resistance (Sprague, 1968). The presence of mayflies and stoneflies in the contaminated stream was probably because they survived spraying while in the egg stage. Toxicity studies did not provide data that would support the apparent insecticide resistance supposedly incorporated in the population.

Similar changes to DDT resistance in aquatic insects have been observed elsewhere. On the north shore of the St. Lawrence River ten years of aerial spraying for blackfly control has led to a 10-fold increase of DDT tolerance in the larvae of Simulium venistum, and similar blackfly control programs near Tokoyo, Japan, have induced a 13-fold resistance to DDT in Simulium aokii (Suzuki, et. al., 1963).

An aquatic invertebrate complex located in an area of drainage ditches in southern Mississippi with a long history of pesticide contamination demonstrated high tolerance limits to selected pesticides (Naqui and Ferguson, 1968). Six species of Cyclopoid

copepods, a clam (Eupera singleyi) and Tubifex tubifex from this high pesticide use area did not show any mortality at the 48 hour TL_m established for nine insecticides for control animals. The potential effect of increased tolerance in these invertebrates species could be to increase the amount of pesticide residue available to higher trophic levels.

Overview of Polychlorinated Biphenyl - PCB

The presence of the industrial contaminant, polychlorinated biphenyl (PCB), in the aquatic environment has been recently recognized and characterized (Risebrough, 1966). Since the discovery of PCB in natural waters a review of the present state of knowledge concerning environmental contamination by the pollutant indicates that it may be one of the more widespread contaminants (Veith and Lee, 1970). The significance of this contamination has not yet been evaluated due in part to the lack of systematic analytical procedures for the quanitative and qualitative determinations of the components of chlorinated biphenyl mixtures. A review was recently written to summarize the current status of the analytical determination of polychlorinated biphenyl in environmental samples (Risebrough, et. al., 1969). The environmental occurrence, uses and present toxicological aspects of PCB's were recently reviewed by a number of workers (Peakall and Lincer, 1970; Gustaffson, 1970; Risebrough, 1970).

The Monsanto Company is the sole manufacturer of PCB's in the United States and markets eight formulations of chlorinated biphenyls under the trademarks Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262 and 1268 with the last two digits indicating the percent chlorine of

each formulation (Gustaffason, 1970). Aroclor 1248 and 1254 are the materials produced in the greatest quanities and are used as a dielectric fluid in capacitors and in closed-system heat exchangers.

Toxicity and Biological Concentration of PCB

Chronic and acute toxicity studies of PCB on aquatic organisms is quite limited. Due to the low water solubility of PCB's, tests to obtain 96 hour LC_{50} values do not adequately reflect their toxicities to fish and aquatic invertebrates (Stalling and Mayer, 1972). Aroclors 1221 through 1268 have 96 hour LC_{50} values ranging from 1,170 to 50,000 ug/liter for Cutthroat trout. The acute oral toxicities of Aroclors 1242 through 1260 was greater than 1500 mg/kg in Rainbow trout. Intermittent-flow bioassays of Aroclor 1242, 1248 and 1254 to Bluegills resulted in 15-day LC_{50} values of 54, 76 and 204 ug/liter respectively. Chronic tests have been conducted to determine the effects of Aroclors 1242 and 1254 on Fathead Minnow reproduction, all fish exposed to greater than 8.3 ug/liter of each Aroclor died. Accumulation of Aroclors 1248 and 1254 by Bluegills chronically exposed to 2-10 ug/liter was from 26,300 to 71,400 times the exposure levels for both PCB's.

Studies involving aquatic invertebrates and PCB's are somewhat limited, however a few studies have been undertaken to determine the toxicity of the specific mixtures of PCB's. Aroclor 1248 was shown to be the most toxic series to Daphnia magna and preliminary studies indicate that levels above 5 ug/liter are not safe for reproduction (Nebeker and Puglisi, 1971). After 48 hours exposure to 300 ug/liter, Daphnia concentrated Aroclor 1254 48,000 times. The level of Aroclor

1248 that did not affect scud (Gammarus pseudolimnaeus) reproduction was comparable to that for daphnids. The 96 hour LC_{50} values for 1248 and 1254 were 52 ug/liter and 2,400 ug/liter respectively. Scuds were more sensitive to Aroclor 1242 with a 96 hour LC_{50} value of 10 ug/liter. After exposing another species of scud (Gammarus fasciatus) to 1.6 ug/liter Aroclor 1254 for 14 days, the PCB was concentrated 27,000 times the exposure level. No further increase in PCB residue resulted after an additional 21 days of exposure (Sanders, 1970). The damselfly, Ishnura verticalis and dragonfly, Macromia sp. were incorporated into static and continuous-flow bioassay tests using 1242 and 1254 in both types of exposure. Four-day LC_{50} values ranged from 200 ug/liter for 1254 in continuous flow tests to 1000 ug/liter for 1254 in static conditions. Little variation in susceptibility was observed between the two species (Stalling and Mayer, 1972).

Studies on the effect of Aroclors 1248 and 1254 on the emergence of the parthenogenetic midge, Tanytarsus dissimilis, have been completed at the National Water Quality Laboratory, Duluth, Minnesota (Nebeker, et. al., 1972). Adults emerged when exposed to concentrations up to 9 ug/liter of 1248, while larvae were present at 18 ug/liter but adult emergence did not occur. Abundant emergence did not occur above 5.1 ug/liter. Aroclor 1254 was more toxic to midges as no emergence occurred above 3.5 ug/liter, and abundant emergence did not occur above 3 ug/liter even though larvae were present. The survival and growth of the midge when tested with Aroclor 1254 was good in control chambers but was reduced by 50% at the lowest test concentration of .45 ug/liter and at 1.2 ug/liter larval cases

were reduced to 35% of the control and pupal cases were reduced to 24% of the control. No larval cases were formed at 33 ug/liter and no pupal cases were constructed at 9 ug/liter. The authors calculated a two-week LC_{50} for 1254 (50% reduction based on control as 100%) as .65 ug/liter for larvae and .45 ug/liter for pupae, indicating that a safe level for midge well-being is below 1 ug/liter of Aroclor 1254.

Biology of the Midge, Chironomus tentans

The "bloodworm" or midge, Chironomus tentans (Diptera: Chironomidae) is a benthic insect which has received particular attention by the aquatic biologist due in part to its availability as a fish food organism. Studies have been conducted as to ascertain the practicability of artifically and naturally propagating midge larvae as a forage crop for fish (Malloch, 1915; Leathers, 1922; Branch, 1923; Johnson, 1929; Sadler, 1934). From these studies a workable knowledge of the functional biology of the insect has been derived. Later efforts have now been principally directed towards two areas; 1) factors affecting the distribution of the midge population (Hilsenhoff, 1966, 1967) and 2) the functional role of the midge in the total aquatic system (Hall, et. al., 1970).

Hilsenhoff's (1967) three year study in Lake Winnebago contributed some insights into the factors that regulate fluctuations in populations of the insect. Chemistry of the water and physical characteristics of the bottom sediments were recorded and evaluated. None appeared to affect C. plumosus populations, but several characteristics of the mud apparently were related to these

populations. Other benthic organisms seemed to exert only a minor influence on populations of C. plumosus. Climatic factors appeared to be very important in determining the distribution and abundance of C. plumosus in Lake Winnebago. Fish predation probably also influenced C. plumosus numbers, but microsporidia and unknown viruses, fungi and bacteria were probably the most important regulators of the populations.

The comprehensive study undertaken by Hall (1970) indicated that Chironomus tentans is an organism of primary importance in the benthic community comprising 50% to 86% of the total biomass in low and high nutrient ponds respectively. C. tentans is also serving as a prey organism for two consumer levels, the predacious Hemiptera and Odonata at one extreme and young of the year to one year old centrachids at the other. Thus this insect can act as a transitory organism of pesticide transfer to at least two routes of distribution in the aquatic community.

INTRODUCTORY NOTE ON METHODOLOGY

In order to investigate the proposed objectives of this study it was necessary to employ many different types of methodology and quanitative instrumentation. To facilitate some coherence of order to the reader, this study will be presented as sections, each section representing a separate area of investigation. The methodology, results and discussion will be incorporated into each section and a final summation discussion will include all the sections that were presented. The specific sections that will be utilized in this presentation are as follows:

1. Uptake, distribution and elimination of DDE by C. tentans.
2. Influence of the presence of Aroclor 1254 on the uptake of DDE by C. tentans.
3. Determination of the mode of uptake of DDE by C. tentans.
4. Metabolism of DDE by C. tentans.
5. Effect of DDE on the egg viability of C. tentans.
6. Field experimentation with C. tentans and DDE.

RESULTS AND DISCUSSION

SECTION I

THE UPTAKE, DISTRIBUTION AND ELIMINATION DYNAMICS OF DDE BY *Chironomus tentans*

Uptake and Distribution of DDE by *Chironomus tentans*

METHODS AND MATERIALS

Approximately 200 last instar larvae of *C. tentans* were obtained from William Cooper, Department of Zoology, Michigan State University. Stock cultures of *C. tentans* were maintained by placing these larvae on a substrate in Min-O-Cool tanks (model MT-500, without compressors, Frigid Units Inc.). The substrate utilized was prepared by placing 50 grams of paper hand towel (Nibroc, Brown Co.) in a Waring blender with 5 grams of chicken feed (Mason Mix) obtained from the Poultry Science Department, Michigan State University, and 1.6 liters distilled water to cover the mixture. The mixture was homogenized for 3 to 5 minutes and placed in culture tanks containing dechlorinated tap water and aerated.

Aluminum screening was placed over the stock cultures and the adults were allowed to mate, depositing their egg masses in the water within the tanks. This method proved very satisfactory since a ready supply of eggs was always available. Egg masses were collected from stock cultures with a pipetting bulb and a two foot length of 9 mm i.d. glass tubing. Analysis of the paper hand towel, chicken feed and stock culture water showed no detectable pesticide

residues. The paper hand towel was also assayed for elemental mercury residues and found to contain no detectable amount (less than 1 ppb).

The developmental time of C. tentans under the laboratory conditions described is 25 to 30 days from egg to adult. Males appeared to emerge first, followed by a synchronous emergence of males and females. After mating, the female deposits one gelatinous egg mass which contains 1500 to 2000 individual eggs (Cooper, personal communication, 1971).

A continuous flow dilution apparatus was used resembling that described by Burke and Ferguson (1968) equipped with a modification in the toxicant introduction system. A Beckman solution metering pump (model 746, 0-2 ml flow) replaced the carboy siphoning system used by Burke. A stock solution of 130 ppm p,p'-DDE (99.9%) was prepared in 100% ethanol. This stock solution was sufficiently concentrated so that the ethanol concentration in the test aquaria never exceeded five ppm.

Tap water passed through a cellulose filter that removed particulate matter and colloidal iron and then through an activated charcoal filter. The dilution water flowed into a 190 liter stainless steel tank which continuously overflowed, thus maintaining a constant head pressure to the dilution apparatus. The dilutor delivered four different concentrations of DDE and one control; DDE concentrations were diluted by a factor of one-half the preceding concentration (1.0, .5, .25 and .12 ppb). The desired concentrations of DDE flowed into three liter glass aquaria (25 x 16 x 17 cm). The flow of each concentration and control was split into two aquaria so that a duplicate of each concentration could be obtained. A

continuous flow rate of 110 ml per minute was delivered into each of the 10 aquaria, giving a 90% turnover rate of water in three hours.

Two egg masses were placed in each of the 10 aquaria with the described substrate (50g) for every experiment and the test aquaria were covered with plexiglass to contain the emergent insects. The test aquaria were immersed in a constant temperature (Min-O-Cool) bath at 21°C during the experiment. A fluorescent light system was suspended two feet above the test containers to provide a 12 hour light-dark cycle.

The diluent water was sampled weekly and had the following characteristics in the test aquaria:

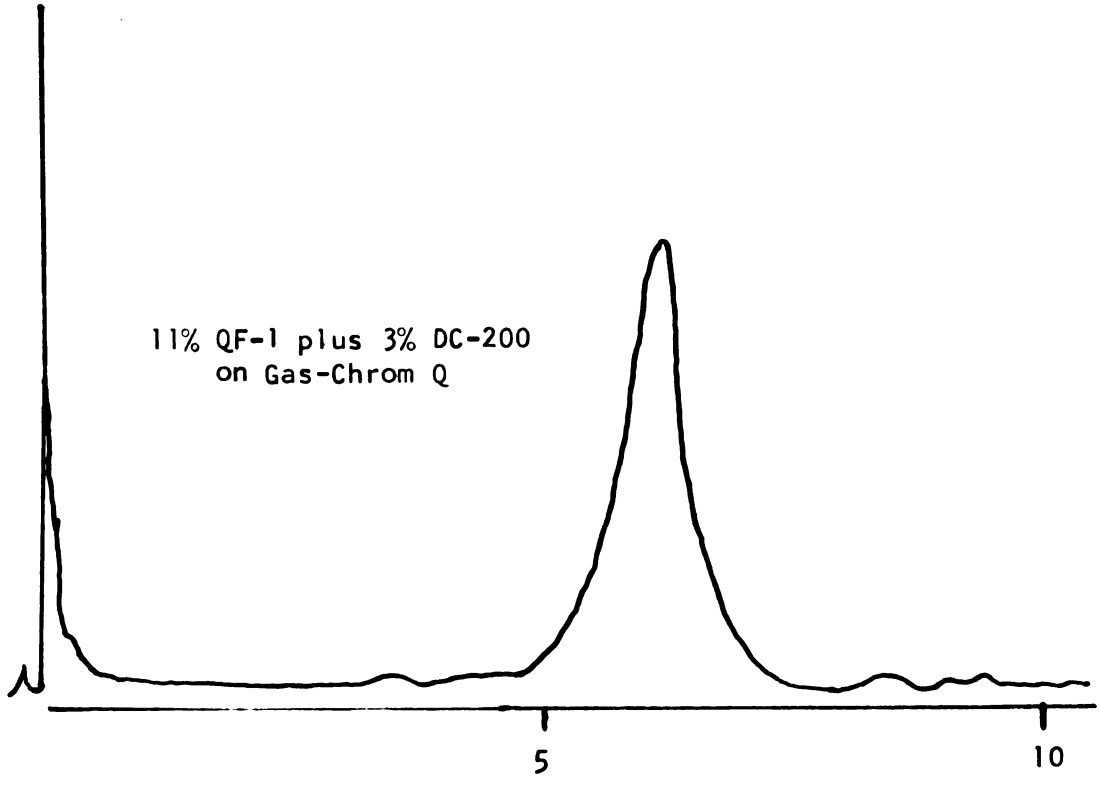
Conductivity	5.95×10^2 umhos/cm^2
Dissolved oxygen	5.52 ppm
Phenophthalein alkalinity	12 ppm as $CaCO_3$
Brom-cresol methyl red alkalinity	330 ppm as $CaCO_3$
Total hardness	347 ppm as $CaCO_3$
pH	7.9

The p,p'-DDE (99.9%) used for this study was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. The identity of this parent compound and DDE residue was confirmed by thin layer and gas-liquid partition chromatography and mass spectrometry. A DuPont Model 21-490 mass spectrometer (DuPont Company, Instrument Division, Monrovia, California) was used. The sample was analyzed at 180-200°C probe and source temperatures, and 70 eV ionizing voltage. No impurities or isomers were detected in the parent material.

A sample of midges comprising at least 50 to 100 individuals were taken from each of the 10 aquaria at specified time intervals and each sample was subsequently divided into three subsamples. The number of individuals in each subsample was recorded, blotted on a dry paper towel and the wet weight was determined to the nearest tenth of a milligram. The subsample was then placed in a teflon-lined screw cap vial with one ml of redistilled hexane and macerated with a glass rod. One ul of this extract was injected into the gas chromatograph for quantitation. A Beckman GC-4 gas chromatograph equipped with a discharge electron capture detector was used for residue analyses. It was fitted with two 6 foot (1.83 m) x 1/16 inch (1.59 mm) boroscilicate glass columns. Two packing materials were employed for residue determination. One material consisted of 11% QF-1 and 3% DC-200 on Gas-Chrom Q (60-80 mesh) and the other column was packed with 4% SE-30 on Chromosorb W (80-100 mesh). Retention times and confirmation of p,p'-DDE residues are presented (Figure 2) for comparison. The instrument was operated at a column temperature of 220°C, and had a helium (99.995%) flow of 30 ml per minute. The injection temperature was 250°C and detector temperature 275°C. DDE standards were injected at the beginning of each run, after every six samples, and at the end of the run. Quantitations were based on peak height. Concentrations were expressed on a wet weight basis for the insects and converted to micrograms of DDE residue per individual.

One liter water samples were taken every other day from the outlets of the aquaria and were extracted successively in two liter separatory funnels with 100, 50, 50, 50 and 50 ml of redistilled

Figure 2. Comparison of two column packing materials used for DDE residue determination in Chironomus tentans, water and substrate.


