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## ABSTRACT

The bioaccumulation of mirex by two species of amphipod was examined in terms of uptake and clearance rates and analyzed mathematically in order to construct a simple model.

The survival of Hyalella azteca was found to be reduced relative to that of Cragonyx pseudogracilis during exposure to mirex in water for a 13-day period. This phenomenon was correlated to greater bioaccumulation of mirex by H. azteca than by C. pseudogracilis, although this may not be the entire explanation. H. azteca was found to exhibit two-compartment kinetics, while C. pseudogracilis was described by single-compartment kinetics. The bioaccumulation differences between the two species were the result of both uptake and clearance differences; uptake into a "fast" compartment of H. azteca; greater uptake into the slow compartment of H. azteca relative to the single compartment of C. pseudogracilis (4500 vs. 2600/day); and greater clearance from the single C. pseudogracilis compartment relative to the slow compartment of H. azteca (0.10 vs. 0.06/day). The fast H. azteca compartment was relatively insignificant from a bioaccumulation standpoint, as determined from predictive equations describing uptake from water. The disparity in net uptake between the two species held over a range of con-

centrations, from 0.1-20.0 ng/ml. This disparity also held over a wide range of body sizes. The relationship of body size to net uptake for both species was described by power functions with exponents of 0.82 and 0.91 for H. azteca and C. pseudogracilis respectively, and indicated a metabolic rate factor was involved. Net uptake was found to be proportional to temperature. This was due to the proportionality of both uptake- and clearance-rates to temperature, which also indicates the involvement of a metabolic rate factor.

Bioaccumulation of mirex from food was uni-compartmental for both species. Feeding rates were the same for both species, as were assimilation efficiencies, which averaged 20%. Clearance of ingested mirex was similar for both species and was of a magnitude similar to clearance of C. pseudogracilis and the H. azteca slow compartment after uptake from water. This similarity may only be coincidental, as clearance rate appeared to be proportional to mirex concentration in food.

Model predictions indicate that both species will receive substantial amounts of mirex through both food and water vectors. Due to the greater importance of the water vector in H. azteca, this species can be expected to accumulate a greater body burden of mirex than C. pseudogracilis.

## RESUME

La bioaccumulation du mirex par deux espèces d'amphipodes fut examinée. La survie de Hyalella azteca fut réduite en comparaison de Crangonyx pseudogracilis durant des expériences de mortalité de 13 jours. Ce phénomène est relié à la bioaccumulation de H. azteca, qui est supérieure à celle de C. pseudogracilis. L'accumulation du mirex de l'eau par H. azteca peut être décrite par un système de deux compartiments, cependant, C. pseudogracilis est décrite par un seul compartiment. La différence de bioaccumulation entre les deux espèces, est le résultat des différences d'absorption et d'élimination; l'absorption par un compartiment "rapide" de H. azteca; l'absorption supérieure par le compartiment "lent" de H. azteca en comparaison avec le compartiment unique de C. pseudogracilis; et l'élimination supérieure par le compartiment unique de C. pseudogracilis en comparaison avec le compartiment "lent" de H. azteca. La contribution du compartiment rapide de H. azteca, est relativement mineure pour la bioaccumulation, comme le témoignent les équations qui décrivent la bioaccumulation du mirex de l'eau. La différence de bioaccumulation entre les deux espèces, demeure constante à des concentrations s'étendant de 0.1-20 ng/ml. Cette différence demeure également constante pour

tous les poids examinés. La relation des poids par rapport à la bioaccumulation pour les deux espèces, est décrite par des équations à exposants, et suggère l'implication du taux métabolique dans l'absorption du mirex. La bioaccumulation est proportionnelle à la température. Celle-ci est la conséquence de la proportionalité entre les taux d'absorption et d'élimination, ce qui confirme la relation entre le taux métabolique et la bioaccumulation.

La bioaccumulation du mirex de la nourriture est décrite par un compartiment unique pour les deux espèces. Les taux d'ingestion de nourriture sont les mêmes pour les deux espèces. L'efficacité d'assimilation est aussi la même pour les deux, et s'échelonne de 10-30%. Les taux d'élimination sont comparables pour les deux espèces. Ils sont comparables au taux d'élimination de l'unique compartiment de C. pseudogracilis après leur exposition au mirex dans l'eau. Cette similarité peut être l'effet d'une coïncidence puisqu'il existe une corrélation entre le taux d'élimination, et la concentration du mirex dans la nourriture.

La cinétique de la bioaccumulation du mirex fut utilisée pour développer un modèle. Ce modèle prédit que les deux espèces accumulent des quantités considérables de mirex à partir de l'eau et de la nourriture. H. azteca accumulerait une quantité supérieure à celui de C. pseudogracilis parce que l'accumulation à partir de l'eau est plus importante pour cette espèce.

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section I  
INTRODUCTION

The advent of the modern industrial society has brought with it, an increasing use of, and dependence on, chemicals. Either, as a direct consequence of their employment, or via secondary means, these chemicals find their way into the biosphere, which in consequence, has become extensively polluted with a wide variety of xenobiotics. The assessment of these chemicals as environmental hazards has traditionally involved measurement of their short-term toxic effects, the most widely used test being the LC50.

The last decade has brought an awareness that some xenobiotics have certain subtle effects which cannot be evaluated by the standard concepts of lethality. The most noteworthy cases include DDT, PCB's, and mercury, which manifested their effects only after the achievement of very high doses. There is an urgent need to understand the relationship between exposure levels, i.e. the level of contamination of the environment, and the dose attained by an organism.

In assessing the long-term, dose related hazard experienced by an animal, the processes involved in bioaccumulation need to be examined. This involves quantification of

the absorption of a pollutant by an organism from its environment, via direct exchange with water, and by assimilation from the food source. This is best studied using a biomathematical approach, a method which has recently been adopted by several authors (Trudel 1980, Harding et al. 1981, Thomann 1981). This approach not only permits prediction of the bioaccumulation hazard for an organism, but also allows determination of the relative importance of the food and water vectors in total bioaccumulation, a subject of considerable controversy over the last decade.

Mirex, perchloropentacyclo [5.3.0.0<sup>2,6</sup>.0<sup>3,9</sup>.0<sup>4,8</sup>.] dioxane, is an organochlorine pesticide which became an environmental contaminant through its use in combatting infestations of plantation pests, especially the imported fire ant Solenopsis richteri, in the American south-east. In the Canadian context, mirex has been found to extensively pollute the Lake Ontario ecosystem, apparently as a consequence of its manufacture at several locations on the lake's south shore. The literature on mirex is extensively covered in Mirex in Canada (1977), and several other reviews (Alley 1973, Waters 1976).

Many fears were raised that mirex would follow the pattern of DDT and the other above-mentioned compounds. This was due to in part, to its great persistence under all environmental conditions. Two other properties of mirex also indicate a compound with great potential for accumulation to

high levels; low toxicity (Ware, 1975), allowing the attainment of high tissue concentrations without toxic effects; and low water solubility (Alley, 1973), leading to a strong tendency for absorption into tissues.

The limited information regarding natural exposure levels of mirex can be summarized as follows. Levels in freshwater have remained in the range 1-18 pg/ml for at least one year after the application of the pesticide (Markin et al. 1974, Spence and Markin 1974). Sediment levels in water bodies near application sites, and in Lake Ontario, range from 1-40 ng/g (Markin et al. 1974, Mirex in Canada 1977).

This study involves two species of freshwater amphipod, Hyalalala azteca, and Cragonyx pseudogracilis. Amphipods are common inhabitants of virtually all bodies of water, and form a vital part of many aquatic ecosystems, especially in shallow waters. They constitute part of the diet for a wide variety of fishes, and often are the main food item (Cooper 1965, Scott and Crossman 1973).

H. azteca is a common amphipod of continental North America, inhabiting all permanent, fresh waters below the tree line (Bousfield, 1958). Though less common than H. azteca, C. pseudogracilis also inhabits a wide range of lakes and rivers in North America, as well as being an introduced species of Europe.

The aim of the present work, as stated above, is to study and compare the accumulation of mirex by the amphipods

4.  
Hyalella azteca and Crangonyx pseudogracilis. To this end,  
the following studies were undertaken:

(a) quantification of mirex uptake rates from contaminated water and it's subsequent clearance.

(b) quantification of mirex uptake rates from contaminated food and it's subsequent clearance

(c) an examination of the effects of body size on bioaccumulation, as well as a determination of the effects of temperature on bioaccumulation by H. azteca.

The data from these studies was formulated into a bioaccumulation model which allows the comparison of the two species, and permits prediction of tissue levels of mirex in each species, under specified conditions.

## section II

## MATERIALS AND METHODS

2.1 GENERAL PROCEDURES2.1.1 Maintenance of Experimental Animals

All amphipods of both species were collected from Kettle Island Bay, Kettle Island, in the Ottawa River below Ottawa, Ontario, Canada, during the summers and autumns of 1979/80.

Collection was conducted by sweeping a 20X40 cm dip net (2 mm mesh) through beds of the submerged aquatic plants, Ceratophyllum, spp. and, Myriophyllum spp. They were then placed in 15 liter containers of river water, and transported to the lab, where they were placed under the same temperature conditions as those existing in the collection area.

Amphipods were initially maintained in 10 liters of Ottawa River water, but were gradually acclimatized to dechlorinated tap water by exchanging 10% of the river water for tap water daily until river water represented less than 20% of the total, whereupon they were transferred to 100% dechlorinated tap water. They were held for a minimum of 2 weeks under these conditions before experimentation.

Holding conditons consisted of 15 liter plexiglass tanks containing 10 liters of water. To this was added sand

Table 1. Characteristics<sup>a)</sup> of the water used for the study

Constituent	Concentration (mg/liter)
orthophosphate	0.52
metaphosphate	0.04
total phosphate	0.56
silica	6.50
Fe	0.10
N-NO <sub>3</sub>	8.00
NO <sub>2</sub>	36.20
O <sub>2</sub>	10-11.00
hardness	41-48.00
pH	7.2-7.4
alkalinity	18-20.00

a) Determinations made according to standard methods as described by Lind (1974)

and plants from the site of collection. Food, in the form of the alga, Scenedesmus quadricauda, was added periodically. A light regime of 12:12 hour light:dark and a temperature of 20°C was maintained for both holding and experimental conditions, except for specific experiments assaying the effect of temperature on kinetic parameters. The two species were maintained separately, as it was observed that C. pseudogracilis exerted a negative effect on H. azteca survival.

#### 2.1.2 Labelled Compound

Uniformly labelled <sup>14</sup>C-mirex, with a specific activity of 11.6 mCi/mM and radiochemical purity of 98%, was obtained from California Bionuclear Corporation (7654 San Fernando Road, Sun Valley, California 91325) in crystalline form, and immediately dissolved in acetone. Radiolabelled mirex was used without a carrier of non-labelled mirex in all experiments.

#### 2.1.3 Residue Analysis

Routine analyses were performed on a Beckman LS-3133 liquid scintillation counter. To follow aqueous mirex concentrations during uptake and clearance experiments, 5 or 10 ml of water was placed in a 20ml glass scintillation vial

with an equal volume of Aquasol-2 (New England Nuclear Corp.), shaken to form a gel, and counted. Levels of  $^{14}\text{C}$ -mirex in algae used in feeding experiments were determined by adding 0.5ml aliquots of the algae to 10ml Aquasol-2, shaking, and counting. Amphipods were lyophilized and individual dry-weights determined on a Mettler, Micro Gram-atic balance ( $\pm 5$  ug). Individuals, or groups of individuals, were placed in scintillation vials along with 200 microliters of distilled water, and 1ml of Beckman Tissue Solubilizer (BTS-450). Vials were then placed in an oven at  $55^{\circ}\text{C}$  for five hours to allow solubilization. After cooling, 10ml of Econofluor (New England Nuclear Corp.) were added to the vials, along with 100 ul, 99% acetic acid to establish neutral pH. These were dark adapted overnight, and subsequently counted.

Liquid scintillation counting was cross-checked, and the presence or absence of metabolites determined on a Hewlett-Packard 5700 gas chromatograph. A six-foot glass column, 2mm inside diameter, packed with 3% OV-101, 80-100 mesh, was used. Injection port temperature was  $260^{\circ}\text{C}$ , column temperature  $240^{\circ}\text{C}$ , and detector temperature  $280^{\circ}\text{C}$ . Gas (95% Argon, 5% Methane) flow was 50ml/min. Detection limit was  $3 \times 10^{-12}$  g. A simple procedure was followed for the extraction of mirex and any metabolites from amphipods. Amphipods were placed in 15ml glass, graduated centrifuge tubes. One ml glass distilled dichloromethane was added,

and the amphipod crushed with a glass rod. The tube was placed on a vortex mixer for 1 minute, the tissue allowed to settle, the dichloromethane drawn off with a pasteur pipett, and the process repeated twice more. The dichloromethane was evaporated in a water bath at 50°C under a stream of nitrogen. Mirex and metabolites were then taken up in 0.5ml pesticide grade hexane and a small volume (1-7ul) injected. Extraction efficiency was 94%. Residue peaks were compared to standards obtained from Dr. D.R. Peakall of the Canadian Wildlife Service, Hull, Quebec.

No formation of mirex metabolites, specifically the 2,8-dihydro and 8-monohydro derivatives, was observed in any of the experiments in which samples were analyzed by GC. Radiotracer technique was thus thought to be sufficient for the detection of total mirex, and was the sole method utilized in the balance of the experiments.

#### 2.1.4 Preparation of Food Stock

The green alga to be used in all feeding experiments, Scenedesmus quadricauda, was obtained from Carolina Biological Co., and cultured on media number 11 of Hughes et al. (1958). For feeding in uncontaminated form, algal culture was filtered through 5um pore size millipore filters, and the resultant algal mass sub-divided and added to experimental containers.

### 2.1.5 Preparation of $^{14}\text{C}$ -mirex Contaminated Water

One-liter of dechlorinated tap-water, and an appropriate volume of  $^{14}\text{C}$ -mirex stock solution were mixed vigorously in 150X75 mm crystallization dishes to obtain a desired concentration. This was allowed to sit for three hours prior to an experiment, to allow equilibration between the water and the glass surface of the container. Mirex levels were determined by removing 5 ml aliquots at the beginning and end of every 24 hour period and measuring their  $^{14}\text{C}$  levels.

### 2.1.6 Preparation of $^{14}\text{C}$ -mirex Contaminated Food

Algal cultures were inoculated to a level of approximately 200,000 cells/ml. After 5 days, when cell density had reached about 800,000 cells/ml, 200ml of the culture were centrifuged, and the algae transferred to a 17ml capped vial along with an appropriate amount of  $^{14}\text{C}$ -mirex. This was placed on a rotary mixer for 2 hours after which it was centrifuged at 3000 rpm for 2 minutes to pellet the algae. The supernatant, along with any unabsorbed mirex, was poured off, and the algae resuspended in 15ml dechlorinated tap-water, to remove loosely bound mirex. This was repeated 5 times or until the amount of mirex in the supernatant was less than 2% of the original dose. Contents of the tube were then passed through a millipore filter (5um pore) to form a cake of  $^{14}\text{C}$ -mirex labelled algae.

### 2.1.7 Data Handling

In the analysis of mirex uptake from water by amphipods, the term "transfer coefficient" is used. This is defined here as the amount of water (g or ml) cleared of its mirex content, by one gram (dry weight) of amphipods, in one hour. Thus, it is a net rate of uptake, normalized for concentration of mirex in the exposure media, the units being hour<sup>-1</sup>.

$$T_c = \frac{\text{ug mirex/g body wt}}{(\text{ug mirex/ml water})(\text{time in hours})} \quad (1)$$

During the course of uptake from water, body burden increases in a non-linear fashion. As the body burden increases, clearance increases until the point is reached at which uptake equals clearance, the condition termed steady-state. The length of time required to reach steady-state is dependant on the chemical used, and in most cases is impractically long from an experimental point of view. Upon transferal to uncontaminated water, uptake ceases, but clearance continues at the same rate as during exposure.

