RESIDUE DYNAMICS AND AVAILABILITY OF 2,4,5,2',4',5'-HEXACHLOROBIPHENYL IN AQUATIC MODEL ECOSYSTEMS

By

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ABSTRACT

RESIDUE DYNAMICS AND AVAILABILITY OF 2,4,5,2',4',5'-HEXACHLOROBIPHENYL IN AQUATIC MODEL ECOSYSTEMS

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The polychlorinated biphenyls (PCB's) are organic compounds of industrial origin which have become ubiquitous contaminants of aquatic systems. Because of their hydrophobic nature, the PCB's are attracted to surfaces which results in their accumulation on bottom sediments. Sorption onto bottom sediments creates a potential reservoir of material which can buffer changes in residue concentrations in the water via equilibrium exchange reactions. Fine textured sediments and sediments with high organic contents are often enriched with hydrophobic compounds. The significance of these substrate-bound residues, their availability to aquatic organisms, and the principal route of uptake by organisms are largely unknown.

The present study was undertaken to determine the residue dynamics and metabolism of a representative PCB isomer in a semi-natural model of a woodland stream ecosystem and in a series of simplified laboratory experiments. The laboratory experiments were designed to determine the

the relative importance of residue accumulation by a representative benthic invertebrate from water, from contaminated food, and from direct contact with contaminated substrates. The bioavailability of substrate-bound residues as a function of substrate type, organic matter content, and particle size distribution was also investigated. It was hoped that the laboratory studies would also support and clarify the results derived from the more complex model streams experiment.

Hexachlorobiphenyl was accumulated rapidly by the substrates and benthic organisms of the model streams and concentrations increased with duration of exposure. Residues in substrates after thirty days of exposure ranged from 630-3200 times the concentrations in the water and were significantly correlated with the organic matter content.

The rate of uptake of the isomer by the biota was biphasic with the most rapid accumulation occurring during the initial three hours of exposure. Maximum residue concentrations in the biota usually occurred after thirty days of exposure. Bioaccumulation factors ranged from about 300,000 in amphipods to 1200 in clams while fish, crayfish, and other macroinvertebrates had intermediate values. There was no evidence that residue concentrations were increased by trophic transfer.

Residues of hexachlorobiphenyl were present in all benthic organisms after sixty days of depuration but

repeated exposure to the isomer did not result in stepwise increases in concentration with each exposure.

Metabolites of hexachlorobiphenyl were not detected in the substrates or biota of the model streams.

Amphipods accumulated hexachlorobiphenyl residues directly from water as a function of time and exposure concentration under both static and continuous flow conditions. Residue levels in amphipods under continuous flow exposure were 3.3-6.7 times higher than under static conditions although bioconcentration factors were similar under both exposure conditions.

The accumulation of residues by ingestion of contaminated food was less important than direct uptake from water although residues accumulated from each pathway appear to be additive.

Accumulation of residues by amphipods was enhanced by direct contact with contaminated substrates although direct uptake of substrate-desorbed residues directly from the water was the major route of uptake. The bioavailability of substrate-bound residues is controlled by the level of organic matter and to a lesser extent by the particle size of the substrates.. Residue levels in amphipods were inversely related to the organic content and directly related to the particle size of the substrate.

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INTRODUCTION

The polychlorinated biphenyls (PCB's) are complex mixtures of chlorinated organic compounds of industrial origin and have become widespread contaminants in the environment. Their structure, chemical properties, commercial uses, acute and chronic toxicity, environmental residues, rates and route of transport and metabolism have been reviewed by Gustafson (1970), Maugh (1972), Hammond (1972), Peakall and Lincer (1970), Peakall (1972), Risebrough and Brodine (1970), Ahmed (1976), Nisbet and Sarofim (1972), and Veith and Lee (1970, 1971a). The toxicity of the PCB's to fish and aquatic invertebrates has been described by Stalling and Mayer (1972), Halter and Johnson (1974), Nebeker et al. (1974), Mayer et al. (1977), Defoe et al. (1978), and Mauck et al. (1978).

In general, the PCB's are hydrophobic, lipophilic and persistent compounds of low water solubility and volatility which behave in the environment in a manner similar to many of the chlorinated hydrocarbon pesticides, especially dichlorodiphenyldichloroethylene (DDE). Because of their hydrophobic nature, the PCB's and other chlorinated hydrocarbons are attracted to surfaces which results in their accumulation on pond and stream bottoms and by inorganic and organic materials

suspended in the water column (Cope 1966; Gerakis and Sficas 1974). Thus, almost all of the PCB's released into fresh water and substantial portions of the chlorinated hydrocarbons entering the sea are adsorbed on bottom sediments (Nisbet and Sarofim 1972; Hom et al. 1974). The bottom sediments of Escambia Bay below an industrial outfall had PCB residues as high as 486 ppm (Duke et al. 1970) while Baltimore Harbor sediments ranged as high as 84.2 ppm (Morgan and Sommer 1979). The worst case of environmental pollution by PCB's is in New York where Hudson River sediments in the Fort Edward area contain PCB concentrations ranging from 6.6 - 6700.0 ppm (Nadeau and Davis 1976). In the Great Lakes there is an estimated 84-100 tons of PCB in the water column and bottom sediments have been proposed as the immediate source of this material (Nisbet and Sarofim 1972; Neely 1977).

Sorption of neutral hydrophobic compounds onto suspended material and bottom sediments is usually attributed to an equilibrium exchange reaction which occurs rapidly and is reversible (Baughman and Lassiter 1978; Paris et al. 1977; Paris and Lewis 1976). This reaction can be described by a simple distribution coefficient which is a function of the differential solubility of the solute in two different solvents. Thus the water solubility of the PCB's which is inversely related to their chlorine content will determine the distribution of the mixture with the result that the lower chlorinated chlorohomologues and isomers will be preferentially dissolved in the water (Hague et al. 1974; Paris et al. 1978).

Adsorption onto sediments creates a reservoir of material which can buffer changes in water concentrations (Frank et al. 1974; Baughman and Lassiter 1978) and may stabilize the compounds against volatilization and microbial degradation (Pionke and Chesters 1973; Oloffs et al. 1972, 1973). Adsorption may also reduce toxicity to aquatic organisms by making the material unavailable for uptake (Ferguson et al. 1965; Terriere et al. 1966; Veith and Lee 1971b). However, several workers have reported that DDT and methoxychlor adsorbed on suspended particulates are a very effective means of controlling filter-feeding simulid larvae for as much as 98 miles downstream from the point of application (Arnason et al. 1949; Fredeen et al. 1953; Wallace et al. 1976).

Sediment characteristics can affect the extent of residue accumulation by sediments which may lead to a heterogeneous distribution of the compounds in the environment. Highest concentrations of hydrophobic compounds are often associated with fine textured sediments (Browman and Chesters 1977; Frank et al. 1974; Hetling et al. 1978; Hargrave and Phillips 1974; Steen et al. 1978; Karickhoff and Brown 1978; Routh 1972; Richardson and Epstein 1971). In addition, organic content is often inversely related to particle size with the result that fine textured sediments are often high in organic matter (Hargrave 1972; Odum and de la Cruz 1967).

Hydrologic conditions affect the distribution of sediments and thus influence the distribution of PCB residues in the aquatic environment. Higher concentrations of DDT

and PCB have been detected in the sediments of deep basins of the Great Lakes than in nearshore sands and gravels (Leland et al. 1973; Glooschenko et al. 1976). Impoundments may also produce conditions conducive to dieldrin buildup in the benthos as silt settles out (Morris and Johnson 1971). Significant differences in sediment pesticide levels were found between sampling points in ponded and fast flowing waters which were attributed to greater amounts of organic matter and fine particles in the sediments of the ponded areas (Dimond et al. 1974). The bed load (the shifting mass of detritus and sediment which forms the interface between water and bottom materials) of Ontario streams contained 3 - 6 times more DDT, dieldrin and endosulfan than did bottom sediments (Miles 1976).

Residue levels of chlorinated hydrocarbon insecticides in stream sediments are often correlated with the organic matter content (Pionke and Chesters 1973; Richardson and Epstein 1971). Hetling et al. (1978) found that PCB residues in Hudson River sediments were higher when associated with either muck or sand which contained wood chips than in sand without wood ships. In Lake Michigan sediments, Leland et al. (1973) reported that the DDT residue concentrations were significantly related to the organic carbon content but Glooschenko et al. (1976) were not able to establish such a relationship for either DDT or PCB's.

The association of chlorinated organics with fine textured sediments has often been attributed to increased

surface area available for adsorption. Even among organic detritus particles, higher concentrations of DDT were noted in small particles than in larger undecayed pieces (Odum et al. 1969). However, Aroclor 1254 was found to be evenly distributed in particle size fractions of Escambia Bay sediment (Nimmo et al. 1971) while PCB concentrations in Great Lakes sediments were not clearly correlated with sediment texture (Glooschenko et al. 1976). More work is needed to elucidate the role of the various substrate characteristics in the accumulation of chlorinated organics by bottom sediments.

A number of workers have speculated that benthic organisms can accumulate hydrophobic organics from contaminated substrates and thus provide a means of entry into aquatic food chains (Holden 1965; Odum et al. 1969; Leshniowsky et al. 1970; Yule and Tomlin 1971; Veith and Lee 1971b; Reinert 1972; Herbes 1977; Karickhoff and Brown 1978; Weininger 1978). There is little experimental evidence to substantiate this hypothesis although some observations from survey data do lend some support to the idea. Wallace and Brady (1971) observed that most of the organisms with high dieldrin concentrations in their study were near the bottom of the food chain and were either detritus or omnivorous filter feeders. In another study, benthic feeding fish contained larger amounts of dieldrin than species whose feeding habits were less benthically oriented (Morris and

Johnson 1971). Hindin et al. (1968) observed that organisms which ingested sediment had the greatest concentrations of chlorinated organics in their body tissues. Accumulation of PCB's by snails has also been attributed to ingestion of contaminated Hudson River sediments by Nadeau and Davis (1976). Matsumura and Benezet (1973) observed that the highest residues of TCDD were accumulated by mosquito larvae which were also the only benthic feeding organisms in their studies with contaminated substrates. Thus, the potential for increased accumulation of residues due to direct exposure to contaminated substrates appears to exist and should be examined in greater detail.

Many aquatic organisms exhibit species specific preferences for a given range of substrate particle sizes which is often exhibited in different distribution patterns for various macro- and microhabitats (Shelford and Eddy 1929; Wene and Wickliff 1940; Pennak and Van Gerpen 1947; Bell 1969; Chutter 1969; Hynes 1970; Cummins and Lauff 1969; Barber and Kevern 1973; Reice 1974; Higler 1975). In fact, Cummins and Lauff (1969) have proposed that substrate particle size may represent a common denominator in habitat selection by benthic stream invertebrates.

Food resources are also partitioned on the basis of particle size and whether they are active, stationary or in suspension (Cummins 1973, 1974; Petersen and Cummins 1974; Cummins et al. 1973). Community structure may also depend

on the distribution of detritus within aquatic ecosystems as Egglishaw (1969, 1964) has shown a definite correlation between numbers of organisms present in substrate samples and the quantity of detritus available for consumption.

Because of the tendency of hydrophobic organics to accumulate in bottom sediments, benthic organisms are exposed to higher concentrations than are pelagic species. In addition, those organisms which are attracted to, or feed on, fine textured sediments of high organic content may be exposed to the highest concentrations. Whether these residues are available to the organism is largely unknown and needs to be evaluated as does the mechanism and route of uptake by sediment dwelling organisms. It is vitally important to our understanding of aquatic toxicology that we determine whether sediments can be considered sinks and safely ignored or if they will continue to be long-term sources of contaminants to the overlying water and to benthic and pelagic organisms.

The commercial PCB's are very complex mixtures and are difficult to analyze. In order to simplify analysis and data interpretation a representative isomer was chosen for study. The isomer selected was 2,4,5,2',4',5'-hexachlorobiphenyl which is a major component of the technical PCB's with chlorine contents greater than 50% (Jensen and Sundstrom 1974). Hexachlorobiphenyls comprise

4%, 34% and 38% of Aroclors 1248, 1254 and 1260 respectively (Hammond et al. 1972). Chiou et al. (1977) reported that 2,4,5,2',4',5'-hexachlorobiphenyl is soluble in water at 0.95 ug/liter (24°C) and has an octanol/water partition coefficient (log) = 6.72.

The primary objectives of this study were to determine; 1) the residue dynamics and metabolism of the isomer in the water, substrate and benthos of a laboratory model of a woodland stream, 2) the respective roles of uptake from water and from contaminated food, 3) the availability of residues to the benthos from contaminated substrates, 4) the relative importance of substrate type, organic matter content and particle size in the availability of residues from contaminated substrates to the overlying water and to the benthos.

MATERIALS AND METHODS

The fate and behavior of 2,4,5,2',4',5'-hexachlorobiphenyl (HCB) in aquatic systems were studied in two phases; 1) a year-long model streams study and 2) a series of short-term laboratory studies during which exposure conditions were systematically altered.

The model streams study was designed to determine the accumulation, depuration and metabolism of HCB residues in various abiotic and biotic components of seminatural laboratory streams. The laboratory streams represented a complex, multi-species ecosystem which retained many of the natural characteristics of a small woodland stream.

The short-term laboratory studies were designed to determine the relative importance of HCB uptake from exposure to contaminated water, food, or substrate and the effect of substrate type, organic content, and particle size on the availability of substrate-adsorbed HCB to a representative benthic macroinvertebrate.

Model Streams - Description

The laboratory streams were constructed in the fish hatchery building of the Michigan Department of Natural

Resources located at Paris, Mecosta County, Michigan. The site provided a natural stream water supply and an abundance of aquatic organisms for colonization of the laboratory streams.

The hatchery was supplied with water from Cheny Creek, a woodland stream of approximately 5.9 m^3/minute discharge which originates from several springs within a fenced watershed adjacent to the hatchery. Water was diverted to the hatchery through an underground pipe that leads from a concrete diversion dam and headbox 320 m upstream. Trash screens within the headbox removed large debris but allowed the free entry of aquatic organisms and particulate material drifting in the water supply.

Within the hatchery building, the laboratory streams were constructed by modification of existing concrete troughs. An upper tier of six troughs, each 3.96 m x 0.58 m x 0.27 m, served as constant-head reservoirs for a lower tier of identical sized troughs that served as the laboratory stream channels (Figure 1). Water entered each reservior trough through paired pipes (3.17 cm diameter) and then drained by gravity through paired 5.08 cm diameter pipes to the matching stream channel below. Water flow to each stream was maintained at 60 liters/minute by adjustment of a gate valve at the head of each reservoir trough.

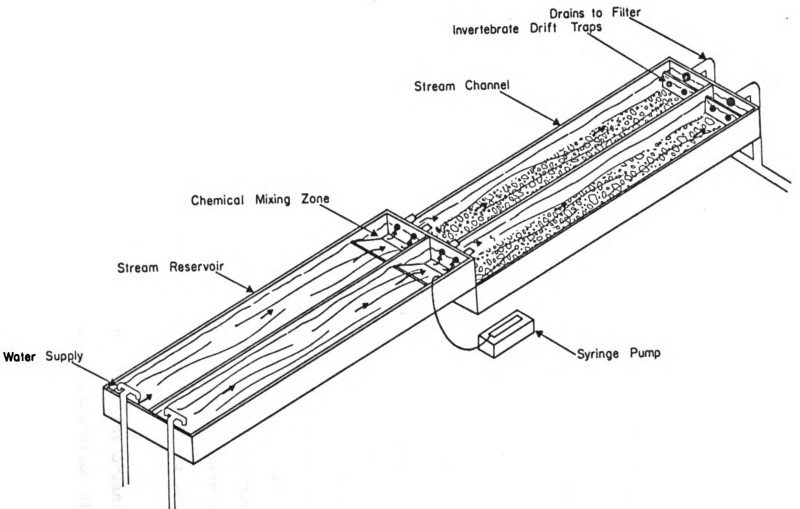

Figure 1. Diagram of one pair of laboratory streams. The direction of water flow is indicated by arrows.

A single drain (10.2 cm diameter) at the end of each stream channel diverted the water to a common filter box which contained alternate layers of activated carbon and polyurethane foam. The filtered effluent was discharged to the Muskegon River.

The stream channels were illuminated by 48 high-intensity, cool-white fluorescent lamps attached to plywood sheets suspended above the streams. Light intensity at the stream water surface was 900 ± 50 foot candles. The lights were wired through an automatic time switch which was adjusted at two-week intervals to conform with the natural day length of the local area (Table A1).

Water temperatures in the laboratory streams followed the natural daily and seasonal fluctuations of Cheny Creek which ranged from a maximum of 15.5°C in May to a minimum of 0.5°C in December (Table A2). Data for dissolved oxygen, pH, alkalinity, and hardness are also presented in Table A2.

In June 1975, natural substrate from Buckhorn Creek, Mecosta County, Michigan, was transferred to the stream channels. Equal quantities of substrate which consisted of silt, sand, gravel, and rubble (maximum 10 cm diameter) were uniformly distributed in each stream channel to a depth of 5 to 10 cm.