11% QF-1 plus 3% DC-200
on Gas-Chrom Q
5
10
Retention time (minutes)

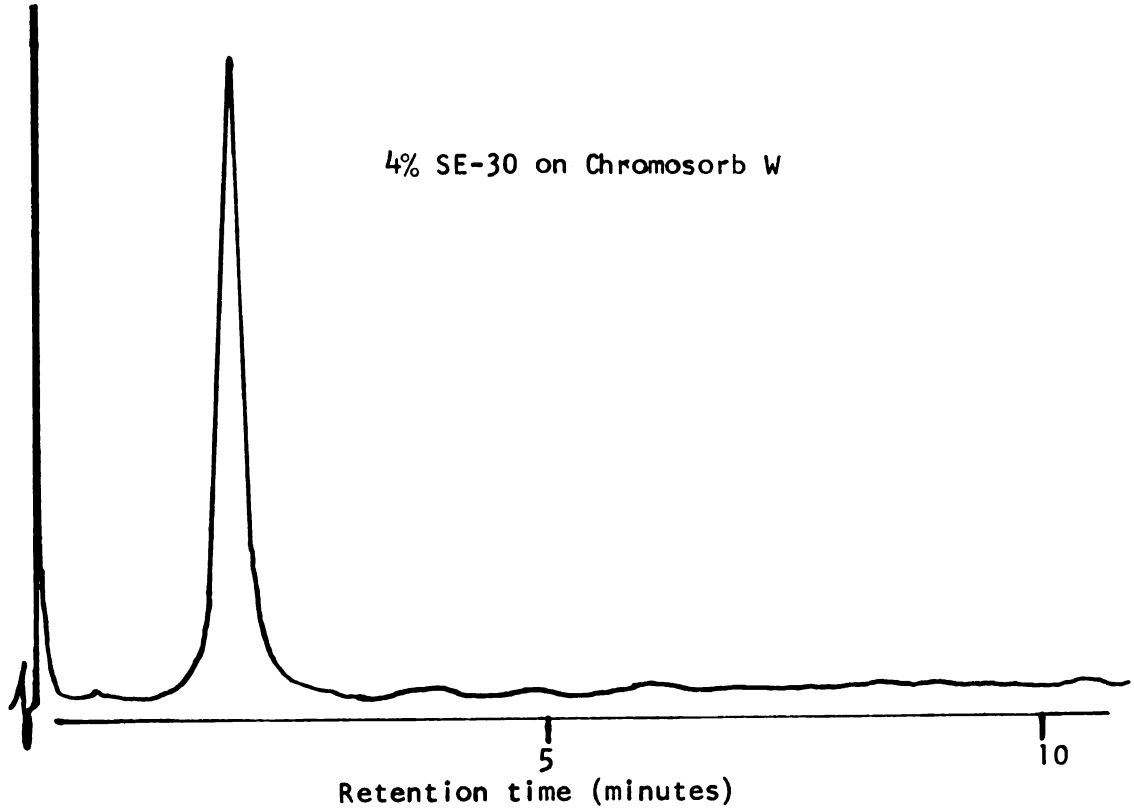

4% SE-30 on Chromosorb W
5
10
Retention time (minutes)

Figure 2

hexane. The combined extract was dried over anhydrous sodium sulfate and concentrated to 5 ml for introduction into the gas chromatograph.

The substrate was analyzed for DDE residue by filtering it through No. 1 Whatman filter paper using a Buchner funnel. The filtrate and retained substrate were oven dried (80°C) for two hours and weighed, extracted three times with redistilled hexane and introduced into the gas chromatograph for quantitation.

Recovery of DDE from the water was 82%, based on six spiked samples. The recovery of DDE, utilizing the maceration technique with hexane, from C. tentans larvae, pupae and adults ranged from 79% to 86%, based relative to that efficiency achieved by Soxhlet extraction with acetone-hexane (1:1) for four hours. Three different concentration ranges of DDE were used for experimental purposes (Table 1).

Table 1. Concentration ranges of p,p'-DDE in water (ppb) that Chironomus tentans was exposed to.

I		II		III	
ppb	S.E.[1]	ppb	S.E.	ppb	S.E.
.53	.038	1.1	.228	2.2	.156
.29	.015	.55	.054	1.1	.071
.20	.013	.25	.046	.56	.063
.07	.004	.10	.008	.21	.023

[1] Standard Error

RESULTS

Uptake of DDE

In every experiment uptake of DDE from eggs through last instar larvae demonstrated a dose-dependent relationship. Equilibrium concentrations in the last instar larvae were plotted against the corresponding water concentration and fitted with a least squares line (Figure 3).

The last instar larvae appeared to concentrate DDE approximately 14,000 to 20,000 times over the concentration of DDE in the water. The slopes of the lines and regression coefficient are presented with the concentration of the insects in ppm and water concentration in ppb. The correlation coefficients (r) of .997, .998 and .990 indicate a very close fit and a highly significant correlation between the concentrations. ($F_{exp} = 208.1$, $F_{exp} = 164.3$, $F_{exp} = 536.7$, $F_{.995} = 9.28$.)

Semilogarithmic plots of DDE accumulation by C. tentans from egg through adult versus days of exposure at different DDE concentrations in the water are presented (Figures 4, 5, 6 and 7). Each point on the curve represents the mean of the three subsamples and two duplications for each time period.

Figure 3. Relation between concentration in the last instar larvae Chironomus tentans and the concentrations in the water from which they were removed. Roman numerals indicate results of three separate experiments and each point represents the mean residue value of three samples (each sample consisting of 50 individual larvae).

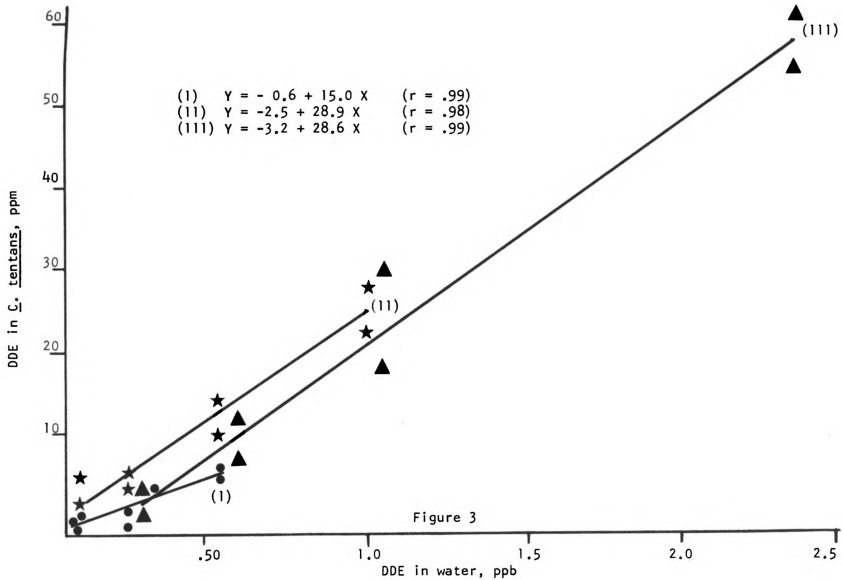

(1) Y = - 0.6 + 15.0 X (r = .99)
(11) Y = -2.5 + 28.9 X (r = .98)
(111) Y = -3.2 + 28.6 X (r = .99)
DDE in C. tentans, ppm
DDE in water, ppb
60
50
40
30
20
10
.50
1.0
1.5
2.0
2.5
(1)
(11)
(111)

Figure 3

Figure 4. Accumulation of DDE residues over time by Chironomus tentans from egg to adult when exposed to a concentration of 1.1 ± .20 ppb DDE in water.

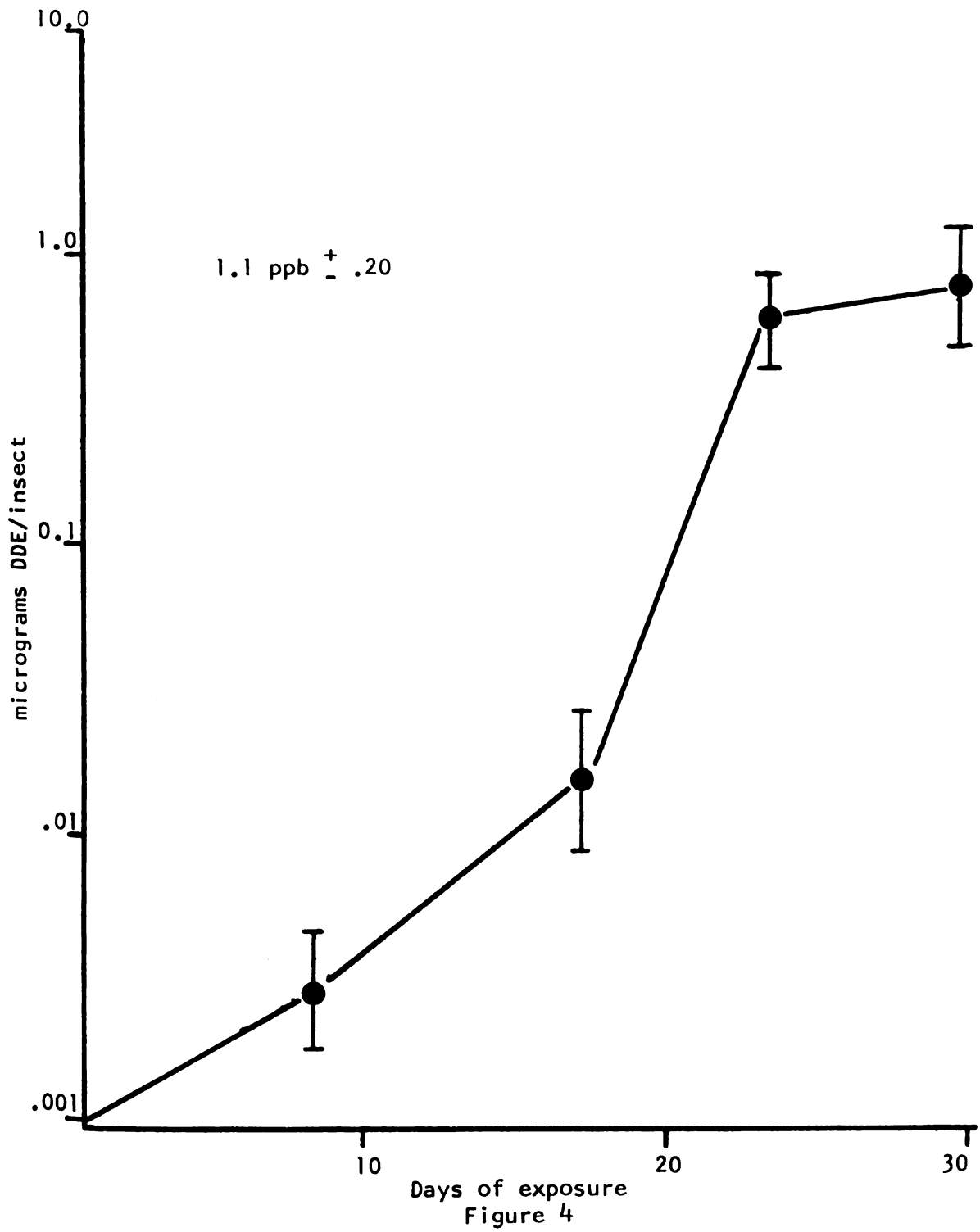

10.0
1.0
0.1
.01
.001
micrograms DDE/insect
1.1 ppb ± .20
10
20
30
Days of exposure

Figure 4

Figure 5. Accumulation of DDE residues over time by Chironomus tentans from egg to adult when exposed to a concentration of .55 ± .05 ppb DDE in water.

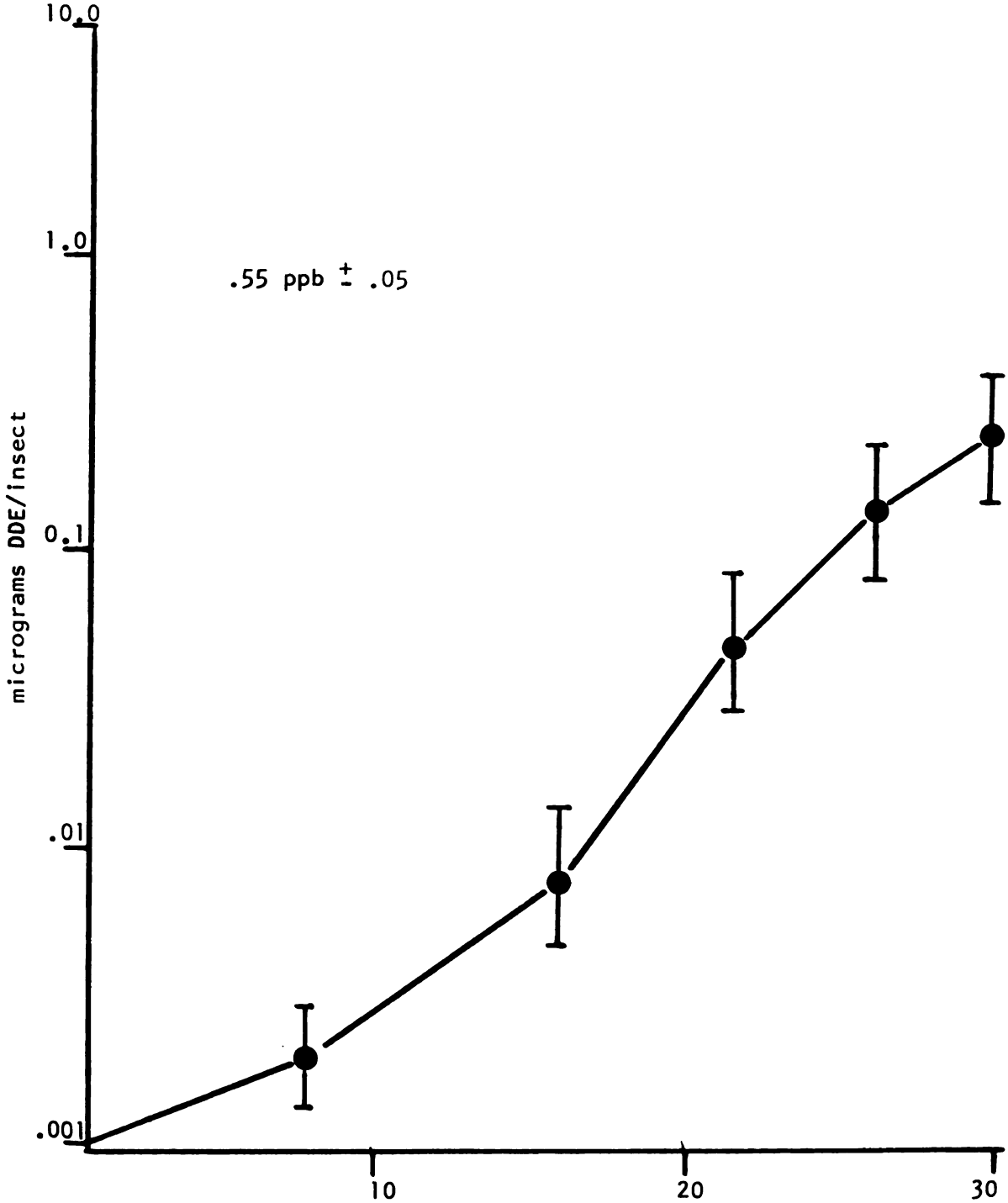

10.0
1.0
.55 ppb ± .05
0.1
.01
.001
micrograms DDE/insect
10
20
30
Days of exposure

Figure 5

Figure 6. Accumulation of DDE residues over time by Chironomus tentans from egg to adult when exposed to a concentration of .25 ± .04 ppb DDE in water.