An exponential model, Euzic (1972), Moriarty (1975), was utilized in compartmental analysis of the data obtained in this study. The general form of the equation for accumulation from water is

$$\frac{dY}{dt} = k_{01}W - K_{10}Y \quad (2)$$

Where  $k_{01}$  and  $k_{10}$  are rate constants of uptake and clearance respectively,  $W$  is mirex concentration in exposure medium,

and  $Y$  is mirex body burden. When integrated, equation 2 becomes the bioaccumulation equation

$$Y_t = \frac{k_{01}}{k_{10}} W (1 - e^{-k_{10}t}) \quad (3)$$

Steady-state body burden (where uptake is equalled by clearance) is achieved as time increases and the bracketed term approximates 1. The time to reach steady-state is calculated by setting the parenthetic term equal to a number close to 1 (.95) and solving for  $t$ .

For clearance, where  $W$  equals zero, the solution to equation 2 describes the single compartment clearance function,

$$Y_t = Y_0 e^{-k_{10}t} \quad (4)$$

where  $Y_0$  is the body burden at the end of exposure. These equations apply to situations involving a single compartment of pollutant, a compartment being defined as a pool of pollutant having uniform kinetics (i.e. having a single fractional clearance rate). For situations best described by two compartments, equations are determined separately for each compartment, the sum of the two describing total accumulation. In the apparent two-compartment situation, clearance data was inspected to ensure that mirex loss after 24 hours postexposure involved only a single compartment. The best-fitting single exponential curve was then determined for the data beyond 24 hours postexposure by the method of least squares, using the equation

$$\ln B_t = \ln B_0 - kt \quad (5)$$

where  $B_t$  is the mirex content of the slow-clearing compartment in ug mirex/g amphipod at time  $t$  (days),  $B_0$  is the mirex content of the slow compartment at one minute postexposure, and  $k$  is the fractional clearance rate in day<sup>-1</sup>.

To determine the clearance equation for the fast compartment, the mirex content of this compartment at time  $t$  postexposure,  $A_t$ , was estimated by subtracting the estimated mirex content of the slow compartment,  $B_t$ , from the observed total body burden of the amphipod,  $Y_t$ .

$$A_t = Y_t - B_t \quad (6)$$

The method of least squares was then used to determine a clearance equation for the fast-clearing compartment.

Uptake during exposure to contaminated food is a rapid, linear phenomenon (as long as feeding time is less than the time needed for food to pass through the gut), dependant on the feeding rate of the organism. When removed from the contaminated food source, and given uncontaminated food, body burden will rapidly decline, corresponding to the egestion of food containing unassimilated mirex. That mirex assimilated from the gut, will be cleared at a rate proportional to body burden. This rate is the slope of the clearance curve.

## 2.2 SPECIFIC PROCEDURES

### 2.2.1 Mortality experiments

Ninety amphipods of each species were removed from culture aquaria and placed in 1 liter dechlorinated tap-water under non-feeding conditions for 24 hours prior to commencement of an experiment, to allow voidance of the gut. One-liter solutions of  $^{14}\text{C}$ -mirex in dechlorinated tap-water were made up and allowed to stand for 3 hours prior to beginning an experiment. Concentrations were at ten-fold intervals ranging from 0.01-100.0 ng/ml, plus two controls (one with acetone, one without). Fifteen animals of each species were placed in separate containers at each concentration and observations made continuously for the first 2 hours, and then at 4, 8, and 24 hours, and daily thereafter until significant mortality was observed in at least one concentration. Solutions were replaced and dead animals removed every 24 hours.  $^{14}\text{C}$ -levels were determined at the beginning and end of every 24 hour period for the duration of the experiment. Results were plotted as % mortality. Animals alive at the end of a test were analyzed for their  $^{14}\text{C}$  content.

### 2.2.2 Bioaccumulation from Water

Forty to sixty animals, either H. azteca or C. pseudo-gracilis, were removed from culture aquaria and placed in crystallization dishes containing 1 liter of dechlorinated

tap water for 24 hours without food, to allow clearance of the gut. This was essential as, under the non-feeding conditions of the uptake experiments, amphipods would consume their own feces. Since feces are organic, they tend to adsorb large amounts of mirex, and ingestion of these particles caused considerable distortion in preliminary experiments. Subsequent to the non-feeding period, animals were transferred to 1 liter solutions of mirex and exposed for 96 hours. Solutions were replaced every 24 hours to prevent exposure concentrations falling more than 15%. Samples of 10-15 animals were taken at 24 hour intervals, passed through 4 washes of 200ml dechlorinated tap water, and their mirex content determined. Aliquots of water were removed at the beginning and end of each 24 hour period to follow concentration in the exposure media. All exposures were carried out under static conditions.

To study release of mirex from amphipod tissues, animals were prepared and exposed as above, the exposure period however, being reduced to 24 hours in most cases. After exposure, all animals were passed through washes as above, and placed in 2500ml of dechlorinated tap water with food (Scenedesmus quadricauda). In general, amphipod samples were taken at 0, 6, 12, and 24 hours for the first day postexposure, and then at 2, 4, 8, 12, and 16 days. Water was replaced at each sampling time, and at least daily for the duration of the clearance period. Analysis of mirex content was ac-

completed using radioassay technique, except for one series of experiments in which the degree of mirex metabolism by amphipods was assessed by gas chromatography after 4 days of uptake, and on the final day of clearance.

### 2.2.3 Passive Uptake of Mirex---Dead Control

Ten individuals of each species were removed from holding conditions, and prepared for experimentation in the usual manner. After the preparatory period, animals were killed by placing them in dechlorinated tap water which had been heated to 50°C. Death was immediate. These animals were then exposed to a 1 liter solution of  $^{14}\text{C}$ -mirex at a concentration of 1 ng mirex/ml water, for a single 24 hour period.

### 2.2.4 Effect of Duration of Exposure on Uptake and Release of Mirex

Eighty amphipods were removed from culture aquaria and prepared for experimentation in the usual manner. For these experiments, only one concentration, 1 ng mirex/ml water, was used. The group of animals was exposed for the selected time period, and then passed through a series of 4 washes. Uptake was determined from a sample taken at one minute post-exposure. This sample also served as zero time point of mirex clearance. Clearance was determined from a series of

measurements made over the next 16 days. Exposure times of 6, 12, and 24 hours were examined for H. azteca, while those for C. pseudogracilis were 12, 24, and 48 hours.

#### 2.2.5 Effect of Temperature of Exposure on Mirex Uptake and Release

For uptake studies, 40 H. azteca per temperature were removed from culture aquaria and prepared for experimentation. These animals had been collected at temperatures approximating those of the proposed experiments ( $\pm 2^{\circ}\text{C}$ ), and acclimatized to experimental temperatures for 4 weeks. Relative absence of C. pseudogracilis, especially at the colder temperatures, precluded study of temperature effects with this species. Exposure media was prepared from dechlorinated water at the correct temperature, and a concentration of 1 ng mirex/ml water used throughout. Samples of animals were taken at 24 hour intervals over a 96 hour period, and analyzed for  $^{14}\text{C}$  content.

For the study of retention, groups of 60 animals which had been collected and maintained as described above were removed from culture aquaria and prepared for experimentation. Animals were exposed at a concentration of 1 ng mirex/ml water, for a single 24 hour period, then taken through a series of 4 washes and transferred to 2500ml water with food at the proper temperature. Samples were taken periodically over a 12 day period and radioassayed.

### 2.2.6 Effect of Body Size on Uptake of Mirex from Water

Twenty-five individuals of each species were prepared for experimentation as per the general procedure. H. azteca size ranged from 0.1-0.6 mg dry weight, while the range for C. pseudogracilis was 0.2-1.0 mg. One liter solutions of  $^{14}\text{C}$ -mirex were prepared for each species, at nominal concentrations of 1 ng mirex/ml water. After the preparatory period, the two species were introduced into separate containers, and allowed to accumulate  $^{14}\text{C}$ -mirex for a 24 hour period, after which they were sacrificed, and their  $^{14}\text{C}$  content determined. The actual mirex concentration in the exposure media was determined from water samples taken at the beginning and end of the exposure period.

### 2.2.7 Effect of Mirex Concentration in water on Uptake and Release of Mirex

Forty to sixty H. azteca or C. pseudogracilis were removed from culture aquaria and prepared for experimentation as described previously. Amphipods were exposed to concentrations ranging from 0.1-20.0 ng mirex/ml water. Animals were removed in groups of 10 at selected time intervals and analyzed individually, except for the lowest concentration (0.1ng/ml) for H. azteca, and the two lowest concentrations (0.1, 0.3 ng/ml) for C. pseudogracilis. In these latter three cases, sample sizes were increased to 15, and analysis was performed on groups of three or five.

The effect of exposure concentration on clearance was determined for H. azteca only. Sixty animals were exposed for 24 hours to a concentration of either 1 or 20 ng mirex/ml water. Clearance was subsequently determined from a series of samples taken over a 16 day period.

#### 2.2.8 Bioaccumulation from Food

To determine the various parameters of uptake of mirex from food, and its subsequent clearance, 60-80 H. azteca or C. pseudogracilis were removed from culture conditions 48 hours before an experiment was to begin. These were placed in 1 liter of dechlorinated tap water, to which was added uncontaminated Scenedesmus quadricauda, the algae used in all feeding experiments. After 24 hours feeding on uncontaminated algae, amphipods were transferred to 1 liter of dechlorinated tap water without food for 24 hours to ensure sufficient feeding activity during the experiment. Preliminary experiments determined that feeding rates, assimilation efficiencies, and fractional clearance rates were not affected by periods of starvation ranging from 0-24 hours. Subsequent to this period, mirex-contaminated algae was added and the amphipods allowed to feed for 30 minutes, except for the experiment investigating effect of feeding time on uptake. Animals were then passed through 4 rinses of 200 ml dechlorinated tap water, and placed in 2500 ml water with

uncontaminated food, for clearance periods of up to 16 days. Water and food were replaced daily, and 10-amphipod samples taken periodically, usually at 0, 0.5, 1, 2, 4, 8, 12, and 16 days.

#### 2.2.9 Determination of Voidance Time, Feeding Rate, and Assimilation efficiency

To determine the rate at which ingested food particles pass through the gut, amphipods of both species were handled in the above described manner, but allowed to feed on mirex-contaminated algae over a 90 minute period. Samples of 8 animals were taken at 20, 40, 60, and 90 minutes, and assayed for their  $^{14}\text{C}$  content.

Feeding rates were examined to determine inter-specific variation, and effect of contaminant level. The  $^{14}\text{C}$  levels in amphipods sampled immediately after a feeding period, were analyzed in relation to  $^{14}\text{C}$  counts/algal cell, which had been determined subsequent to exposure of algae to  $^{14}\text{C}$ -mirex, and feeding rates (cells/g amphipod/hr) determined.

To determine the efficiency of mirex assimilation from the gut, the regression for assimilated mirex was extrapolated back to time zero to give the size of this compartment at the end of the feeding period. Comparing this value to the total body burden after feeding, gives an estimate of assimilation efficiency.

2.2.10 Effect of Toxicant Level in Food on Uptake and Clearance

Food, in the form of the alga, Scenedesmus quadricauda, was added to 17 ml tubes with 25, 50, or 100 ul of mirex stock solution, to obtain a food source with the desired mirex concentration. The contaminated algae was collected on a millipore filter and added to a crystallization dish (100 X 50 mm) containing 250 ml dechlorinated tap-water, and amphipods of one or the other species.

## section III

## RESULTS

3.0.11 Mortality

The toxic effects of mirex in aqueous solutions were determined at concentrations of 0.01, 0.10, 1.0, 10, and 100 ng mirex/ml water (ppb) by daily determinations of mortality over a 13 day period.

Results (Fig. 1) demonstrate greater sensitivity of H. azteca, which showed increasing mortality with increasing concentration up to 50% at the highest mirex concentration tested. In contrast, C. pseudogracilis was relatively insensitive, exhibiting little toxic response to the range of concentrations tested.

In the case of H. azteca mortality was not observed until the fifth day of exposure, and, even at the highest concentration, significant mortality (>10%) did not occur until seven days of continuous exposure had been completed. Mortality then increased gradually to day 14 of the experiment, at which time exposure was terminated, and some of the remaining animals analyzed for their mirex content. Data for the two highest concentrations (Table 2) indicates that H. azteca accumulates twice as much mirex as does C. pseudogracilis at the same water concentration.

Figure 1. Survival of H. azteca and C. pseudogracilis exposed to mirex for a 13-day period at 20°C at various concentrations in water, control (□), 0.1 (Δ), 1.0 (○), 10 (○), and 100 (■).

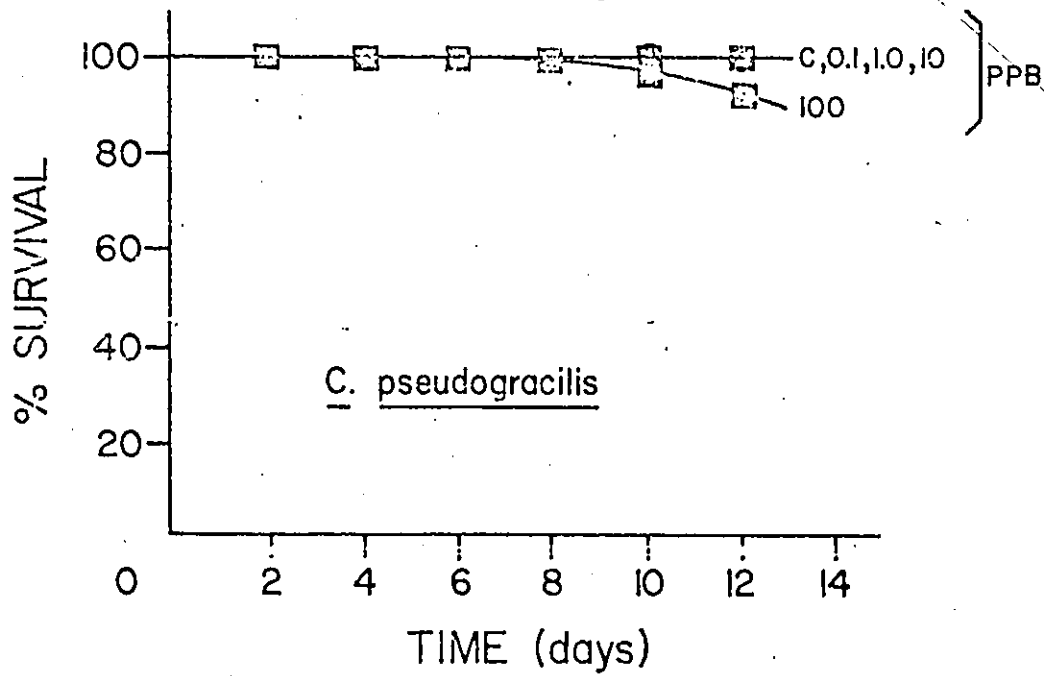
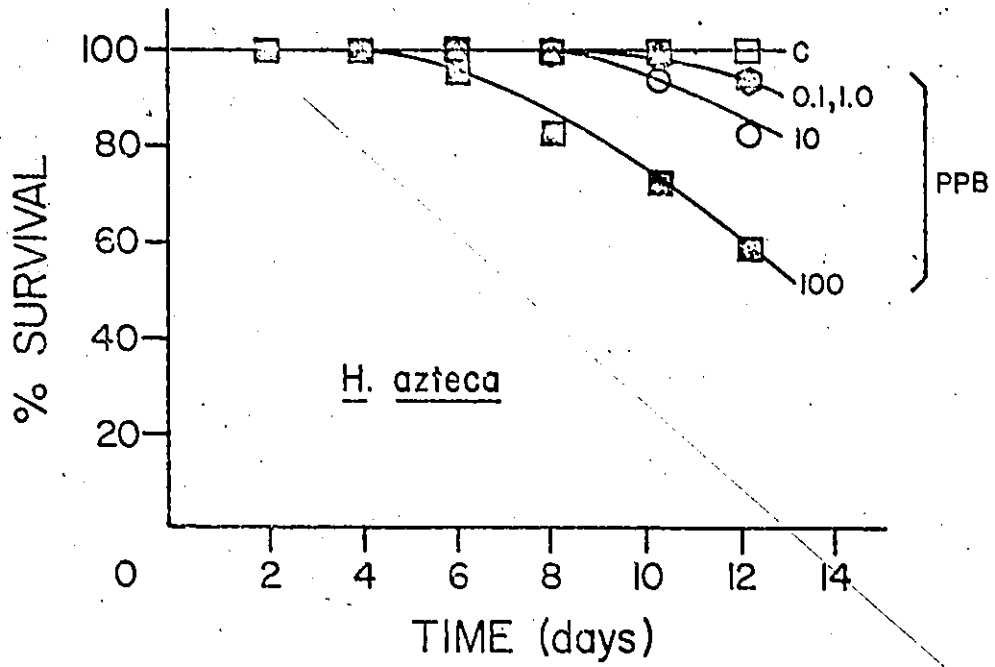


Table 2. Uptake of mirex by amphipods surviving a 15-day exposure to mirex in water

Concentration <sup>a</sup> (ng mirex/ml water)	Species	Number of amphipods analyzed	body burden (ug mirex/g dry wt)	Transfer coefficient
118	<u>H. azteca</u>	4	305.3(110)	10
112	<u>C. pseudogracilis</u>	5	166.3(17.1)	5
12	<u>H. azteca</u>	7	179.9(32.9)	58
11	<u>C. pseudogracilis</u>	6	88.1(7.6)	28

a) average of concentrations determined twice daily by radioassay of exposure solutions

### 3.0.12 Uptake from water and clearance of mirex

The accumulation of mirex by the two species of amphipod was initially studied at a concentration of 1 ng mirex/ml water (ppb), by taking samples daily during a 4 day period.