Biological colonization resulted from the organisms introduced with the substrate and from immigration of

organisms drifting in the water supply. A diverse community of algae, invertebrates, and bryophytes was in evidence eight weeks after substrates were added.

Larger stream organisms, i.e., fish, clams, and crayfish, were added to the stream communities prior to each chemical treatment. These organisms were collected from nearby streams and acclimated to the Cheny Creek water for a minimum of two weeks prior to stocking in the streams. The test animals were not fed either before or during their residence in the test streams, but were free to forage within the biological communities existent within the streams.

Model Streams-Experimental Procedure

The model stream experiments were planned to determine the fate and residue dynamics of HCB in the seminatural streams through one annual period. The design of the streams and the water supply allowed "natural" seasonal fluctuation in temperature, photoperiod, water chemistry, and biological community structure, but required control of stream flow, light intensity, and the stocking and replacement of larger stream organisms, i.e., clams, fish and crayfish.

One year was allowed for biological colonization of the streams and pretreatment measurements. In the second year, four replicate treatments were timed to coincide with the

fall, winter, spring, and summer seasons. During each experimental period (season), HCB was metered into the test streams during a 30-day treatment phase which was followed by a 60-day depuration phase. This sequence was repeated for each season except a depuration phase was not included in the summer experimental period.

Two streams were treated with HCB, and one was designated as a solvent control. The control stream received acetone equivalent to the amount which was added to the test streams with the chemical stock solutions.

The chronological sequence of the major experimental events is listed in Table 1.

Radiolabeled and unlabeled 2,4,5,2',4',5'-hexachlorobiphenyl were obtained from Pathfinder Laboratories, Inc., St. Louis, Missouri. The uniformly ^{14}C-ring-labeled compound had a specific activity of 14.06 mCi/mM. Gas and thin layer chromatography indicated that the compounds were >98% pure. Stock solutions were prepared by mixing appropriate quantities of labeled and unlabeled HCB in acetone to provide a concentration of 127.12 mg/liter and a specific activity of 8.60 dpm/ng.

Syringe pumps (Harvard model 975) which were equipped with 100 ml capacity glass syringes and teflon needles delivered the stock solution to mixing chambers at the lower end of each reservoir trough. Water turbulence in

Table 1. The chronological sequence of major events in the model streams study.

Dates		Event
Start	Finish	
06/07/75	06/08/75	Streams stocked with substrates and organisms
08/02/75	08/10/76	Pretreatment monitoring
08/11/76	09/08/76	Fall Experimental Period - Treatment Phase
09/09/76	11/09/76	Fall Experimental Period - Depuration Phase
11/10/76	12/09/76	Winter Experimental Period - Treatment Phase
12/10/76	02/07/77	Winter Experimental Period - Depuration Phase
02/09/77	03/10/77	Spring Experimental Period - Treatment Phase
03/11/77	05/09/77	Spring Experimental Period - Depuration Phase
05/11/77	06/08/77	Summer Experimental Period - Treatment Phase
06/08/77	06/09/77	Streams dismantled

the mixing chambers dispersed and mixed the stock solution with diluent water before discharge to the stream channels. A 1.27×10^6-fold dilution of the stock solution gave a nominal stream HCB concentration of 100 ng/liter. Acetone was metered into the control streams at the same rate as in the test streams (0.69 ul/liter).

Syringes were filled daily and the quantities of stock solution used during the previous 24 hours were recorded. Syringe failure occurred on several occasions but pumping rates were always reestablished within a 12-hour period.

Concentrations of HCB in water, substrate, and selected plants and animals were determined from samples collected during each experiment. Substrate was collected from all streams on days 5, 15, and 30 of each treatment phase and days 15, 30, and 60 of each depuration phase. Crayfish and sculpins were collected on days 15 and 30 and clams were removed on day 30 of the treatment phases. Water, benthic invertebrate, and plant samples were collected from the streams at 3, 6, 12, 24, 48, and 96 hours, and on days 8, 16, and 30 of each treatment phase.

During the depuration phase of each experiment, samples were collected on days 4, 8, 16, 32, and 60.

Biological samples usually included amphipods, snails, stoneflies, mayflies, aquatic earthworms, filamentous algae and bryophytes, but it was occasionally necessary to omit certain groups and/or to sample others depending upon their

seasonal availability. Samples of watercress, an aquatic macrophyte, which was abundant in the lower sections of the streams were subdivided into leaf, stem, root, and seed pod components when possible.

Small invertebrates were collected by removing individual organisms with forceps and small dip nets. A sample of 10-20 organisms of a single species was placed in a glass vial and stored at -20°C until analyzed. Larger organisms, including fish, clams, and crayfish, were wrapped in aluminum foil and frozen.

Substrate cores were collected with a 10.2 cm diameter core sampler which was pushed to the stream bottom. The entire core was lifted with a stainless steel end-plate, placed in a plastic bag and stored at -20°C.

Composite 2 liter water samples were collected by siphoning equal quantities of water from the upper, middle, and lower portions of each stream. The samples were extracted within a few hours after collection.

Laboratory Studies - Objectives

Laboratory studies were conducted to determine the residue dynamics of HCB under simplified exposure conditions. Organisms were exposed to the chemical in water under static and continuous flow conditions and to a contaminated food source in order to determine the contribution of each exposure condition to the accumulation of body residues.

Static tests are often used to simulate exposure to a spill or other single dose introduction whereas continuous flow conditions would be analogous to a constant input of a toxicant in a stream, river or well mixed lake. Experiments with contaminated food as a source of residues would give some indication of the potential for transfer through food chains and would also indicate whether uptake from food or from water was the dominant pathway of residue accumulation.

The availability of substrate-bound residues to benthic organisms was also evaluated. The purpose of these experiments was to determine the potential of contaminated sediments as long term sources of toxicants in aquatic environments and the extent to which residue levels in the overlying water and in benthic organisms are controlled by substrate characteristics. Of primary interest in this regard were the respective roles of substrate type, organic matter content and particle size distribution.

It was hoped that the laboratory studies would also support and clarify the results and conclusions derived from the more complex model streams experiments.

Laboratory Studies - Experimental Procedures

Laboratory studies were conducted in the Fisheries Research Laboratory on the campus of Michigan State University. Well water of the following characteristics was used

in all tests; total alkalinity as $CaCO_3$, 270-330 mg/liter; hardness as $CaCO_3$, 329 mg/liter; pH, 7.4-7.8; dissolved oxygen, 9.1-10.0 mg/liter; and temperature, 10-14°C.

The organism selected for laboratory study was the amphipod, Gammarus pseudolimnaeus Bousfield, which is widespread and common in unpolluted clear waters including springs, spring brooks, and streams. Generally, freshwater gammarids are cold-stenothermal, photonegative, thigmotactic, (Holsinger 1976), omnivorous, scavengers which utilize both plant and animal materials (Pennak 1953).

Amphipods were chosen as the test organism because they were an important component of the model streams community in terms of abundance and biomass. They are also important in the diet of stream fish and were observed to occupy a variety of substrate types within Cheny Creek and the model streams. In addition, they were; available in large numbers, easy to maintain in laboratory culture, and durable when handled.

Amphipods were collected from Cheny Creek and cultured in the laboratory in a circular stream of the type used by McIntire et al. (1964). A paddlewheel circulated the water and a continuous inflow of well water maintained water temperature at 12-14°C. Photoperiod (16 hours light, 8 hours dark) was provided by natural daylight which was supplemented by fluorescent lights suspended over the stream. Amphipods were fed preleached maple leaves (Acer sp.) and

Purina Trout Chow #2. Amphipods could be maintained indefinitely when cultured in this manner.

Accumulation from Water - Static Exposure

Amphipods were exposed to HCB under static conditions in 2 liter glass beakers which contained 1.5 liters of well water. Known quantities of HCB in 1.0 ml acetone were added to each beaker below the water surface and stirred to create nominal concentrations of 0.100, 0.200, and 0.400 ug/liter. Amphipods (15-20/beaker) were added to the beakers which were then placed in water baths (12-16°C).

At 24, 48, 96, and 192 hours of exposure, three beakers per concentration were removed from the water baths. Water samples (1 liter) were siphoned from each beaker into 4 liter amber glass bottles which contained 100 ml of hexane. The bottles were shaken for 30 seconds and set aside at room temperature until extracted. Samples were usually extracted within one week of collection. Amphipods were removed by dip net, placed in glass vials, and frozen until analyzed.

Mechanism of Accumulation

The mechanism of accumulation of HCB residues by amphipods under static conditions was studied at a nominal HCB concentration in water of 0.200 ug/liter. During trial 1, water samples (1 liter) and amphipods (14-20) were collected from each of three replicate beakers at 24, 48,

96, and 192 hours of exposure. Water samples were treated as described. Amphipod samples were divided into two lots; the individuals of one lot were quickly dipped five times in a scintillation vial containing 10 ml of acetone, the other lot was dipped in distilled water. Both lots were placed in separate glass vials and frozen. The acetone was retained for later analysis by liquid scintillation techniques. During trial 2, the entire experiment was repeated as described but additional samples were taken after 2, 6, and 12 hours of exposure.

Accumulation from Water - Continuous Flow Exposure

Accumulation of HCB by amphipods from water under continuous flow conditions was tested in 20 liter glass aquaria at nominal HCB concentrations of 0.100, 0.200, and 0.400 ug/liter. The aquaria were equipped with standpipes which maintained the water level at 9 liters. Harvard syringe pumps (Model 975), which were equipped with 100 ml glass syringes, delivered known quantities of HCB in acetone at 1.45 ml/hour into the bottom of 2 liter beakers which served as mixing chambers. Diluent water (100 ml/minute) was added to each mixing chamber from which the water overflowed through a standpipe into a glass splitter box which in turn delivered the water into duplicate test aquaria. The flow rate of 50 ml/minute per aquarium provided a 90% replacement time of about 7 hours (Spraque 1969).

Hexachlorobiphenyl was injected into the system for 24 hours to allow the system to equilibrate after which 50-60 amphipods were added to each aquarium.

At 24, 48, 96 and 192 hours of exposure, water samples (1 liter) were siphoned from each aquarium and treated as described; 8-10 amphipods were dipnetted from each aquarium at these times and frozen until analyzed.

Accumulation from Food

Accumulation of HCB residues by ingestion of contaminated food was determined by placing 120 amphipods into each of six 20 liter aquaria. Each aquarium contained 9 liters of water which was continuously replaced at 50 ml/minute. Water from each of the aquaria overflowed via a standpipe into an identical set of six aquaria which contained 60 amphipods each.

The food which was used in this study was Purina Trout Chow #2 pellets. The food was ground with a mortar and pestle and then contaminated with HCB at concentrations of 1.45, 14.5, and 145 ug/g dry weight by adding known quantities of stock solution in hexane to 1 g lots of food. Excess solvent was removed by evaporation under vacuum and under a nitrogen stream. Uncontaminated food was treated with the hexane only.

One particle of the contaminated food was added daily for each amphipod in the upper set of aquaria. Amphipods were allowed access to the contaminated food particles for

two hours after which any unconsumed food was removed. Organisms in the lower set of aquaria were fed only uncontaminated food which was also removed after two hours. The number of food particles added to both sets of aquaria was adjusted for the number of amphipods remaining after previous sampling activities.

Hexachlorobiphenyl accumulation by amphipods in the upper set of aquaria was assumed to be from the contaminated food and/or by direct uptake from water of HCB that had desorbed from the food. Residues in the amphipods from the lower set of aquaria were assumed to be from water only.

At 24, 48, 96, and 192 hours of exposure and prior to feeding, 16-20 amphipods were removed from each of the upper aquaria. Each amphipod sample was divided into two lots; one was used for whole body residue analysis and the second for separate determinations of residues in the body tissues and as unabsorbed gut contents. Amphipods (8-10) were also collected at these times from each of the lower aquaria.

Water samples (1 liter) were obtained from each aquarium on a similar schedule but were taken two hours after the food was presented.

Availability from Substrates

The availability of HCB to amphipods from artificially contaminated natural substrates was studied to determine the role of differences in substrate type, particle size distribution, individual particle size fractions, and the quantity

of organic matter within each soil type or particle size fraction. The HCB availability experiments may be separated into two phases based on whether intact or sieved substrates were used; each phase will be treated separately because of procedural differences.

Intact Substrates. The availability of HCB from unfractionated substrates was studied in 20 liter glass aquaria which were arranged in two tiers of six aquaria each. Each of the upper tier aquaria contained 800 g of substrate and 9 liters of water which was replaced by clean well water at the rate of 50 ml/minute (90% replacement time = 7 hours, Spraque 1969). Flow rates were checked daily and adjusted as necessary. Water levels in the upper aquaria were maintained by surface overflow standpipes; excess water from each upper aquarium flowed through a short length of glass tubing into one of the lower aquaria which did not contain substrates. Water levels in the lower aquaria were maintained at 9 liters by surface overflow standpipes through which excess water was discharged to floor drains. Accumulation of HCB by amphipods in the upper aquaria was assumed to be the result of direct uptake from water of HCB that had desorbed from the substrate, substrate ingestion, and/or by direct contact of amphipods with the substrate while accumulation in the lower tanks was assumed to result from water uptake only.

Two different natural substrates were used in the availability studies. One was obtained from a riffle in Cheny Creek, Mecosta County, Michigan while the other was dredged from a pond in the Fenner Arboretum, Ingham County, Michigan. Prior to use, both substrates were dry sieved so that only particles <2.0 mm were utilized. Cheny Creek substrate consisted primarily of washed coarse and medium sand (organic content = 0.67%) while the Arboretum Pond substrate consisted primarily of medium and fine sand (organic content = 4.61%).

At the start of each experiment, 800 g (dry wt.) of Cheny Creek or Arboretum Pond substrate were added to each of three replicate aquaria. To each substrate, 800 ug of HCB were added in excess acetone until the substrates were saturated. The acetone was allowed to evaporate until the substrates were dry. The nominal HCB concentration in substrates was thus 1 ug/g; radiolabeled HCB represented 1% of the total HCB. Well water was slowly added so as to minimize disturbance of the substrates, continuous flow of clean well water was initiated (50 ml/minute), and the system allowed to equilibrate for 7 - 15 days prior to the addition of 50-60 amphipods per aquarium.

Four trials were conducted as described below. During each trial 7-10 amphipods were dipnetted from each aquarium after 24, 48, 96, and 192 hours of exposure, placed in glass vials, and frozen until analyzed.

During trial 1, only the upper tier of aquaria was used. The system was allowed to equilibrate for 12 days prior to amphipod exposure. Water samples (1 liter) were taken after 24, 48, 96, and 192 hours of equilibration; because residue levels were below the limit of detection (0.0145 ug/liter) water sampling was discontinued during the period of amphipod exposure.

For trial 2, the lower tier of aquaria was installed and the substrates from trial 1 were reutilized. No water samples were taken.

Prior to use in trial 3, Cheny Creek and Arboretum Pond substrates were combusted at 500°C for eight hours to remove all organic material. The substrates were then distributed to the aquaria, contaminated, and allowed to equilibrate for 15 days. Water samples were taken after 24, 48, 96, and 192 hours of equilibration and after 24, 48, 96, and 192 hours of amphipod exposure.

For trial 4, only Cheny Creek substrates were utilized. Experimental design was modified in that the two tiers of aquaria contained only four aquaria each; only the upper aquaria contained substrate. The standpipes on two of the upper aquaira were adjusted so that only 0.8 liters of water covered the substrates (water to substrate ratio = 1:1, wt./wt.) while the standpipes in the other two aquaria maintained a water level of 9 liters (water to substrate ratio = 11.25:1, wt./wt.). Upper aquaria overflowed into

lower aquaria which contained 9 liters of water. The system was allowed to equilibrate for seven days. After 24, 48, 96, and 192 hours of amphipod exposure, two water samples were obtained from each of the 9 liter upper tier aquaria to determine if a vertical gradient in HCB concentration existed; one was siphoned from just below the surface and the other from just above the substrate. No water samples were obtained from the 0.8 liter aquaria. One middepth water sample was obtained from each of the four lower tier aquaria.

Nominal HCB concentration in the substrates was 1 ug/g for all trials but the percentage of radiolabeled HCB was increased from 1% in trials 1-3 to 2.44% in trial 4 to lower the limits of HCB detectability.

At the end of trials 2, 3, and 4 the substrates were removed from the aquaria, placed in aluminum pans, and frozen until analyzed for HCB residues. Excess unextracted substrate from replicate aquaria of trials 2 and 3 which remained after residue determination were composited by substrate origin (Cheny Creek or Arboretum Pond) and trial, then fractionated by dry sieving to determine particle size distributions; the organic matter content and HCB residue concentrations in each particle size fraction were also determined.

Sieved Substrates. The availability of HCB to amphipods as a function of substrate particle size and organic matter content was determined in 2 liter glass beakers. The beakers were equipped with glass standpipes which maintained the water level at 1.5 liters. Well water flowed continuously into each beaker at 30 ml/minute which gave a 90% replacement time of about two hours (Spraque 1969).