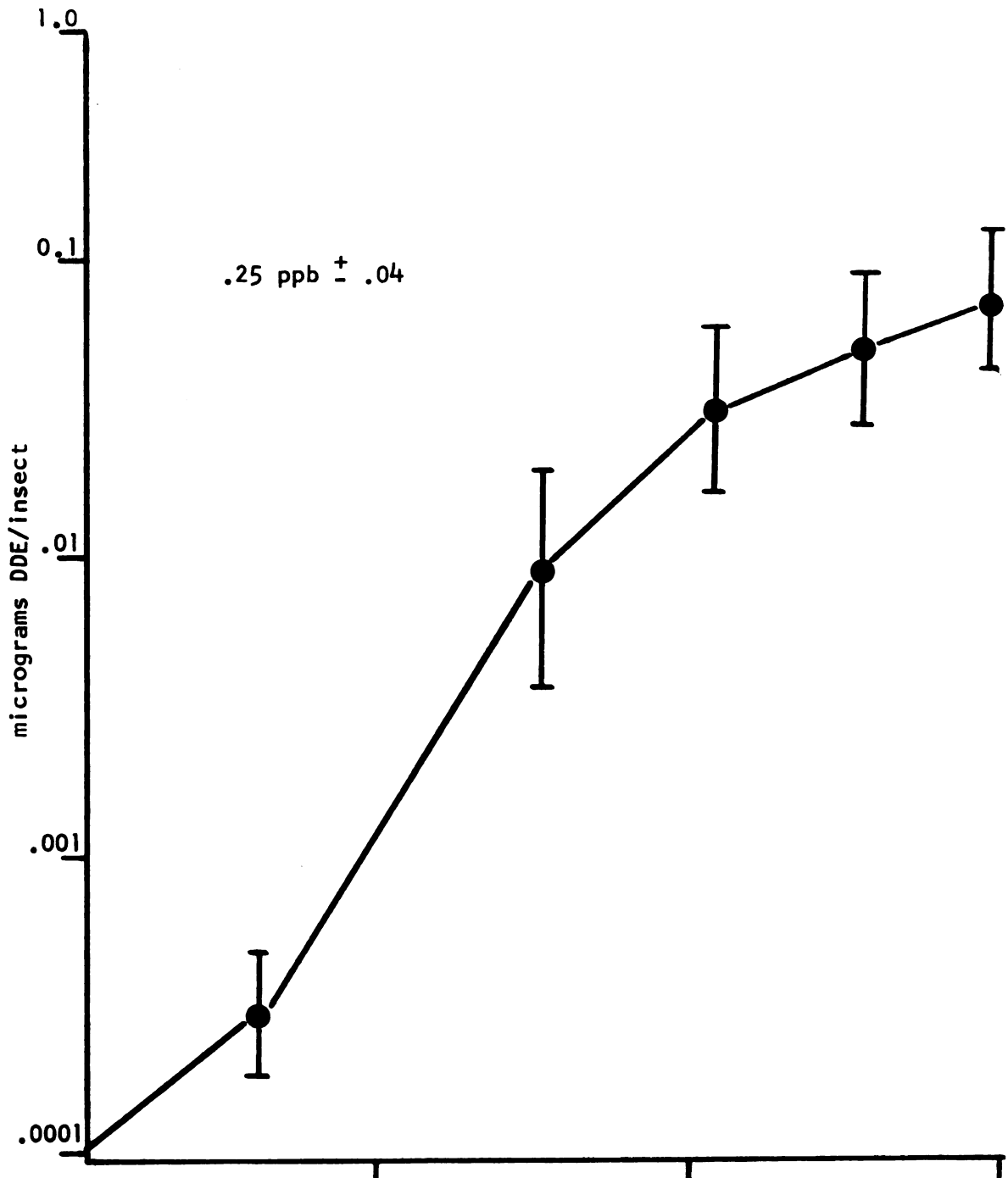

1.0
0.1
.01
.001
.0001
micrograms DDE/insect
.25 ppb ± .04
10
20
30
Days of exposure

Figure 6

Figure 7. Accumulation of DDE residues over time by *Chironomus tentans* from egg to adult when exposed to a concentration of .10 ± .08 ppb DDE in water.

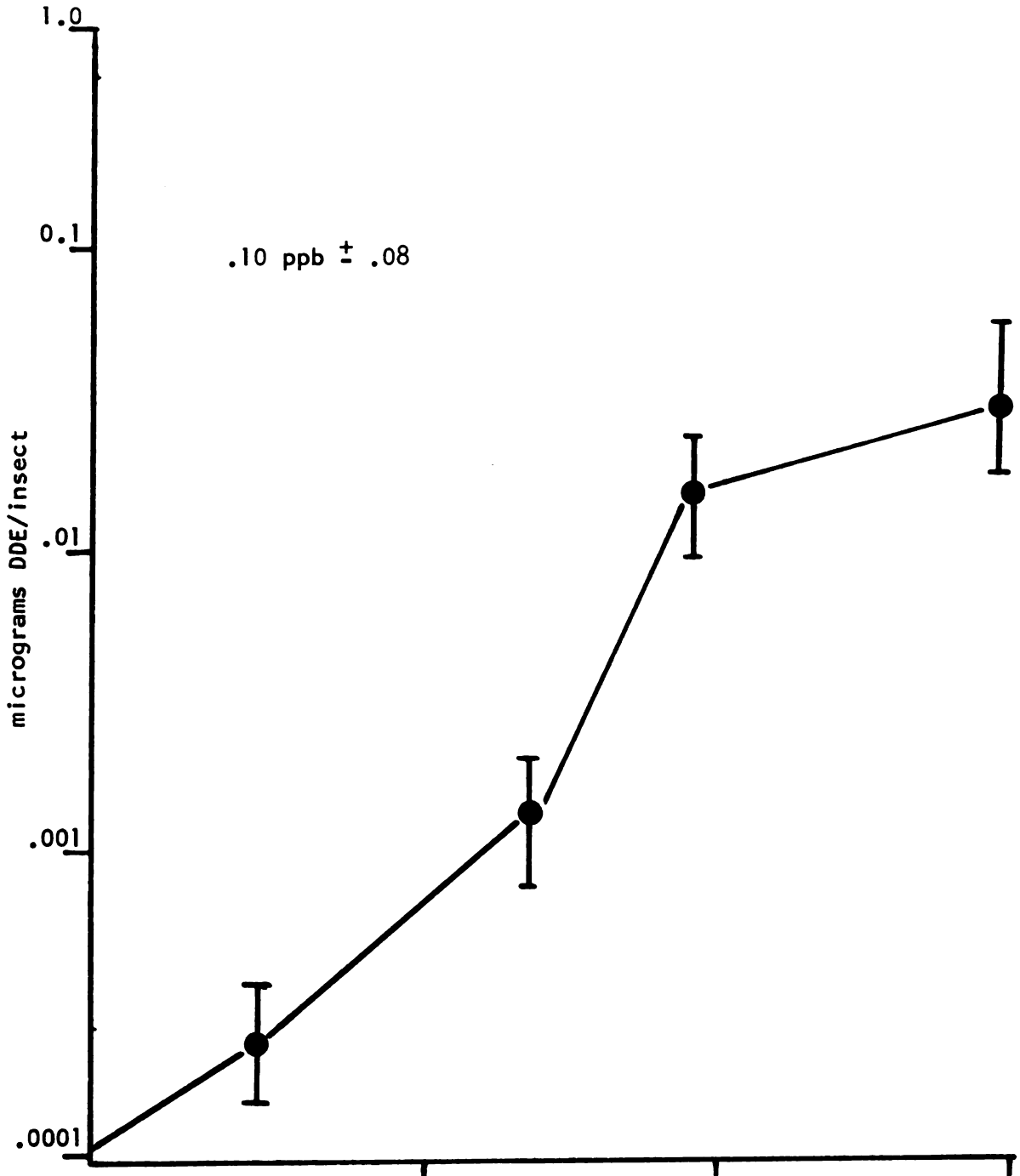

1.0
0.1
.01
.001
.0001
micrograms DDE/insect
.10 ppb ± .08
10
20
30
Days of exposure

Figure 7

Generally there was a period of approximately linear increase in DDE residue accumulation followed by an "equilibrium" state during which the DDE level did not increase with continued exposure. Equilibrium, or a plateau effect, was generally reached in 22 to 25 days after initial exposure to DDE. There was some indication that insects exposed to the higher concentrations of DDE (.50 to 2.2 ppb) had a shorter developmental period. These insects went from egg to last instar in 19 to 21 days as opposed to 22 to 25 days when exposed to the lower concentration (.06 to .29 ppb) and controls.

It was thought that the substrate could act as a DDE reservoir and accumulate the DDE in the water; consequently the larval DDE levels would be an expression of what was contained within the substrate. However, analysis of the substrate showed that DDE quickly partitioned between the substrate and water. There was not much accumulation of DDE residue by the substrate over time, with equilibrium levels being reached in five days. The relationship between DDE residue in the substrate and DDE in the water is presented (Figure 8).

Distribution of DDE Residues

When the larvae were allowed to pupate and emerge as adults, an idea of the route of DDE distribution could be obtained. A summary of the DDE residue distribution, expressed on a microgram per individual insect basis, in each developmental stage is presented (Table 2).

Figure 8. Relation between DDE concentration in the water and corresponding concentration in the substrate that *Chironomus tentans* was reared on.

Figure 8

Table 2. Summary of distribution of residue in Chironomus tentans exposed to varying concentrations of p,p'-DDE.

DDE in Water (ppb)		2.2 ± .15		1.1 ± .20		.56 ± .06		.21 ± .02	
Days of Exposure	Stage	[1]ug/insect	[2]S.E.	ug/insect	S.E.	ug/insect	S.E.	ug/insect	S.E.
7	Larva	.29	.013	.03	.009	.01	.004	.03	.005
16	Larva	.47	.09	.27	.006	.02	.007	.06	.003
24	Larva	,89	.18	.46	.23	.27	.018	.19	.08
29	Larva	1.92	.32	.89	.12	.36	.067	.29	.026
33	Pupa	2.14	.43	.96	.19	.38	.09	.25	.06
36	Adult	2.53	.27	1.18	.26	.44	.21	.26	.04
	Exuvia	.03	.006	.02	.009	.02	.005	.01	.007
	Egg Mass	.78	.18	.24	.09	.13	.08	.03	.004
Pupal Residue as Exuvia (%)		1.4		2.5		4.9		4.4	
Adult Female Residue as Egg Mass (%)		30.8		20.3		29.5		11.6	

[1]Values represent means of three subsamples from two replications.

[2]Standard error.

The exuvia did not demonstrate a major route of DDE residue elimination from the pupa as only 1.4 to 4.4% of the total residue was lost in the exuvia. An inverse relationship exists between DDE loss by way of the exuvia and the DDE level in the water. The insects treated with lower concentrations of DDE (.56 and .21 ppb) appeared to eliminate a greater percentage (4.9 to 4.4%) of their DDE pupal residue via the exuvia.

A significant proportion of the DDE residue in adult females was eliminated by the process of egg deposition. Egg masses possessed 11 to 30% of the total DDE residue contained in the adult females.

There was a positive relationship between the amount of DDE in the adult female and the concentration of DDE in the water to which the adult (as egg to larvae) was exposed. Adult females clearly reflected the amount of DDE (ppb) in the water on a microgram per individual insect basis. The concentration of DDE residues in adults represents adult females that have not deposited their egg masses.

The rationale for expressing the DDE residue on a microgram per individual insect basis instead of a ppm basis is due to the loss of weight by last instar larvae going into pupation and a further loss of weight from pupa to adult (Jonasson, 1965). This weight loss would then reflect a higher concentration when using parts per million as a means of describing the residue accumulated.

DISCUSSION

To assess the significance of biologically active compounds in the aquatic environment one must correlate the response of each organism with the concentration of the compound in the water.

Concentrations of DDE utilized in these experiments are comparable to those found in environmental samples (.5 - 20.0 ppb) (Lichtenberg, et. al., 1970). However there appeared to be a greater amount of DDE residue accumulated by the midges in the laboratory as compared to residues found in aquatic insects in nature. Crouch and Perkins (1968) reported .26 ppm DDE in aquatic insects in an Oregon stream one week after the spraying of DDT and Meeks (1968) has shown that blood worms accumulated .98 ppm (wet weight) CL^{36}-DDT after one week when exposed to .3 to 3.0 ppb in a fresh-water marsh. However in both of these studies there was a decrease in the amount of DDT available to the aquatic organisms over a period of time, whereas in this study the insects were exposed to a continuous input of DDE.

Chironomids appear to play a major role in a benthic community due in part to their particular size and availability (Hall, et. al., 1970). Due to their importance in benthic communities chironomids could serve as a transitory organism between pesticides in aqueous solution and substrate to predators. The uptake relationship shows that chironomids are capable of accumulating DDE residues far greater

than the amount of DDE present in the water.

The midges accumulated DDE exponentially with increasing concentration of the compound in the water. This phenomenon would mean that small increases of DDE in the water would result in a comparatively large increase in accumulation of the compound by the midge. The last instar larvae never reached an upper limit of DDE accumulation with the concentrations of DDE used in this study. This same relationship was demonstrated by Wilkes and Weiss (1971). They found that the upper limits of DDT accumulation were never reached with dragonfly naiads, even when exposed to 20 ppb DDT.

The midges had an accumulation factor of DDE of approximately 14,000 to 20,000 times over what was present in the water. This is much higher than Wilkes and Weiss (1971) found with dragonfly naiads which had concentrated DDT 2700 times. A difference in results of this magnitude might be explained by the fact that the midge possesses a membranous cuticle and thus in contrast to an anisopteran chitinized cuticle possibly could have absorptive processes involved in accumulation. It is apparent that when this type of magnification is coupled with the consumption of these midges there will be a significant transfer of this biological active compound to higher trophic levels.

The rate of concentration or accumulation of DDE is a function of the level of intake and time of exposure and is also dependent upon the rate of detoxication, excretion and total elimination. This process of accumulation is especially dramatic in fish where rates of detoxication and elimination are very low. DDT and DDE can be absorbed directly from ppb concentrations in the water and accumulated

to levels of 10^5 to 10^6 times those of intake (Reinbold, et. al., 1971). Thus the rate of accumulation in the aquatic biota is proportional to the concentration of DDE in the water times the time of exposure.

The total concept of biological concentration of trace quanities of pesticides in the aquatic environment is comprised of a series of components of which elimination dynamics or excretion is one. The significance of studying elimination dynamics lies in two areas: 1) a study of this type would exemplify an intermittent dose of a bioactive compound in the environment and an idea of elimination could be formulated and 2) the rate of elimination can indicate how the compound is incorporated or bound to body tissue and also metabolic processes can be better understood.

Elimination Dynamics of DDE by *Chironomus tentans*

METHODS AND MATERIALS

In another set of experiments the midge was exposed to a different series of DDE concentrations (.26, 1.8, .10 and .06 ppb) for the purpose of investigating the dynamics of DDE elimination. All experimental conditions were similar to those utilized in the uptake and distribution studies. Samples of contaminated midges were collected at 7, 15, 20, 26 and 31 days after egg hatching occurred. The samples were subdivided, the number of midges recorded and weighed to the nearest tenth of a milligram and quanititation of DDE residue was identical to the procedure used in the uptake studies. After 15 days of DDE exposure, introduction was stopped and an idea of elimination rates could be investigated. A 15 day exposure to DDE was chosen because this time interval represents one-half the average developmental time of the insect (30 days) and thus the insect has 15 days to supposedly eliminate the accumulated DDE burden. Water and substrate were also monitored for DDE after introduction was stopped. The experiment was terminated with the onset of pupation, thus this study involves only larval elimination of the DDE accumulation. Because only the larval

stages were involved in these experiments and thus no weight loss with pupation and emergence to adults, the DDE residue could be expressed on a ppm wet weight basis.

RESULTS AND DISCUSSION

At the end of the 15 day DDE exposure period the larvae had accumulated residues of 1.23, 4.5, .33 and .15 ppm when exposed to water concentrations of .26, .18, .10 and .06 ppb, respectively (Table 3). At all levels of exposure when the introduction of DDE stopped the DDE concentrations in the midges dropped rapidly for the first ten days and then remained nearly constant for the next five days. A semi-log plot of DDE residue values following cessation of DDE introduction were nearly linear with a negative slope (Figure 9). The equation of this relationship is as follows:

$$(1) \quad Y = (A)\ (-B^{X})$$

where:

Y = concentration of DDE in the midge (ppm, wet weight)

A = regression intercept (ppm)

X = days following cessation of DDE introduction

B = relative rate of residue loss

or for the rectified data:

$$(2) \quad \text{Log } Y = \log A - (\log B)\ X$$

Log B, the regression coefficient, estimates the logarithmic rate of residue loss. DDE residue half-life regression coefficient from each exposure level were calculated and half-lives determined

Table 3. Concentration of DDE (ppm, wet weight) in Chironomus tentans exposed to various concentration of DDE in water for 15 days.

	Exposure Levels of DDE in Water (ppb)			
	.26	.18	.10	.06
Days	Concentration in C. tentans larvae (ppm)			
7	.48	.29	.19	.11
	.61	.21	.17	.09
	.56	.26	.16	.12
Mean	.55	.25	.17	.10
15	1.4	.40	.26	.12
Stop DDE Introduction	1.21	.46	.31	.18
	1.09	.51	.42	.16
Mean	1.23	.45	.33	.15
20	.69	.19	.26	.09
	.79	.31	.20	.06
	.63	.26	.37	.12
Mean	.70	.25	.27	.09
26	.19	.10	.06	.02
	.12	.07	.02	.09
	.16	.11	.09	.01
Mean	.15	.09	.05	.04
31	.11	.06	.04	.09
	.07	.09	.09	.02
	.09	.10	.01	.01
Mean	.09	.08	.05	.04

Figure 9. Elimination of DDE from Chironomus tentans following a 15 day DDE exposure period.