Figure 2 demonstrates the apparent linear uptake of mirex observed for both H. azteca and C. pseudogracilis over the 4-day exposure period. H. azteca however, accumulated mirex at a greater rate, it's body burden being 3 times that of C. pseudogracilis over the entire period of exposure. The transfer coefficient, which measures the amount of water cleared of it's mirex content per hour, remains above 200 (with one exception) for H. azteca and near 100 for C. pseudogracilis. Exposure concentrations (Table 3) remained relatively constant and were similar for both species ( $0.95 \pm 0.02$  ppb for H. azteca,  $0.92 \pm 0.05$  ppb for C. pseudogracilis).

Heat-killed amphipods of both species were exposed to an aqueous solution of mirex. Results (Table 3) indicate that absorption of mirex under these conditions is approximately equal for both species, but substantially slower than by live animals. The degree of reduction however is much greater for H. azteca, which accumulates only 12% of the live dose when dead, while C. pseudogracilis accumulates 43% of it's live dose when dead.

Figure 2. Accumulation of mirex from water by H. azteca (O), and C. pseudogracilis (●), at a concentration of 1 ng mirex/ml water and 20°C. Each point represents the mean and S.D. of ten animals.

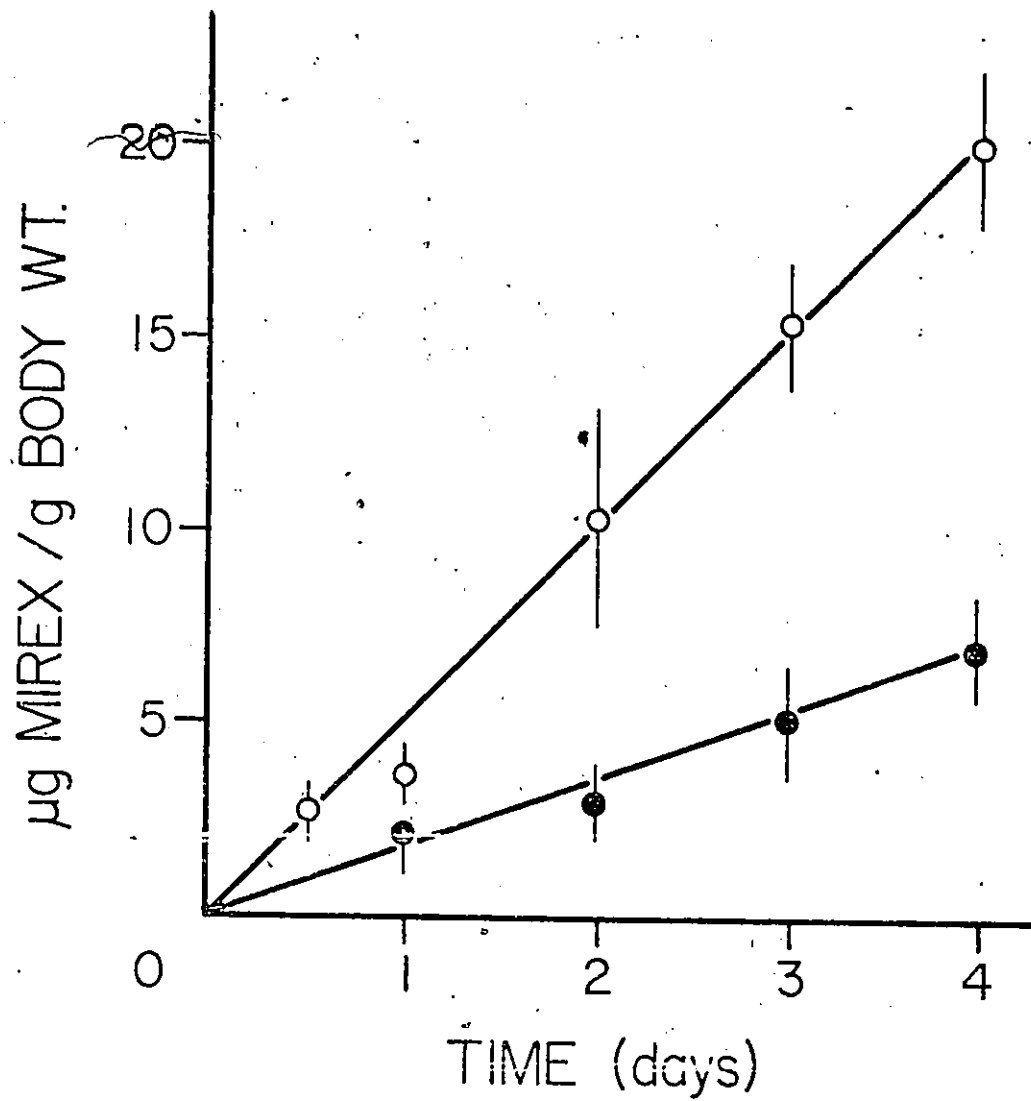


Table 3. Effect of duration of exposure on uptake of mirex from water by H. azteca and C. pseudogracilis.

Duration of exposure (hours)	<u>H. azteca</u> <sup>a</sup>			<u>C. pseudogracilis</u> <sup>a</sup>		
	Mean mirex concentration in exposure soln. (ng mirex/ml)	Mirex content <sup>b</sup> in amphipods (ug mirex/g body wt)	Transfer coefficient (hour <sup>-1</sup> )	Mean mirex concentration in exposure medium (ng mirex/ml)	Mirex content <sup>b</sup> in amphipods (ug mirex/g body wt)	Transfer coefficient (hours <sup>-1</sup> )
12	0.94	2.7±0.8	239	-	-	-
24	0.94	3.9±0.7	173	0.95	3.0±2.5	129
(24) <sup>c</sup>	(1.00)	0.6±0.1	(26)	(1.00)	0.8±0.2	(36)
48	0.94	10.4±3.1	228	0.85	3.8±2.4	96
72	0.95	17.0±4.3	249	0.93	5.8±2.5	86
96	0.99	20.2±2.1	213	0.94	7.6±2.5	84

a) dry weights of animals used: H. azteca, 0.271±0.035mg; C. pseudogracilis, 0.237 ±0.016 mg

b) mean ± s.d. of 10 animals

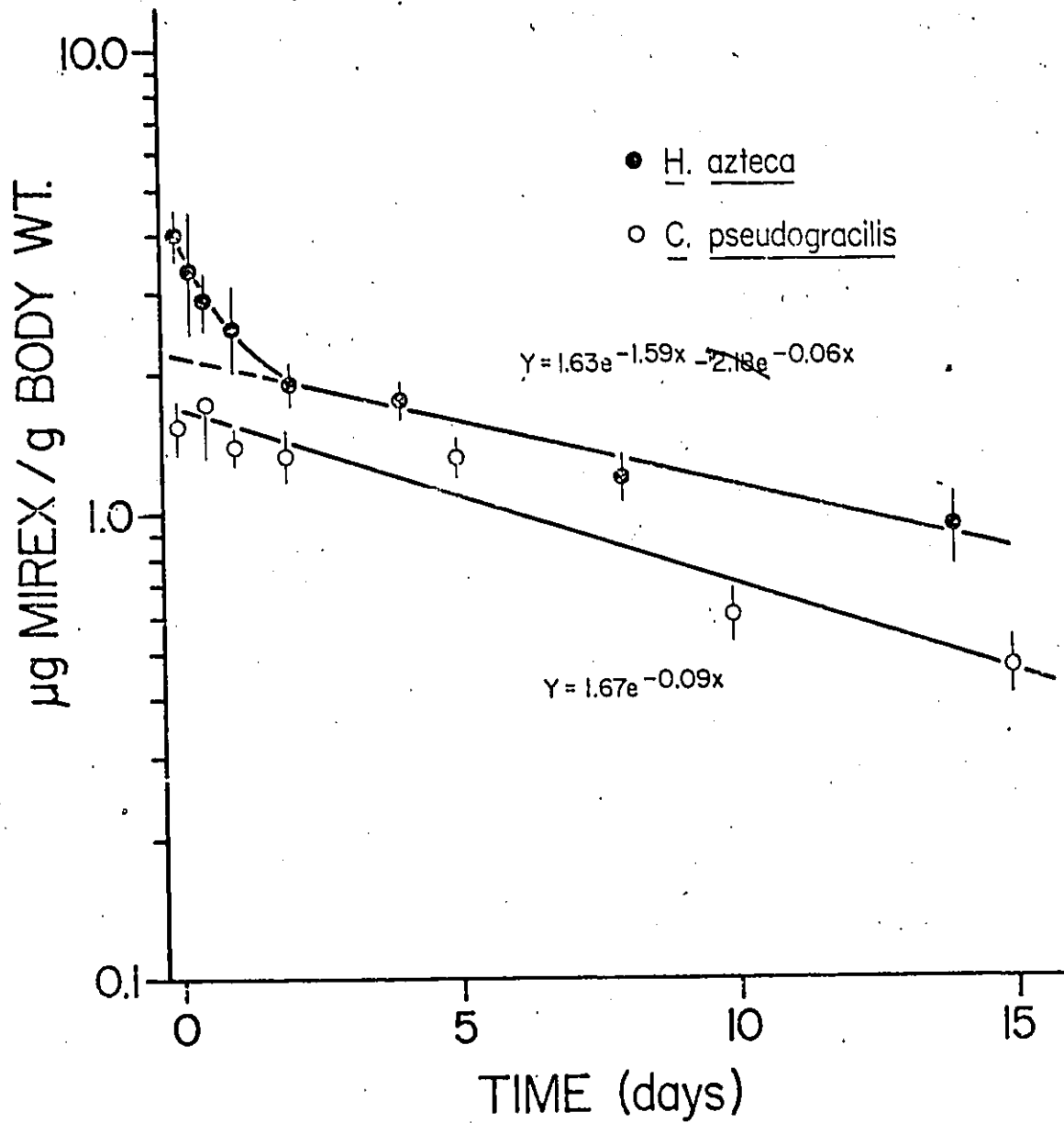
c) parenthetic terms are for separately run dead control

Clearance of mirex by the two species was examined after an exposure of 24 hours at a concentration of 1 ppb mirex in water. Samples were removed at 0, 6, 12, and 24 hours for the first day postexposure and then at 2, 4, 8, and 14 days for H. azteca. C. pseudogracilis was sampled at 0, 12, and 24 hours, and at 2, 5, 10, and 15 days.

H. azteca exhibits a biphasic clearance curve suggestive of two compartments (Fig. 3), an initial fast-clearing phase with a fractional clearance rate of 1.54/day, followed in approximately 2 days by a slower-clearing component having a fractional clearance rate of 0.06/day. The equation describing H. azteca clearance of mirex gives the Y-intercepts of the fast and slow clearing compartments, 1.63 and 2.18 ug mirex/g dry wt respectively. If we are dealing with a first order kinetic process and independent compartments, these may represent the actual sizes of two compartments at the beginning of the clearance period, and thus the sizes after 24 hours of exposure under the stated conditions. Accumulation by the slow-clearing compartment after 24 hours would therefore represent about 60% of total accumulation by H. azteca.

The clearance of mirex from the tissues of C. pseudogracilis is best described by a monophasic equation (Fig. 4) having a fractional-clearance rate of 0.09/day (9%/day). This compartment is similar to the slow H. azteca compartment, the fractional-clearance rates being not significantly different (Ancova  $p < .05$ ).

Figure 3. Whole body clearance of mirex by H. azteca and C. pseudogracilis after exposure in water at a concentration of 1 ng mirex/ml water for 24 hours at 20°C. Each point represents the mean and S.D. of 10 animals.



Although accumulation for both species (Fig. 2) could be fitted to linear equations, this would imply that uptake is occurring without concurrent clearance. Bioaccumulation equations which account for clearance not only describe results more accurately, but also can be used to predict body burdens for any exposure period, and have been used by several authors to describe bioaccumulation (Moriarty 1975, Harding and Vass 1977). The general bioaccumulation equation is,

$$Y_t = \frac{k_{01}}{k_{10}} W (1 - e^{-k_{10}t}) \quad (7)$$

where  $Y_t$  is body burden at time  $t$ ,  $k_{01}$  and  $k_{10}$  are the uptake rate constant and clearance rate constants respectively,  $W$  is the mirex concentration in water, and  $t$  is time of exposure in days. To compare the uptake of mirex by H. azteca and C. pseudogracilis, it will be necessary to estimate the uptake rate indirectly from data on accumulation and clearance. By rearrangement of the above equation, one can solve for  $k_{01}$ , the uptake rate constant

$$k_{01} = \frac{Y_t k_{10}}{W(1 - e^{-k_{10}t})} \quad (8)$$

where  $W=1$  ng/ml,  $t=1$  day,  $k_{10}=0.09$ , and  $Y=1.67$ .

For results presented in Figure 3, where  $W=1$  ng/ml,  $t=1$  day,  $k_{10}=0.09$ , and  $Y_t=1.67$ , the uptake rate constant for C.

pseudogracilis is  $1.746 \times 10^3$  and overall bioaccumulation is described by

$$Y_t = 19.4 (1 - e^{-0.09t}) \quad (9)$$

H. azteca requires a multiple exponential expression to describe its biphasic bioaccumulation, with uptake rate constants of  $3.256 \times 10^3$ /day and  $2.246 \times 10^3$ /day for fast and slow phases, and overall equation

$$Y_t = 2.05 (1 - e^{-1.54t}) + 37.4 (1 - e^{-0.06t}) \quad (10)$$

One can infer from these results, that as time increases, and the steady-state body burden is approached in equations 9 and 10, the parenthetical terms will approximate 1, leaving the steady-state body burden achieved at the stated exposure conditions (1 ppb, 20°C): approximately 20 ug mirex/g body wt (ppm) for C. pseudogracilis, and 40 ug mirex/g body wt for H. azteca. At steady-state, the fast compartment contributes minimally (5%) to total body burden, having reached its steady-state concentration of 2.05 ppb after 2 days. The slow compartment continues to accumulate mirex for about 50 days to its steady-state concentration of 37.4 ppb.

### 3.0.13 Effect of duration of exposure on uptake from water and clearance of mirex

In order to test whether or not kinetic parameters determined in this study are independent of exposure duration, and to establish the independence of compartments, groups of animals of each species were exposed for varying lengths of time to a concentration of 1 ppb and subsequent clearance determined. H. azteca was exposed for 6, 12, and 24 hours, C. pseudogracilis for 12, 24, and 48 hours, and subsequent clearance determined.

Mirex content of H. azteca increased over the 24 hours of exposure (Table 4). This increase was not directly proportional to duration of exposure, there being a decline in the rate of uptake with increasing duration of exposure. This decline could not have been due to a decrease in exposure levels, as measured concentrations were the same for all exposure durations (Table 4). This decline would however be expected if one of the compartments of H. azteca were approaching equilibrium.