Cheny Creek and Arboretum Pond substrates were wet and dry sieved (twice each) into five particle size fractions per substrate. The particle size fractions, in millimeters, used in the experiment were; 1.0-0.50, 0.50-0.25, 0.25-0.12, 0.12-0.06 and less than 0.06.

Prior to an experiment, four aliquots of known weight of each size fraction were placed in individual beakers. A known quantity of HCB (2.44% ^{14}C) in acetone (50-100 ml) was added to each beaker, swirled to insure uniform mixing, and the beakers set aside for 3-5 days for acetone evaporation. Water (1400 ml) was slowly added to each beaker so as to minimize substrate disturbance and allowed to stand for 12-24 hours. Continuous flow was then initiated and the system allowed to equilibrate for an additional 24 hours at which time 15 amphipods were added to each beaker.

At 24, 48, 96, and 192 hours of amphipod exposure, water samples (1 liter) were siphoned from one beaker of each particle size fraction, the excess water was discarded, and amphipods were separated from the substrates. Amphipods and substrates were frozen until analyzed for HCB residue

concentrations.

Five trials were conducted using this design; differences in experimental conditions are summarized in Table 2.

Prior to trials 3 and 4, the particle size fractions of each substrate were combusted at 500°C for eight hours to remove organic materials. During trial 5 preleached, dried maple leaves were chopped in a meat grinder and a Wylie mill, then dry sieved twice to obtain the five particle size fractions.

Amphipods were not fed during any of the trials but were free to graze on organic materials within the substrates.

Sample Extraction and Analysis

Water samples (2 liters) from the model streams study were extracted with three successive additions of hexane (200, 100, 100 ml); the combined extracts were evaporated under vacuum to 20 ml and three 0.5 ml aliquots were counted in scintillation vials which contained 15 ml scintillation fluor (3a20 fluor, Research Products International Corp.). Water samples (1 liter) from laboratory experiments were handled in the same manner but hexane volumes were proportionately reduced. Final HCB concentrations were corrected for isotope dilution. Recovery of HCB from fortified water samples averaged 106.2%.

Substrate samples were thawed and air dried, then 50 g subsamples were extracted in glass chromatographic columns with acetone:hexane (1:1, v/v), (U.S.E.P.A. 1977), and

Table 2. Summary of procedural differences for experiments on HCB availability from sieved substrates.

	Trial Number				
	1	2	3	4	5
	Substrate Origin and Condition				
	Cheny	Arboretum	Arboretum	Cheny	Maple leaves
	natural organic levels	natural organic levels	inorganic materials only	inorganic materials only	high organic content
	Substrate Dry Weight (g)				
Fraction (mm)					
1.00-0.50	100	100	100	100	15
0.50-0.25	100	100	100	100	15
0.25-0.12	100	100	100	100	15
0.12-0.06	15	20	100	15	15
< 0.06	10	20	100	10	15
	Nominal HCB Concentrations (ug/g)				
1.00-0.50	1.0	1.0	1.0	1.0	1.0
0.50-0.25	1.0	1.0	1.0	1.0	1.0
0.25-0.12	1.0	1.0	1.0	1.0	1.0
0.12-0.06	1.0	1.0	1.0	1.0	1.0
< 0.06	1.0	1.0	1.0	1.0	1.0

prepared for counting as described above. Recovery from fortified samples averaged 82.7%.

Small invertebrate organisms were blotted to remove excess moisture, then homogenized in glass tissue grinders containing 2 ml acetone. The homogenates were centrifuged for 5-10 minutes, the supernatant transferred to centrifuge tubes, evaporated to 2.0 ml under a nitrogen stream, and prepared for counting. The extraction efficiency of fortified amphipod samples was 101%; the extraction efficiency was considered to be the same for other small invertebrates.

Clams, crayfish, and fish were individually weighed, and then homogenized in a mortar and pestle with anhydrous sodium sulfate. The homogenate was extracted for four hours with 200 ml hexane in a Soxhlet apparatus; the extract was concentrated to 2 ml volume, and cleaned up on a Florisil column (700 mm x 22 mm glass chromatographic column which contained 10.2 cm Florisil activated at 130°C.). The samples were eluted from the columns with 200 ml of ethyl ether in hexane (6:94, v/v). The eluent was concentrated to 2 ml volume and three 0.5 ml aliquots prepared for counting. Recovery from fortified samples of fish, crayfish, and clams averaged 90.47, 69.94 and 76.80%, respectively.

Plant materials, i.e., algae, bryophytes, periphyton, and watercress, were oven dried (50°C) for 24 hours and then 100 mg (dry weight) subsamples were combusted in a Nuclear Chicago, semiautomated combustion apparatus. The $^{14}CO_2$

was captured in a 10 ml solution of 2-methoxyethanol: ethanolamine (2:1, v/v). A 5 ml aliquot was pipetted into a vial containing 10 ml scintillation cocktail (3a20 fluor: 2-methoxyethanol, 2:1, v/v) and counted. Hexachlorobiphenyl residue recovery from fortified samples averaged 71%, 39.1%, and 50% for algae, bryophytes, and watercress respectively. The reported concentration values were not corrected for extraction efficiency.

All samples were counted for 10 minutes on a Nuclear Chicago Mark I liquid scintillation counter. Counting efficiency was determined with a series of quenched standards and the external standard method for quench correction.

Metabolite Analysis

A 0.5 ml aliquot of the extract of samples collected after 24 and 48 hours and 30 days of exposure to HCB in the model streams was concentrated to 0.1 ml from which 10-30 ul were spotted on a Silica Gel GF_{250} thin layer plate. The plate was eluted to 10 cm with redistilled hexane and developed under U.V. light; identified spots were compared to co-chromatographed standards. The developed thin layer plates were exposed to Medical No Screen X-Ray film (Kodak) for 30-60 days after which the films were developed. Areas of the film exposed to radioactivity appeared as dark spots on the film. Suspected metabolites were then counted by scraping 1 cm zones from the thin layer plate into scintillation vials to which 15 ml of 3a20 fluor was added. The

vials were capped, labeled, refrigerated, and counted as outlined above. No attempt was made to identify suspected metabolites other than comparison to known standards which were co-chromatographed on the thin layer plate.

RESULTS AND DISCUSSION

The Model Streams

The laboratory streams served as excellent models of a riffle segment in a small natural stream. In essence, the model stream channels were modified extensions of Cheny Creek. The flow-through design of the system permitted the continuous import and export of small organisms and of dissolved and particulate inorganic and organic materials. Water chemistry, temperature, and the biotic communities in the laboratory streams were similar to those found in Cheny Creek.

The trophic status of the model stream communities was modified by physical constraints which were imposed on the laboratory system. Stream flow-discharge was regulated at a constant 60 liters/minute which eliminated periodic spates and in turn permitted the establishment of an autotrophic community dominated by filamentous algae and watercress throughout much of the year. The important effect of controlled flow-discharge regimes on the dominance of primary production in running water has been described by Cummins (1974). Additionally, the screened water intake to the streams prevented entry of large aquatic organisms and coarse organic materials (e.g., leaves) which are usually associated with heterotrophic conditions in small first-order streams.

Chemical Fate

Water. The mean concentration of hexachlorobiphenyl (HCB) in the water samples from each treatment phase ranged between 0.04 and 0.18 ug/liter (Table 3). Eighty-five percent of the measured values were equal to or less than the nominal value (0.100 ug/liter) and 15% exceeded this value. The concentration in stream 3 was usually higher than in stream 2, but the concentrations in both streams followed the same pattern of variation except for a few isolated samples.

Five individual samples which had concentrations exceeding 0.20 ug/liter were probably the result of HCB associated with particulate material suspended in the stream water. Excluding these few values, the mean HCB concentrations in samples from all treatment phases were 0.04 and 0.05 ug/liter for streams 2 and 3, respectively.

During the depuration phases, the HCB concentrations in the stream water decreased below the limits of detection (0.006 ug/liter) within 4 to 8 days after treatment ended.

Only 40 to 50% of the nominal concentration of HCB was found in the water samples; of the remainder, most was probably adsorbed onto stream sediments and biota, but some may have accumulated at the air-water interface and was either discharged in the effluent or volatilized to the atmosphere. Oloffs et al. (1972) reported that significant losses of chlorinated hydrocarbons occurred by evaporation

Table 3. Mean concentrations of HCB (ug/liter) in water from treated streams during the treatment phase of each experimental period.

	Experimental Period			
	Fall	Winter	Spring	Summer
STREAM 2				
Number of samples	9	9	9	9
Mean	0.043	0.045	0.149	0.051
Standard deviation	0.044	0.034	0.150	0.107
STREAM 3				
Number of samples	10	9	9	9
Mean	0.072	0.069	0.180	0.049
Standard deviation	0.096	0.066	0.376	0.023
Mean	0.057	0.057	0.165	0.050

from natural waters in the absence of sediments but atmospheric losses were negligible when natural sediments were present (Oloffs et al. 1973). Low solubility compounds like HCB would be expected to adsorb rapidly onto suspended particulates and onto bottom sediments (Baughman and Lassiter 1978). Haque et al. (1974) concluded that the concentration of PCBs in water will be reduced whenever water comes in contact with a solid surface and that the type and degree of surface area, and the organic content of the solid will be important factors.

Higher concentrations of HCB in stream 3 may have been caused by undetected leakage of stock solution from the base of the syringe which supplied stream 2. Both syringes emptied at equal rates and required equal amounts of replacement stock solution when refilled daily. Small chemical deposits were observed on both syringes at the point where the plunger entered the barrel. Because of the very concentrated nature of the stock solution, even very slight loss differences between the syringes could have affected the concentration in the water.

Substrate. Hexachlorobiphenyl concentrations in the stream substrates averaged less than 0.1 ug/g sediment dry weight (Table 4). Mean HCB concentrations for a particular sampling day varied between streams and with season of treatment; the concentrations ranged from 0.11 to 0.182 ug/g during the 30-day treatment phases and from 0.004 to 0.073

Table 4. Concentrations (ug/g dry wt.) and regression equations for accumulation and depuration of HCB in the stream substrates. Each value represents the mean of four samples.

Experimental Period	HCB Concentration Treatment Phase (days) 5	15	30	Regression Equation
Fall	0.011	0.037	0.182	y=-0.041+0.007X
Winter	0.019	0.037	0.036	y=0.020+0.0006X
Spring	0.049	0.060	0.074	y=0.044+0.001X
Summer	0.029	0.051	0.081	y=0.019+0.002X
Mean (Std. dev.)	0.027 (0.024)	0.046 (0.030)	0.093 (0.125)	
	Depuration Phase (days) 15	30	60	
Fall	0.017	0.027	0.027	y=0.176-0.002X
Winter	0.035	0.004	0.014	y=0.047-0.0004X
Spring	0.073	0.071	0.053	y=0.088-0.0004X
Summer	-----	-----	-----	----------
Mean (Std. dev.)	0.041 (0.039)	0.034 (0.040)	0.031 (0.026)	

ug/g during the 60-day depuration phases.

The accumulation and depuration of HCB residues in stream substrates were time dependent as concentrations increased progressively during the treatment phase and decreased with time during each depuration phase. Equilibrium was not approached during the treatment phases except perhaps in winter when residue concentrations on days 15 and 30 were nearly equal. Higher concentrations occurred in stream 3 substrates than in stream 2 which was consistent with the difference in HCB concentration in the water of the two streams.

The partition coefficients for HCB residues in the substrates (HCB concentration in substrates (ng/g)/mean seasonal HCB concentration in water (ug/liter)) on day 30 of the fall, winter, spring and summer treatments were 3193, 632, 448, and 1620 respectively. These values are in general agreement with data in the literature for other chlorinated hydrocarbons. Baughman and Lassiter (1978) reported that partition coefficients for two tetrachlorobiphenyl isomers ranged from 420-1270 for four natural sediments tested in the laboratory. Concentrations of DDT and its metabolites in the bottom mud of a drainage ditch from an agricultural area were 820-13,000 times the concentration in the water (Miles and Harris 1971). The bottom muds of a lake which had been treated with toxaphene one year previously contained concentrations 1000 times those detected in the water (Johnson

et al. 1966) while contemporary Lake Erie sediments accumulated aldrin levels 620 times higher than water concentrations within 20 minutes of exposure in a laboratory test (Leshniowsky et al. 1970).

The mean percent organic matter of model stream substrates varied from 0.88 to 2.71%. When HCB residue calculations were based on substrate organic matter content, concentrations increased linearly with duration of treatment and decreased in a nearly linear manner during depuration (Figure 2). Hexachlorobiphenyl residues and organic matter content were highly correlated in day 30 samples (r= 0.91) and less so in samples obtained after other durations of treatment or depuration (Table 5). The importance of sediment organic matter content in the adsorption or accumulation of hydrophobic organic compounds has been reported by a number of investigators (Karickhoff et al. 1979; Steen et al. 1978; Herbes 1977; Dimond et al. 1974; Leland et al. 1973).

Regression equations were computed for each phase of treatment and depuration for HCB residue concentrations in the substrate as a function of time (Table 4). The maximum rate of accumulation (slope) occurred during the fall treatment phase and was slightly higher than the rates occurring in other treatment phases. This may be due to the previously uncontaminated nature of the substrates at the beginning of the fall treatment phase, whereas the substrates at the

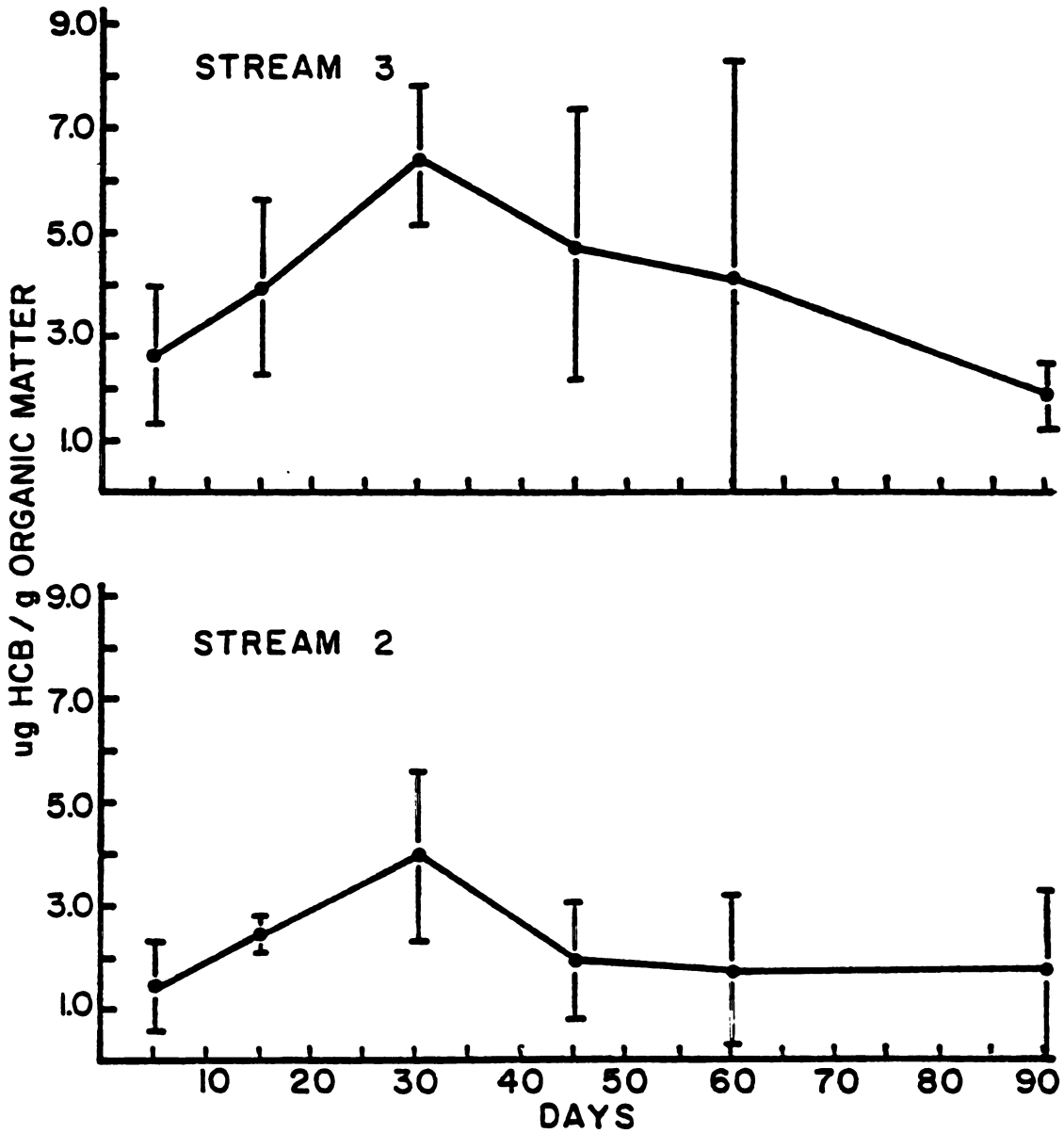

Figure 2. Uptake and depuration of hexachlorobiphenyl in the organic matter of the laboratory stream substrates. Data points are averages for each stream which were then averaged across experimental periods. Vertical bars indicate standard errors about the mean.