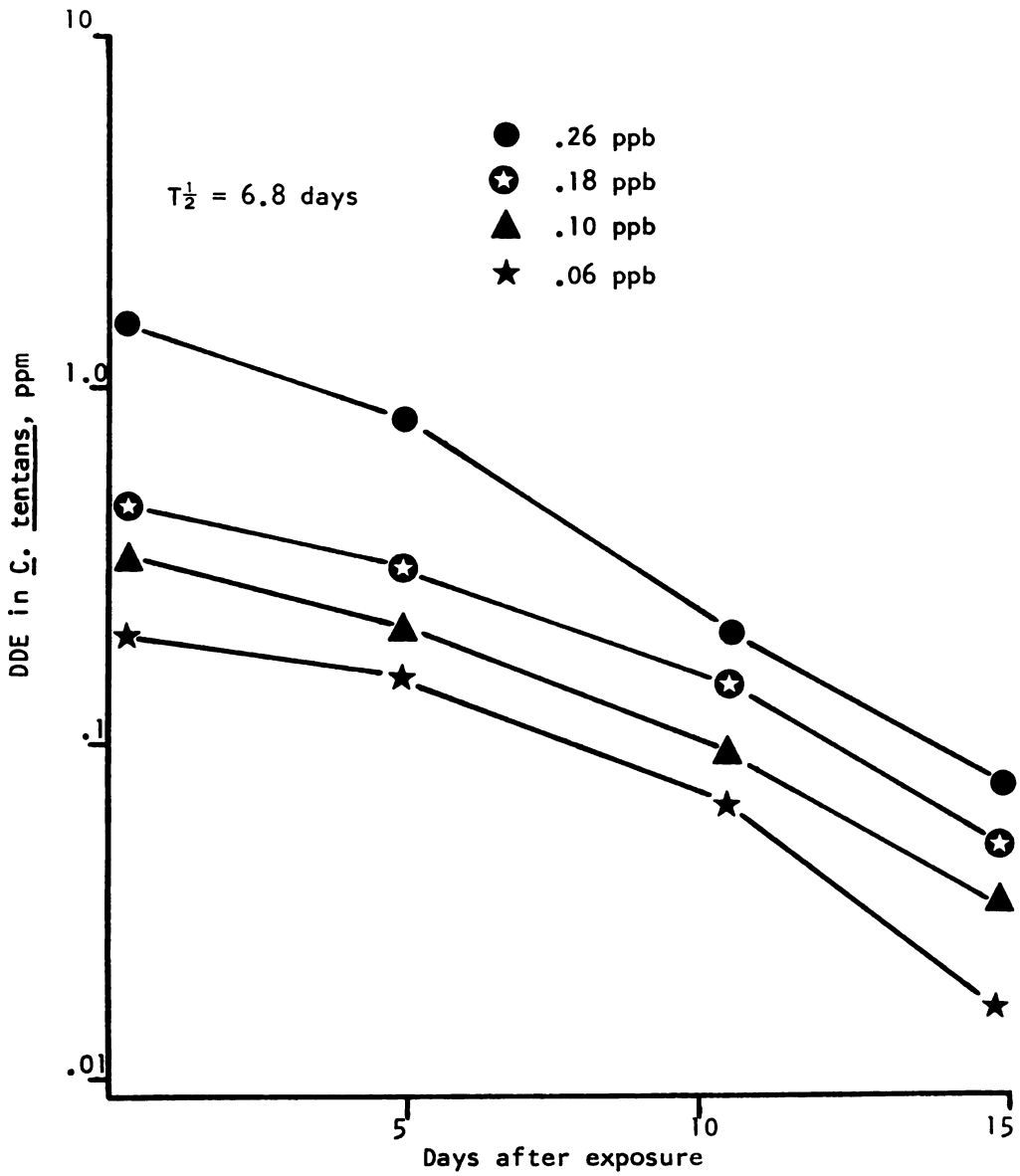

10
1.0
.1
.01
$T\frac{1}{2}$ = 6.8 days
.26 ppb
.18 ppb
.10 ppb
.06 ppb
DDE in C. tentans, ppm
5
10
15
Days after exposure

Figure 9

(Table 4). The elimination rates of the larvae when exposed to different DDE exposure levels did not differ significantly. Only midge larvae exposed to .26 ppb had an elimination rate significantly different from the others. This absence of differences in losing DDE from body tissue at the test concentrations used is likely due to the fact that the exposure levels of DDE (.18, .10 and .06 ppb) were not different enough from each other to elicit a significant response in the rate of elimination. Upon application of equations (1) and (2) the mean log of DDE concentration in C. tentans can be said to decrease at an estimated uniform rate of .42 with an average half-life of 6.8 days.

The concentrations of DDE in the water at the various experimental levels dropped sharply after the 15 day exposure period. Five days after the cessation of the introduction concentrations of DDE were less than .10 ppb in all experimental aquaria. Ten days after stoppage all test aquaria had less than .05 ppb. However, the substrate retained some DDE after introduction was terminated. Typically there was a rapid initial loss followed by a subsequent equilibria reached. The amount of DDE residue retained by the substrate is likely an expression of the amount of sorptive sites that are available. With the concentration of residue in the water decreasing there should have been a parallel reduction in substrate concentration. However, due to the design of the experimental aquaria, a mixing between the water-substrate interface was not accomplished and adsorption was responsible for maintaining DDE residue in the substrate. The amount of DDE retained by the substrate is negligible when viewing the elimination rates of the larvae.

Table 4. Mean calculated regression coefficients estimating half-life and elimination rates of DDE from Chironomus tentans following a 15 day exposure to various concentrations of DDE (ppb) in water.

Exposure level (ppb) in water	Logarithmic rate of loss (regression coefficient)	Half-life (days)
.26	.63a[1]	7.9a
.18	.48b	6.3b
.10	.27b	6.2b
.06	.32b	6.9b

[1]Means followed by unlike letters are significant at the 5% level by Tukey's W procedure.

The value of 6.8 days for the half-life elimination rate of DDE for C. tentans and a rate of loss of .42 suggests a relatively rapid rate of loss when compared to rates obtained for fish and other aquatic organisms. When goldfish were exposed to an 18 ppm DDT diet a mean logrithmic rate of loss and a residue half-life value of 0.0725 and 29.5 days, respectively, was obtained (Grzenda, et. al., 1970). Freshwater mussels when exposed to a concentration of .62 ppb DDT in lake water demonstrated a regression coefficient of 0.148 and a half-life of 13.6 days (Bedford, 1970). This lower half-life value expressed by the midge suggests that there is a minimal incorporation of DDE into body tissue and thus the majority of the body burden is in the form of adsorped DDE. This study could also imply very little selective storage of DDE by the larval midge being that residue was lost at such a rapid rate. Another factor that aids in the understanding of elimination dynamics is the rate at which the gut (fore, mid and hind) is loaded and unloaded. Some preliminary studies have been conducted on a midge in the subfamily Diamesinea and found to have a gut loading time of 10 minutes (King, persomal communication. 1972). Loading rates of this magnitude suggests a rapid movement of food material through the gut and thus a significant process by which DDE could be eliminated.

SECTION II

INFLUENCE OF THE PRESENCE OF AROCLOR 1254 ON THE UPTAKE OF DDE BY *Chironomus tentans*

Influence of Aroclor 1254 on the Uptake of DDE by *Chironomus tentans*

METHODS AND MATERIALS

A continuous-flow dilution apparatus was designed and constructed to deliver two test concentrations, a combination of both and one control for investigating the effect of the presence of PCB (Aroclor 1254) on DDE uptake (Figure 10). Dilution reservoirs were constructed of 3/16" (4.5 mm) glass and cemented with e-poxy resin. The toxicant introduction system consisted of two solution metering pumps (Beckman Instruments, model 746, 0-2 ml) each delivering a measured amount of toxicant in ethanol (DDE or PCB) into their respective dilution reservoirs. Aroclor 1254 and DDE were sufficiently concentrated so that the ethanol concentration never exceeded 5 ppm. A 200 ml/minute flow rate of each treatment (toxicant, combination and control) was maintained to their individual 35 liter all-glass experimental aquaria. The turnover rate of the aquaria was 85% in 12.6 hours. Experimental concentrations of toxicants used in this series of experiments were 1 ppb DDE, 1 "ppb" Aroclor 1254 and a combination of both (1 ppb Aroclor and 1 ppb DDE). Three egg masses were placed into each aquarium for experimental purposes.

Figure 10. Schematic of experimental dilution apparatus used for exposing midge larvae to DDE, PCB and combinations of both.

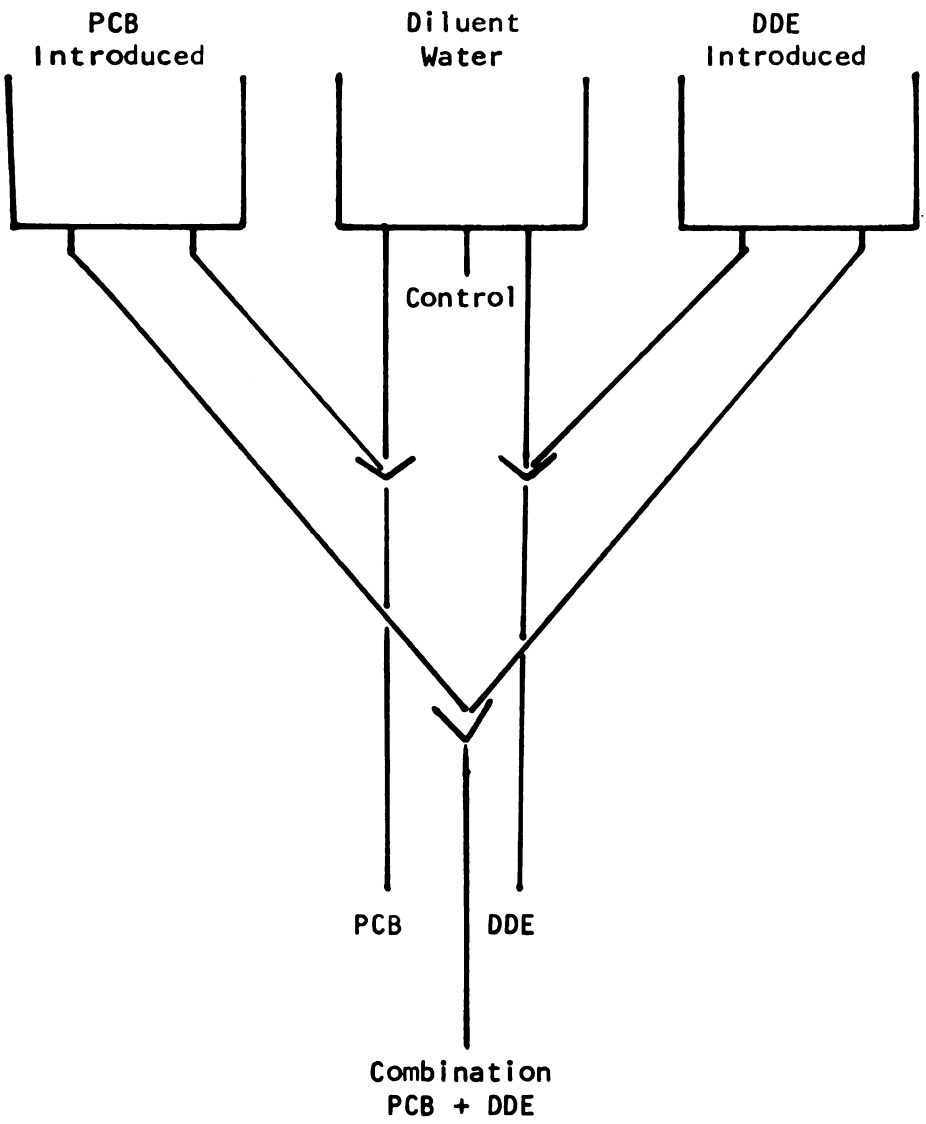

PCB
Introduced
Diluent
Water
DDE
Introduced
Control
PCB
DDE
Combination
PCB + DDE

Figure 10

Midge larvae, pupae and adults taken from the test aquaria were prepared for residue analysis in the same manner described earlier. A Beckman GC-4 gas chromatograph with a discharge electron capture detector fitted with a six foot (1.83 m) borosilicate column packed with 3% SE-30 and 6% QF-1 on Chromosorb W (80-100 mesh) was used for residue analysis.

Typically there were 16 definite peaks on the chromatogram obtained from Aroclor 1254 when this column material was used. The first four and last three peaks were excluded when quantitating PCB residue due to the lack of reproducibility inherent in these peaks. The total peak height of the middle nine peaks were used for quantitation purposes and compared to standards for an "estimate" of total Aroclor 1254 residue in the samples. There was fairly good chromatographic separation of DDE from the PCB, however the DDE and its corresponding PCB isomer were never completely resolved (Figure 11). Quantitation of DDE was also based on peak height and compared to standard concentrations.

One liter water samples were taken periodically from the experimental aquaria. The water samples in this experiment were subjected to a different scheme of extraction than described earlier. Polyurethane DipSco foam plugs were placed at the bottom of pyrex columns, 2.4 x 50 cm, and the water sample was passed through the foam plug. The foam plug was then eluted with 200 ml of redistilled hexane and the extract concentrated to a 5 ml volume for introduction into the gas chromatograph for quantitation. The polyurethane plugs were washed in acetone-hexane (1:1) for 12 hours prior to use in the column extraction and no extraneous peaks were present. Recovery of

Figure 11. Gas chromatogram of p,p'-DDE and Aroclor 1254 showing resolution that was obtained on 3% SE-30 and 6% QF-1.

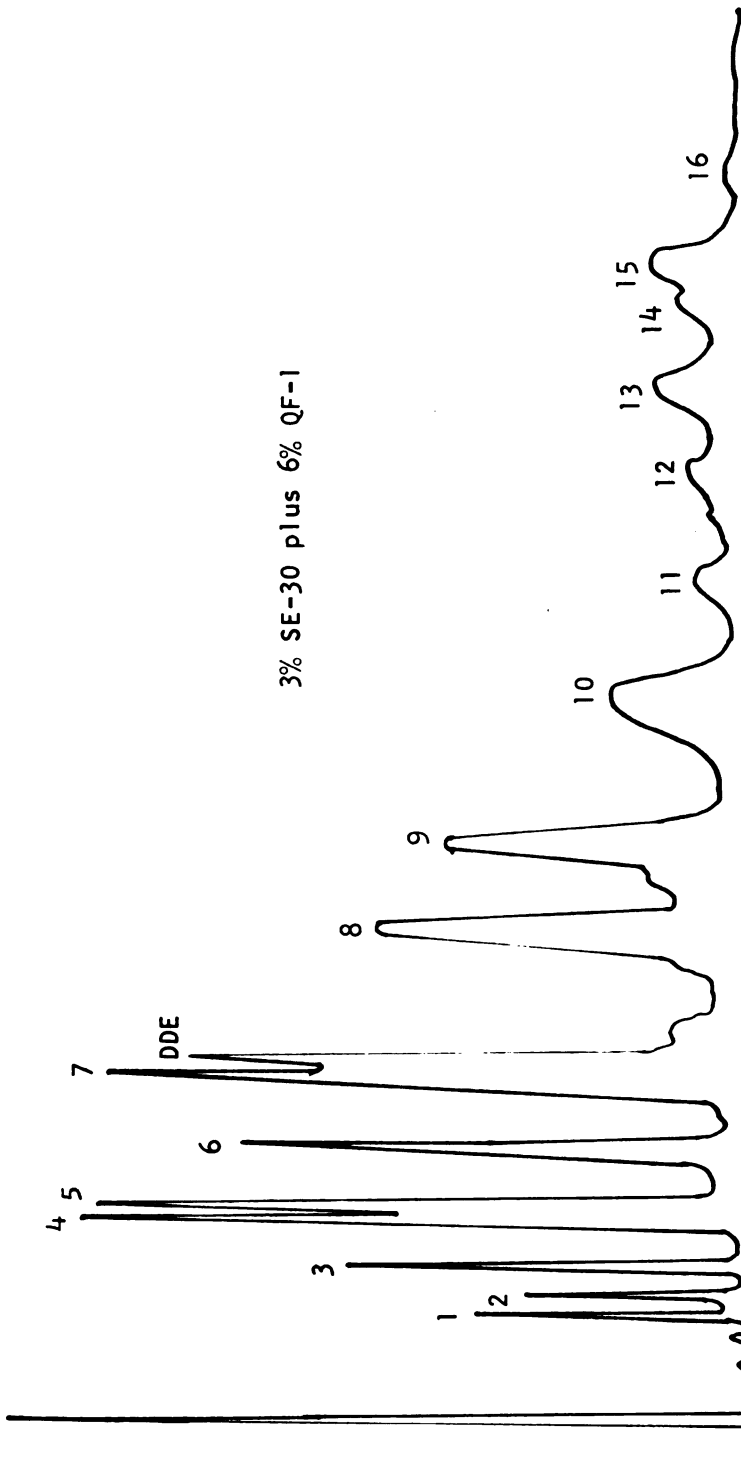


Figure 11

DDE and Aroclor 1254 from water was 83.2 $\pm$ 3.2% and 86.6 $\pm$ 4.9% respectively. Each percent recovery was based on six spiked samples.