Biphasic clearance was observed with H. azteca for all exposure durations (Figure 4). The fast-clearing compartment of H. azteca had a fractional-clearance rate ranging from 1.26-1.96/day, with no apparent correlation to duration of exposure. The size of this compartment increases with increasing exposure time, from 1.59ppm at 6 hours, to 2.49ppm at 24 hours. As a percentage of total body burden it steadily decreases in size with increasing exposure time from 53% at 6 hours, to 38% at 24 hours (Table 4).

Figure 4. Whole body clearance of mirex by H. azteca after exposures varying from 6 to 24 hours at a concentration of 1 ng mirex/ml water at 20°C. Each point represents the mean and S.D. of 10 animals.

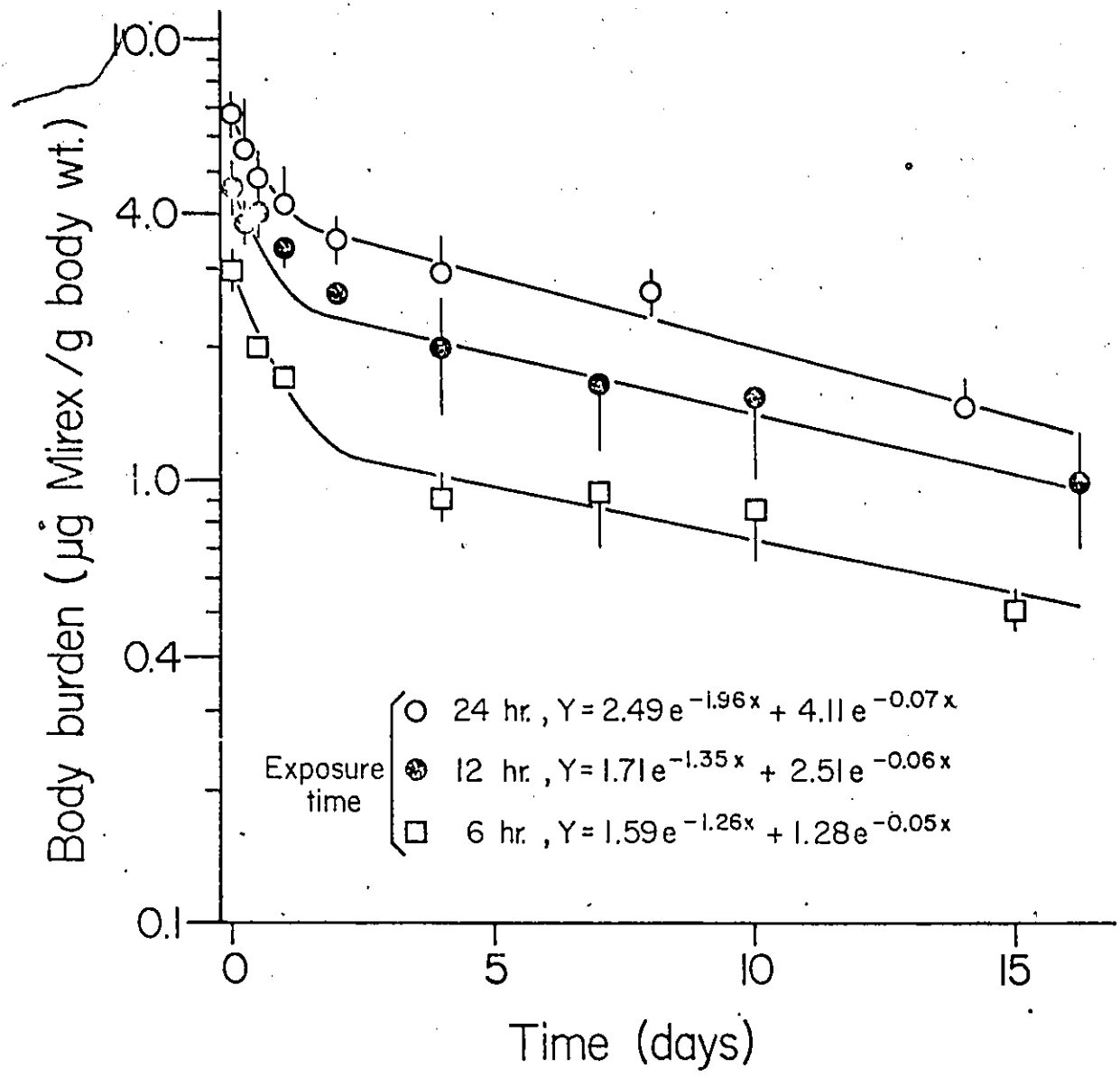


Figure 5. Whole body clearance of mirex by C. pseudogracilis after exposures varying from 12 to 48 hours at a concentration of 1 ng mirex/ml water at 20°C. Each point represents the mean and S.D. of 10 animals.

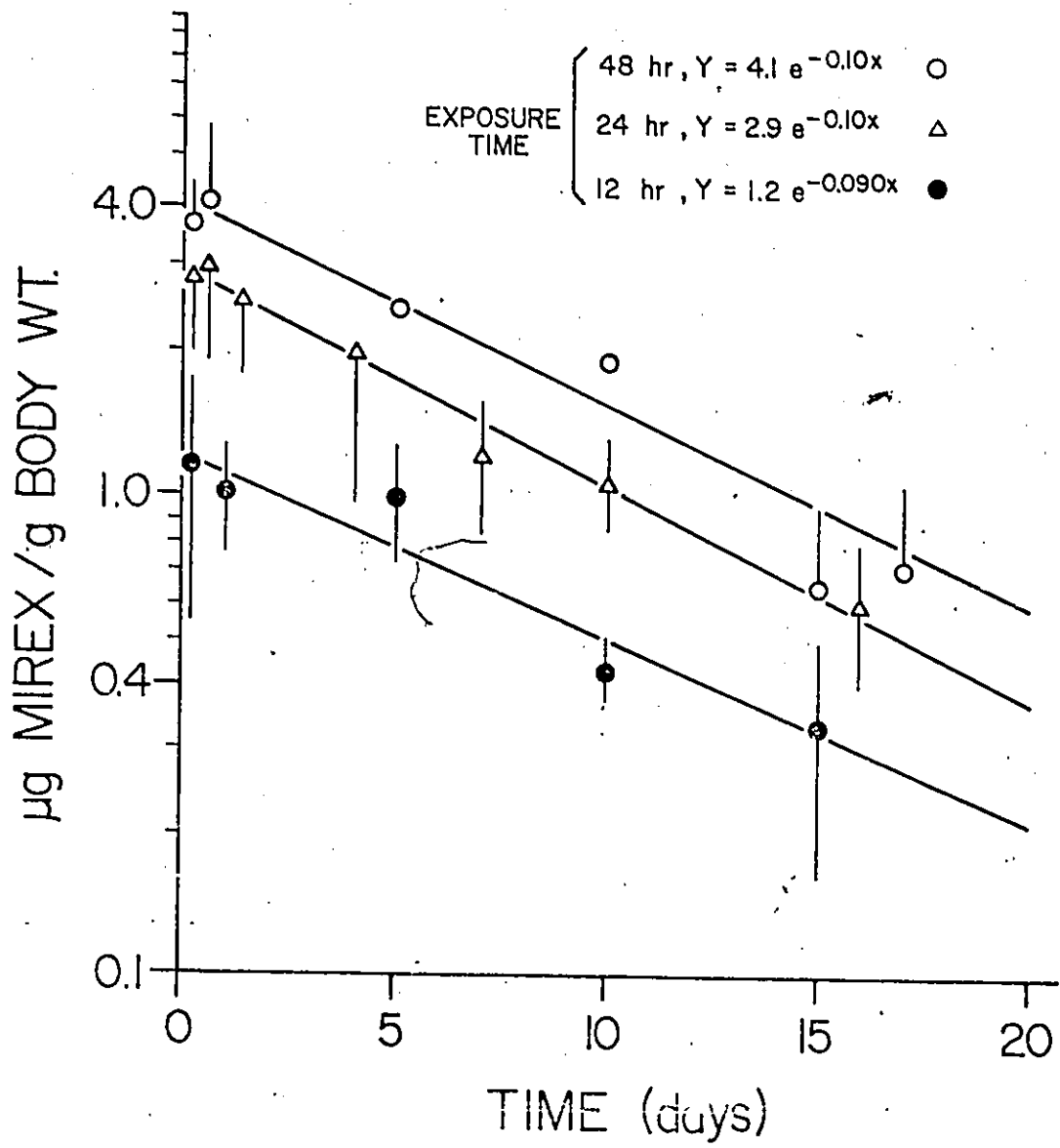


Table 4. The effect of duration of exposure to aqueous solutions of mirex on compartmental kinetics of *H. azteca* and *C. pseudotrachealis*.

Duration of exposure (days)	Mirex content of amphipods at one minute postexposure (ug mirex/g dry wt)	fast-compartment			slow compartment <sup>c</sup>		
		Compartment size (ug mirex/g dry wt)	Uptake rate constant $k_{01}$ (day <sup>-1</sup> )	Fractional clearance rate $k_{10}$ (days <sup>-1</sup> )	Compartment size (ug mirex/g dry wt)	Uptake rate constant $k_{02}$ (day <sup>-1</sup> )	Fractional clearance rate $k_{20}$ (days <sup>-1</sup> )
<i>H. azteca</i>							
0.25	2.87±0.34	2.59(55%)	74±4	1.26	1.28(45%)	5152	0.05
0.50	4.26±0.69	1.75(41%)	48±3	1.35	2.51(59%)	4246	0.06
1.0	6.60±0.83	2.19(38%)	5680	1.96	4.11(62%)	4318	0.07
<i>C. pseudotrachealis</i>							
0.50	1.20±0.61	-	-	-	1.20	2454	0.09
1.0	2.90±0.90	-	-	-	2.90	3047	0.10
2.0	4.10±0.85	-	-	-	4.10	2262	0.10

(a) Mirex concentration was 1 ng mirex/mL water throughout all exposure periods

(b) Dry weights of animals used in experiments: *H. azteca*, 0.448±0.105 mg; *C. pseudotrachealis*, 0.338±0.101 mg

(c) Numbers in brackets represent the size of the compartment as a percentage of total body burden

(d) Calculated using uptake and clearance data in the bioaccumulation equation (#3, materials and methods)

(e) For purposes of comparison, the single *C. pseudotrachealis* compartment is placed here

Clearance from the slow-clearing compartment of H. azteca varied little, being 0.6-0.7/day, with no correlation to duration of exposure. The slow-compartment increased both in absolute size (1.28ppm at 6 hr to 4.11ppm at 24hr), and as percentage of total body burden (47% at 6hr to 62% at 24 hr). It also exhibited an increase in size directly proportional to duration of exposure. These results indicate that the two phases of accumulation by H. azteca can be treated as independent compartments.

Calculated uptake rate constants for the slow-compartment (Tab. 4) were only slightly (20%) lower than those of the fast-compartment, the average uptake rates being 4855/day vs. 5969/day for slow- and fast-compartments respectively. As was noted for fractional clearance rates, no correlation between uptake rates and duration of exposure is apparent.

Accumulation into the single C. pseudogracilis compartment was similar to the slow-compartment of H. azteca, in that it's size was proportional to time of exposure, increasing from 1.2ppm at 12 hours, to 4.5ppm at 48 hours. In terms of actual amount of mirax taken into the animal, twice the exposure time was required by C. pseudogracilis to reach the same body burden as in the H. azteca slow-compartment. Calculated uptake rate constant averaged 2588/day, which is approximately half that for the H. azteca slow-compartment. The difference in fractional-clearance rate between the sin-

gle C. pseudogracilis compartment, and the slow compartment of H. azteca, which had been noted in previous experiments, was observed for all exposure times.

#### 3.0.14. Effect of water concentration of mirex on uptake and clearance

Accumulation from water was examined over a range of concentrations (0.1-20 ppb) during 96 hour exposures to <sup>14</sup>C-mirex. Accumulation over the range 0.1-1.0 ppb is plotted in Fig. 6 and confirms the pattern observed previously in which uptake followed linear trends (although it is not thought to be linear, for previously stated reasons), and was 2-3X greater by H. azteca than C. pseudogracilis under equivalent exposure conditions. Through this range of concentrations, body burden is directly proportional to exposure concentrations for both species, an increase in exposure concentration resulting in an equivalent increase in accumulation.

Accumulation was also examined at concentrations above 1 ppb (and therefore above the water solubility of mirex), and demonstrated non-proportionality with concentration. Results in Figure 7 indicate no further increase in accumulation occurs when exposure concentration is increased above 5 ppb. This relationship was similar for both species.

Figure 6. Accumulation from water of mirex by H. azteca, a, and C. pseudogracilis, b, exposed to 0.1(▲), 0.3(■), and 1.0(●) ng mirex/ml water at 20°C. Each point represents the mean and S.D. of 10 animals.

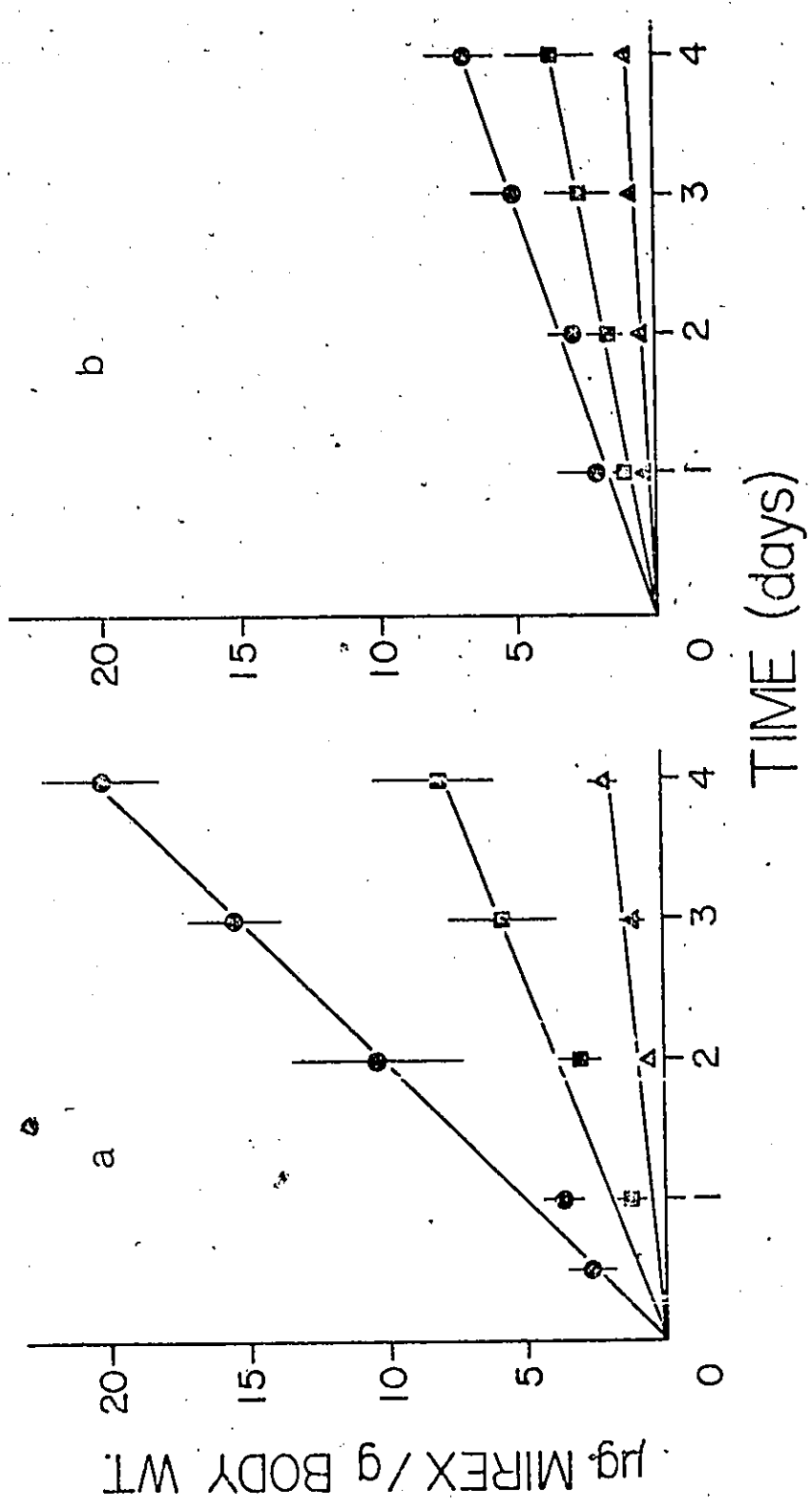


Figure 7. Accumulation of mirex by H. azteca and C. pseudo-  
gracilis exposed to concentrations ranging from 0.1-20.0 ng  
mirex/ml water. Each point represents the mean and s.d. of  
10 animals. H. azteca (●), C. pseudogracilis (○).