Table 5. Mean HCB concentrations (ug/g dry wt.) and organic matter content (%) in the laboratory stream substrates and correlation (r) of residue concentration with organic matter. Standard deviation in parentheses.

Sampling Day	Number of Samples	Organic Content (%) Mean	Organic Content (%) Range	HCB (ug/g) Mean	HCB (ug/g) Range	r
Treatment Phase						
5	16	1.51 (0.65)	0.71-2.59	0.027 (0.024)	0.006-0.112	0.32
15	16	1.54 (0.68)	0.65-2.75	0.046 (0.030)	0.008-0.104	0.45
30	16	1.56 (0.96)	0.85-4.73	0.093 (0.125)	0.014-0.544	0.91***
Depuration Phase						
15	12	1.19 (0.47)	0.78-2.53	0.041 (0.039)	0.005-0.132	0.72**
30	12	1.07 (0.27)	0.66-1.56	0.034 (0.040)	0.002-0.139	0.42
60	12	1.81 (0.78)	0.70-3.35	0.031 (0.026)	0.007-0.099	0.17

**P < 0.01
***P < 0.001

beginning of each subsequent treatment phase contained some residual HCB from the previous treatment. The y intercept values for the regression equations reflect the carry-over of residual HCB concentrations from one experimental period to the next. Hexachlorobiphenyl concentrations in the spring depuration phase were higher than in the winter phase but the rates of depuration were essentially the same.

Biota. All residue data for the biota are presented on a wet weight basis. As plant materials were analyzed on a dry weight basis, a dry weight to wet weight conversion was necessary to facilitate comparison with other data. Water content of aquatic plant materials was considered to be 92%; this value is intermediate between those given by Westlake (1965), Wetzel (1975), and Dr. Clarence McNabb (Department of Fisheries and Wildlife, Michigan State University, personal communication).

The stream biota rapidly accumulated HCB residues during the initial 24 hours of the fall treatment phase (Table 6). Highest residue concentrations were found in Baetis mayfly nymphs while the lowest concentrations were observed in plant materials.

During subsequent treatment periods, HCB concentrations increased after three hours of exposure, but then showed a slight decrease after six hours. Residue levels in the biota appeared to stabilize at this time as there was little difference in HCB concentrations within a given species in

Table 6. Hexachlorobiphenyl concentrations (ug/g wet wt.) accumulated by the benthos during the initial 24 hours of each treatment phase. Each value is a mean obtained by averaging values from the individually treated streams. Except for the fall treatment, the pre-exposure concentrations are day 60 values from the prior depuration period.

Organism	Treatment Phase	Pre-exposure conc. (ug/g)	Duration of Exposure (hours) 3	6	12	24
Bryophyte	Spring	0.41	0.00*	0.12*	0.18*	0.20*
	Summer	0.02	0.15	0.28	0.21	0.97
	Mean	----	0.07	0.20	0.19	0.59
Algae	Winter	0.07	0.04*	0.03*	0.14*	0.20*
	Spring	0.03	0.09	0.81	1.10	1.61
	Summer	0.07	0.27	1.01	0.29	0.17*
	Mean	----	0.13	0.62	0.51	0.66
Periphyton	Winter	----	0.13	0.00	0.29	0.00
	Spring	----	0.16	0.09	0.26	0.31
	Summer	----	0.07	0.05	0.13	0.27
	Mean	----	0.12	0.05	0.23	0.19
Gammarus pseudolimnaeus	Fall	0.00	1.65	2.25	1.95	2.89
	Winter	1.89	3.55	2.63	3.27	1.65
	Spring	6.97	8.37	6.23	4.73	6.90
	Summer	3.25	7.94	7.17	6.63	6.37
	Mean	----	5.38	4.57	4.15	4.45
Physa sp.	Fall	0.00	0.77	0.91	----	1.77
	Winter	0.69	1.07	0.93	1.03	0.83
	Spring	1.61	2.35	1.51	2.70	1.73
	Summer	0.59	0.33	0.65	0.73	0.87
	Mean	----	1.13	1.00	1.49	1.30
Hydrobia sp.	Fall	0.00	0.51	0.43	----	0.33
Baetis sp.	Fall	0.00	13.27	6.11	----	17.75*
	Winter	0.83	----	4.05	----	8.45
	Mean	----	----	5.08	----	13.10
Nemouridae	Spring	4.99	6.77	4.69	5.23	6.03

* Stream 2 samples only

the 6-, 12-, and 24-hour samples.

Concentrations of HCB residues in the stream biota increased with time during the treatment phase and decreased during the depuration phase (Table 7). In plant samples, highest concentrations were generally observed on day 30 of the treatment phase while concentrations in the fauna were usually the highest on day 4 of the depuration phase. Very high residue levels were detected in samples of Baetis mayflies and snails of the genus Hydrobia; residue levels in the mayflies were highly variable, possibly as a result of their small sample weights. Samples of bryophytes and periphyton had consistently low concentrations of HCB when compared to other benthic samples; concentrations in the bryophytes were similar to those in periphyton regardless of exposure duration.

Residue concentrations were similar from season to season on a given sampling day for any particular component of the benthos with the possible exception of algae which had higher concentrations during the winter and spring treatment phases than during the summer treatment. Residue concentrations in samples from stream 3 were consistently higher than similar samples from stream 2 (Table 8). However, when the two streams were compared, residues were distributed among the various components of the benthos in a similar manner.

Table 7. Mean HCB concentrations (ug/g wet wt.) in the benthic flora and fauna during the treatment and depuration phases of the model streams study. Each value was obtained by averaging the mean values for each experimental period (season).

	Treatment Phase (days)						Depuration Phase (days)				
Organism	1	2	4	8	16	30	4	8	16	33	60
Bryophyte	0.51	0.43	0.51	0.93	0.38	1.46	0.56	0.30	0.45	----	0.21
Algae	0.66	1.65	3.40	6.69	4.03	11.81	7.00	5.27	3.03	0.45	0.06
Periphyton	0.19	0.18	0.40	0.43	0.59	0.94	----	----	----	----	----
Gammarus pseudolimnaeus	4.45	6.80	6.15	8.70	8.23	11.60	13.55	11.77	10.41	5.99	4.04
Physa sp.	1.30	2.64	1.90	2.95	3.45	5.02	4.39	3.29	2.54	1.12	0.96
Lumbriculidae	----	----	1.40	2.52	1.97	2.90	----	3.75	2.80	2.07	1.45
Hydrobia sp. Fall only	0.33	2.30	1.83	----	13.00	23.66	22.61	24.17	----	----	----
Baetis sp. Fall only	17.75*	53.91	64.42	42.19	84.76	37.72	----	----	----	----	0.83

* Stream 2 sample only

Table 8. Mean HCB concentrations (ug/g) in the benthos of individual streams on day 30 of the treatment phase. Except where indicated the mean values were obtained by averaging single values for a composite sample across experimental periods. Values for clams, crayfish and fish are the grand means of the experimental period means of single individuals.

	Stream Number	
	2	3
Primary Producers		
Algae	7.55	15.78
Bryophytes	0.59	2.33
Periphyton	0.38	1.49
Herbivores		
Physa sp.	4.85	5.28
Nemouridae	14.70	30.63
Detritivores		
Gammarus pseudolimnaeus	6.92	16.29
Orconectes virilis+	3.03	5.45
Lumbriculidae	1.84	3.96
Strophitis rugosus+	0.31	0.38
Carnivores		
Cottus bairdi+	2.22	3.52

+ Grand means

Residual concentrations of HCB were detectable in all benthic species after 60 days of depuration and were higher in invertebrate samples than in plant materials (Table 7). Despite the presence of HCB residues in the benthos from prior treatment phases, there was no indication of a consistent stepwise increase in residue concentration as a result of multiple exposures to the chemical. There was no evidence of HCB increasing through trophic level magnification (Table 8).

The rate of accumulation of HCB residues was most rapid during the initial three hours of treatment and was slowest during the day 1 to day 30 interval (Table 9). Good reproducibility was observed in the rate of accumulation within a given component of the benthos for the different seasonal treatment phases. Arthropods had the highest short-term uptake rates followed by mollusks while plant materials had the slowest short-term uptake rates. Baetis mayflies, Hydrobia snails and algae had the highest long-term (day 1 - day 30) uptake rates.

Long-term rates of accumulation and depuration of HCB residues in the various organisms were also compared using linear regression equations (Table 10). The values which were used in the regression analysis were the mean concentrations for a given duration of treatment or depuration averaged over seasons and did not include samples taken prior to 24 hours of exposure.

Table 9. Rate of accumulation of HCB (ug/g/hour) in the stream benthos over several intervals of time for each treatment phase. Values were calculated as the change in HCB concentration divided by the corresponding time interval (hours)

Organism	Time		
	Hours 0-3	Hours 3-24	Days 1-30
Gammarus pseudolimnaeus			
Fall	0.55	0.06	0.02
Winter	0.55	-0.09	0.01
Spring	0.47	-0.07	0.01
Summer	1.56	-0.07	0.00
Nemouridae			
Spring	0.59	-0.03	----
Baetis sp.			
Fall	4.42	0.21	0.03
Physa sp.			
Fall	0.26	0.05	0.01
Winter	0.13	-0.01	0.00
Spring	0.25	-0.03	0.01
Summer	-0.09	0.03	0.01
Hydrobia sp.			
Fall	0.17	-0.01	0.03
Algae			
Winter	-0.01	0.01	0.01
Spring	0.02	0.07	0.03
Summer	0.07	-0.01	0.00
Bryophytes			
Spring	-0.14	0.01	0.00
Summer	0.04	0.04	0.00
Periphyton			
Winter	0.04	-0.01	0.00
Spring	0.05	0.01	0.00
Summer	0.02	0.01	0.00

Table 10. Regression equations calculated for the accumulation and depuration of HCB in the stream benthos over time. Correlation coefficients (r) indicate goodness of fit. Data points for calculating the equations were the mean concentrations for a given sampling day.

Organism	Accumulation (Days 1-30) Regression Equation	r	Depuration (Days 1-60) Regression Equation	r
Bryophyte	y = 0.41 + 0.03x	0.80	y = 0.479 - 0.004x	0.69
Algae	y = 1.39 + 0.33x	0.91	y = 5.99 - 0.117x	0.89
Periphyton	y = 0.20 + 0.02x	0.78	No data	
Gammarus pseudolimnaeus	y = 5.61 + 0.20x	0.91	y = 13.42 - 0.17x	0.94
Physa sp.	y = 1.76 + 0.11x	0.94	y = 3.40 - 0.05x	0.98
Hydrobia sp.*	y = -0.03 + 0.80x	0.99	Insufficient data	
Baetis sp.*	y = 47.65 + 0.24x	0.10	Insufficient data	
Nemouridae	y = 5.76 + 0.31x	0.90	y = 16.35 - 0.19x	0.78

* Data from the fall treatment phase only

Hydrobia snails accumulated HCB most rapidly (greatest slope) followed in decreasing order by algae, Nemouridae, Baetis, Gammarus, Physa, bryophytes, and periphyton. Data for all organisms showed a reasonably good fit to the regression lines with the exception of Baetis. The high y-intercept values for Baetis, Nemouridae, and Gammarus reflect rapid short-term accumulation and/or high residues from previous treatments. Hexachlorobiphenyl was eliminated by the benthos in a linear manner during each 60-day depuration phase; Nemouridae had the most rapid rate of depuration.

Based on the data presented, accumulation of HCB appears to occur in two phases; a rapid short-term uptake followed by a slower long-term accumulation. Matsumura (1975) suggests that such a biphasic relationship can be explained by quick absorption of the chemical by the insect cuticle which is then followed by a slower penetration into the body. Lipid solubility of the chemical will determine the extent of initial accumulation by cuticular lipids whereas aqueous solubility will determine the rate and amount of penetration through cuticular lipids to an aqueous body fluid (Matsumura 1975). Chitin has been shown to be the major factor influencing the uptake of DDT by insects and other arthropods (Richards and Cutkomp 1946) and may explain why the arthropods accumulated more HCB than mollusks, fish, or plant samples.

The elimination of HCB residues from the benthos is probably the result of slow partitioning of the surface-bound residues into the surrounding water. Deeper tissues

in the organisms would lose residues at a slower rate and thus remain contaminated for a longer time. If elimination occurs in this manner then surfaces of the organism would be relatively unsaturated at the end of the 60-day depuration phase and would allow a rapid surface sorption of HCB when the subsequent treatment phase was initiated.

Hexachlorobiphenyl residues were unequally distributed in the various tissues of the watercress plants (Table 11). All tissues accumulated HCB residues but for any given duration of treatment the leaves had the highest concentrations followed in decreasing order by roots, stems, and seed pods. Concentrations in plant tissues failed to show a consistent trend with time during the treatment phases but residue levels decreased on successive sampling days during the depuration phases.

The distribution of residues in the watercress tissues may result from differences in surface area available for sorption. Partitioning of HCB into the plant tissues directly from water was probably the primary mechanism of accumulation but translocation from the substrates and storage in the various tissues may have been an important route for contamination. Meeks (1968) reported that submerged vegetation usually had higher residues of DDT than emergent plants in a field study while Witcamp (1960) reported that the quantity of radionuclides per unit of surface area increased as the leaf surface area increased.

Table 11. Hexachlorobiphenyl concentrations (ug/g wet wt.) in tissues of watercress plants (Nasturtium officinale). Each value is the grand mean of experimental period means.

Plant Tissue		Treatment Phase Days 5	10	20	30	Depuration Phase Days 4	15	32	60
Leaves	Mean	0.94	3.00	4.42	3.15	----	4.39	0.51	0.78
	(Standard error)	----	(1.63)	(3.18)	(0.89)	----	----	----	(1.13)
Stems	Mean	0.20	0.72	0.69	0.75	----	0.39	0.18	0.14
	(Standard error)	----	(0.65)	(0.53)	(0.21)	----	----	----	(0.05)
Roots	Mean	0.93	1.41	1.35	2.61	----	0.39	0.14	0.10
	(Standard error)	----	(0.73)	(0.41)	(2.07)	----	----	----	(0.06)
Seed Pods	Mean	----	0.57	0.55	0.55	----	----	----	----
	(Standard error)	----	(0.63)	(0.29)	(0.12)	----	----	----	----

Bioaccumulation. The terms bioconcentration and bioaccumulation have often been used synonymously in the literature. For the purpose of clarity, the term bioaccumulation will be used in this report when uptake of residues by organisms may have occurred through multiple routes of exposure (i.e., food, water and/or direct contact with contaminated substrates) while the term bioconcentration will be used only when residue levels are known to have resulted from direct uptake from water.

Bioaccumulation factors were calculated for each group of organisms in the model streams as the ratio of the 30-day HCB concentration in the organisms to the mean HCB concentration in water for each stream (Table 12). In general, amphipods had the highest bioaccumulation factors which ranged from 66,000 to 300,000 while clams had the lowest bioaccumulation factors which ranged from 1300 to 16,000. The remainder of the benthos accumulated HCB residues between 10,000 to 100,000 times the mean measured water concentrations.

Values for the bioaccumulation factors were similar for a given organism during the fall, winter and summer treatments but were lower during the spring treatment primarily as a result of higher concentrations detected in the water rather than a decline in residue levels in the benthos.

The bioaccumulation factors for the benthos of stream 3 were generally higher than those from stream 2; this observation is consistent with the other data on HCB residues in the water, substrates, and benthic organisms.

Table 12. The range of HCB bioaccumulation factors for organisms in the laboratory streams. Ranges reflect seasonal variations.

Organism	Stream 2	Stream 3
Algae (*Rhizoclonium* sp.)	21,000 - 140,000	41,000 - 253,000
Bryophyte (*Fontinalis* sp.)	6,600 - 8,000	6,000 - 60,000
Periphyton	2,000 - 12,000	4,800 - 31,000
Snail (*Physa* sp.)	26,000 - 149,000	44,000 - 78,000
Amphipod (*Gammarus pseudolimnaeus*)	66,000 - 233,000	69,000 - 298,000
Crayfish (*Orconectes virilis*)	22,000 - 125,000	18,000 - 134,000
Annelid (Lumbriculidae)	15,000 - 47,000	18,500 - 104,000
Clam (*Strophitis rugosa*)	2,000 - 11,000	1,300 - 16,100
Fish (*Cottus bairdi*)	16,500 - 55,000	22,000 - 77,000

Direct comparison of these results with those of others is difficult due to the paucity of published studies with individual PCB isomers. However, some meaningful comparisons can be made with laboratory and field studies on closely related compounds. Metcalf et al. (1975) have conducted model ecosystem studies with tri-, tetra-, and pentachlorinated biphenyls. In their results, algae generally had the lowest "ecological magnification factors" (5,400-18,000) whereas snails were the highest (5800-59,600) and fish ranged between 6,400 and 12,100. These authors concluded that ecological magnification factors increased with increasing chlorine content of the isomers. The lower range of the model streams bioaccumulation factors (Table 12) tends to overlap the values presented by Metcalf et al. (1975) but the upper range of the model streams data is approximately one order of magnitude higher. These results are expected because of the higher chlorine content and the lower water solubility of HCB.