In addition to the continuous-flow experiments this investigation also utilized DDE-^{14}C in a series of short term static experiments. Two appraoches were used in elucidating the influence of PCB on the uptake of DDE: 1) exposing the larvae to a series of concentrations of PCB (.01, .0 and 1.0 ppb) with a standard concentration of DDE-^{14}C in each treatment, and 2) pre-loading the larvae with PCB (.01, 1 and 100 ppb) for nine hours and then exposing these same larvae to only DDE-^{14}C.

Uniformly ring labelled p,p'-DDE-^{14}C with a specific activity of 2.4 mCi/mM (.05 mCi, MW 318) obtained from Mallinckrodt Nuclear was used for experimental purposes (18,675 dpm/ug). Two ml of the DDE-^{14}C ethanolic stock solution was added to two liters of diluent water (water used in past uptake studies). One-hundred ml of this treated water was added to 100 mm diameter pyrex crystallizing dishes which served as experimental aquaria. To each dish then was added .1 ml ethanolic Aroclor 1254 which would give a resultant series of concentrations of .01, .10 and 1.0 ppb in the 100 ml of water. Ethanol alone (.1 ml) was added to a series which served as the control. There were four replicates of each treatment and the design was completely randomized. Five fourth instar C. tentans larvae (21 $\pm$ 3 mm total length) were placed in each experimental dish. After four hours exposure the larvae (five larvae are treated as a sample) were removed, blotted dry and weighed to the nearest tenth of a milligram.

The sample of larvae was then digested for two days in 1 ml of 1 M methanolic Hyamine-Hydroxide. Fifteen ml of scintillation fluid (4 g BBOT in 1 liter of redistilled toluene) were added to the digested sample and counted on a Nuclear Chicago Mark I liquid scintillation counter. The samples were corrected for quenching through the use of an automatic external standardization feature of the instrument.

The techniques used in the pre-loading experiment were no different than those just described. The only difference being that the larvae were initially exposed to .01, 1.0 and 100 ppb Aroclor 1254 for nine hours and then placed for four hours in water that contained only DDE-^{14}C. There were four replicates of each treatment and the design was completely randomized.

RESULTS

After a 32-day exposure to a continuous flow dilution system adult emergence occurred and the experiment was terminated, thus an idea of the effect of PCB on DDE uptake could be obtained. Typically there was a gradual linear uptake of DDE (0-11 days) then a rapid exponential period (11-23 days) and a subsequent leveling off or plateau (24-31 days) at which time pupation and emergence occurred (Figure 12). This picture of DDE uptake with PCB present is not significantly different than DDE alone. PCB or DDE alone or in any combination with each other demonstrated no significant differences in C. tentans uptake of DDE. The midge appeared to concentrate "total" Aroclor 1254 to the same limits as DDE thus suggesting that the midge has no isomeric concentration selection as might be suggested by the chlorine distribution of the biphenyl rings.

The same relationship was also observed in the static tests. The simultaneous exposure of Aroclor 1254 and DDE had no significant effect on the uptake of DDE-^{14}C by fourth instar C. tentans larvae after four hours (Table 5). Pre-loading or pre-exposing the larvae to Aroclor 1254 and then subjection to DDE-^{14}C also did not show any significant difference in DDE-^{14}C uptake when compared to the control (Table 6).

Figure 12. Uptake relationship of DDE and Aroclor 1254 alone and in combination with each other by Chironomus tentans from egg to adult.

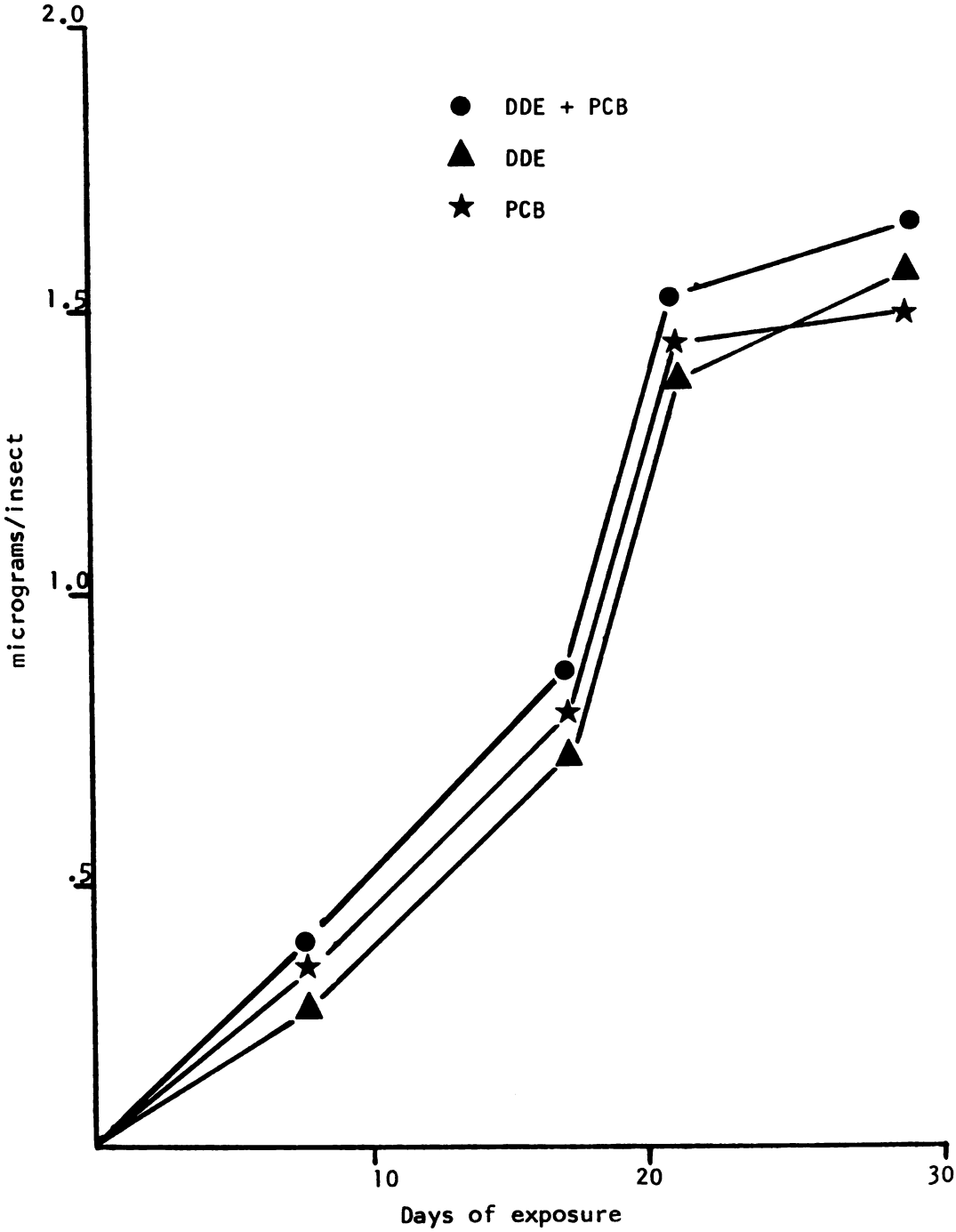

2.0
1.5
1.0
.5
micrograms/insect
DDE + PCB
DDE
PCB
10
20
30
Days of exposure

Figure 12

Table 5. Summary of the effect of the presence of Aroclor 1254 on the uptake of DDE-^{14}C by fourth instar larvae Chironomus tentans after four hours exposure.

Aroclor 1254	dpm DDE-^{14}C/mg				
(ppb)	I	II	III	IV	Mean
Control	118	145	95	92	112.5a[1]
.01	138	88	116	116	114.5a
.10	96	92	137	112	109.6a
1.00	110	104	69	113	99.0a

[1]Means followed by unlike letters are significantly different at the 5% level by Tukey's W procedure.

Table 6. Summary of the effect of pre-exposing Chironomus tentans fourth instar larvae to various concentrations of Aroclor 1254 on subsequent uptake of DDE-^{14}C.

Aroclor 1254 pre-exposure concentration (ppb)	dpm DDE-^{14}C/mg				
	I	II	III	IV	Mean
Control	115	102	116	126	114.7a[1]
.01	117	79	115	110	105.2a
1.00	115	100	130	84	107.2a
100.00	124	120	113	121	119.7a

[1]Means followed by unlike letters are significantly different at the 5% level by Tukey's W procedure.

SECTION III

MODE OF UPTAKE OF DDE BY

Chironomus tentans

Mode of Uptake of DDE by *Chironomus tentans*

METHODS AND MATERIALS

The purpose of this study was to investigate the accumulation of p,p'-DDE from aqueous solution and contaminated substrate by fourth instar *Chironomus tentans* larvae as a function of exposure time and to differentiate between possible physical and biological modes of DDE sorption by comparing the DDE accumulation of live and dead insect larvae.

Five dead and live fourth instar *C. tentans* larvae (20.0 $\pm$ 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 (chicken feed and paper towel homogenate) used for rearing the larvae and the other contained no substrate. Uniformly ring-labelled p,p'-DDE, specific activity 2.4 mCi/mM, was delivered in thanol to four liters of diluent water so that a resultant 100 ml test water had approximately 2.76×10^5 dpm. A 100 ml aliquot of ntaminated water was then added to each side of the partition. e substrate-water system was allowed to equilibrate for two hours fore the experiment was initiated.

Larvae were sacrificed by refrigeration at $4^{o}C$ for two hours. Care was taken to prevent larval freezing so that ice cyrstal formation and cell lysis would not occur. They were then allowed to come to room temperature before introduction into the test aquaria.

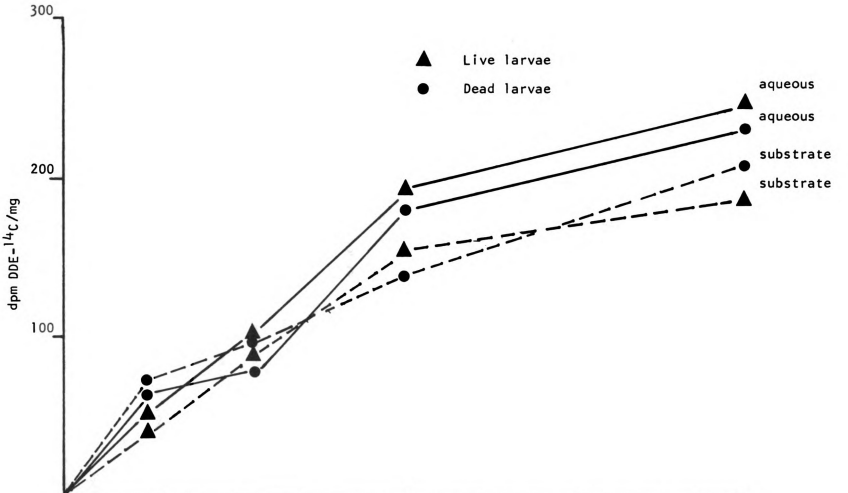
After exposure to the DDE-^{14}C contaminated water and substrate the larvae were dried, weighed and subjected to digestion in 1 ml of 1 M Hyamine-Hydroxide for two days and counted on a Nuclear Chicago Mark 1 scintillation counter. Each larval sample was counted three times and quenching was corrected by an automatic external standardization feature of the instrument. Exposure periods were 1, 2, 4 and 8 hours, and each treatment was replicated three times. One additional aquarium was maintained with the prescribed substrate and a Chorella sp. algal population in order to ascertain that C. tentans larvae would effectively construct tubes and feed in an eight-hour time period. At the end of the experiment larvae from this aquarium were dissected and the presence of green algal cells in the gut verified that feeding had occurred.

RESULTS

The effect of exposure time and means of exposure (aqueous vs. substrate) on the accumulation of DDE by live and dead C. tentans larvae is depicted (Figure 13). Each point of this figure represents the mean of three samples with each sample consisting of five larvae. Both live and dead larvae demonstrated a time related accumulation of DDE-^{14}C. Generally with both live and dead larvae there was a period of linear uptake (0-2 hours) followed by an equilibrium or plateau which was reached at 6-8 hours after initial exposure. Although there was a significant difference in larval uptake of DDE-^{14}C as a function of time there were no accumulation differences between live and dead larvae, furthermore no differences of uptake were demonstrated when live and dead larvae were subjected to an aqueous and substrate means of contamination of DDE-^{14}C. 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, being that no differences in uptake were attributed to contamination by the feeding process and thus the DDE body burden has its origin

Figure 13. Relationship between live and dead Chironomus tentans larvae exposed to an aqueous and substrate contamination of DDE-^{14}C for eight hours.


300
200
100
dpm DDE-^{14}C/mg
Live larvae
Dead larvae
aqueous
aqueous
substrate
substrate
1
2
3
4
5
6
7
8
Hours of exposure
Figure 13

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 and thus adsorption should be considered as a possible mode of uptake of environmental contaminants similar in nature to DDE. In order to better understand this adsorptive capacity of midge larvae another series of experiments was conducted to facilitate the understanding of the larval cuticle and the role it imparts in the sorption mechanism.

Five live fourth instar larvae (21 $\pm$ 3 mm total length, 11.1 $\pm$ 3.9 mg wet weight) were placed in 100 mm diameter crystallizing dishes with 100 ml of diluent water containing approximately 1.7 x 10^5 dpm of uniformly labelled DDE-^{14}C. Larvae were exposed for 1, 2, 4 and 8 hours. The sample (five larvae per sample) of exposed larvae were subjected to a cuticle wash or dip into 1 ml of redistilled hexane for five seconds and then followed the extraction and quantitation procedure described earlier. Each treatment was replicated three times in a completely randomized design.

Uptake of DDE-^{14}C by the larvae demonstrated a time dependent relationship as seen before with a plateau reached in eight hours (Figure 14). 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 model 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 exposure. The peak in DDE concentrations obtained by the cuticle wash after two hours exposure exceeded that of the insect after eight

Figure 14. Relationship of uptake of DDE-^{14}C by Chironomus tentans when subjected to a cuticle wash of hexane.

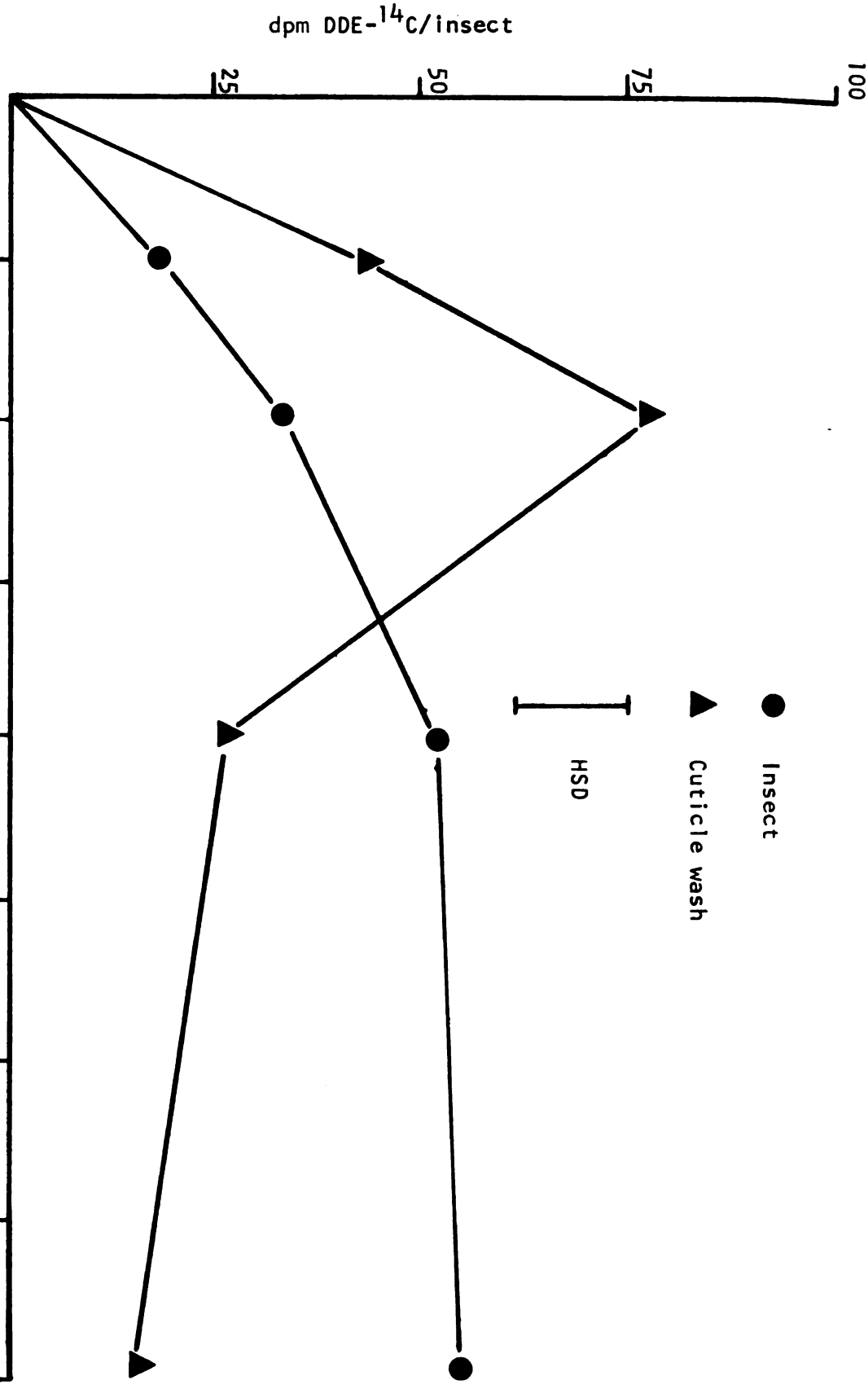

dpm DDE-^{14}C/insect
25
50
75
100
1
2
3
4
5
6
7
8
Hours of exposure
Insect
Cuticle wash
HSD

Figure 14

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 equation (Davson and Danielli, 1952):

$$(3) \quad s = C_o V \ (1-e^{-PAt/v})$$

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 (^{o}C)

C_o = initial concentration of material applied on the cuticle

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 exposure and exponential to the penetration time. The midge 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 away from the expected theoretical diffusion was found with the American cockroach (Periplaneta americana) cuticle when malathion was applied (Matsumura, 1963). On the other hand, 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 someway altering the cuticular properties of the insect and thus the cuticle is not functioning in the same manner throughout the experimental time period of exposure. The process of DDE uptake is adsorption onto the cuticle and subsequent diffusion or incorporation into the body over time.