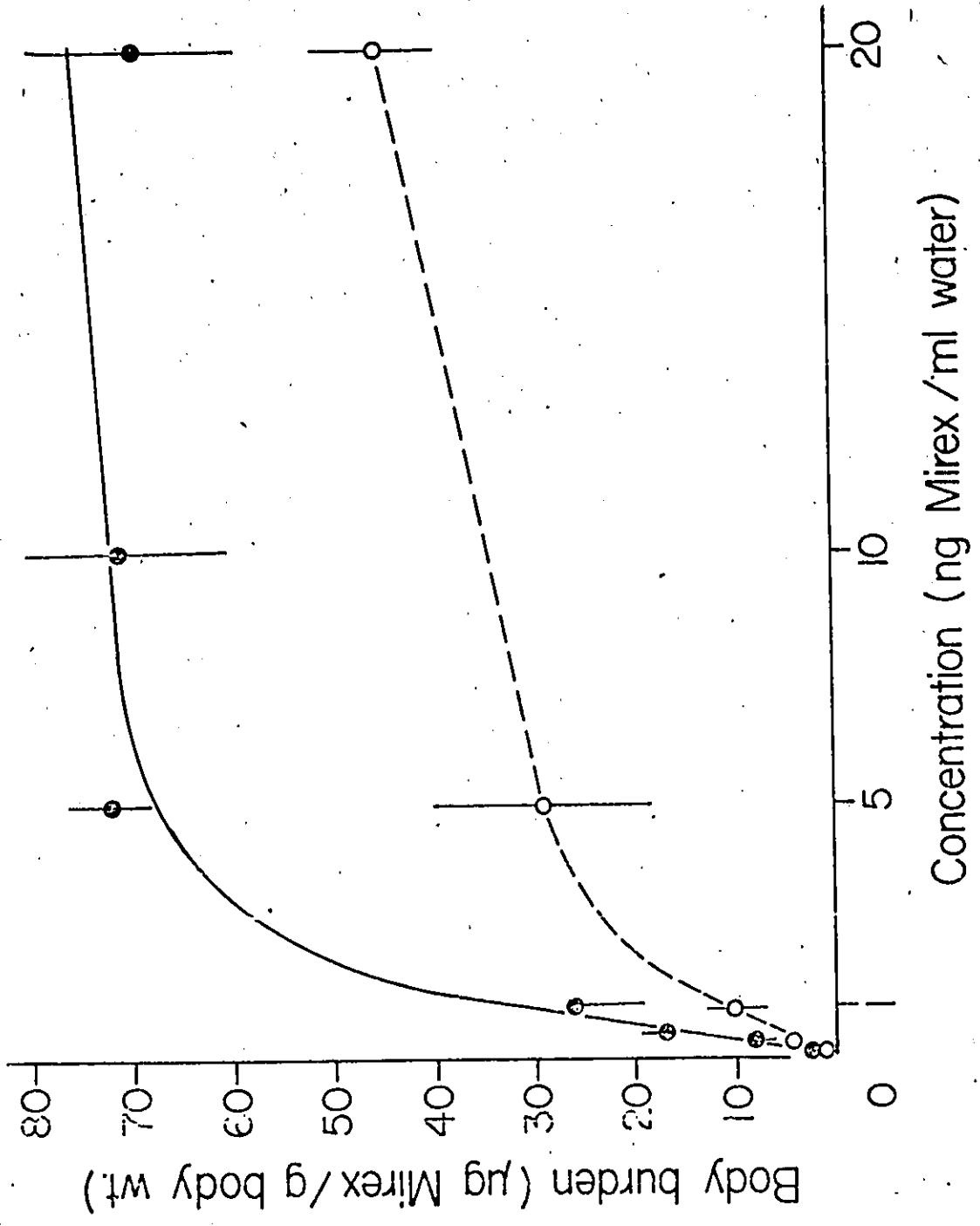
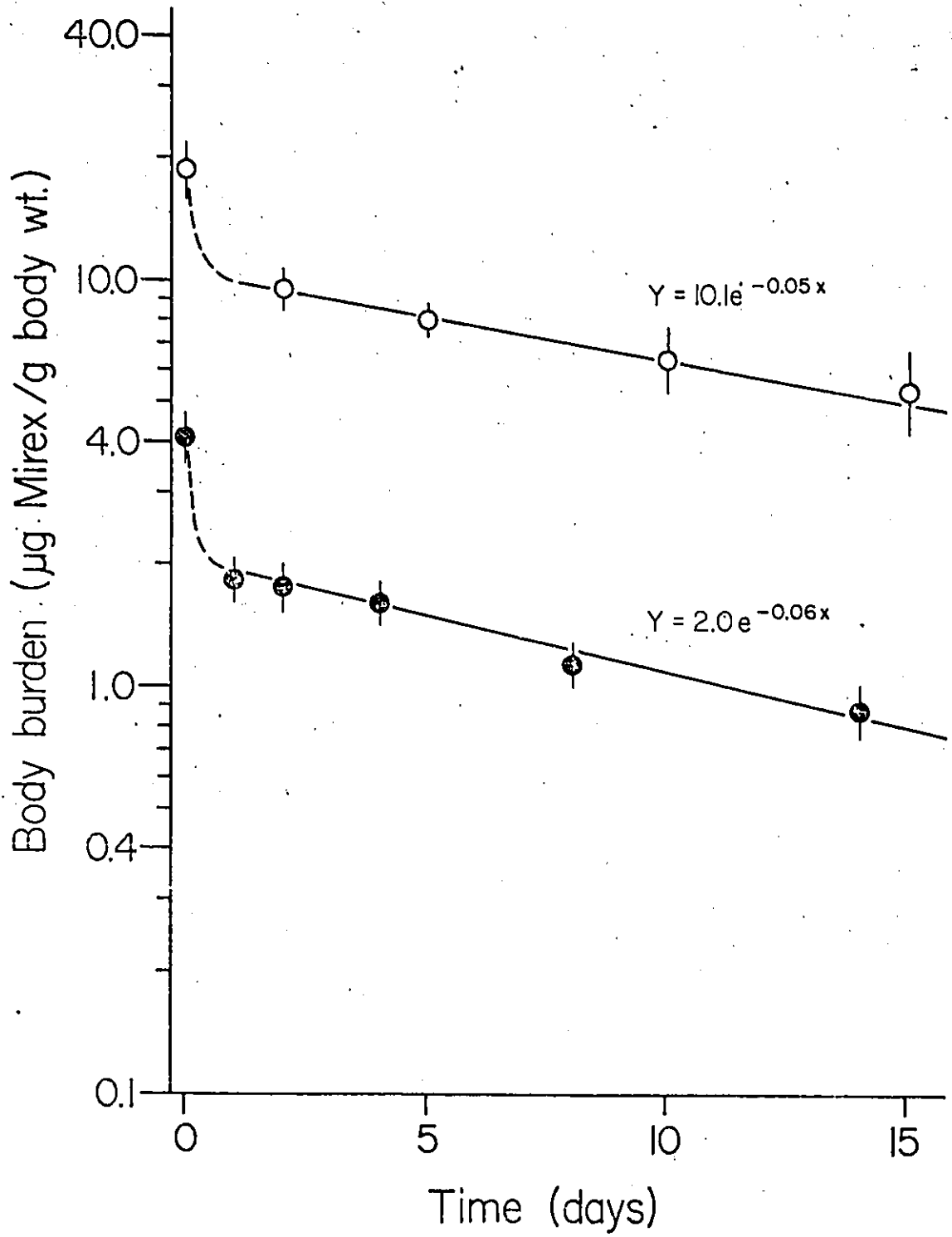


Figure 8. Clearance of mirex by H. azteca after exposure to two water concentrations, 1.0 (●) and 20 (○) ng mirex/ml water at 20°C. Each point represents the mean and S.D. of 10 animals.

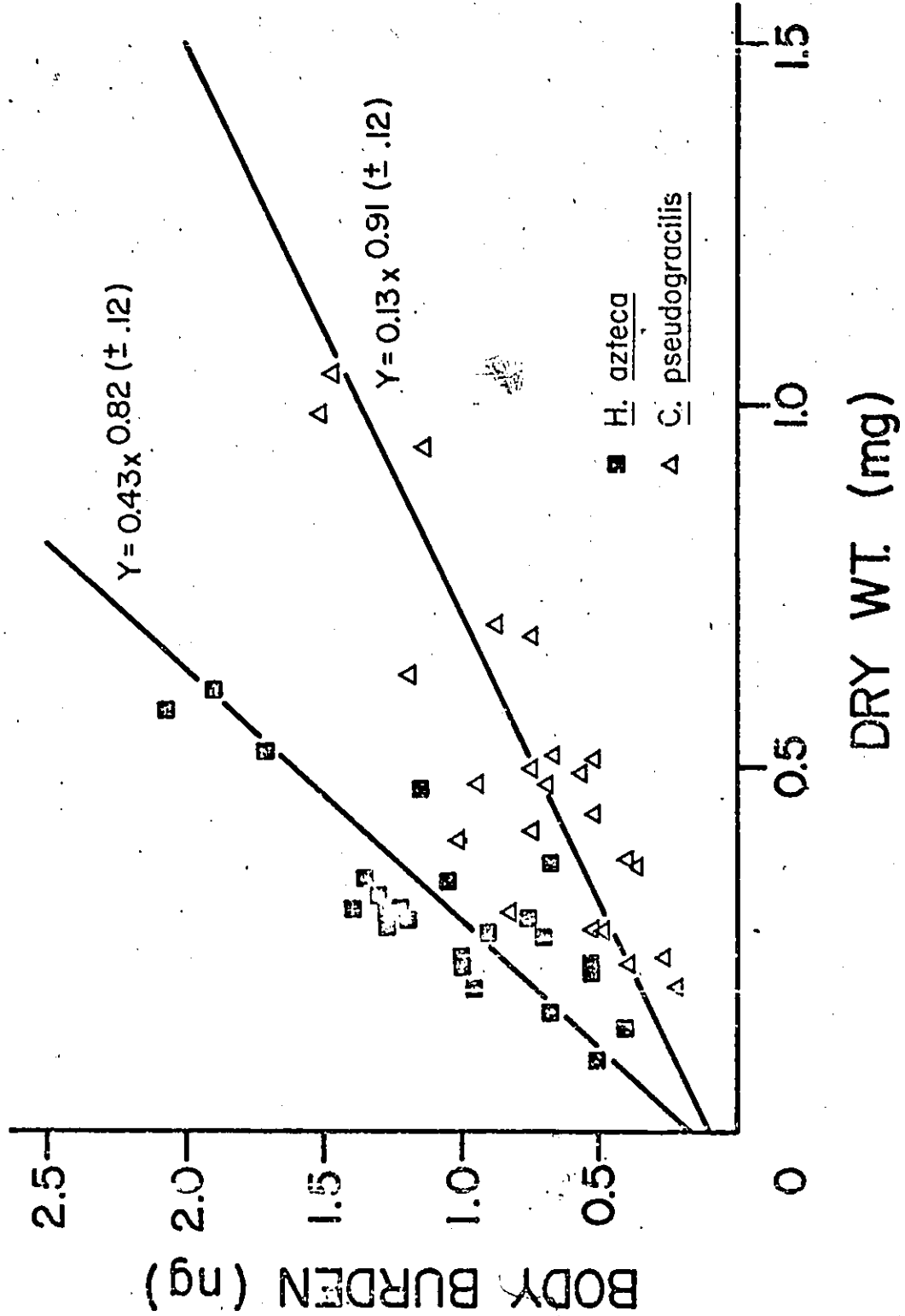


The effect of exposure concentration on subsequent clearance was examined for H. azteca only, at two concentrations, 1 and 20 ppb (Fig. 8). Total accumulation (time 0 of clearance) reflects the reduced uptake at exposure above 1 ppb, there being only a 5-fold increase in body burden. Insufficient samples were taken to determine the kinetics of the fast clearing compartment. The slow clearing compartments are very similar in both cases with fractional-clearance rates of 0.05 and 0.06/day for 20 and 1 ppb exposures respectively. The Y-intercepts of the slow-compartments, which estimate the size of the compartment at the end of exposure, are also similar, being 53% of total body burden for the 20 ppb exposure, and 50% at 1 ppb.

### 3.0.15 Effect of Body size on uptake of mirex

To determine the effect of body size on accumulation of mirex from water, groups of animals ranging from 0.097-0.609mg for H. azteca, and 0.200-1.048mg for C. pseudogracilis were exposed for 24 hours to a 1 ppb solution of mirex in water, and <sup>14</sup>C accumulation determined. Body burden was plotted against dry weight in Figure 9, and described by power functions determined from log-log transformations of the data. The exponents of the equations, 0.82±0.12 for H. azteca and 0.91±0.12 for C. pseudogracilis,

Figure 9. Effect of body size on accumulation of mirex from water by H. azteca and C. pseudogracilis exposed for 24 hours to a concentration of 1 ng mirex/ml water at 20°C. Each point represents a single amphipod. Body size range for H. azteca: 0.120-0.609 mg (dry wt); for C. pseudogracilis: 0.200-1.048 mg (dry wt).



indicate that smaller individuals of both species accumulate more mirex on a body weight basis, than those of larger sizes. An exponent of 1 would indicate an exactly proportional relationship between accumulation and body weight. The relationship is similar for both species, maintaining the interspecific difference over the entire range of body sizes examined.

**3.0.16 Effect of temperature on uptake from water and clearance of mirex by *H. azteca***

To determine the effect of temperature on accumulation of mirex by *H. azteca*, groups of animals which had been acclimatized to 5, 10, and 20°C, were exposed at these temperatures to a 1 ng/ml (ppb) solution of mirex in tap-water for 72 hours, and their mirex content radioassayed daily. Clearance of mirex at these temperatures was determined on similarly acclimatized amphipods, after an exposure of 24 hours to a 1 ppb mirex solution.

Results in Figure 10 indicate that accumulation of mirex by *H. azteca* is proportional to temperature. A two-fold increase in temperature resulted in a 3-fold increase in accumulation, both from 5-10°C, and 10-20°C.

Clearance of mirex at all temperatures (Fig. 11) was biphasic as observed previously under various conditions. The relative sizes of the compartments were maintained at

Figure 10. Effect of temperature on accumulation of mirex from a water concentration of 1 ng mirex/ml water by H. az-  
toca at 5°C (□), 10°C (○), and 20°C (●). Each point represents the mean and S.D. of 4 groups of 3 animals.