Differences in the design and operation of the model streams and the Metcalf ecosystems may also account for the higher concentrations found in the model streams. The primary differences were: flow-through vs. static design, direct toxicant introduction vs. trophic transfer, different species, and different exposure durations (e.g., fish in the model streams were exposed for 30 days compared to 3 days in the model ecosystems).

In the laboratory model streams, clams consistently had the lowest bioaccumulation factors compared to other benthic organisms. Duke et al. (1970) indicated that oysters were excellent indicators of Aroclor 1254 contamination in seawater, despite the fact that they contained lower residue concentrations than fish, shrimp, and crabs. Vreeland (1974) reported that oysters concentrated HCB 48,000 times the level in seawater. This is much higher than the value calculated for the freshwater clams and is probably due to differences in exposure conditions. Freshwater mussels concentrated DDT and dieldrin from lake water 2,400 and 1,200 times respectively (Bedford and Zabik 1973) which agrees more closely with the values for HCB.

Fish in the model streams had 30-day bioaccumulation factors which ranged from 16,500 to 77,000; the variation reflects seasonal and stream differences. These values are higher than those reported for fish ($\sim$12,000) exposed to tetra- and penta-chlorobiphenyl in model ecosystems (Metcalf et al. 1975) but are much lower than values reported for fathead minnows exposed to Aroclor 1248 and 1260 (120,000 and 270,000 respectively) (Defoe et al. 1978). Stalling and Mayer (1972) reported that bluegill sunfish had bioconcentration factors ranging from 26,300 to 71,400 when chronically exposed to Aroclors 1248 and 1254 while Branson et al. (1975) calculated a bioconcentration factor of 28,700 for rainbow trout exposed to 2,2',4,4'-tetrachlorobiphenyl.

These last two studies agree well with the values for bioaccumulation attained by sculpins in the model stream study.

Metabolite Analysis

Unlabeled and ^{14}C-labeled standards of HCB which were spotted on thin layer plates and developed with hexane had a mean R_f value of 0.41 (S.D. = 0.03). Residues in sample extracts were not visible on the chromatographs when viewed under U.V. light but were detected on x-ray film after exposure for 30 to 60 days. Hexachlorobiphenyl residues from sample extracts had the same R_f value as did co-chromatographed unlabeled and radiolabeled standards.

Positive radioautographs resulted from samples of Gammarus, Hydrobia, and filamentous algae taken 24 hours after the initiation of the first treatment and from Baetis mayflies and Physa snails within 48 hours of their initial exposure. Sculpins, crayfish, and substrate samples taken after 15 days of exposure and samples of all types taken after 30 days of exposure had positive radioautographs while sample extracts of control stream benthos and substrates were not visualized on the x-ray film. No metabolites were detected on the radioautographs of 342 individual extracts encompassing samples of water, substrates, and benthic organisms.

To verify the absence of metabolites, thin layer chromatographs of samples taken on day 30 of the summer

treatment phase were divided into ten 1 cm zones after development of the radioautograph. The individual zones were scraped into scintillation vials and radioactivity determined by liquid scintillation counting as already described. Radioactivity above background levels was most often isolated from zone 4 although, on occasion, activity was detected in zones 1, 5, 6, 7, and 8. However, similar results were obtained when radiolabeled HCB standards were scraped and counted. Radioactivity from ^{14}C-HCB standards which was detected in zones other than 4 was considered to represent trailing of the compound as the solvent front moved across the plate. Analysis of the ^{14}C-HCB standard by gas chromatography resulted in only one peak which had the same retention time as standards of known purity.

Isensee and Jones (1975) used the same technique for the analysis of TCDD metabolites and also encountered trailing in radiolabeled standards which they attributed to TCDD adsorption to the plate. Based on similar chromatographic behavior of HCB standards and sample extracts it was concluded that HCB was not metabolized by any of the components of the model stream ecosystem. Hutzinger et al. (1972) found no evidence of reductive dechlorination or hydroxlation of 2, 2',4,4',5,5',-hexachlorobiphenyl in pigeons, rats, or brook trout, although Jensen and Sundstrom (1974) found a phenolic metabolite of the same isomer in rat feces.

No degradation of Clophen A 50 (a PCB manufactured in Germany) by Ephemera danica (Ephemeroptera) was observed after nine days of exposure (Sodergren and Svensson 1973). Highly chlorinated isomers and mixtures of chlorinated biphenyls can be degraded by acclimated activated sludges or by isolated bacterial strains but degradation occurs very slowly (Tucker et al. 1975; Furukawa and Matsumura 1976).

Laboratory Studies

Accumulation from Water - Static Exposure

Hexachlorobiphenyl residues disappeared rapidly from the water of the test beakers with 38-52% of the nominal concentrations recoverable from the water after 24 hours; only 7-13% of the nominal concentrations were present at 192 hours (Figure 3).

Amphipods accumulated HCB as a function of the concentration in water and duration of exposure and appeared to be approaching equilibrium by 96 hours at the lowest concentration (Figure 4). The most rapid uptake (ug/g/hour) occurred during the initial 24 hours when water concentrations were highest; slowest rates were observed between 96 and 192 hours when concentrations in water were lowest (Table 13). When the uptake rates were adjusted for HCB concentrations in the water, they were comparable for each nominal concentration at a given sampling time and less variable between sampling times at a given concentration

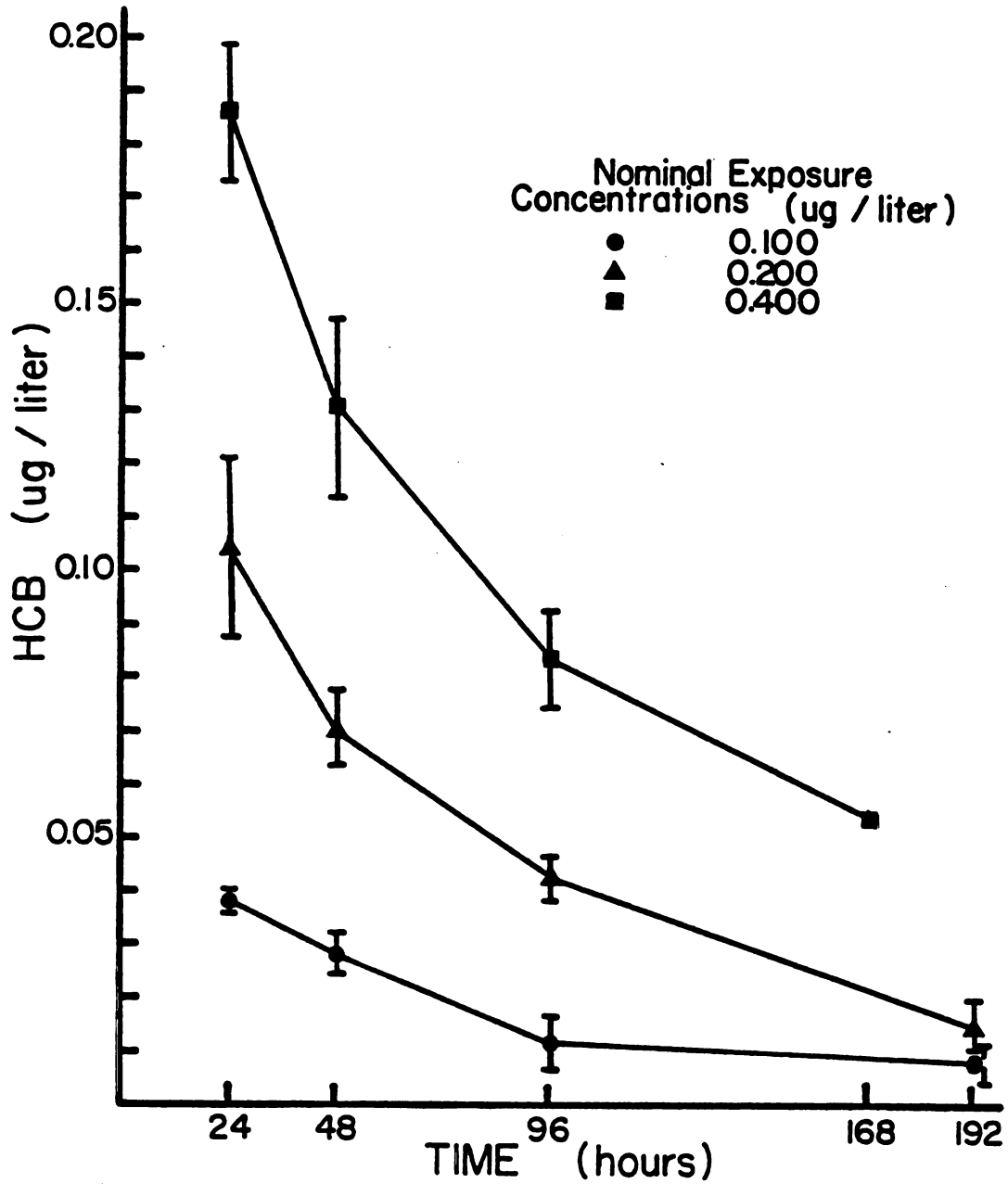

Figure 3. Disappearance of HCB from water at three nominal concentrations under static conditions. Vertical bars indicate one standard deviation above and below the mean.

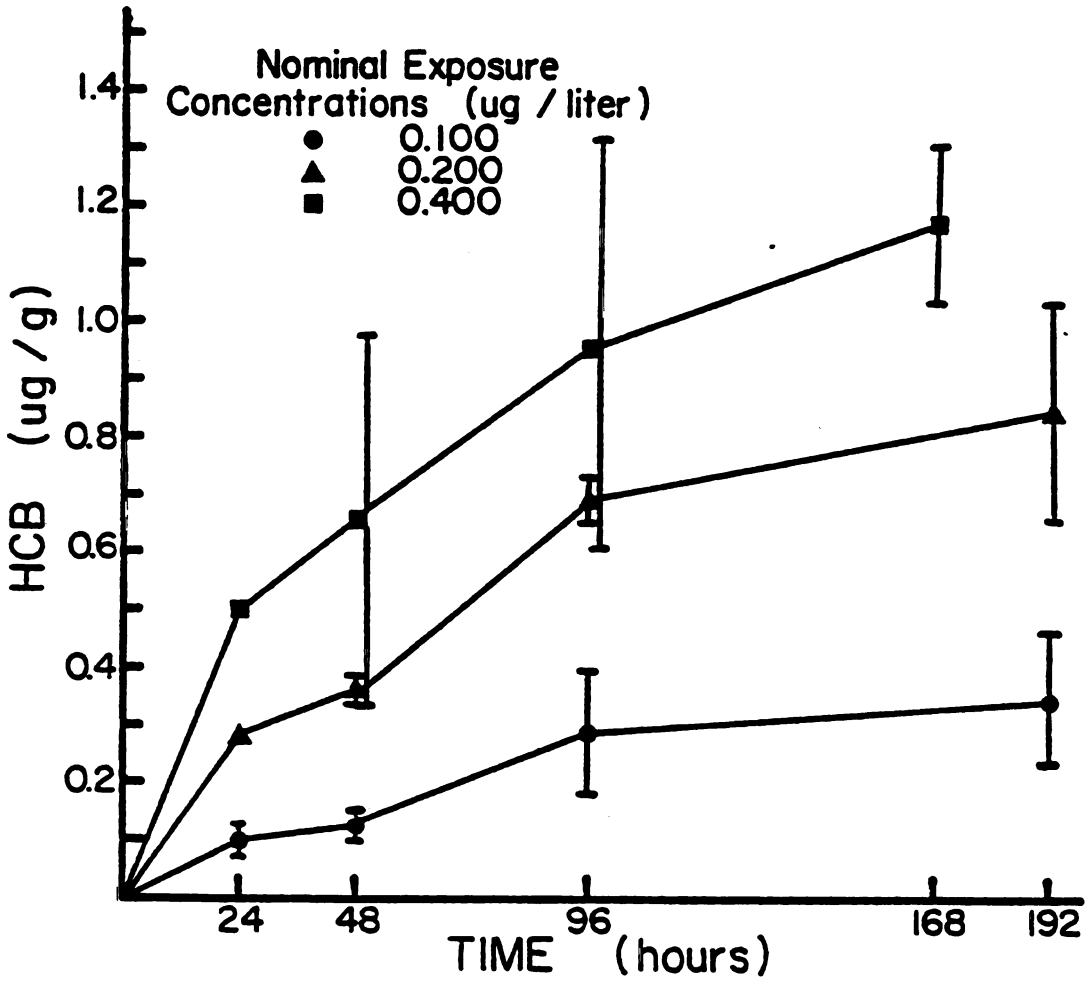

Figure 4. Accumulation of HCB by amphipods exposed to three nominal concentrations in water under static conditions. Vertical bars indicate one standard deviation above and below the mean.

Table 13. Rate of accumulation of HCB by amphipods exposed to three concentrations in water under static conditions

Nominal conc. & time (hour)	HCB conc. (ug/g)	Uptake rate* (ug/g/hour)	HCB conc. in water (ug/liter)	Adjusted uptake rate** (liter/g hour)
0.100 ug/liter				
24	0.105	0.004	0.038	0.11
48	0.028	0.001	0.028	0.04
96	0.163	0.003	0.011	0.31
192	0.059	0.001	0.007	0.09
	C. V.+ =50.0%		C. V.=85.7%	
0.200 ug/liter				
24	0.290	0.012	0.104	0.12
48	0.080	0.003	0.070	0.05
96	0.330	0.007	0.042	0.16
192	0.150	0.002	0.014	0.11
	C. V. =83.3%		C. V.=45.5%	
0.400 ug/liter				
24	0.510	0.021	0.186	0.11
48	0.150	0.006	0.130	0.05
96	0.310	0.006	0.083	0.07
168	0.210	0.003	0.053	0.06
	C. V. =88.9%		C. V.=42.9%	

* Uptake rate = HCB concentration (ug/g)/time (hours)
** Adjusted uptake rate = uptake rate (ug/g/hour)/water concentration (ug/liter)
\+ Coefficient of variation

(Table 13). Adjusted uptake rates were elevated during the initial 24 hours, depressed at 48 hours and elevated again at 96 and 192 hours.

Bioconcentration factors (concentration in amphipods/ concentration in water) were independent of exposure concentration during the initial 48 hours but ranged from 50,000-60,000 at 192 hours (Table 14). The bioconcentration factors would be expected to increase until equilibrium was established.

Mass balance calculations indicate that there was a decrease in total recovery of HCB with time (Table 15). Of the HCB recovered after 192 hours, 84-90% was associated with the amphipods.

Declining residues of hydrophobic compounds in the water of static systems have been reported by a number of investigators (Weidhaas et al. 1960; Holden 1962; Gakstatter and Weiss 1967; Wilkes and Weiss 1971; Crosby and Tucker 1971; Gillespie et al. 1975). Rapid losses of hydrophobic compounds from water have been attributed to adsorption onto container walls and accumulation at the air water interface by Bowman et al. (1959) while longer term losses are due to evaporative codistillation (Acree et al. 1963; Bowman et al. 1959).

Mechanism. The mechanism of accumulation of residues under static conditions was studied at a nominal concentration in water of 0.200 ug/liter. Residues disappeared from

Table 14. Bioconcentration of HCB by amphipods exposed to three concentrations in water under static conditions.

Exposure Time (hours)	Nominal Exposure Concentration (ug/liter) 0.100	0.200	0.400
24	2763	2788	2763
48	4750	5286	5092
96	26909	16667	11627
192	50714	60714	22245*

* 168 hour sample

Table 15. Recovery of HCB residues in amphipods and water during static experiments.

Nominal Concentration and Time (hours)	Total Recovery (%)	% of Nominal Quantity		% of Recovered HCB in Amphipods
		Amphipods	Water	
0.100 ug/liter				
24	55.1	17.1	38.0	31.0
48	54.3	26.3	28.0	48.4
96	45.1	34.5	10.7	76.4
192	44.3	37.3	7.0	84.2
0.200 ug/liter				
24	80.1	28.2	52.0	35.1
48	71.9	37.1	34.8	51.6
96	75.7	54.7	21.0	72.3
192	69.9	62.9	7.0	90.0
0.400 ug/liter				
24	61.2	14.6	46.6	23.8
48	53.3	20.8	32.5	39.0
96	49.5	28.9	20.7	58.3
168	58.4	45.1	13.3	77.3

the water as described for the static tests; concentrations in water ranged from 68% of nominal at two hours to about 9% after 192 hours.

Amphipod samples which had been rinsed with acetone prior to freezing had consistently lower concentrations in body tissue extracts than samples rinsed with water (Figure 5). However, the difference between the acetone-rinsed and water-rinsed amphipods was significant ($P < 0.05$) only at 24 hours of Trial 1 and at 12 hours of Trial 2 as determined by one-tailed t-tests.

When the total radioactivity that was recovered for each amphipod sample was tabulated the percent that was found in the rinse acetone ranged from 81% at two hours to about 7% after 192 hours (Figure 6). The percentage contribution of the acetone rinse to total recovered radioactivity declined most rapidly during the initial 24 hours.