As the exposure time increases the cuticle is altered and a decrease in the amount of residue is adsorped with a slower rate of diffusion into the body. This explanation appears to describe the two relationships depicted in Figure 14: 1) the apparent plateau reached so quickly 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.

The diffusion equation (3) describes the importance of the area of cuticle exposed to the diffusing material in the amount of absorption that takes place. Also from the studies conducted thus far, the area of the larval cuticle exposed to DDE appears to determine to a large extent the amount of residue accumulated by the larvae. For these reasons an experiment was conducted to investigate the surface area to DDE accumulation relationship and further demonstrate the importance of the role of cuticular uptake of DDE by larval C. tentans.

Chironomus tentans larvae were randomly selected from the stock culture tanks which had a distribution of first through fourth instar larvae. Ten of these larvae were then randomly selected and placed in a 100 mm diameter crystallizing dish which contained approximately 4.26×10^6 dpm of DDE-^{14}C in 100 ml aliquot of diluent water. The insects were exposed for 1, 2, 4 and 8 hours to the experimental DDE concentration. The insects were removed at the prescribed time and each insect 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 ml of 1 M methanolic Hyamine-Hydroxide for two days, a scintillation fluid added and counted. There were three replicates of each treatment and the design was completely randomized. Surface areas were calculated by treating the larvae as a cyclinder and then using the equation:

$$(4) \quad S = 2\pi rh + 2\pi r^2$$

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

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 15, 16, 17 and 18). The correlation coefficients (r) at each exposure period of

Figure 15. Relationship of surface area to uptake of DDE-^{14}C by Chironomus tentans larvae after one hour exposure.

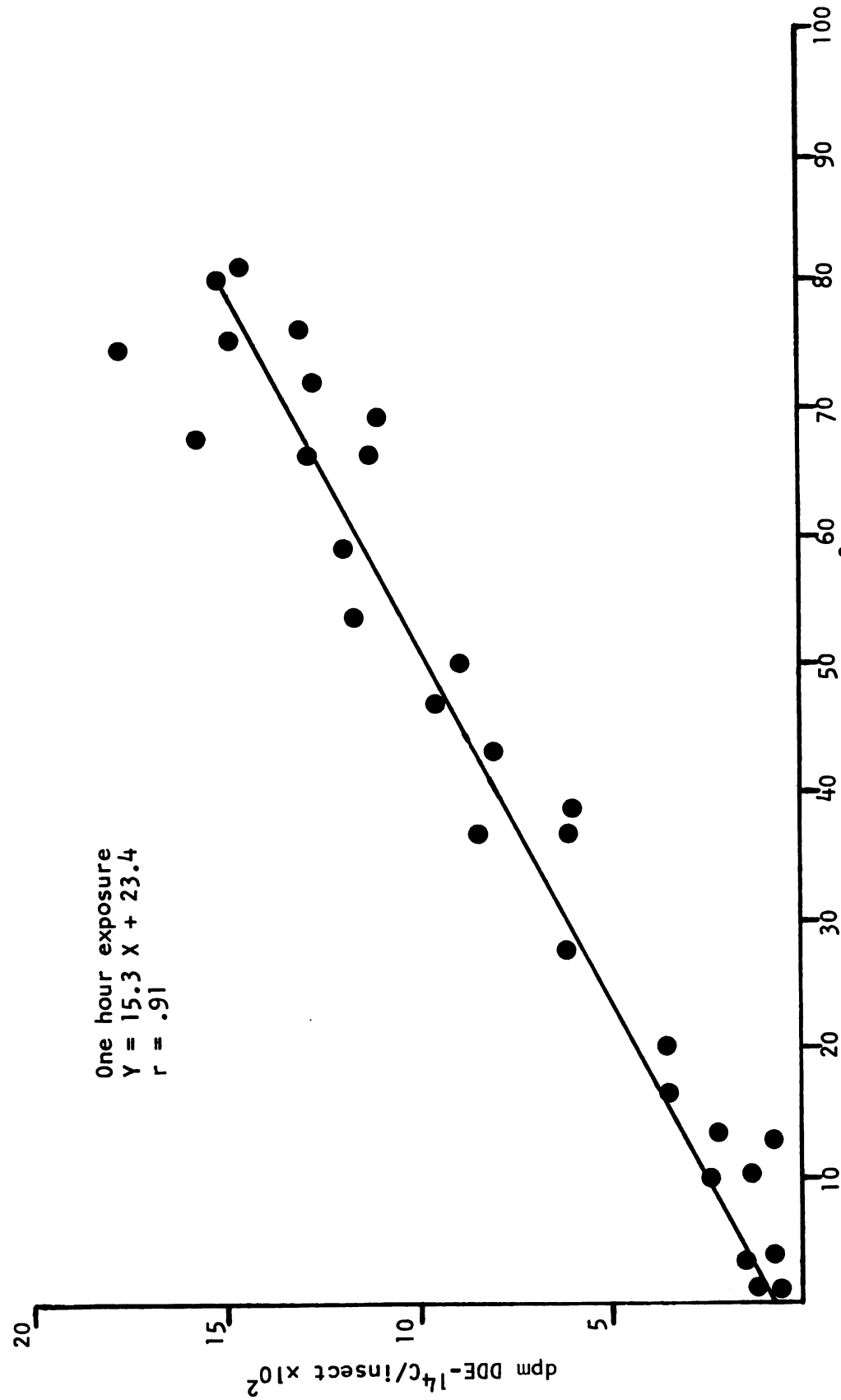

One hour exposure
Y = 15.3 X + 23.4
r = .91
dpm DDE-^{14}C/insect x10^2
Surface area, mm^2
20
15
10
5
10
20
30
40
50
60
70
80
90
100

Figure 15

Figure 16. Relationship of surface area to uptake of DDE-^{14}C by Chironomus tentans larvae after two hours exposure.

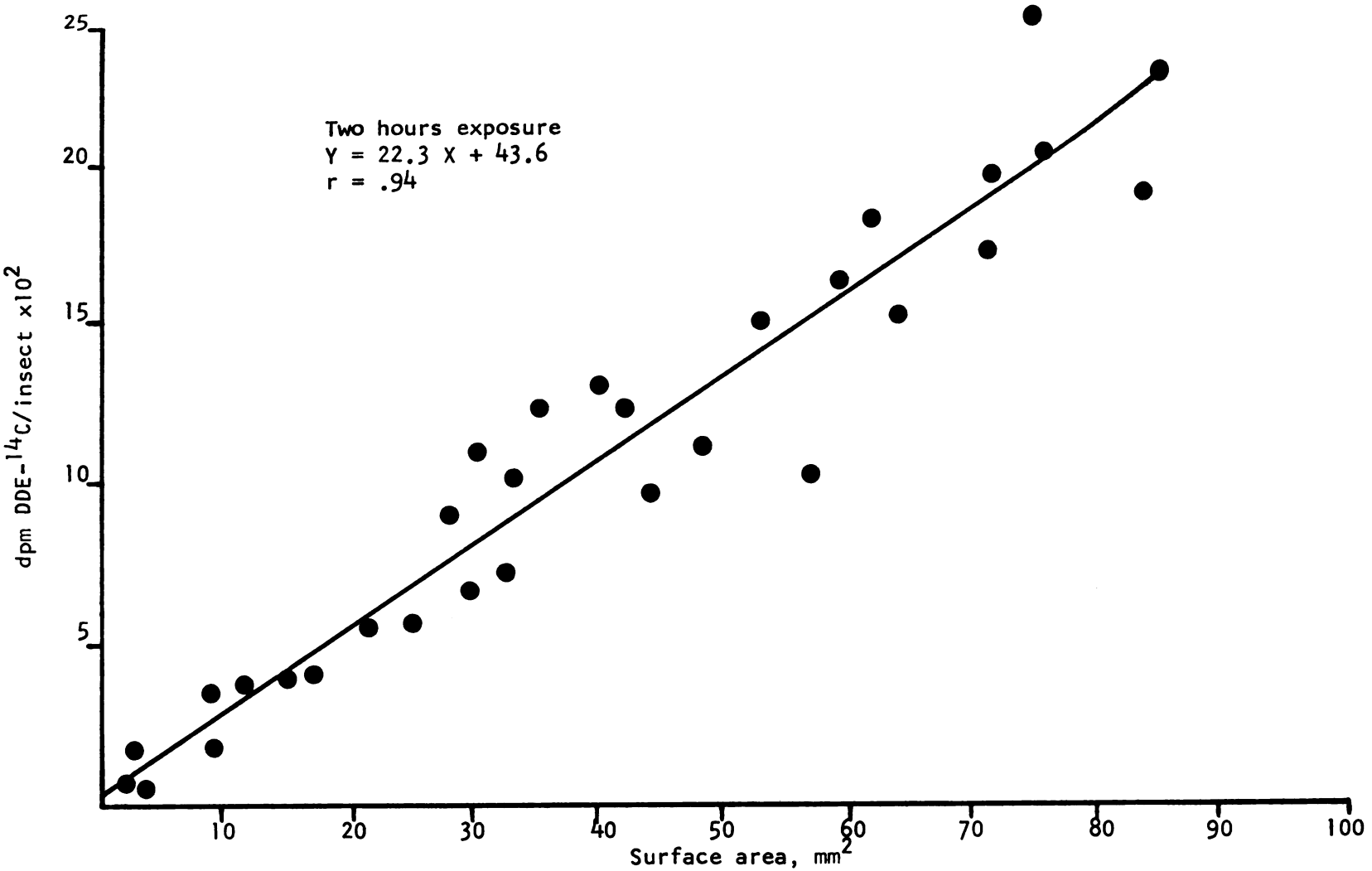

Two hours exposure
Y = 22.3 X + 43.6
r = .94
dpm DDE-^{14}C/insect x10^2
25
20
15
10
5
10
20
30
40
50
60
70
80
90
100
Surface area, mm^2

Figure 16

Figure 17. Relationship of surface area to uptake of DDE-^{14}C by Chironomus tentans larvae after four hours exposure.

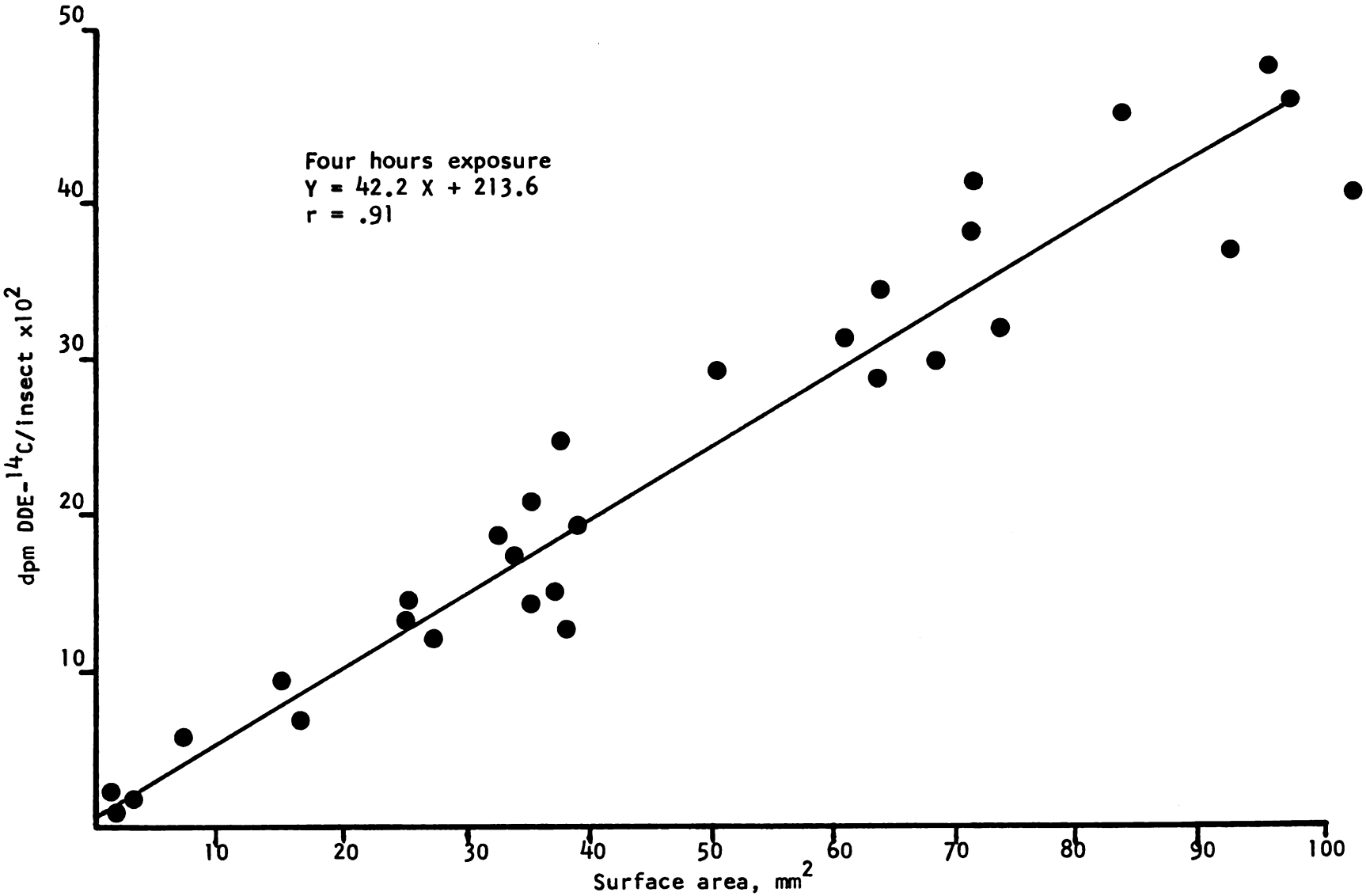

Four hours exposure
Y = 42.2 X + 213.6
r = .91
dpm DDE-^{14}C/insect x10^2
Surface area, mm^2
50
40
30
20
10
10
20
30
40
50
60
70
80
90
100

Figure 17

Figure 18. Relationship of surface area to uptake of DDE-^{14}C by Chironomus tentans larvae after eight hours exposure.