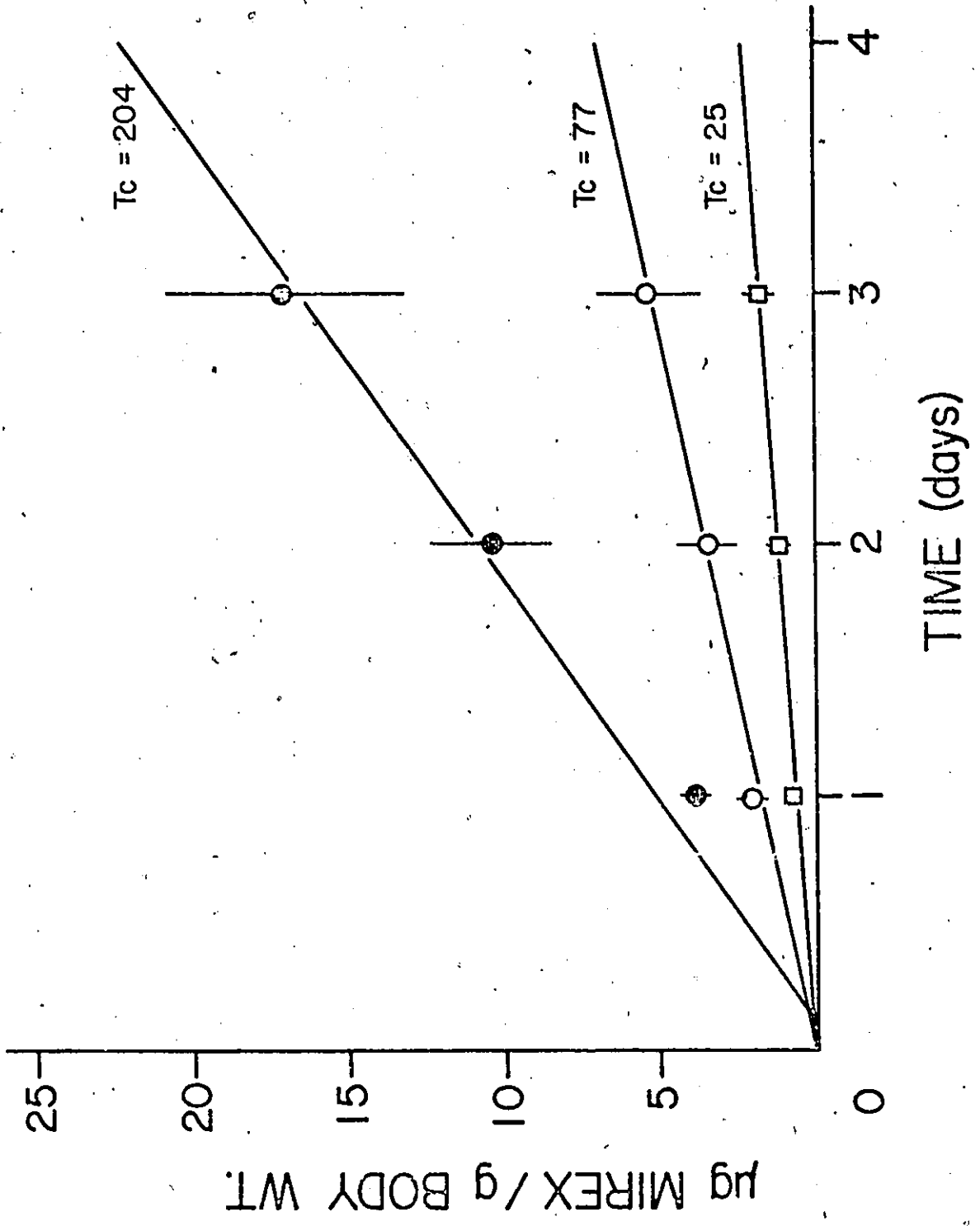


Figura 11. Effect of temperature on clearance of mirex by H. azteca exposed to a 1 ng/ml solution of mirex in water. Each point represents the mean and S.D. of 5 groups of 3 animals.

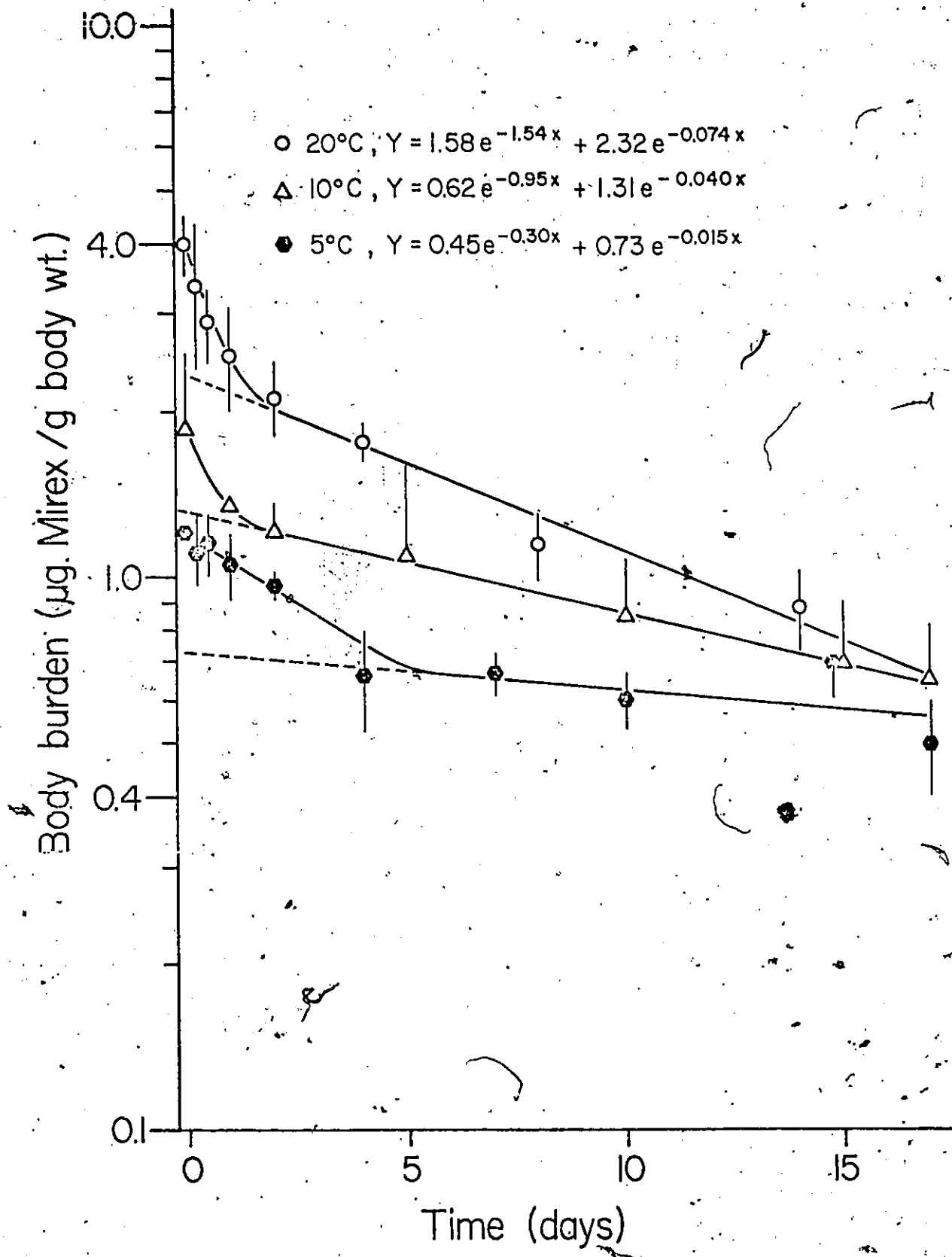


Table 5. The effect of temperature on bioaccumulation kinetics of *H. azteca* exposed for 24 hours to 1 ng mirex/ml water.

Temperature <sup>a</sup> of exposure (°C)	Mirex content <sup>b</sup> of amphipods at one minute postexposure (ug mirex/g dry wt)	fast-clearing compartment			slow-clearing compartment		
		Uptake rate constant $k_{01}$ (day <sup>-1</sup> )	Fractional clearance rate $k_{10}$ (day <sup>-1</sup> )	Compartment size (ug mirex/g dry wt)	Uptake rate constant $k_{02}$ (day <sup>-1</sup> )	Fractional clearance rate $k_{20}$ (day <sup>-1</sup> )	Compartment size (ug mirex/g dry wt)
5.0	1.18±.05	521	0.30	0.45(38%)	735	0.015	0.73(62%)
10	2.93±.05	960	0.95	0.62(34%)	1336	0.040	1.31(66%)
20	3.90±.50	3097	1.54	1.58(40%)	2407	0.074	2.32(60%)

(a) All temperatures ± 0.5°C

(b) Mean dry weight of amphipods used: 0.407±0.067

(c) Numbers in parentheses represent size of compartment as a percentage of total body burden

all temperatures, the fast-clearing compartment representing 34-40% of total body burden (Table 7). This indicates that the influence of temperature is similar on both compartments. Fractional clearance rates also reflect this, since in both compartments they are directly proportional to temperature, increasing from 0.015/day at 5°C, to 0.074/day at 20°C in the slow compartment, and from 0.30/day at 5°C to 4.54/day at 20°C in the fast compartment. Estimated uptake rate constants behave similarly, increasing from 735/day at 5°C to 2407/day at 20°C in the slow compartment, and from 521/day at 5°C to 3097/day at 20°C in the fast compartment.

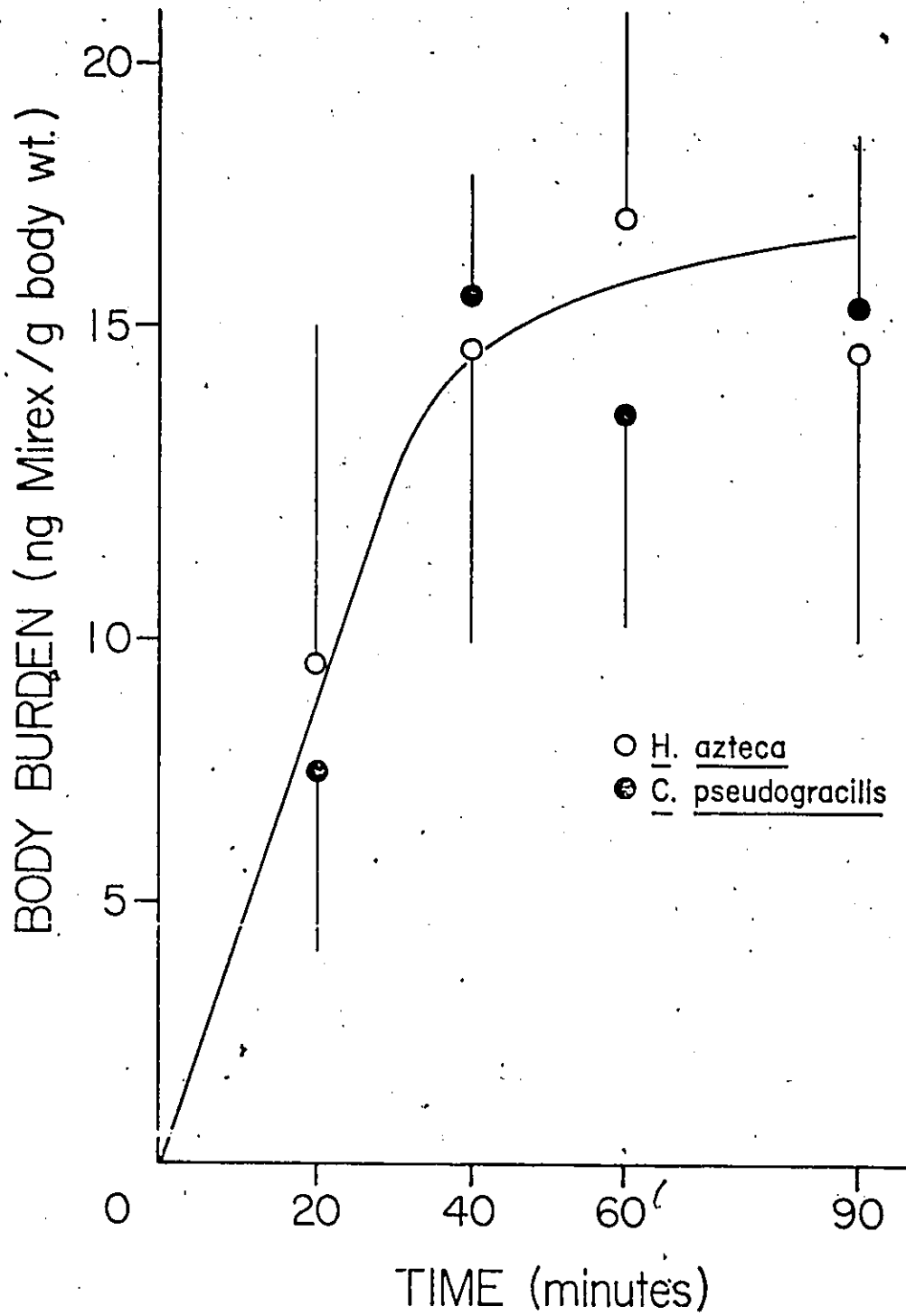
3.0.17 Effect of duration of exposure to contaminated food on uptake of mirex

Amphipods of both species were exposed to mirex-contaminated algae, and allowed to feed for up to 90 minutes. Samples of amphipods were taken at 20, 40, 60, and 90 minutes, and radioassayed for their mirex content. Figure 12 indicates rapid uptake of mirex over the first 40 minutes of exposure, the body burden doubling between 20 and 40 minutes, followed by a sharp decline in the rate of increase of the body burden. Since amphipods are continuous feeders, and assuming no change in feeding rate over the course of the experiment, the initial rapid uptake corresponds to ingestion of mirex-contaminated food until the gut is filled. The point at which body burden no longer increases, should correspond to the first egestion of food containing unassimilated mirex. To estimate assimilation efficiency of mirex by amphipods, exposure to food must be less than the time required to fill the gut. Therefore, exposure to contaminated food was limited to 30 minutes in all subsequent experiments.

3.0.18 Effect of concentration of mirex in food on bioaccumulation parameters

To examine bioaccumulation of mirex from food, and to determine the effect of mirex concentration in the food on

Figure 12. Body burden of mirex attained by H. azteca and C. pseudoqracilis allowed to feed on mirex-contaminated algae for periods of time varying from 20-90 minutes. Each point represents the mean and S.D. of 8 animals.



bioaccumulation parameters, groups of amphipods of both species were exposed to contaminated food at several mirex concentrations. They were allowed to feed for 30 minutes, after which they were transferred to chambers containing uncontaminated food, and clearance of the ingested mirex followed for 15 days.

Results in Table 6 indicate that the feeding rate of H. azteca is unaffected by concentration of mirex in food, averaging  $6.4 \times 10^8$  cells/hour/g amphipod. Similarly, C. pseudogracilis demonstrated no correlation of feeding rate to concentration of mirex in food, averaging  $7.5 \times 10^8$  cells/hour/g amphipod, a rate not statistically different from that of H. azteca (t-test,  $p < 0.05$ ).

Results in Table 6 show that while H. azteca demonstrated fairly wide variation in assimilation efficiency (13-29%), no correlation was apparent with the concentration of mirex in food. C. pseudogracilis exhibited a similar variation in assimilation efficiency (13-23%) with no correlation to mirex concentration in food.

Clearance of ingested mirex by H. azteca appeared to fit a bi-compartmental pattern. The initial fast-clearing phase however, is at least partly due to the egestion of algae containing unassimilated mirex, and disguises the presence or absence of a true (somatic) fast-clearing compartment. Clearance data was therefore described by single compartment equations and, from a sampling point of view,

Figure 13. Retention of an ingested dose of mirex for a 48 hour period following exposure by H. azteca. Each point represents the mean and S.D. of 10 animals.