Uptake rates were a function of water concentration and when adjusted for differences in water residue levels they were elevated at each time interval during the initial 24 hours (Table 16). Rates were depressed between 24 and 96 hours but were again elevated at 192 hours.

Accumulation from Water - Continuous Flow Exposure

Mean concentrations of HCB in water for the eight day exposure period were 0.031, 0.056, and 0.098 ug/liter respectively at the 0.100, 0.200, and 0.400 ug/liter nominal test concentrations (Figure 7). Mean values were only

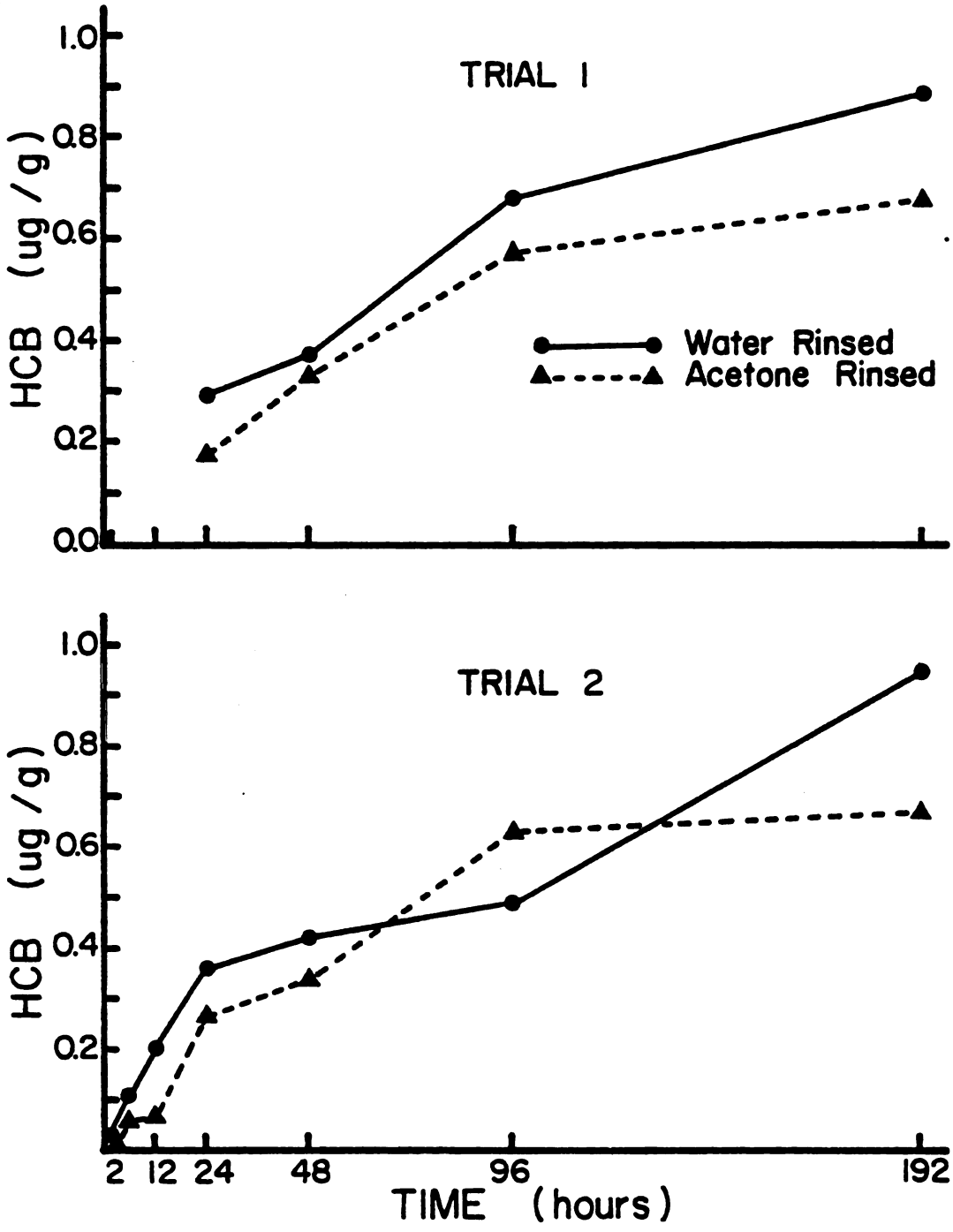

Figure 5. Residue concentrations of HCB in acetone extracts of amphipods which had been rinsed quickly in water or acetone at the time of sampling. Each data point represents the mean of three samples.

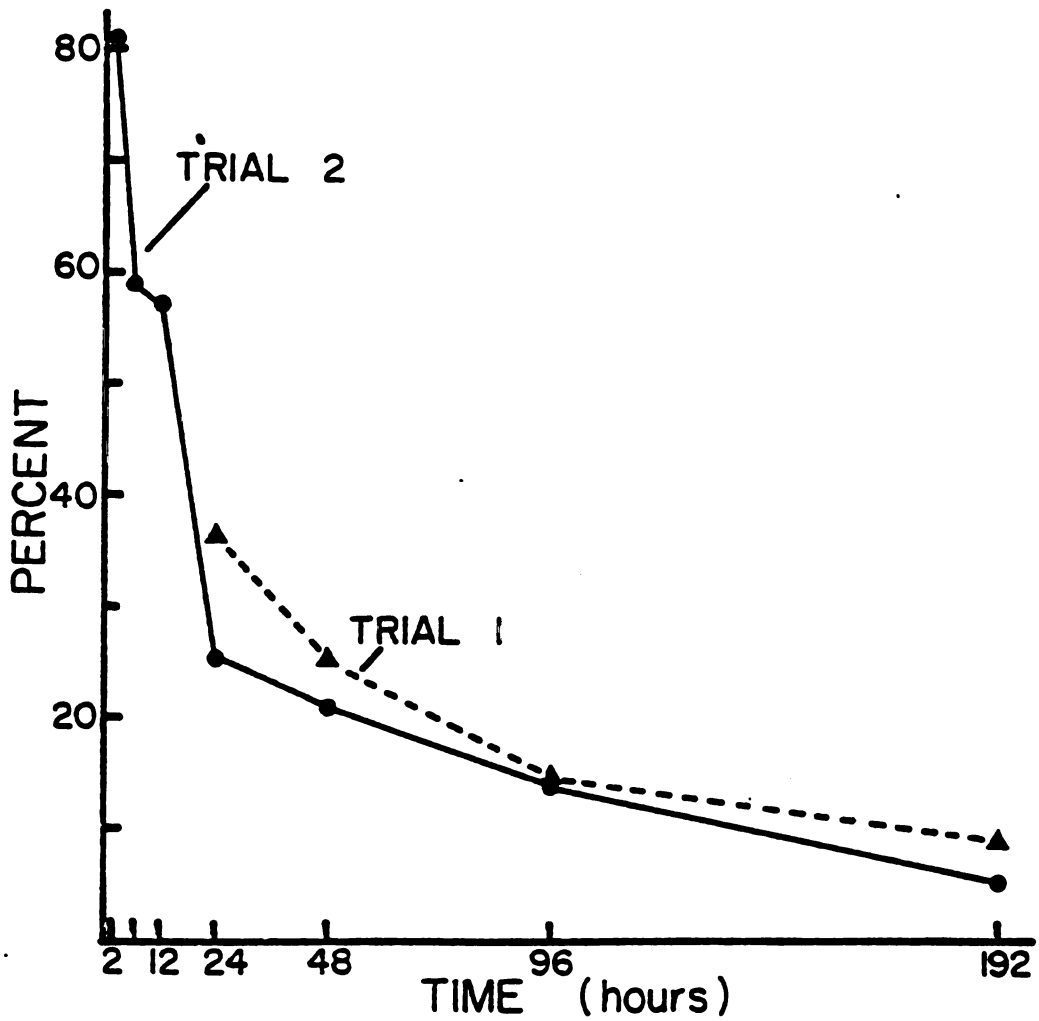

Figure 6. Percent of total extracted radioactivity that was found in the acetone rinse of amphipods exposed to 0.200 ug HCB/l.

Table 16. Rate of accumulation of HCB residues by amphipods exposed to 0.200 ug/liter under static conditions during experiments on the mechanism of uptake.

Time (hours)	HCB conc. (ug/g)	HCB conc. in H_2O (ug/liter)	Uptake rate (ug/g/hour)	Adjusted uptake rate (liter/g hour)
2	0.03	0.136	0.0150	0.11
6	0.11	0.128	0.0200	0.16
12	0.20	0.097	0.0150	0.15
24	0.36	0.090	0.0133	0.15
48	0.42	0.056	0.0025	0.04
96	0.49	0.035	0.0015	0.04
192	0.95	0.016	0.0048	0.30

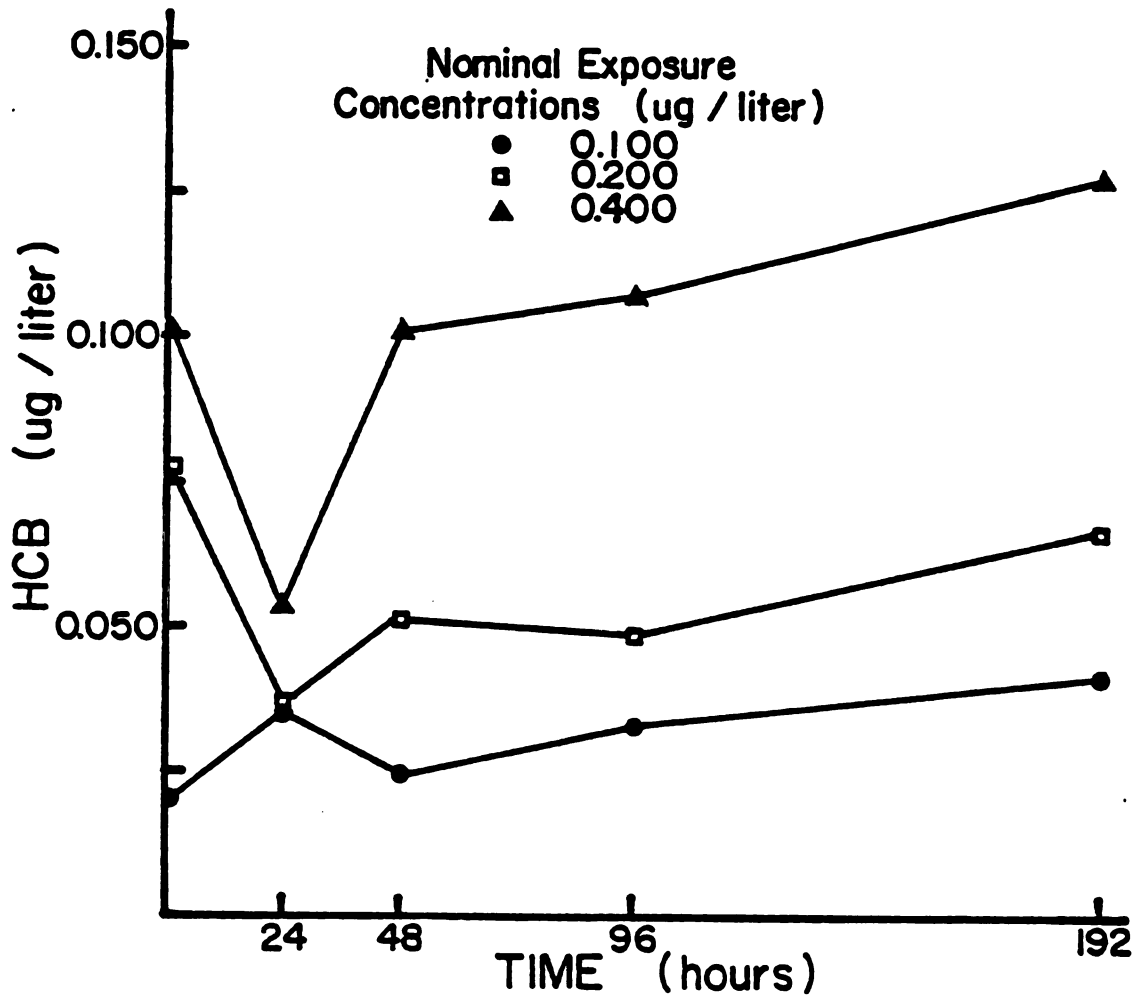

Figure 7. Concentrations of HCB in water for three nominal test concentrations during continuous flow exposure.

24.5-31.0% of the nominal concentrations; the remainder was probably adsorbed onto the walls of the mixing chambers, splitter boxes and test aquaria. Inadequate mixing, which would result in accumulation at the air-water interface (Bowman et al. 1959; Oloffs et al. 1972) and discharge via the surface overflow standpipes, also cannot be discounted. A similar loss of material was observed in the model streams. Lower concentrations in the water at 24 hours were due to syringe pump failure.

Residue levels in amphipods increased linearly with time and were a function of exposure concentration (Figure 8). Data showed excellent fit to the regression lines at each concentration. Residues in amphipods at 192 hours were 3.3 - 6.7 times higher than concentrations in amphipods after an equivalent time exposure to the same nominal concentrations under static conditions.

Bioconcentration factors for HCB accumulation by amphipods under continuous flow exposure ranged from 5400-10,200 after 24 hours to 42,600-61,900 after 192 hours (Table 17). Bioconcentration factors were generally similar for all concentrations at a given sampling time but had a tendency to increase with increasing exposure concentration. Calculated values at 96 and 192 hours agree well with values after similar intervals in the static tests but both sets of data underestimate the actual bioconcentration potential which would continue to increase until the HCB residues in amphipods

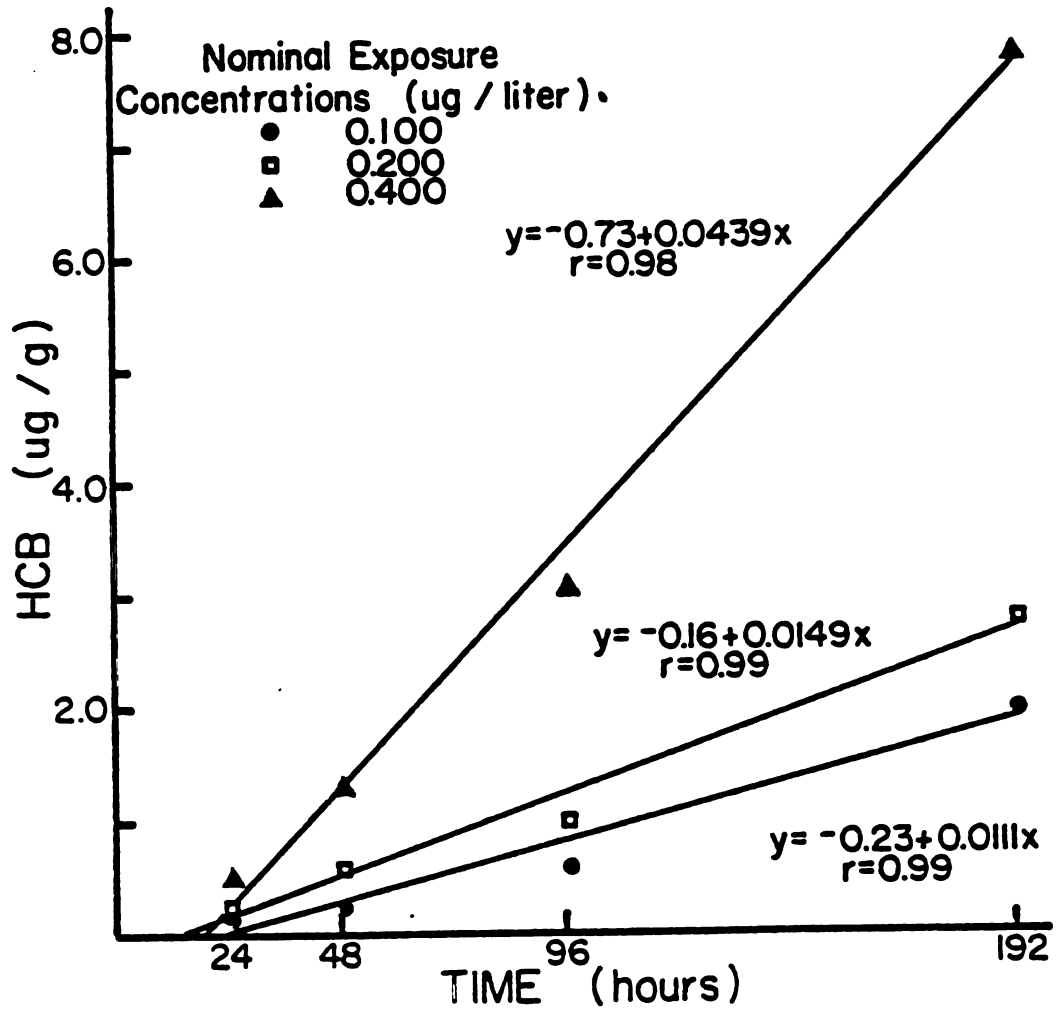

Figure 8. Accumulation of HCB by amphipods exposed to three nominal concentrations in water with continuous flow conditions.

Table 17. Bioconcentration of HCB by amphipods exposed to three concentrations in water under continuous flow conditions.