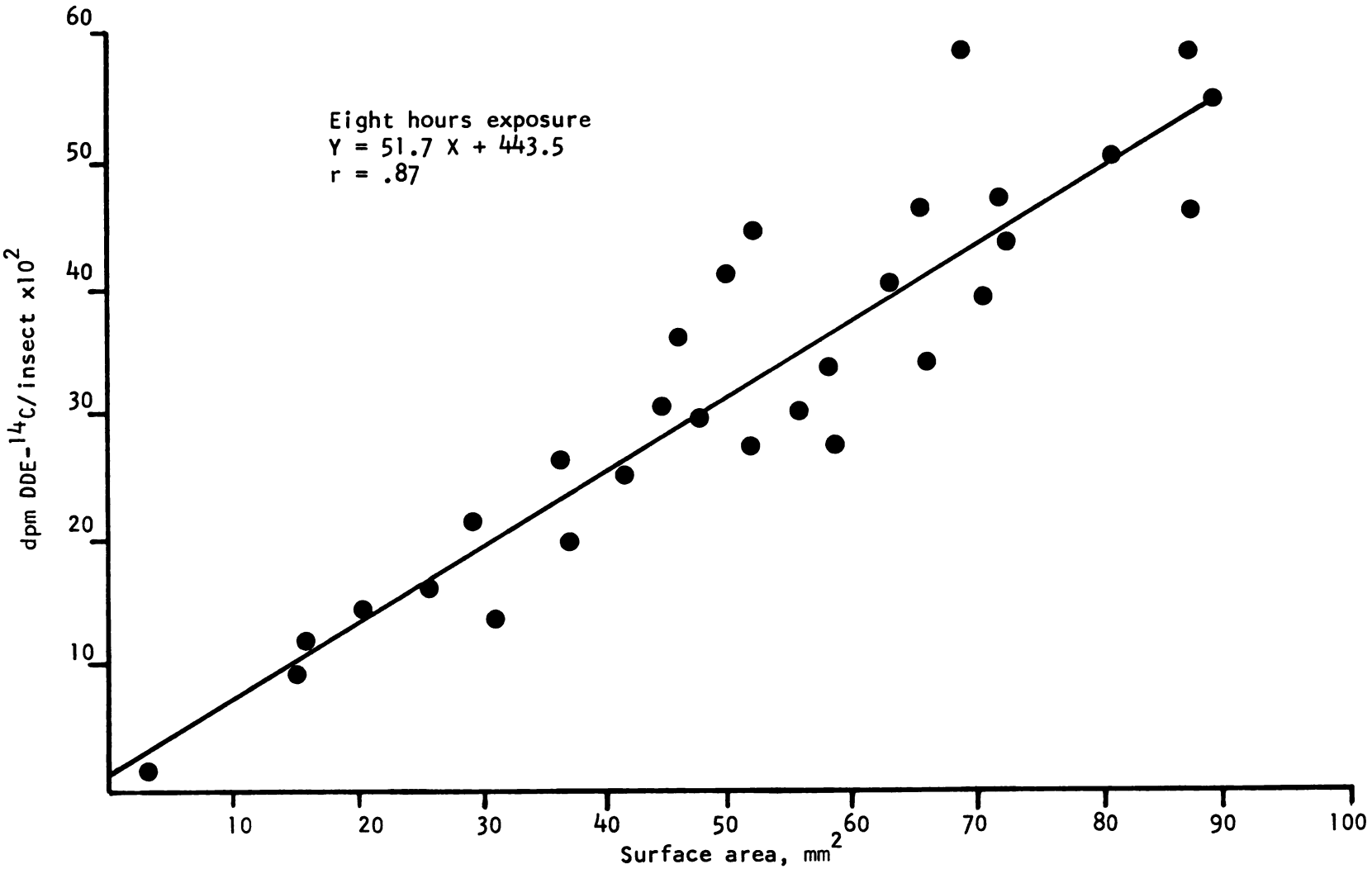

Eight hours exposure
Y = 51.7 X + 443.5
r = .87
dpm DDE-^{14}C/insect x10^2
60
50
40
30
20
10
10
20
30
40
50
60
70
80
90
100
Surface area, mm^2

Figure 18

1, 2, 4 and 8 hours were .91, 194, .91 and .87 respectively and indicated a very close fit and highly significant relationship. Thus, it appears that there is a direct proportional relationship between the size of the larvae and the amount of DDE-^{14}C accumulated.

The surface area of the cuticle is then a very significant factor when considering 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.

In order to further develop the significance of accumulation of DDE by adsorption of the larval cuticle another experiment was conducted 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 the synthetic waters, here denoted as "soft" (I), followed that described by Cairns (1969) and served as the control. The other synthetic water, denoted as "hard" (II) paralleled that of the soft (I) except the magnesium and calcium ion concentrations were increased by two fold each. The molar concentrations and chemical properties of the two synthetic dilution waters are described (Table 7).

Table 7. Molar concentration and chemical properties of synthetic dilution waters used for experimental purposes.

	Molar Concentration	
Chemical	Soft (I)	Hard (II)
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$_2$ (umhos/cm^2)	1.71×10^{2}	3.61×10^{2}
pH	7.81	8.15
Hardness as ppm $CaCO_3$	60.0	136.2

DDE-^{14}C was added in ethanol to the two stock synethic 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. Three-fourth instar larvae (20.2 $\pm$ 2.6 mm total length, and 11.3 $\pm$ 3.9 mg wet weight) were then placed in the test aquaria. Exposure times were 1, 3, 9 and 18 hours and each sample was subjected to a cuticle wash with hexane. Each treatment was replicated three times.

There was a significant difference in the amount of DDE-^{14}C accumulated by the midge under the experimental conditions to which they were subjected (Figure 19). The larvae that were exposed to the synthetic dilution water which contained higher calcium and magnesium ions accumulated almost two times as much DDE after 18 hours then those exposed to the synthetic water which served as the control. The cuticle in both types of water (I and II) behaved as in previous studies with a peak in adsorption being reached in the early periods of exposure and a gradual decrease over time. However the hard water exposed cuticle adsorped almost three times more DDE than the "soft" (control) water exposed midge after one hour exposure.

This investigation was not pursued any further than described here. No attempt was made to ellucidate the mechanism by which water hardness determines DDE uptake by C. tentans larvae. It appears however that the calcium and magnesium ion concentrations are in some way altering the integrity of the cuticle and the adsorptive and permeability properties of the cuticle are thus altered and a resultant accumulation of DDE is observed. Studies on the stonefly,

Figure 19. Effect of exposing Chironomus tentans to two different synthetic waters varying in calcium and magnesium concentrations on uptake of DDE-^{14}C.

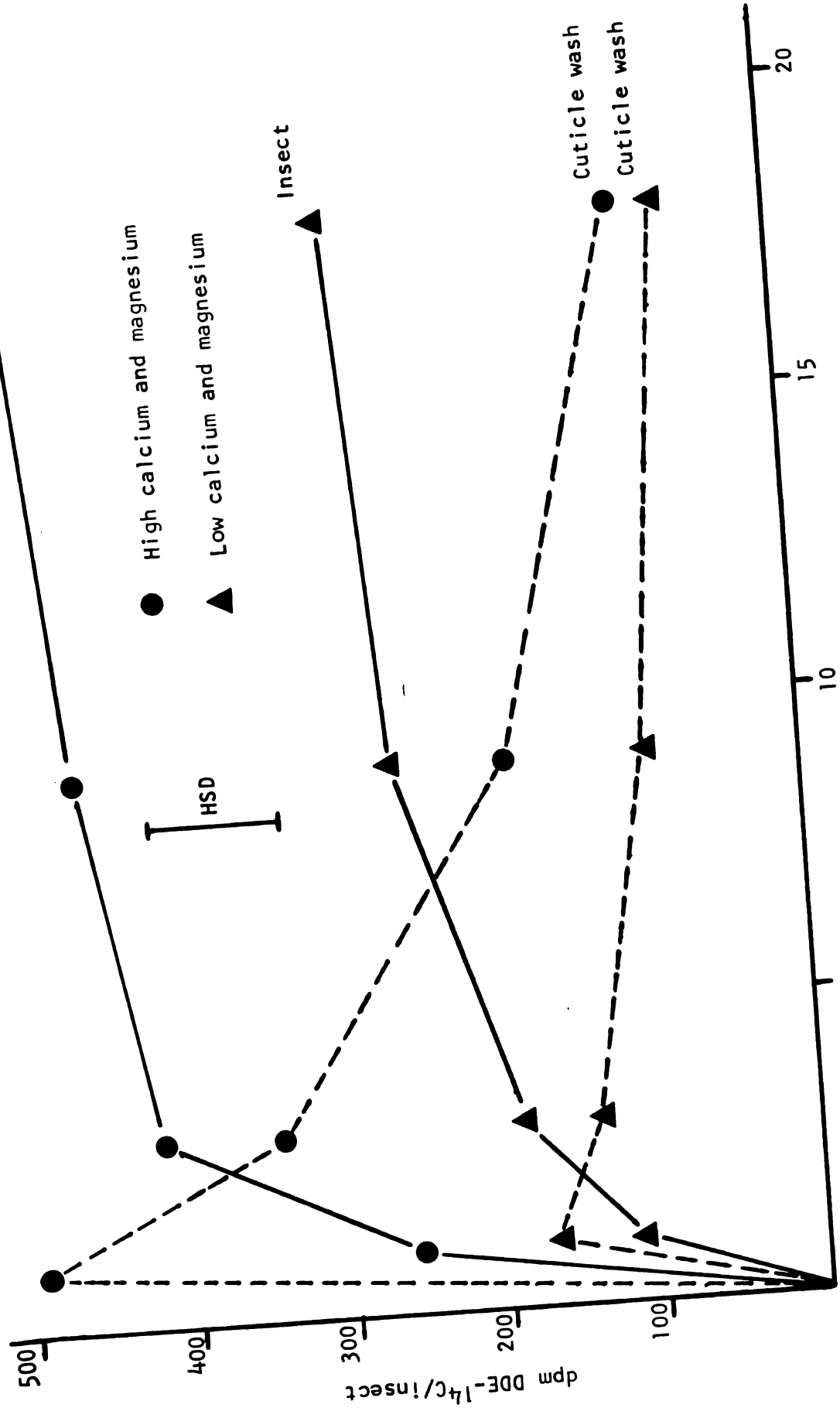

Insect
High calcium and magnesium
Low calcium and magnesium
Insect
Cuticle wash
Cuticle wash
HSD
500
400
300
200
100
dpm DDE-14C/insect
5
10
15
20
Hours of exposure

Figure 19

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). Findings such as this would then indicate that any alteration in the surface lipid composition would affect the adsorption of non-polar compounds. The water hardness experiment does however again support the concept that accumulation of DDE is significantly related to the cuticular adsorption process.

The results obtained from this study might aid in the understanding of some of the differences found in accumulation rates of chlorinated hydrocarbon pesticides by fish and other aquatic organisms in eutrophic and oligotrophic lakes (Hamelink, et. al., 1971). No other studies have been conducted in this area of water chemistry and pesticide uptake relationships. Studies of this nature also support the use of prescribed standard dilution waters proposed by Cairns (1969) for utilization in fish and invertebrate bioassays. I suspect that many of the unresolved conflicts between investigators might not have developed had they used some standard synthetic dilution water.

SECTION IV

METABOLISM OF p,p'-DDE BY *Chironomus tentans*

Metabolism of p,p'-DDE by *Chironomus tentans*

The purpose of this investigation was to determine if *C. tentans* midge larvae are responsible for the degradation of DDE in the aquatic environment. It seems likely to assume that with the decreased use of DDT, and the degradative process to DDE, that there should be a greater amount of DDE found in the aquatic environment than actually has been reported (Zabik, *et. al.*, 1971). Consequently some biological or physical process must be responsible for the dissipation of DDE. For this reason the midge was investigated as to its capacity to metabolize p,p'-DDE.

Approximately seven grams of third to fourth instar larvae were exposed to 30 ppm p,p'-DDE (in ethanol) for one week in a 45 liter all glass aquaria maintained at 21°C together with the defined substrate. Immediately after removal the midges were blotted dry, placed in vacuum bottles, frozen with dry-ice acetone and lyophilized. After 24 hours the tissues were thoroughly homogenized and placed in a Soxhlet apparatus and extracted with 10% ethyl ether in hexane (v/v) for 24 hours and then with chloroform-methanol (2:1, v/v) for another 24 hours. These treatments were sufficient to extract DDE and all potential metabolites (Abou-Donia and Menzel, 1968). The solvents were evaporated under vacuum and the residues redissolved

in hexane for clean up. Pyrex columns, 24 x 50 cm, fitted with a fritted-glass disk, were packed with 10 g of Florisil-Celite (5:1, w/w) with a layer of anyhdrous sodium sulfate above and below the column packing. The Florisil, activated at 649°C by Floridin, Inc., was deactivated with approximately 10% distilled water. The column was eluted with 300 ml of hexane and 100 ml methanol. The column eluates were evaporated to dryness and taken up in benzene. The concentrated extracts were chromatographed by direct injection into a gas-liquid chromatograph or applied directly to thin-layer chromatography (TLC). Electron capture gas chromatography was employed utilizing three columns: 5% QF-1, 3% SE-30 and 10% DC-200. For analysis of potential polar metabolites two-dimensional TLC on silica gel G was used. The solvent systems were 10% ethyl ether in hexane and 50% chloroform-methanol (2:1) in hexane. Infrared spectroscopy utilizing potassium bromide pellets was also employed in confirmation analysis.

Gas-liquid chromatography did not resolve any metabolites or degradative products other than the parent compound. No additional residues were found on the thin-layer plates, other than the parent DDE, thus confirmation of the absence of metabolism of DDE by the midge larvae was observed. Infrared spectroscopy of the non-polar extract resulted in a spectra which was identical to p,p'-DDE.

The utilization of infrared spectroscopy, gas-liquid and thin-layer chromatography in this metabolic study resulted in the absence of metabolism of p,p'-DDE by C. tentans larvae.

SECTION V

THE EFFECT OF DDE ON THE EGG VIABILITY OF *Chironomus tentans*

The Effect of DDE on the Egg Viability of *Chironomus tentans*

The occurrence of DDE in surface waters and the organic substrate acting as a partitioning reservoir would mean that this compound could act as a potential biological hazard in exhibiting deleterious effects on aquatic insect populations. This study was designed to determine the effect of p,p'-DDE residues on the egg viability of the aquatic midge, *Chironomus tentans*.

Experimental cultures of the midge, *Chironomus tentans*, were maintained in four aerated 45 liter all-glass aquaria with the defined substrate. To two of the aquaria, a solution of p,p'-DDE in ethanol was added to give a resultant concentration of 30 ppb in the water. The remaining two aquaria received an equivalent amount of ethanol and served as a control. The ethanol concentration in the aquaria never exceeded five ppm. Three egg masses of *C. tentans* were placed in each of the four aquaria. After a developmental time of approximately one month (28-35 days) the adults emerging from these four experimental aquaria were collected separately and allowed to mate in 250 ml flasks. The egg masses from these DDE exposed and control females were collected and observed. These experimental egg masses were then incorporated into four different experimental treatments:

1) H/E*-water that contained no DDE and one egg mass that was obtained from a DDE exposed female (contaminated egg mass).
2) H*/E-water that contained 20 ppb DDE and one egg mass that was obtained from a control female.
3) H*/E*-water that contained 20 ppb DDE and one egg mass that was obtained from a DDE exposed female (contaminated egg mass).
4) H/E-control-water that contained no DDE and one egg mass obtained from a control female.

These experimental egg masses were placed in aerated four liter all-glass aquaria with the described substrate. Each treatment was replicated three times in a completely randomized design and the experiment repeated twice. The number of adults emerging from the treatment was used as a measure of egg viability.

RESULTS AND DISCUSSION

There was a significant reduction in the number of C. tentans adults emerging from aquaria that contained DDE contaminated egg masses (Table 8).

However, the presence of 20 ppb p,p'-DDE in the water with control (uncontaminated) eggs did not show a significant reduction in the number of adults emerging. The combination of DDE treated water and DDE contaminated eggs also demonstrated no significant difference from DDE contaminated eggs alone.

The egg masses obtained from DDE exposed females were of a less gelatinous consistency and had a shriveled appearance compared to that of the control eggs. The number of individual eggs in both DDE contaminated and uncontaminated (control) egg masses appeared to be the same. From the uptake and distribution studies it was shown that approximately 30-34% of an adult female burden of DDE residue is lost to the extruded egg mass, thus a significantly high amount of DDE residue is being transferred to these eggs when an adult emerged from exposure of 30 ppb in the water.

The results obtained suggest that sublethal concentrations of p,p'-DDE in the aquatic environment could have deleterious effects on Chironomidae populations. Although the initial concentration of p,p'-DDE that the stock culture of C. tentans was exposed to was

Table 8. Summary of the effect of p,p'-DDE on the egg viability of Chironomus tentans.

Treatment	Number of Emergent Adults	
	Experiment Number	
	1	2
No DDE in water-DDE contaminated egg mass (H/E*)	167a[1]	209a
DDE in water-uncontaminated egg mass (H*/E)	385b	402b
DDE in water-DDE contaminated egg mass (H*/E*)	190a	228a
No DDE in water-uncontaminated egg mass (H/E)	432b	408b

[1]Means followed by unlike letters are significantly different at the 5% level by Tukey's W procedure.

higher (30 ppb) than found in the aquatic environment the idea that egg viability is related to pesticide burden is demonstrated. These findings emphasize the need for further study on the subacute exposure of biologically active compounds to aquatic insects in relation to their reproductive physiology.

SECTION VI

FIELD STUDIES WITH CHIRONOMIDAE AND DDE

Field Studies with Chironomidae and DDE

A field study was initiated in the summer of 1971 in order to evaluate the significance and applicability of laboratory results obtained in the area of uptake and accumulation of DDE by the midge. The Red Cedar River is a warm-water stream located in south-central Michigan. It flows through farmland and woodlots, several small towns, and through the Michigan State University campus before emptying into the Grand River at Lansing, Michigan. The Red Cedar was selected as the experimental lotic system to be studied due to its close proximity to the Michigan State University campus and more important the understanding of the distribution of pesticide contamination in the river (Zabik, et. al., 1971). The river was shown to be contaminated with DDT and metabolites and became progressively more contaminated in a downstream direction. The chemical, physical and morphological characteristics are described in this same study.