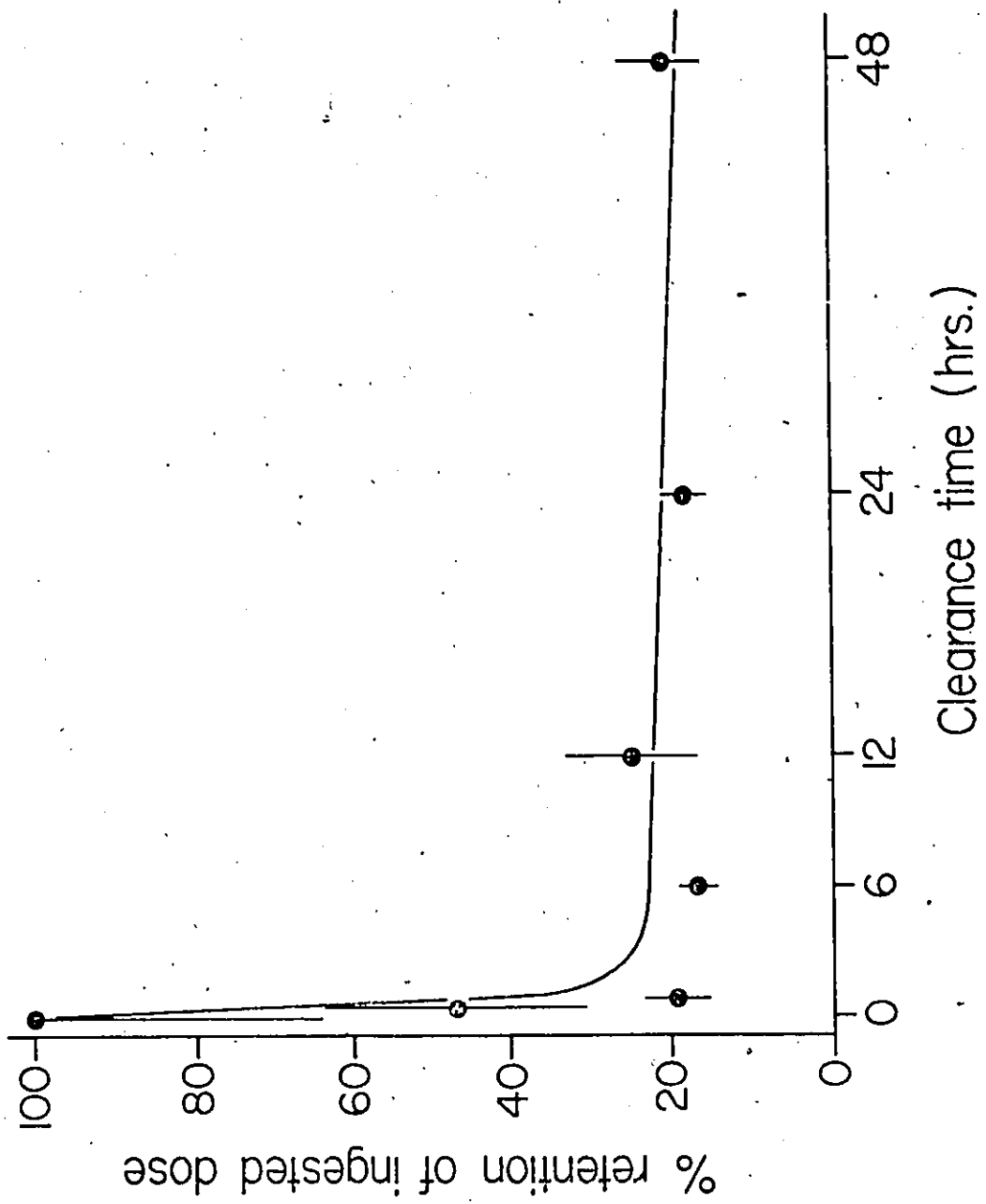


Figure 14. Clearance of ingested doses of mirex by H. azte-  
ca at 20°C fed S. quadricauda having mirex concentrations of  
64, 137, and 203 ug mirex/g dry wt. Each point represents  
the mean and S.D. of 10 animals.

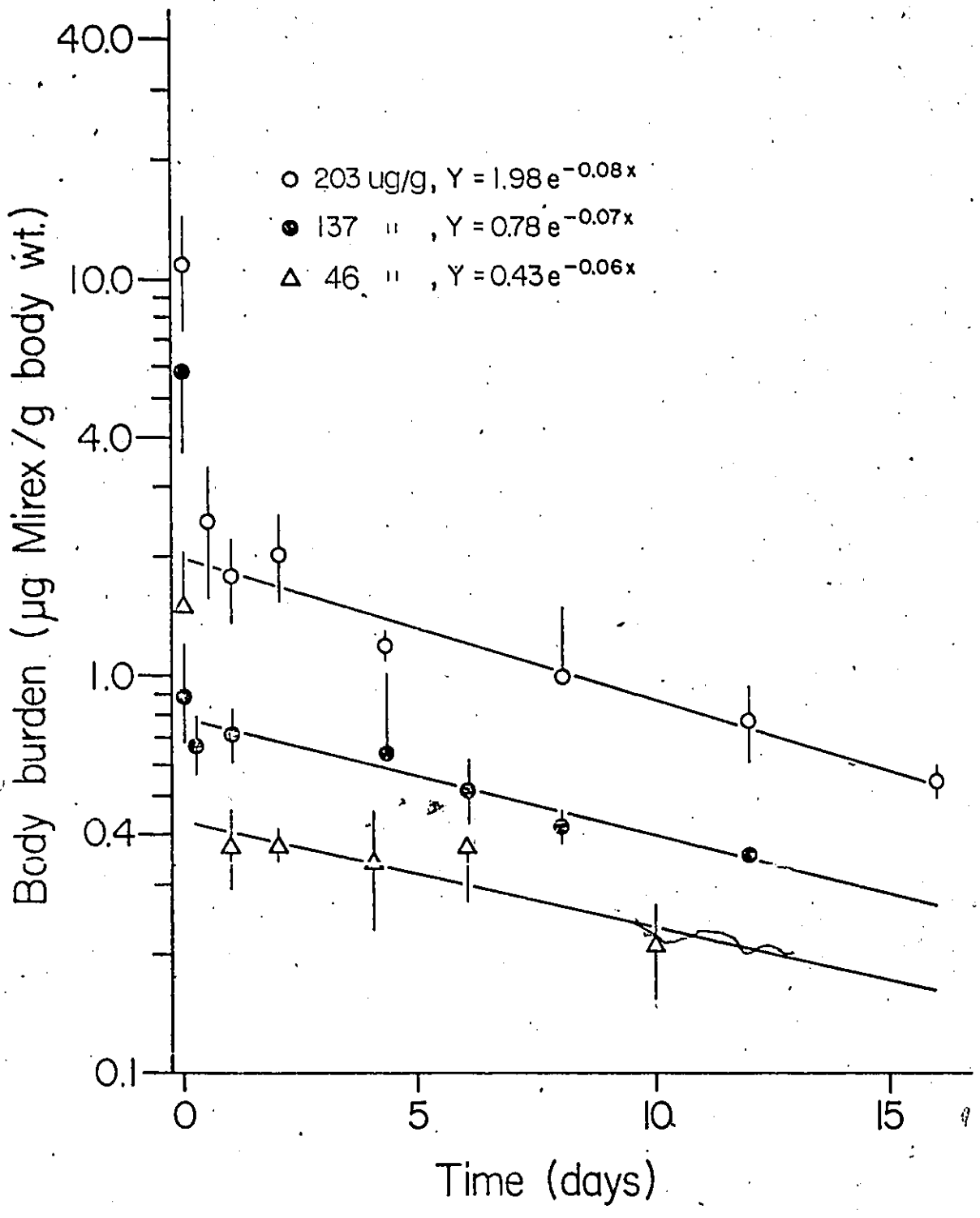


Figure 15. Clearance of ingested doses of mirex by C. pseudo-  
doqracilis at 20°C fed S. quadricauda having mirex concen-  
trations of 86, 115, and 176 ug mirex/g dry wt. Each point  
represents the mean and S.D. of 10 animals.

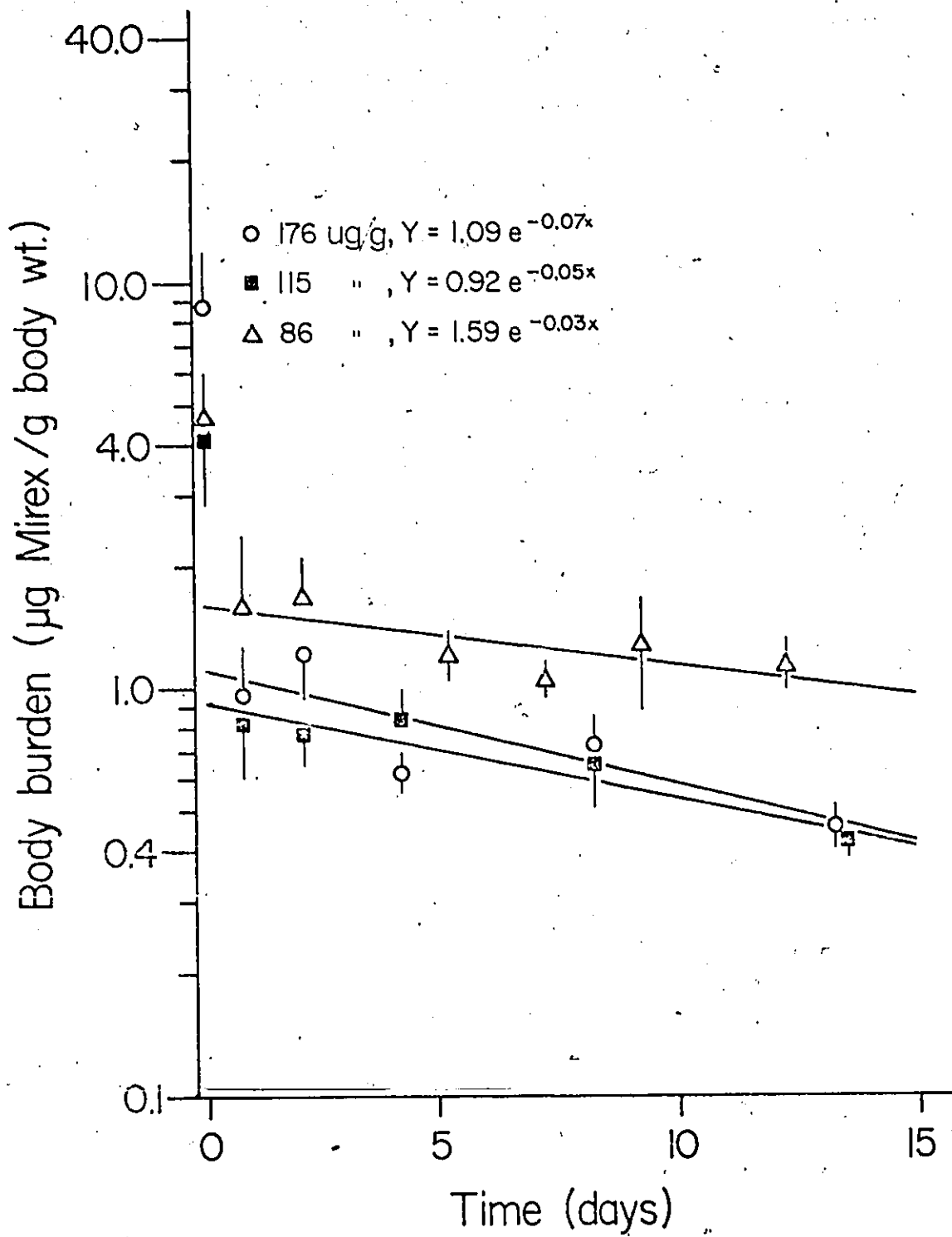


Table 4. Effect of concentration of mirex in food on parameters of bioaccumulation by H. azteca and C. pseudogracilis.

Concentration in food (ug mirex/g dry wt)	Feeding rate (cells/hour/g amphipod)	Assimilation Efficiency (%)	k (day <sup>-1</sup> )
<u>H. azteca</u>			
203	6.4±2.0 x 10 <sup>8</sup>	18.3	0.08
137	7.2±2.8 x 10 <sup>8</sup>	13.2	0.07
46	5.5±2.1 x 10 <sup>8</sup>	28.7	0.06
<u>C. pseudogracilis</u>			
176	9.1±2.8 x 10 <sup>8</sup>	13.0	0.07
115	5.8±1.4 x 10 <sup>8</sup>	23.0	0.05
82	7.7±2.6 x 10 <sup>8</sup>	22.1	0.03

the initial phase was ignored, except for one experiment in which clearance was examined in detail over the first 2 days (Fig. 13). The initial clearance phase was completed in the first hour postexposure, with clearance then continuing at a much slower rate, similar to that observed in subsequent, longer-term experiments. Results of these longer term experiments (Fig. 14) indicate that for H. azteca, a trend may exist which correlates fractional clearance rate to either mirex concentration in the food or assimilated mirex dose. In this set of results, fractional clearance rate increased from 0.06 to 0.08/day with an increase in mirex food concentration from 46 to 203 ppm, although this increase in clearance rate was not statistically significant, (Ancova,  $p < 0.05$ ).

C. pseudogracilis also demonstrated biphasic clearance, similar to that of H. azteca ( Fig. 15). A more pronounced correlation of fractional clearance rate to mirex concentration in food occurred for this species, increasing from 0.03 to 0.07/day with an increase in mirex-food concentration from 82-176 ppm. This increase could not be correlated to increasing assimilated dose as was the case with H. azteca, since assimilated dose was not a function of mirex concentration in food. As with H. azteca, the rates were not significantly different. The test of significance (Ancova) however, has certain limitations, which will be discussed below.

## section IV

## DISCUSSION

4.0.19 Bioaccumulation Models

In pollutant bioaccumulation studies, a model must be devised to use the data. In the pharmacological framework, the most widely used one is the mamillary model, in which a central compartment exchanges with peripheral compartments and the environment, with no direct exchange occurring between peripheral compartments and the environment. This model has the advantage of attempting to be physiologically correct, as well as describing accumulation of a compound. Moriarty (1975) however points out that the estimation of rate constants between the central and peripheral compartments is difficult if not impossible and so far only theoretical or partial models have been attempted (Ruzic, 1972).

For the purposes of describing bioaccumulation, physiological validity is not necessarily required, a model describing pollutant flux in the organism of interest being adequate. The approach taken here is similar to that of Harding and Vass (1977), and Trudel (1980). Direct uptake of mirex from water is seen as a one or two phase process (as discussed below), each compartment taking up mirex directly from water. Uptake of mirex from food is determined by ingestion rate and mirex concentration in the food. This is modified by an assimilation efficiency, which measures

the proportion of mirex absorbed through the gut wall. Clearance of mirex from tissues after uptake from both food and water is assumed to be a first order process.

#### 4.0.20 Bioaccumulation from water

The studies described here have shown that accumulation of mirex from water is best described by a two compartment model for H. azteca, and a one compartment model for C. pseudogracilis. The presence of a fast compartment in H. azteca, or perhaps more correctly, it's apparent absence in C. pseudogracilis, since a two compartment system is more common, has some fundamental implications for populations of the two species. It was shown that although the fast compartment will constitute a significant portion of total body burden in short term exposures, it reaches a low steady state level relatively quickly, and therefore has little long term bioaccumulation potential. The presence of a fast compartment in H. azteca is the most striking difference between the two species, and it is tempting to attribute the observed greater sensitivity of H. azteca to the presence of this compartment. It was however determined from bioaccumulation equations, that the fast compartment reaches steady-state in about two days, while initial mortality of H. azteca was not observed until 6 days of exposure at the highest concentration. It would therefore appear unlikely that the fast compartment is responsible for the greater sensitivity

of H. azteca. This conclusion however must be qualified by the knowledge that mirex is often noted to cause delayed mortality (Alley, 1973). A more likely, if less obvious, explanation of the sensitivity differences between the two species will be discussed below. In conclusion, the fast compartment of H. azteca is important in short term bioaccumulation, and cannot be totally disregarded from a toxicological point of view.

The dead control was designed to test the validity of the fast compartment. Within the limitations of the procedure, it did show that the fast compartment was not a simple adsorption/desorption problem, and can be regarded as a real compartment. Thus H. azteca fits into the generally observed pattern of biphasic pollutant kinetics, and indicates that it is C. pseudogracilis which is atypical.

Blood is often assumed to be the tissue equivalent of a fast compartment, and has been indicated as such in at least two studies on insects (Moriarty and French 1971, Benezet and Forghash 1972). Such a simple designation as this cannot apply here since both species have extensive circulatory systems. Morgan et al. (1972) and Schoor (1973) have shown that DDT in blood is almost always associated with the soluble lipoprotein fraction. Maliwal and Guthrie (1981) have also shown this, as well as demonstrating that certain specific classes of lipoprotein had far greater capacity for binding and storing organochlorines. A difference in lipo-

protein content, either in a quantitative or qualitative sense, could therefore account for the presence of a fast compartment in H. azteca and its absence in C. pseudogracilis.