Exposure Time (hours)	Nominal Exposure Concentration (ug/liter)		
	0.100	0.200	0.400
24	5,428	8,055	10,189
48	10,740	12,157	13,465
96	17,879	21,458	29,065
192	49,024	42,576	61,811

were in equilibrium with concentrations in the water. Bioaccumulation factors for amphipods from the model streams averaged 98,889, 151,111, 136,666 and 193,333 at 24, 48, 96 and 192 hours respectively; these values are substantially higher than bioconcentration factors determined in the laboratory and probably reflect multiple routes of accumulation (i.e., from water, food and substrate contact).

As expected, the rate of residue accumulation by amphipods was concentration dependent but when adjusted for differences in exposure concentration the data were similar between test concentrations and were less variable over time within test concentrations (Table 18). Adjusted uptake rates were elevated during the initial 24 hours and again during the 96-192 hour interval.

The data from the static and continuous flow exposures indicate that accumulation of HCB residues by amphipods is rapid during the initial 24 hours and is, at least initially, due to adsorption onto the external surfaces of the organism. This conclusion is supported by the high uptake rates during this period for both the static and continuous flow exposures and by the percentage of radioactivity associated with the acetone rinse during the early hours of the static exposure. These results are also consistent with data from the model streams study.

Johnson and Kennedy (1973) reported that both live and autoclaved bacteria accumulated 80-90% of the total residue levels of DDT and methoxychlor within 30 minutes of exposure

Table 18. Rate of accumulation of HCB by amphipods exposed to three concentrations in water under continuous flow conditions.

Nominal conc. & time (hour)	HCB conc. (ug/g)	Uptake rate* (ug/g hour)	HCB conc. in water (ug/liter)	Adjusted uptake rate** (liter/g hour)
0.100 ug/liter				
24	0.19	0.008	0.035	0.23
48	0.10	0.004	0.027	0.15
96	0.20	0.004	0.033	0.13
192	1.42	0.015	0.041	0.36
	C.V.+ =62.5%		C.V.=	45.5%
0.200 ug/liter				
24	0.29	0.012	0.036	0.34
48	0.33	0.014	0.051	0.27
96	0.41	0.009	0.048	0.18
192	1.78	0.019	0.066	0.28
	C.V. =30.8%		C.V.=	25.9%
0.400 ug/liter				
24	0.54	0.023	0.053	0.42
48	0.82	0.034	0.101	0.34
96	1.75	0.037	0.107	0.34
192	4.74	0.049	0.127	0.39
	C.V. =30.5%		C.V.=	10.8%

* Uptake rate = HCB concentration (ug/g)/time (hours)
** Adjusted uptake rate = uptake rate (ug/g/hour)/water concentration (ug/liter)
\+ Coefficient of variation

which would indicate a passive process. No difference was found in the accumulation of DDE by live and dead chironomid larvae at various time intervals for up to eight hours (Derr and Zabik 1974); both live and dead larvae exhibited a time dependent accumulation which was related to cuticle surface area. The importance of chitin in the accumulation of DDT was discussed previously and is further supported by Khan et al. (1976) who found that unpeeled shrimp had a greater PCB adsorption potential than peeled shrimp. Early accumulation is thus a passive process and is based on the physical and chemical properties of HCB and the external surfaces of the organism.

Uptake over longer periods of time however appears to involve an active process. Wilkes and Weiss (1971) reported that live and dead dragonfly nymphs accumulated DDT at similar rates at low concentrations but these workers observed a divergence in accumulated residues as exposure times and concentrations increased. They attributed the greater accumulation by live nymphs to biological processes. Crosby and Tucker (1971) reported that heat killed daphnia accumulated only about 50% of the residues in live daphnia in a 24 hour exposure and that uptake occurred principally through the carapace. Wildish and Zitko (1971) also concluded that accumulation of Aroclor 1254 by marine amphipods occurred as an active process across the general integument while Kenaga (1975) concluded that the first period of residue

accumulation is due to adsorption and that later uptake is by absorption.

Adsorption followed by absorption may also explain elevations in the uptake rates that were observed during the first 24 hours and again at intervals beyond 96 hours in the data presented above. Wilkes and Weiss (1971) observed the same type of uptake rate fluctuation while Derr and Zabik (1974) attributed slower uptake after an initial rapid uptake period to an alteration of the cuticle.

Accumulation from Food

Hexachlorobiphenyl was detected in water two hours after food was presented to amphipods; concentrations in the water were a function of HCB concentration in the food and the quantity of food presented (Table 19). Residues in water resulted from desorption from the contaminated food and changed continuously because of two experimental design factors; 1) food was removed after two hours, 2) the quantity of food presented was adjusted for amphipod removal. These design features produced a spike in concentration each day which was followed by a relatively rapid decline in water concentrations. Therefore, it was not possible to calculate a mean daily water concentration over the duration of the experiment. However, the concentration in water at a given time could be approximated by $Ct=C_o e^{-rt}$ where Ct and C_o were the concentrations at time t and two hours respectively;

Table 19. Concentrations of HCB in water (ug/liter) of the upper aquaria two hours after food was added. Each value represents the mean of two observations.

Time (hours)	Concentration in Food (ug/g) 1.5	14.5	145.0
24	N.D.*	0.0102	0.0729
48	N.D.	0.0049	0.0349
96	N.D.	N.D.	0.0313
192	N.D.	N.D.	0.0083

L.O.D. = 0.0029 ug/liter

* None detected

r=0.33, the percent of water volume replaced per hour and e=natural logarithm.

Concentrations of HCB in amphipods which were exposed to the combination of contaminated food and water or to contaminated water alone increased with duration of exposure and as a function of HCB concentration in the food (Figure 9). However, amphipods which were exposed to the combination had HCB concentrations 4-20 times higher than those exposed to contaminated water only; uptake from food became less important as the exposure duration increased (Table 20). After eight days of exposure, concentrations in amphipods ranged from 0.09 - 0.13 times the concentrations in the food.

Because of fluctuating water concentrations, it was impossible to calculate a meaningful bioaccumulation factor. However, it was possible to back calculate from amphipod concentrations to water concentrations using the bioconcentration factors which were determined in the continuous flow exposure. The backcalculated water concentration would be the mean concentration which would have to be maintained over the specified time interval in order to produce the measured HCB concentration in amphipods.

Water concentrations were backcalculated for 24 hour and 192 hour samples (Table 21). At 24 hours water concentrations that were measured after food was present for two hours in the upper tanks (Table 19) were lower than the backcalculated values; values for the 192 hour sample reveal even more

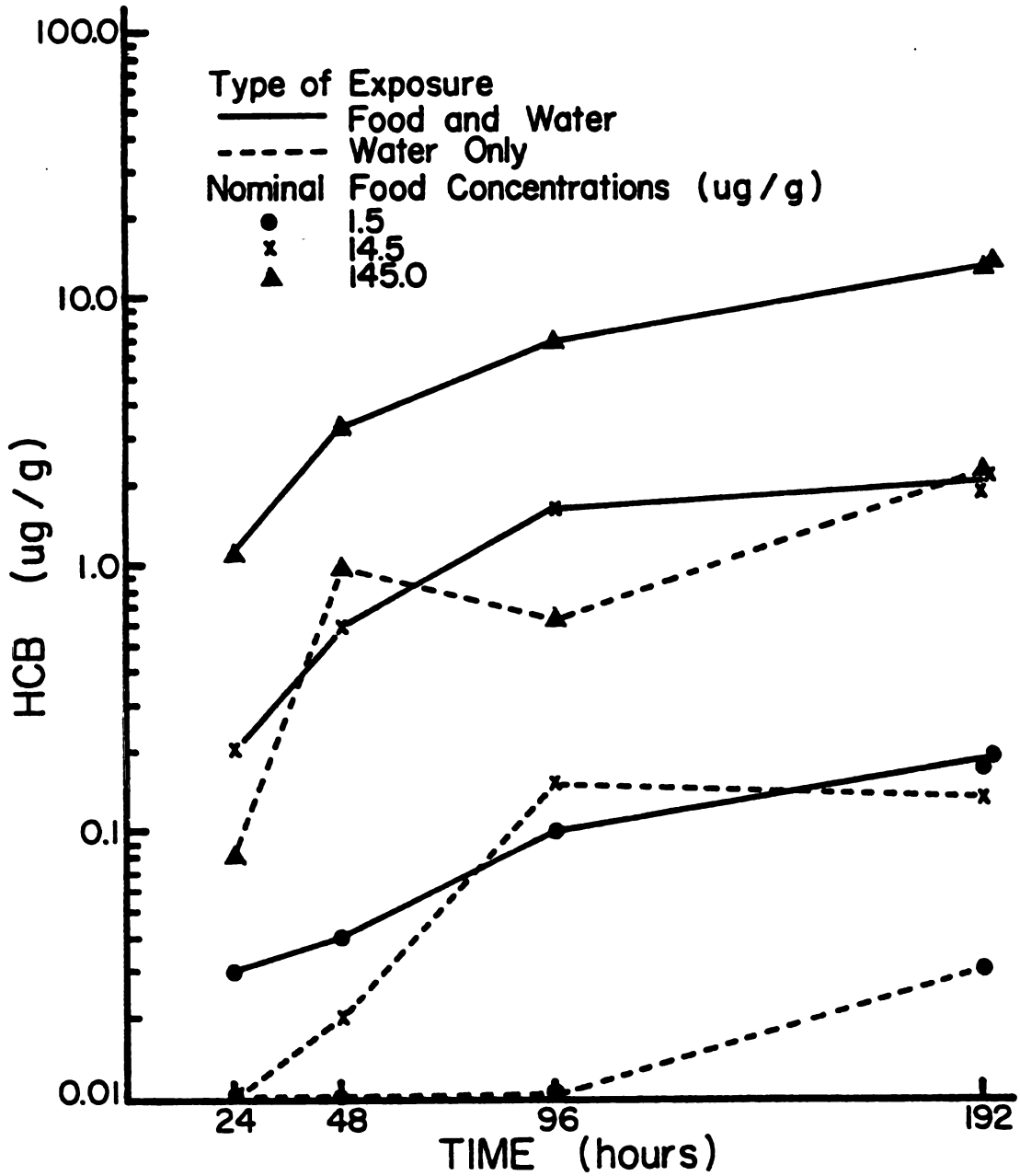

Figure 9. Accumulation of HCB by amphipods which were exposed to contaminated food and water and to contaminated water only. Each data point represents the mean of two replicate samples.

Table 20. Ratio of HCB residues in amphipods from upper and lower aquaria (upper/lower). Accumulation in upper tanks was from food and water whereas accumulation in lower tank was from water only.

	Concentration in Food (ug/g)		
Time (hours)	1.5	14.5	145.0
24	-	20.0	14.0
48	4.0	29.5	3.5
96	10.0	10.9	10.9
192	5.7	14.4	5.7

Table 21. Water concentrations (ug/liter) which would be required in upper and lower aquaria if HCB accumulation by amphipods was from water only. Values were backcalculated using the bioconcentration factors from the continuous flow study.

Time and Food Concentration (ug/g)	Upper Aquaria		Lower Aquaria	
	Bioconcentration Factor			
	Min.	Max.	Min.	Max.
24 hours	5000	10,200	5000	10,200
145	0.224	0.110	0.016	0.008
14.5	0.040	0.020	0.002	0.001
1.5	0.006	0.003	-	-
192 hours	50,000	80,000	50,000	80,000
145	0.225	0.159	0.044	0.028
14.5	0.037	0.023	0.003	0.002
1.5	0.003	0.002	0.001	0.001

disparity between backcalculated and measured concentrations in the water (compare Tables 19 and 21).

To accumulate their measured HCB body burdens from water only, amphipods which were exposed to the contaminated overflow water from the two upper aquaria treated with 14.5 and 145.0 ug HCB/g of food would have required water concentrations of 0.001-0.002 and 0.008-0.016 ug/liter respectively during the initial 24 hour period. These values agree with estimated mean concentrations in the lower tanks of 0.002 and 0.015 ug/liter respectively. The estimated values were calculated from the previously given equation. However, at 192 hours backcalculated water concentrations in the lower tanks seem high and could not have been maintained for the duration of the experiment. Thus, the actual bioconcentration factors were probably higher than those predicted from the continuous flow experiment. If, however, the concentrations in the water-exposed amphipods are assumed to represent the minimum accumulation from water, then residue accumulation from contaminated food would represent a maximum of 5.7 to 14.4 times the uptake from water alone.

Relatively few studies have been conducted on aquatic invertebrates to determine the importance of dietary intake of hydrophobic organic compounds. Reinert (1972) concluded that uptake of dieldrin from water was the principal mechanism of accumulation by Daphnia magna; Crosby and Tucker (1971) reached the same conclusion with regard to DDT accumulation by Daphnia. Derr and Zabik (1974) reported

that live chironomid larvae exposed to DDE in food and water accumulated the same residue levels as dead larvae (uptake from water only) when tested for up to eight hours. Kenaga (1972) probably best summarized the problem when he stated that the amount of pesticide intake by an organism is determined by the availability and concentration of the compound in the diet or in the surrounding environment as well as by the quantity of the contaminated food consumed and the quantity of the compound penetrating the organism through such cellular barriers as the cuticle, lungs, digestive system, gills, etc. In the present study, the role of ingestion in the accumulation of residues is subordinate to direct uptake from water although residues accumulated from each pathway appear to be additive.

Residue Availability from Substrates

Intact Substrates - Trials 1 and 2. While the system was equilibrating during Trial 1, only 13 of 24 water samples had radioactivity above background levels but none were above the statistically significant limit of detection (0.0145 ug/l, $P < 0.10$, (Seelye 1975)). For this reason, water sampling was discontinued during the remainder of Trial 1 and all of Trial 2.

Amphipods accumulated HCB as a function of exposure time and there was no difference in accumulation by amphipods as a function of exposure to the different substrates (Figure 10). Residue levels in amphipods were similar within

Figure 10. Accumulation of HCB residues by substrate-exposed and water-exposed amphipods for two different intact substrates. Substrates contained naturally occurring levels of organic matter.

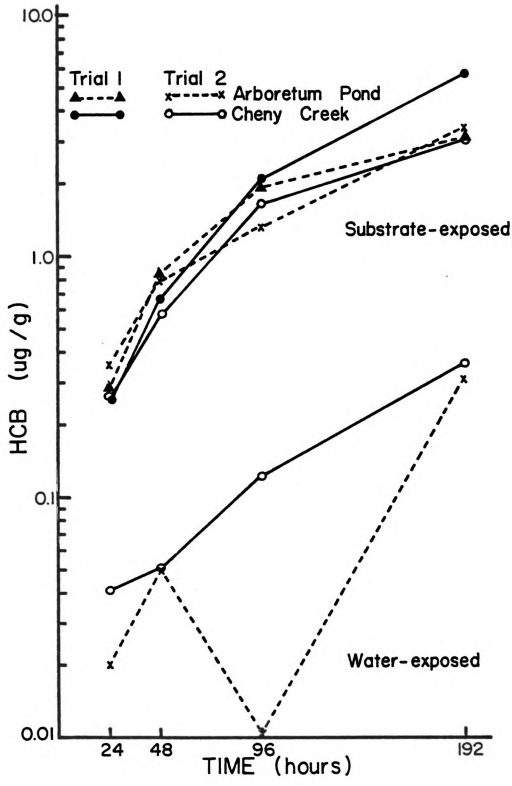
10.0
1.0
0.1
0.01
HCB (ug/g)
Trial 1
Trial 2
Arboretum Pond
Cheny Creek
Substrate-exposed
Water-exposed
24
48
96
192
TIME (hours)

Figure 10.

substrates of the same origin during Trials 1 and 2 despite the fact that the substrates had depurated for 21 days between the respective sampling times of each trial.

Residue levels in amphipods exposed only to the contaminated water, which overflowed from the upper aquaria (water-exposed), were consistently lower than residues in amphipods exposed directly to the contaminated substrates (substrate-exposed). After 192 hours, substrate-exposed amphipods had accumulated residue concentrations that were 8.8 - 10.5 times higher than in water-exposed organisms.

The extent of bioconcentration from water could not be calculated because HCB was not detected in water samples. However, residue levels in substrate-exposed amphipods on day 8 of Trial 2 were only 5.6 and 3.8 times higher than concentrations in the Cheny Creek and Arboretum Pond substrates respectively. These values would be reduced to 5.0 and 3.4 in the respective systems when compensated for known uptake from water.

Cheny Creek substrate consisted primarily of coarse and medium sand and had a mean organic matter content of 0.67% (Table 22). The two size fractions which were less than 0.125 mm represented less than 0.3% of the total soil dry weight but had the highest organic matter contents and HCB concentrations. However, they contributed little to the total HCB burden of the substrates. Hexachlorobiphenyl concentrations were significantly correlated with the

Table 22. Particle size distribution, organic matter content and HCB concentrations in various fractions of Cheny Creek and Arboretum Pond substrates at the end of Trial 2.