Five sampling stations were established throughout the experimental area of the river from just above the Williamston Waste Water Treatment Plant downstream to the Michigan State University campus to monitor DDE residue only. The stations were selected to represent highly contaminated, low contaminated and recovery zone DDE residue levels in the river. The stations are denoted and described as:

I. Located approximately 120 meters above the outlet of the Williamston Waste Water Treatment Plant and 300 meters below the Williamston impoundment. This station represents water with "low" pesticide residue levels.

II. Located approximately 20 meters below the Williamston Waste Water Treatment Plant outlet (140 meters downstream from Station I) and represents an increase in chlorinated hydrocarbon residue in water and sediments.

III. Located approximately eight kilometers downstream from Station II, at the M-43 bridge. The site was downstream from the bridge in backwater eddy relatively high in organic debris. This represents a recovery or intermediate zone of pesticide contamination.

IV. Located at the Hagadorn Road Bridge approximately 12 kilometers downstream from Station III. Between Station III and IV the river runs through two residential communities (Haslett and Okemos).

V. Located above the old East Lansing Waste Water Treatment Plant (now Department of Fisheries and Wildlife Limnological Research Laboratory, Michigan State University) approximately 800 meters below Station IV. This site was selected to represent a highly contaminated pesticide area due to the presence of such high organic matter deposits.

At each station a site was selected that supported a relatively high midge population, usually the sites were located in slow moving backwater eddys that contained sediments with a high organic matter.

A cylindrical emergence trap covering 1.2 m^2 was then placed over the resident midge population. Three midge sampling periods were employed throughout the study while water samples were taken weekly for residue analyses. There were many genera of adult midges taken in the emergence traps and identification to generic level would have been to tedious for the amount of benefit returned for accomplishing such a task. The majority of the collected midges were however in the subfamily Chironominae with a few Orthocladinae being represented. After collection the midges were counted and weighed and prepared for DDE quanitation by gas chromatography and residue concentration expressed as micrograms DDE per individual midge.

Generally there was an increase in DDE contamination of surface water in a downstream direction with Station II having the highest residue level (Table 9). Stations III and IV were intermediate in DDE residue and thus served as recovery zones from the input of DDE below the Williamston Waste Water Treatment Plant. The Chironomids reflected the quanity of DDE in the water on a ug/insect basis at every station. The emergence trap at Station V was lost after the first sampling period (July 1) and thus no further samples were taken at that station. As the DDE in the water increased in a downstream direction the body burden of DDE in the insect also increased. Results of this field study were closely similar to those results obtained in the laboratory uptake studies. The laboratory results suggested that the adult midge's body burden of DDE on a ug/insect basis clearly reflects the concentration of DDE in the water on a ppb basis (i.e., 1.6 ug/insect when exposed to 1.1 ppb DDE in water). This same relationship was observed in the Red Cedar study, thus the

Table 9. Summary of mean DDE residue in Chironomidae as compared to water in Red Cedar River July, 1971, residues expressed as ug DDE/insect.

		Station				
Date		I	II	III	IV	V
July 1	Chironomidae	.19(68)[1]	2.14(37)	.41(20)	.82(29)	.24(45)
	Water (ppb)	.37	1.93	.36	.26	.51
July 13	Chironomidae	.09(29)	1.69(82)	.73(12)	1.20(21)	---
	Water	.26	3.21	.61	.96	---
July 26	Chironomidae	.28(26)	3.15(6)	.43(13)	.21(71)	---
	Water	.19	1.51	.29	.31	

[1]Number of insects from which mean was derived.

laboratory studies are indeed applicable to a field situation and confidence can be put in these studies.

SUMMATION DISCUSSION

The majority of effort in the area of pesticide and aquatic invertebrate study has been in the total ecosystem approach. In studies such as these the components of the system in the field are defined, a pesticide of known concentration applied and the compound is monitored through the various components, each representing a trophic level, until the pesticide reaches the ultimate consumer in this conceptualized system. In each of the various components, rates of accumulation and degradative processes are observed and inferences are made as to the total impact of this compound on this defined ecosystem. Recently Metcalf (1971) has developed a laboratory model ecosystem for the evaluation of pesticide biodegradability and ecological magnification, and has proposed the use of such a system for the evaluation of present and future pesticides. Field studies and model ecosystems serve a great capacity in determining the ultimate fate of the compound in the system but have a limited scope when evaluating the effects, if any, ellicited by the compound at each trophic level. No statements can be made as to elimination rates, factors affecting bio-accumulation and the effect of the presence of other compounds when working from the ecosystem approach. It was for these reasons that this study was implemented.

This investigation was generally descriptive in nature in that a wide variety of questions were asked concerning the rate of accumulation, elimination dynamics, metabolism and possible reproductive physiology effects of DDE on Chironomus tentans. One of the most significant aspects resulting from this study is the mechanism by which DDE is accumulated from the aqueous environment. Wallace (1971) was rather surprised with the relatively low levels of dieldrin found in the hellgramite, Corydalis cornuta, from a high dieldrin contaminated section of stream in South Carolina. Gut analysis revealed this animal to be a predator on other aquatic insects, including Trichoptera and Simuliidae larvae. With the reported increase in concentration of chlorinated hydrocarbons up the food chain (Naqui 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 (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 the cuticular properties of Simuliidae and Hydropsychidae are different than Corydalis and thus 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 (Matsumra, 1963). This adsorption and diffusion concept would support the findings of Keith (1966), Peterle (1966) and Chadwick and Brocksen (1969) that aquatic organisms may not necessarily show

pesticide concentrations relative to their position in the food chain.

The proposal submitted by Hamelink (1971), that of exchange equilibria determining the degree to which chlorinated hydrocarbons are biologically magnified, can also be incorporated into the adsorption-diffusion mechanism. The exchange equilibrium depends on the differences in solubility of the pesticide in water and fats. If the assumption that DDE, DDT, dieldrin, toxaphene and lindane have similar solubilities in fats is met, 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. Conversly, if we assume that there is no difference in the above compounds solubility in water than 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 amount of DDT accumulated by invertebrate component of Hamelink's (1971) study. The invertebrates captured in the pools were primarily ostracods, mites, dragonfly naiads and some Hyallella azteca and Chaoborus sp. When all the invertebrates 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% more DDT residue than

the ostracods, while the odonates contained about half of the residue of the ostracods. Thus the epicuticular lipid layer could indeed be of different properties in each invertebrate group and therefore be exhibiting different accumulation rates with regard to DDT (Armold, et. al., 1969).

Hamelink (1971) also suggests that the degree of biological magnification and persistence of DDT in the lentic ecosystems appears to be promoted by oligothrophic 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) readily stimulates the adsorption-diffusion mechanism and thus an increase in accumulation results. Oligotrophic lakes are characteristicly 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.

The concept of the adsorption-diffusion mechanism could also be considered and used as another parameter in the designing of new synthetic non-persistent pesticides. Metcalf (1964, 1966, 1967, 1968 and 1971) and Kapoor (1970, 1972) have done extensive study in the areas of structure-activity relationships of organophosphate and carbamate insecticides and metabolic pathways of DDT analogs which are readily attacked by the multifunction oxidase system. However no attempt has been made to synthesize organo-chlorine type

compounds that are unable to be readily adsorped by organisms in the aquatic environment. A compound could possibly be synthesized that possessed the organo-chlorine insecticidal properties analogous to materials such as methoxychlor, methioclor and methylchlor for the terrestrial system and would then be unable to be accumulated in the aquatic environment should indirect contamination occur.

One of the more practical aspects obtained from this study is the utilization of C. tentans larvae as a contaminated food source for fish and other predators in laboratory and field studies dealing with pollutants and the aquatic ecosystem. Factors associated with the biology of the insect such as ease of collection and maintenance in the laboratory, relatively high abundance and a major food source for many benthic predators makes the chironomid an ideal organism for representing a "natural" food source for laboratory pesticide studies. Studies in the past have used methods of injection and contaminated food pellets as a source of pesticide introduction into fish and problems have been encountered using these methods (Gakstatter and Weiss, 1967). Injection of pesticides suspended in Ringer's solution does not ideally represent an introduction of pesticide into the fish in the field situation and the variability in uniformly contaminating food pellets with pesticide has led some investigators to force feed contaminated gelatinized capsules to fish. This study has shown that C. tentans larvae demonstrate a high degree of reproducibility in the amount of DDE they accumulate over a prescribed time period and a known concentration of material that they were exposed to. Research has been recently initiated at Michigan State University using Aroclor 1254 contaminated C. tentans

larvae as a food source for a fathead minnow reproductive study and excellent reproducibility in dosing the fish with the PCB has been achieved (Eckenrode, personal communication. 1972). Cairns (1969) proposes the utilization of a standard test fish for bioassay experimentation. I submit that the use of C. tentans larvae due to its ease of handling in the laboratory, a natural food material and predictibility of accumulating pesticide residue be incorporated into laboratory procedures as a contaminated food source for fish-pesticide studies. I feel the benefits of using such a "standard food source" would far exceed any problems arising from the laboratory maintenance of these insects.

The utilization of chironomids as a possible mechanism of pesticide removal from lentic ecosystems has been considered as a future aspect of the applicability of this research. The lentic ecosystem which should be considered as a candidate body of water for this type of approach is the sewage oxidation pond due to the following aspects:

1. By previous study it has been shown that the largest amount of pesticide contamination entering the Red Cedar River comes from the waste water treatment plant (Zabik, et. al., 1971), and being the Red Cedar is located in an urbanized community with secondary treatment facilities these results are closely applicable to other urbanized communities.
2. The use of sewage oxidation ponds as a means by which municipal sewage is processed is increasing. In Michigan alone there are five of these treatment facilities projected for use by 1975 (Eyer, personal communication. 1972).

3. These ponds are capable of maintaining extremely high populations of the midge due to the ideal organic substrate and other environmental factors, numbers of 8700/m^2 have been recorded from such ponds in California.
4. Sewage oxidation ponds, due to their small size and isolation, are capable of being managed or manipulated economically in such a way as to favor production of midges and not alter the beneficial microbial component of the system (Hall, et. al., 1970).

To achieve a better understanding of the practicality of such a pesticide removal mechanism a preliminary study was conducted in the summer of 1972 at a series of five sewage oxidation ponds located at Belding, Michigan, Ionia County (T8N, R8W, Section 25). Grab samples of water and sediments were taken at the various ponds to obtain an idea of the distribution of pesticide residue in each pond and evaluate the significance of pesticide removal through the dynamic system. Chironomid larvae were also collected, identified and analyzed for chlorinated hydrocarbon residues. The majority of midge larvae collected were C. plumosus which comprised approximately 95% of the benthic fauna. Quanitative sampling with an Ekman dredge in one pond revealed average population estimates of 3800 larvae/m^2.

No observable differences were found between ponds in the amount of pesticides residue contained in each, indicating that residue loss from the treatment is minimal when the water is moved from one pond to another. Sediments contained 1.21 - 4.9 ppm DDE while water had .24 - 1.27 ppb DDE. There was present some trace quanities of

PCB similar in chromatography characteristics to Aroclor 1248. The absence of differences between pond residue could be attributed to the low number of samples that were taken. A yearly profile of chlorinated hydrocarbon residue in the pond system would be needed in order to evaluate the effect the pond facility had on pesticide breakdown and reduction. It is interesting to note at this point the paucity of information dealing with the contamination and distribution of pesticide residues in sewage oxidation pond systems.

The research accomplished with DDE and C. tentans larvae has shown that the results are applicable in theory to such a postulated approach of pesticide removal from sewage oxidation ponds. The conceptual mechanism of pesticide removal by emergent chironomids will be developed in this discussion. The results of practical application derived from this study are as follows: 1) rates of uptake and elimination were determined and more important correlation of the amount of DDE residue concentrated by the midge larvae with the concentration in the water and 2) factors affecting DDE uptake by midge larvae such as water hardness (calcium and magnesium effects), surface area-accumulation relationships and the presence of Aroclor 1254 on DDE uptake.

It is a basic assumption in this postulated approach to pesticide removal that any increase in the population of the midge would ultimately result in an increased amount of contaminant removed from the pond. From other studies dealing with the biology and factors affecting population distribution of the midge the population aspect can be incorporated into the removal mechanism (Hilsenhoff, 1966, 1967). Aspects in the area of population density that should be

considered are: 1) developmental time and factors affecting population turnover rates and 2) physical and chemical requirements of the insect and population density of the insect as related to substrate composition.

An idea of the possible means by which pesticide removal could be optimized by manipulation or management of the pond could be accomplished by combining the residue accumulation and population density relationships. A total management scheme of the sewage oxidation pond system might include such practices as: 1) removing predators, 2) providing ideal substrate for the midge, 3) oxygenating the water and thus decreasing the developmental time and increasing the turnover rate of the population, also oxygenation of the water might increase the magnitude of the codistillation process, 4) placing activated carbon and polyurethane filters at some point in the dynamic system might also be incorporated into the management scheme to remove some pesticide residues and 5) addition of calcium and magnesium to the system for increasing the adsorptive properties of the midge for chlorinated hydrocarbon residues. These proposed practices for a total management scheme of sewage oxidation ponds for removing pesticide by trapping emergent insects must all be evaluated as to their individual effects on the total management program.

The increased use of water resources for the treatment of waste products in an oxidation pond system would make the proposed mechanism a likely candidate for consideration and evaluation.

SUMMARY AND CONCLUSIONS

1. The midge, Chironomus tentans, was exposed from egg to adult to various concentrations of DDE in water under continuous flow dynamic conditions.
2. The C. tentans larvae concentrated the DDE approximately 15,000 fold over that present in the water.
3. The DDE accumulation by the midge demonstrated a dose-dependent relationship.
4. The exuvia did not demonstrate a major route of residue elimination as only 1.4 to 4.9% of the pupal burden was lost via the exuvia. The process of egg deposition eliminated 11.6 to 30.9% of the adult female burden of DDE residue.
5. The half-life of DDE in C. tentans larvae is 6.8 days.
6. The presence of the PCB (Aroclor 1254) did not affect the rate of uptake of DDE either in continuous flow or static experiments. Preloading the larvae with Aroclor 1254 did not affect DDE uptake.
7. There was no difference in live and dead larval uptake of DDE and there was no difference in uptake of DDE when live and dead larvae were exposed to an aqueous and substrate source of DDE.

8. Surface area of cuticle is directly related in DDE uptake from aqueous exposure.
9. The primary mode of uptake of DDE by C. tentans is an adsorptive-diffusion mechanism.
10. The calcium and magnesium ion concentration affects the adsorption-diffusion mechanism and an increase in the calcium and magnesium ion concentration in the water results in greater uptake of DDE.
11. There was no metabolism of p,p'-DDE by C. tentans larvae.
12. The exposure of C. tentans to 30 ppb in the water resulted in a decrease in the number of emergent second generation adults obtained from these exposed insects.

PROPOSALS FOR FUTURE RESEARCH

With most investigations, as many or more questions are generated which require further research as the number of questions answered in the proposed study. The following areas of investigation are proposed for future study:

1. The nature, chemical and physical properties of the larval cuticle should be looked at and compared to other aquatic insect cuticles to further evaluate the role of the insect cuticle in the biological concentration of pesticides.
2. The calcium and magnesium ion effects seem to suggest selective diffusion. Other inorganic ion effects should be investigated and the mechanism of increased uptake with increased inorganic ion concentration should be ellucidated.
3. The effect of the presence of two or more compounds on egg viability of C. tentans.
4. The practicality of using emergent C. tentans adults as a mechanism of pesticide removal from sewage oxidation ponds should be evaluated both in laboratory and field situations.

APPENDICES

APPENDIX I

Concentrations of DDE delivered in water by dilution system during one experiment demonstrating consistency of delivery.

		DDE in Water (ppb)			
Sample No.	Date	I	II	III	IV
1	April 1	2.31	1.15	.60	.23
2	April 5	2.14	1.14	.60	.22
3	April 6	2.09	1.11	.59	.21
4	April 7	2.41	1.19	.62	.21
5	April 13	2.42	1.31	.61	.21
6	April 15	2.11	1.31	.62	.22
7	April 17	2.10	1.26	.63	.25
8	April 19	2.16	1.10	.61	.26
9	April 23	2.07	1.11	.60	.26
10	April 29	2.07	1.12	.59	.27
11	April 30	2.08	1.26	.57	.28
12	May 3	2.08	1.10	.54	.19
13	May 6	2.01	1.10	.54	.21
14	May 9	1.96	1.14	.54	.20
15	May 13	1.86	1.15	.54	.21
16	May 19	1.91	1.16	.51	.21
Mean		2.26	1.15	.56	.21

APPENDIX II

Wet weight of individual Chironomus tentans according to age.

Days	Stage	Weight (mg)[1]	S.E.[2]
7	larva	.73	.18
16	Larva	1.34	.39
20	Larva	6.87	.27
27	Larva	14.68	1.63
29	Pupa	12.87	.67
	Exuvia	1.82	.11
33	Adult	11.23	.78

[1]Means represent average of 50 individuals for each sampling period.
[2]Standard error.

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