The slow compartment of H. azteca and single compartment of C. pseudogracilis exhibit some similarities in terms of size and clearance rate, which indicate the possible homology of the two. When examined in detail however, consistent differences appear which lead to the conclusion that the two compartments are not identical, and have quite different ecological and bioaccumulative potentials. In all experiments, clearance of mirex from the H. azteca slow compartment was 40-50% slower than that by C. pseudogracilis. In experiments in which both species were exposed for the same time period, the size of the H. azteca slow compartment was consistently higher (>50%) than the single C. pseudogracilis compartment. In accordance with these observations, calculated uptake rate constants for H. azteca were approximately double those of C. pseudogracilis, and in combination with the other kinetic parameters, explain the observed greater accumulation of mirex by H. azteca. Additionally, since uptake and clearance rate constants were shown to be independent of duration of exposure, the above kinetic dissimilarities can be used in an extrapolation to long term exposure conditions, and indicate that the slow compartment of H. azteca has the potential to accumulate considerably

greater amounts (>2X) of mirex than C. pseudogracilis. In consequence, H. azteca is placed at greater risk from a toxicological point of view, simply by its higher tissue levels of mirex, and provides a likely explanation of sensitivity differences between the two species observed in this study. The phenomenon of bioaccumulation/sensitivity relationships has been demonstrated, the development of resistant strains within several species of insect being a function of reduced accumulation (Forgash et al. 1962, Sanchez and Sherman 1966, Benezet and Forgash 1972).

The above also has some implications for the biotransfer of mirex (and other compounds since this bioaccumulation difference appears to exist for two other pollutants--unpublished results). The movement of a pollutant to higher trophic levels can be expected to be greater when H. azteca is the predominant amphipod, as compared to a situation in which C. pseudogracilis is the major species. At present, the former situation is most common, H. azteca being the most widespread and numerous freshwater amphipod in North America (Bousfield, 1958).

Although the fractional clearance rates of C. pseudogracilis are not statistically different from those of the slow compartment of H. azteca, the greater clearance by C. pseudogracilis is a reproducible phenomenon, and is probably biologically "real". Also, according to the statistical test (Analysis of covariance), the fractional clearance

rates of both species are not statistically different from zero, which is clearly not biologically correct. For these reasons, the test is not thought to be sufficiently sensitive for the required purposes, and is not used.

Hamelink et al. (1971) postulated that exchange equilibria between water and fat control the degree to which an organism accumulates organochlorines. Although some observations appear to contradict this (Lieb et al. 1974, Gruger et al. 1975, Bjerk and Brevik 1980, Guiney and Peterson 1980), the proposal is now widely accepted. If correct, it implies that the differences observed between the single C. pseudogracilis compartment and the slow compartment of H. azteca are based on dissimilarities between lipid pools. Such differences as these could be a function of lipid pool size or, as discussed above, a consequence of qualitative differences between lipid pools. Also, the greater clearance exhibited by C. pseudogracilis would be expected if turnover of its lipid pool was greater than that by H. azteca. However, since the turnover of lipid stores of amphipods is a function of moulting frequency (Martin, 1965), and since H. azteca moults at twice the frequency of C. pseudogracilis (personal observation), such an explanation would not appear feasible.

The accumulation of mirex from water was not proportional to concentration in water over the entire range tested. It was however proportional at concentrations which are

environmentally realistic i.e. at and below the solubility level of 1ppb in water. Proportionality of uptake to exposure concentration over a discrete range is a widely observed phenomenon (Chadwick and Brocksen 1969, Bedford and Zabik 1973).

The presence of two distinct slopes in the accumulation/concentration curve indicates that at some point above the solubility level in water, the bioavailability of mirex becomes reduced. While this reduction has no direct relevance to the environment, it does have some implications with regards to toxicity testing. To conduct the standard lethality test, the 96 hour LC50, very high concentrations would be required to produce lethality with mirex and these amphipod species. By then attempting to extrapolate these results to more reasonable (environmental) levels, the toxic potential of mirex would be underestimated due to the reduced uptake rate at higher concentrations. A valid test would involve the determination of a toxic body burden, which could then be related to body burdens accumulated at more environmentally realistic levels.

The data indicates that the reduced accumulation effect is mediated solely through an uptake rate phenomenon, since exposure concentration did not affect compartmentalization or clearance. This effect could be mediated by a toxic phenomenon, dying animals having been shown to exhibit reduced uptake (Crosby and Tucker 1971, Harding and Vass 1978).

This explanation is unlikely however, since the reduction in uptake occurs in both species at the same exposure concentration, and it was shown that the two species do not have the same toxic response to mirex.

The observed reduction in uptake rate may involve saturation of the site of uptake. No specialized transport system is involved in absorption of organochlorines, passive diffusion being responsible for movement into the organism (Parke, 1968). It is possible that the membrane at the site of uptake is physically incapable of passing more mirex. However since the concentration at which accumulation levels off appears to be identical for both species, and since differences appear to exist in the site of uptake between the two species, it would be unlikely that saturation of the site of uptake would occur at the same exposure concentration. The close proximity of the concentration at which uptake rate is reduced to the maximum solubility of mirex in water, indicates that only that mirex dissolved in water is available for uptake. Mirex in excess of this may be associated with the carrier acetone, and not available for uptake.

Smaller individuals of both species were observed to accumulate more mirex, in proportion to body weight, than larger ones. This trend was more pronounced for H. azteca than C. pseudogracilis, as measured by the exponents of the power functions used to describe the accumulation/body size

relationships. Greater accumulation by smaller individuals of a species has been observed for a variety of species, with a variety of pollutants (Wildish and Zitko 1971, McLeese et al. 1980, Trudel 1980).

The effect of body size on clearance was not determined in this study, but Trudel (1980), in studying uptake of methyl mercury by Daphnia magna, observed an accumulation/body size relationship similar to that seen here, while also determining that body size had no effect on clearance rates. Trudel found that accumulation differences were the result of body size specific uptake rates, possibly due to the greater surface to volume ratio, or metabolic rate of smaller individuals. Harding and Vass (1978) determined that for the marine crustacean Thysanoessa raschii, a 1:1 relationship of DDT uptake to surface area existed. Harding (1977) found that the surface area-metabolic rate relationship was also a 1:1 ratio, and that in this case the influences of metabolic rate and surface area on bioaccumulation could not be distinguished. The accumulation-body size relationship observed here correlates closely with the respiration rate-body size relationship determined by Sushchenya (1972) for the order Amphipoda.

Temperature, although examined for H. azteca only, was found to exert considerable influence on bioaccumulation. Accumulation was seen to be proportional to temperature, due to the direct proportionality of both uptake and clearance

rate constants to temperature. These types of relationships would be expected if bioaccumulation is a function of metabolic rate (as discussed above for body weight), and metabolic rate were proportional to temperature over the range examined. Due however to the lack of published material on the relationship of metabolic rate of amphipods to temperature, no useful comparison can be drawn.

Studies with a number of pollutants, and a variety of organisms, (Murphy and Murphy, 1971, Reinert et al, 1974b, Fowler, and Unlu 1978), have established a general trend for increase in pollutant accumulation with temperature increase. Lloyd (1979) observed increasing uptake of methyl mercury with temperature by H. azteca. Trudel (1980) found similar increases in accumulation by Daphnia magna of methyl mercury with temperature, but attributed this to uptake phenomenon, since no effect of temperature on clearance was noted.

Under constant exposure conditions, the time to reach steady-state in both compartments will be lengthened in proportion to reduced clearance rates. For the slow compartment, 40 days is the approximate time required to reach steady state at 20°C, while at 5°C, this would require about 200 days. Actual body burden attained at steady-state should not be different, since both uptake and clearance rates are reduced by the same degree as temperature decreases.

Sanders (1969) determined that for the freshwater amphipod, Gammarus lacustris, DDT toxicity was directly proportional to temperature during short-term toxicity tests. If this is related to bioaccumulation differences, such as those observed in the present work, toxicity would eventually be equal over the range of temperatures examined, with temporal differences in the attainment of maximal toxicity.

4.0.21 Bioaccumulation from food

Since kinetic differences between H. azteca and C. pseudogracilis were observed with the water vector, it was reasonable to ask whether or not this phenomenon occurred with the food vector. Results indicate that both feeding rate and assimilation efficiency are not species-specific, while conclusions regarding clearance rates of assimilated mirex are less clear.

Although there was considerable variation in feeding rates with both species, no correlation to concentration of mirex in food was observed, indicating the amphipods did not detect the mirex. Feeding rates seen here, were similar to those of Lloyd (1979) for H. azteca fed on mercury contaminated S. quadricauda, the food item used in the present study, and other algal species. Rates were also within the range of values observed by Hargrave (1970) for H. azteca feeding on a variety of organisms.

Wide variation in assimilation efficiency appears to be an inherent quality exhibited by a wide variety of aquatic invertebrates (Cox 1971, Trudel 1980, Harding et al., 1981). Lloyd (1979), working with H. azteca, found assimilation efficiency to range from 3-18% for inorganic mercury, and 50-90% for methyl mercury. Hargrave (1970) determined assimilation of carbon from green algae by H. azteca to be approximately 50%. From these studies, it appears that assimilation efficiency is a function of the pollutant, rather than the particular species being examined.

Clearance of ingested mirex by both species of amphipod appears to be biphasic. The initial fast-clearing phase was similar in both species, being completed within one hour postexposure. This fast phase probably corresponds to that ingested mirex which is not assimilated from the gut, and is egested as part of the feces. The slower clearing phase would correspond to mirex assimilated into the tissues of the amphipods. This slowly cleared mirex appears to act as a single compartment in both species, as did mirex taken up from water by C. pseudogracilis.

The clearance of the fast phase within one hour corresponds to the time required to fill the gut, which was determined in an earlier experiment to be 40 minutes. Hargrave (1970) also determined a gut passage time in H. azteca of 30-40 minutes. Although the gut clearance phase may conceal a fast-clearing compartment in one or both species, such a compartment would be cleared within one hour postexposure, the time for clearance of the unassimilated mirex. As was discussed for H. azteca compartmentalization in uptake from water, the faster a compartment is cleared, the smaller will be the contribution of the compartment to total body burden at steady-state. The fast-compartment of H. azteca after uptake from water, with a biological half-time for clearance of 12 hours, was estimated to contribute less than 5% to total body burden at steady-state. A compartment which clears totally within one hour, would contribute con-

siderably less, and would thus be unimportant from a bioaccumulation point of view. As was also discussed for the fast compartment of H. azteca after uptake from water however, such a compartment cannot be completely overlooked with regards to it's potential to exert toxic effects.

Clearance of assimilated mirex was similar to that observed after uptake from water in C. pseudogracilis, and the slow compartment of H. azteca. This similarity however, may only be coincidental since clearance rates, although not statistically different ( $p < .05$ ), appeared to be dose dependant, decreasing with decreasing mirex level in the food. This may represent a mechanism whereby both species respond to a high dose of pollutant. Clearance rates for C. pseudogracilis however were not a function of assimilated dose, but were proportional to mirex concentration in the food. The increase in fractional clearance rate with food concentration may represent the desorption of mirex which was not assimilated into internal tissues, but becomes associated with the lining of the gut wall.

#### 4.0.22 Applications to Natural Populations

In this examination of bioaccumulation of mirex by amphipods, it has been assumed that the food and water vectors are the only sources of mirex for the amphipod. Determination of the rates and factors involved in accumulation via these two vectors can lead to an understanding of their relative importance and to some predictions of population consequences. The processes involved in bioaccumulation which have been examined here are; (1) efficiency of uptake from water, (2) efficiency of uptake from food, and (3) whole body clearance.

As was outlined in the Results section, a bioaccumulation equation involving uptake-, and clearance-rate constants can be developed to describe pollutant flux between water and amphipods (equation 5). This can easily be modified to describe accumulation from food, the clearance rate having been empirically determined. The uptake rate will be the product of the experimentally-derived ingestion rate, and assimilation efficiency, and accumulation from food is described by

$$C = \frac{I\alpha}{K} F (1 - e^{-Kt}) \quad (11)$$

where C is body burden,  $\alpha$  is the assimilation efficiency of mirex from food, I is the ingestion rate, K is fractional-clearance rate, and F is the concentration of mirex in food. Equations 5 and 8 together will describe total uptake, and

for any time of exposure, will give the relative contributions of the two vectors. This is most conveniently studied at steady-state conditions ( $t \rightarrow \infty$ ), where the bracketed terms equal 1, and for the following example, this condition is assumed.

Given a water concentration of 1 pg mirex/ml ( $10^{-12}$  g/ml), H. azteca will accumulate a dose of approximately 80 ng mirex/g tissue ( $8 \times 10^{-8}$  g/g tissue). C. pseudogracilis under the same conditions, achieves a body concentration of 23 ng/g. Using the bioaccumulation ratio of 10,000 determined by Hollister et al. (1975) for several species of algae, amphipod food organisms might be expected to attain a concentration of 10 ng mirex/g. Assimilation efficiency for both species was 0.2, and feeding rate approximately 0.3 g algae/g amphipod/day with a fractional clearance rate of 0.06/day assumed for both species. These values substituted into equation 8, gives the steady-state body burdens accumulated from food for both H. azteca and C. pseudogracilis to be approximately 10 ng mirex/g dry weight. It must be noted that, due to the apparent dependence of clearance rate to mirex concentration in food, fractional clearance rate of ingested mirex may be considerably slower than the 0.06/day used here. If so, the amount accumulated via the food vector will increase in proportion to the decrease in the fractional clearance rate.

The above model is vastly oversimplified, as it can only be applied to a segment of the population. To more adequately describe bioaccumulation by the entire population, a bioenergetics approach is required, incorporating metabolic parameters, such as those accounting for body size effects on uptake from water and ingestion rate, and temperature effects. Such approaches have been taken recently by Trudel (1980), and Harding et al., (1981), and describe bioenergetic and bioaccumulation interactions.

The model developed here, has implications regarding the continuing controversy in the literature concerning the relative importance of food and water vectors in accumulation of environmental pollutants. Many studies show that a variety of organisms accumulate a greater proportion from water than from food (Chadwick and Brocksen 1969, Reinert 1972, Epifanio 1973, Jarvinen et al. 1977). Others have demonstrated food to be the dominant vector (Macek and Korn 1970, Reinert et al. 1974a, Fowler et al. 1978, Harding et al. 1981). Results obtained in this study indicate that both food and water can contribute significant amounts to total body burden, and that no generalization can be applied to the importance of vectors in bioaccumulation.

## section V

## SUMMARY

Bioaccumulation of mirex by H. azteca and C. pseudogracilis was defined in terms of uptake rates from both food and water, as well as subsequent clearance of the compound from these two sources. The influence of amphipod age was examined as it relates to mirex bioaccumulation from water. The effect of temperature on bioaccumulation from water was studied with H. azteca. Some of the data was used to formulate a simple model, which allows comparison of the bioaccumulative capabilities of the two species of amphipod. The major points of the work are:

(1) Uptake of mirex from water and subsequent clearance by H. azteca and C. pseudogracilis was adequately described by compartmental kinetic functions. Bioaccumulation by C. pseudogracilis was a single compartment process. H. azteca exhibited two-compartment uptake and clearance, a "slow" compartment somewhat similar to the single C. pseudogracilis compartment, and a "fast" compartment in which both uptake and clearance rates exceeded those of the slow-compartment. Due to its rapid turnover, the fast-compartment is relatively insignificant from a bioaccumulation point of view. However, greater uptake into, and slower clearance from, the slow H. azteca compartment relative to the single C. pseudogracilis compartment, results in a dose accumulated by H. azteca, 3X that accumulated by C. pseudogracilis.

(2) Bioaccumulation was found to vary with the 0.82 and 0.91 powers of dry weight for H. azteca and C. pseudogracilis respectively. These are to be expected if bioaccumulation varies with metabolic rate.

(3) Bioaccumulation of mirex by H. azteca was found to be proportional to temperature. This was seen to be due to the direct proportionality of both the uptake and clearance rates. The effect of decreasing temperature on bioaccumulation will be to lengthen the time required to reach a steady-state body burden. The tissue concentration of mirex at steady-state however, should not vary with temperature, since uptake and clearance rates are affected to the same degree.

(4) Uptake of mirex from food, in terms of feeding rates and assimilation efficiency, was found to be virtually identical for both species. Concentration of mirex in food did not appear to influence either feeding rate or assimilation efficiency. Clearance of assimilated mirex was unicompartamental for both species. Clearance rates were proportional to mirex concentrations in the food, higher concentrations leading to greater clearance.

(5) A bioaccumulation model was constructed from data collected at 20°C, and mirex body burdens achieved at steady-state by each species calculated. This simple model demonstrated that both food and water vectors contribute substantial amounts to total body burden. Due to the relatively

greater importance of the water vector in H. azteca, this species will accumulate more mirex than will C. pseudogracilis.

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