Substrate category and particle size range (mm)*		Percent total soil weight	Percent organic matter	HCB conc. (ug/g)	ug HCB per 800g
Cheny Creek					
very coarse sand	1 - 2	14.61	1.16	1.13	131.4
coarse sand	0.5 - 1	40.21	0.92	0.54	173.0
medium sand	0.25-0.5	42.02	0.54	0.42	145.5
fine sand	0.125-0.25	2.86	2.01	1.10	25.5
very fine sand	0.063-0.125	0.16	18.60	8.14	10.4
silt and clay	< 0.063	0.13	22.87	9.13	9.5
Arboretum Pond					
very coarse sand	1 - 2	7.03	7.37	1.03	58.0
coarse sand	0.5 - 1	12.50	6.59	1.01	101.0
medium sand	0.25-0.5	28.39	2.67	0.54	122.0
fine sand	0.125-0.25	25.23	3.92	0.69	138.7
very fine sand	0.063-0.125	15.92	5.27	0.96	122.3
silt and clay	< 0.063	10.92	5.32	1.26	110.2

* After Cummins (1962)

organic content of the particle size fractions ($r=0.99$, $P < 0.001$).

Arboretum Pond substrate had a mean organic matter content of 4.61% and consisted mostly of medium and fine sand with 26% of the soil dry weight represented by particles less than 0.125 mm. These two fractions contributed 35.6% of the HCB present in the substrate. Residue levels were not significantly correlated ($P > 0.05$) with either the organic matter content or the particle size of the various fractions.

At the end of Trial 2, intact Cheny Creek and Arboretum Pond substrates, which had been depurating for 33 days, retained mean residue concentrations of 0.55 and 0.86 ug/g respectively. Obviously, once contaminated with hydrophobic compounds, sediments may remain so for long periods of time and in so doing provide a reservoir for introduction of toxic compounds into aquatic organisms. Organisms which have direct access to contaminated sediments will accumulate higher residue levels of these compounds than organisms exposed to the compounds in the overlying water only.

Intact Substrates - Trial 3. Residues of HCB were readily detected in the water when the Cheny Creek and Arboretum Pond substrates consisted of inorganic material only (Table 23). During the equilibration period, concentrations in water were higher in the Cheny Creek system than in the Arboretum Pond system but residue levels in the water

Table 23. Hexachlorobiphenyl concentrations in water (ug/liter) overlying Cheny Creek and Arboretum Pond substrates during the equilibration and exposure phases of Trial 3.

Running Time (days)	Time (hours)	Substrate: Cheny Creek	Substrate: Arboretum Pond
		Equilibration Period	
1	24	0.091	0.069
2	48	0.063	0.032
4	96	0.055	0.032
8	192	0.038	0.034
		Exposure Period	
16	24	0.030	0.022
17	48	0.017	0.017
19	96	0.019	0.020
23	192	N.D.*	N.D.

L.O.D.= 0.0145 ug/liter

* none detected

of the two systems were equivalent during the period of amphipod exposure.

Residue levels in both substrate-exposed and water-exposed amphipods were markedly higher than in amphipods from respective exposure conditions during Trials 1 and 2 (Figure 11). This observation is consistent with the higher concentrations of residues in the water during the present experiment. Concentrations in water-exposed amphipods were lower than in substrate-exposed organisms and there was a slight difference in residue accumulation by amphipods as a function of exposure to the different substrates. Differences in the soil characteristics (e.g., particle size distribution) may have made HCB less available from the Arboretum Pond substrates although no differences in HCB concentrations were observed in the water samples.

Bioaccumulation and bioconcentration factors were calculated for substrate-exposed and water-exposed amphipods respectively on the assumption that the HCB water concentrations in the two sets of aquaria were equivalent (Table 24). The factors increased with time for both exposure conditions and were generally higher in the Cheny Creek system than in the Arboretum Pond system. After 192 hours, values for substrate-exposed amphipods were 2.3 and 5.4 times higher than in water-exposed organisms for the Cheny Creek and Arboretum Pond systems respectively.

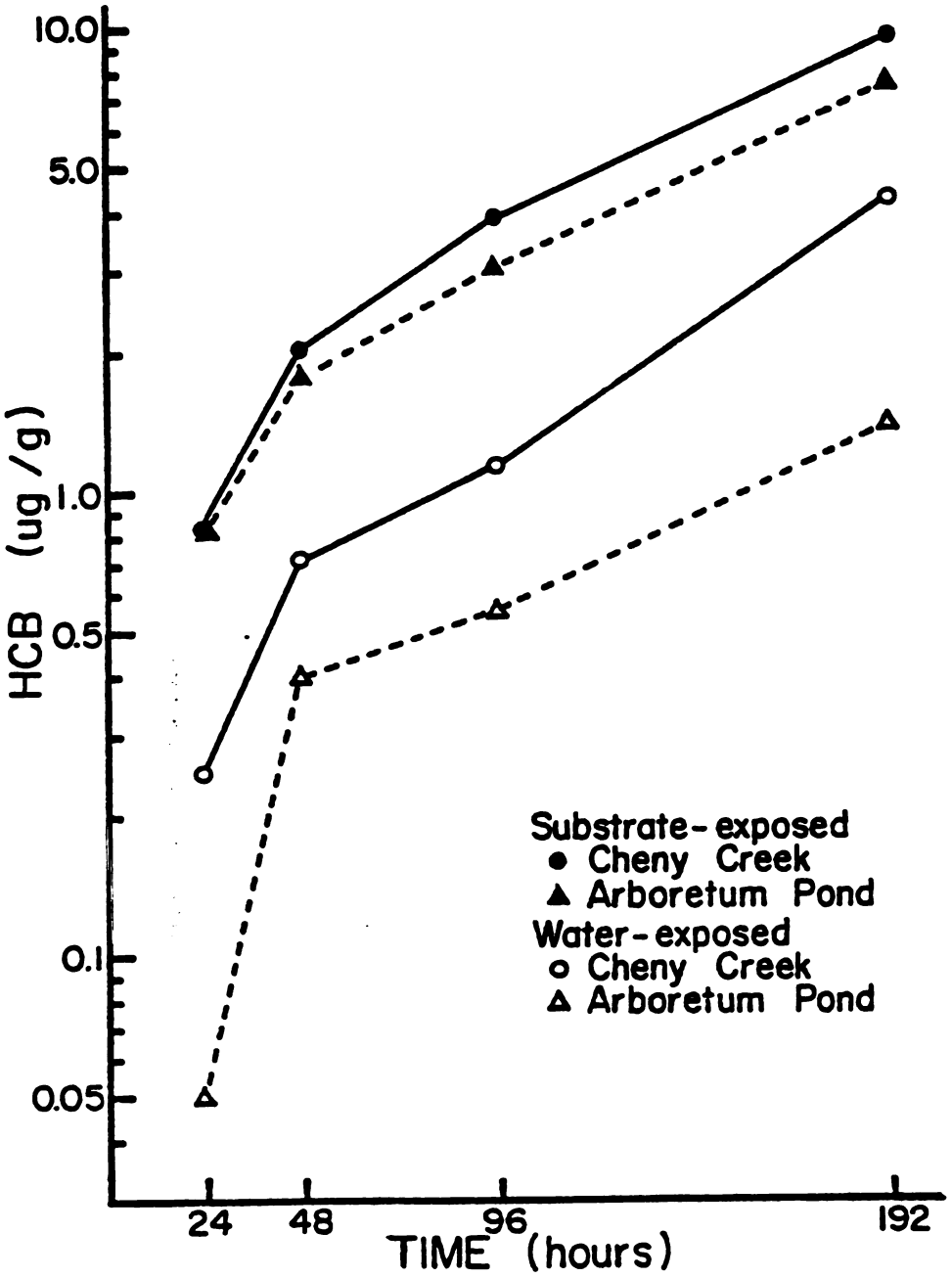

Figure 11. Accumulation of HCB residues by amphipods exposed directly to contaminated inorganic substrates (substrate-exposed) or to residues in contaminated overflow water only (water-exposed).

Table 24. Bioaccumulation and bioconcentration factors for amphipods which were exposed to HCB contaminated substrates and to contaminated water respectively. The factors were computed as the HCB concentration in the organisms (ng/g)/concentration in water (ug/liter).

Substrate and Type of Exposure	Time (hours)			
	24	48	96	192
Cheny Creek				
substrate exposed	27,677	121,176	207,368	1,073,333
water exposed	8,333	42,941	61,053	463,333
ratio*	3.3	2.82	3.40	2.32
Arboretum Pond				
substrate exposed	38,182	104,118	156,500	851,111
water exposed	2,273	23,529	28,000	157,778
ratio	16.80	4.43	5.59	5.39

* ratio = substrate exposed/water exposed

Bioconcentration values for water-exposed organisms were comparable to bioaccumulation values obtained after 192 hours in the model streams study. However, substrate-exposed amphipods had much higher bioaccumulation values than in the model streams which may be explained by exposure to the more highly contaminated substrates in the laboratory study.

The importance of substrate exposure appears to decrease with time for both substrates as judged by decreases in the ratio of bioaccumulation to bioconcentration (Table 24). Direct exposure to the Arboretum Pond substrate appears to be more significant than exposure to the Cheny Creek substrate. After 192 hours, HCB concentrations in substrate-exposed amphipods were 22.5 and 15.0 times the concentrations in the Cheny Creek and Arboretum Pond soils respectively.

The Cheny Creek and Arboretum Pond substrates differed slightly in particle size distribution from those used previously (Table 25). As in Trial 2 the two fractions of Cheny Creek substrate which were less than 0.125 mm represented only a small percentage of the total soil weight and did not contribute a significant amount to the substrate HCB burden despite elevated concentrations. Hexachlorobiphenyl concentrations were not significantly correlated ($P > 0.05$) with particle size.

Arboretum Pond substrates had a smaller percentage of particles less than 0.125 mm when compared to substrates used during Trial 2. Unfortunately, residue data from the

Table 25. Particle size distribution and HCB concentrations in various fractions of Cheny Creek and Arboretum Pond substrates at the end of Trial 3. Substrates had been combusted prior to use.

Substrate category and particle size range (mm)*		% of total soil weight	HCB conc. (ug/g)	ug HCB per 800g
		Cheny Creek		
very coarse sand	1 - 2	16.33	0.78	101.9
coarse sand	0.5 - 1	52.37	0.45	188.5
medium sand	0.25-0.5	28.24	0.26	58.7
fine sand	0.125-0.25	2.85	0.40	9.1
very fine sand	0.063-0.125	0.15	4.42	5.3
silt and clay	< 0.063	0.06	13.45	6.7
		Arboretum Pond		
very coarse sand	1 - 2	7.12	0.66	37.6
coarse sand	0.5 - 1	14.52	0.57	66.2
medium sand	0.25-0.5	46.87	0.48	180.0
fine sand	0.125-0.25	23.12	0.59	109.1
very fine sand	0.063-0.125	6.32	0.61	30.8
silt and clay	< 0.063	2.05	Lost	-

* After Cummins (1962)

smallest particle size fraction were lost but it appears that there was no significant elevation in residue concentration. This assessment is based on the uniform distribution of residues within the other particle size fractions and on the fact that the intact substrate had a mean HCB concentration of 0.51 ug/g. If the HCB quantity in each particle size fraction (exclusive of the smallest) is summed and divided by the total soil weight, the result is a concentration of 0.53 ug/g which agrees well with the measured concentration. Hence, the smallest particle size fraction contributed little to the total HCB burden of the substrate.

The HCB concentrations in each of the particle size fractions of the oxidized substrates were consistently lower than when organic matter was present (compare Tables 22 and 25) and this occurred despite the fact that the Trial 2 substrates had depurated for 18 additional days. Evidently, organic matter, even in small amounts, is important in the retention of HCB residues by hydrosoils. Removal of organic materials increases the residue levels in the water and thereby increases the availability of the residues to aquatic organisms.

Substrate partition coefficients (concentration in the substrate (ng/g)/concentration in the water (ug/liter) at 192 hours of amphipod exposure were 47,800 and 56,800 for the Cheny Creek and Arboretum Pond substrates respectively. These values are much higher than the partition coefficients

determined for the model stream substrates but are much lower than the distribution coefficients reported by Halter and Johnson (1977). The high coefficient values reported by Halter and Johnson (1977) and in the present study reflect the fact that the substrates were artifically contaminated and the contaminant was then allowed to desorb into the water. In this respect the substrates are somewhat analogous to the columns of inert substrates proposed by Veith and Comstock (1975) for continuously saturating water with hydrophobic organic chemicals.

Intact Substrates - Trial 4. Hexachlorobiphenyl concentrations in water decreased with time during the period of amphipod exposure but were higher than in previous trials (Figure 12). The higher concentrations were probably due to the shorter equilibration period that was used during this experiment.

Initially, residue levels were slightly lower in the overflow water than in water overlying the substrates but concentrations in all aquaria were similar by 192 hours. There was no evidence of a vertical gradient in the HCB water concentrations of aquaria with the high ratio of water-to-substrate (data not presented).

Residue levels in substrate-exposed amphipods were higher than in water-exposed amphipods (Figure 13). Substrate-exposed amphipods from aquaria with a high ratio of water-to-substrate (11.25:1) had higher residues at

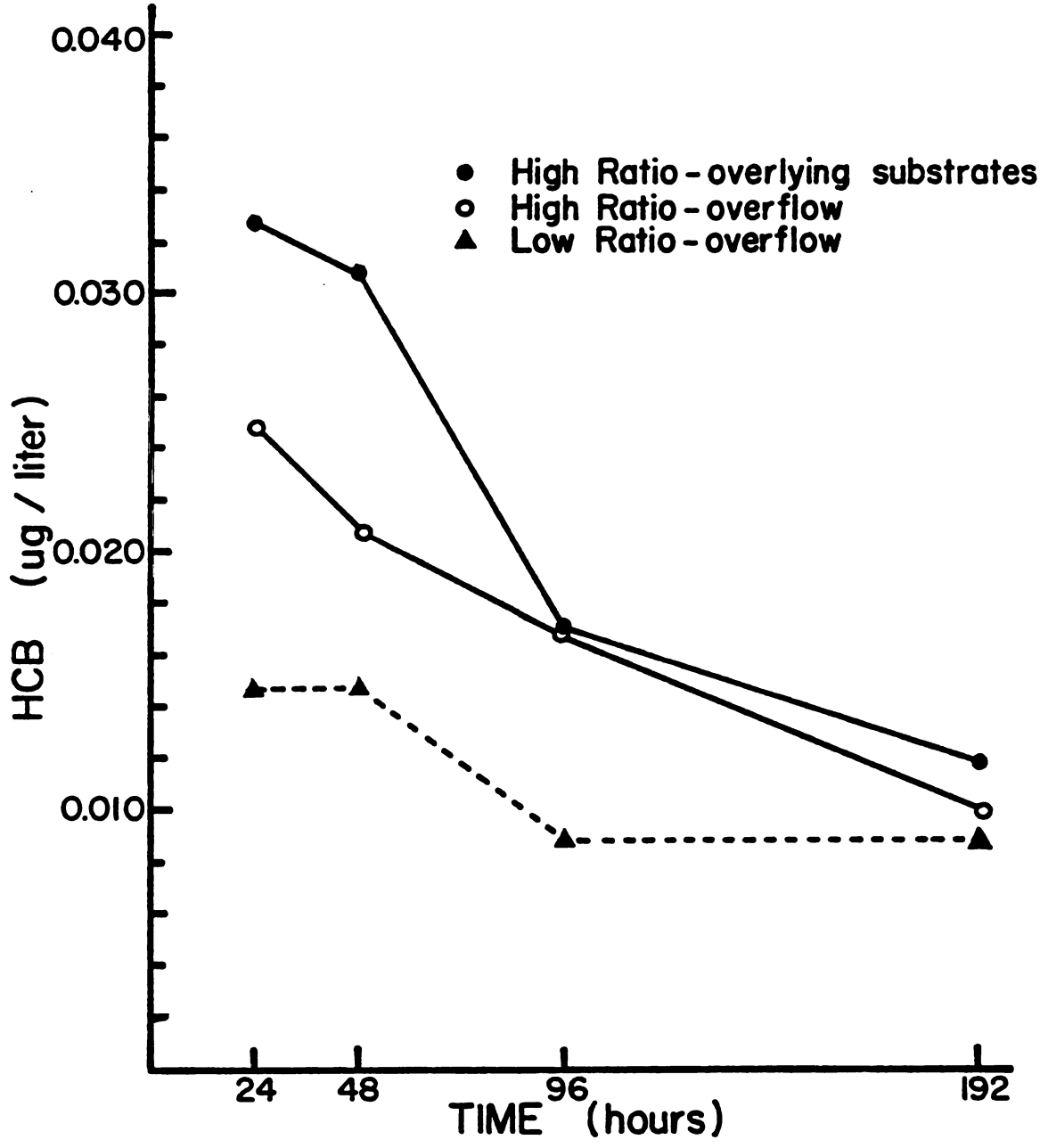

Figure 12. Concentrations of HCB in water (ug/liter) from aquaria at a water-to-substrate ratio of 11.25:1 (wt/wt) and in aquaria receiving the overflow water from both the high and low ratio (1:1, wt/wt) of water-to-substrate aquaria during the period of amphipod exposure.

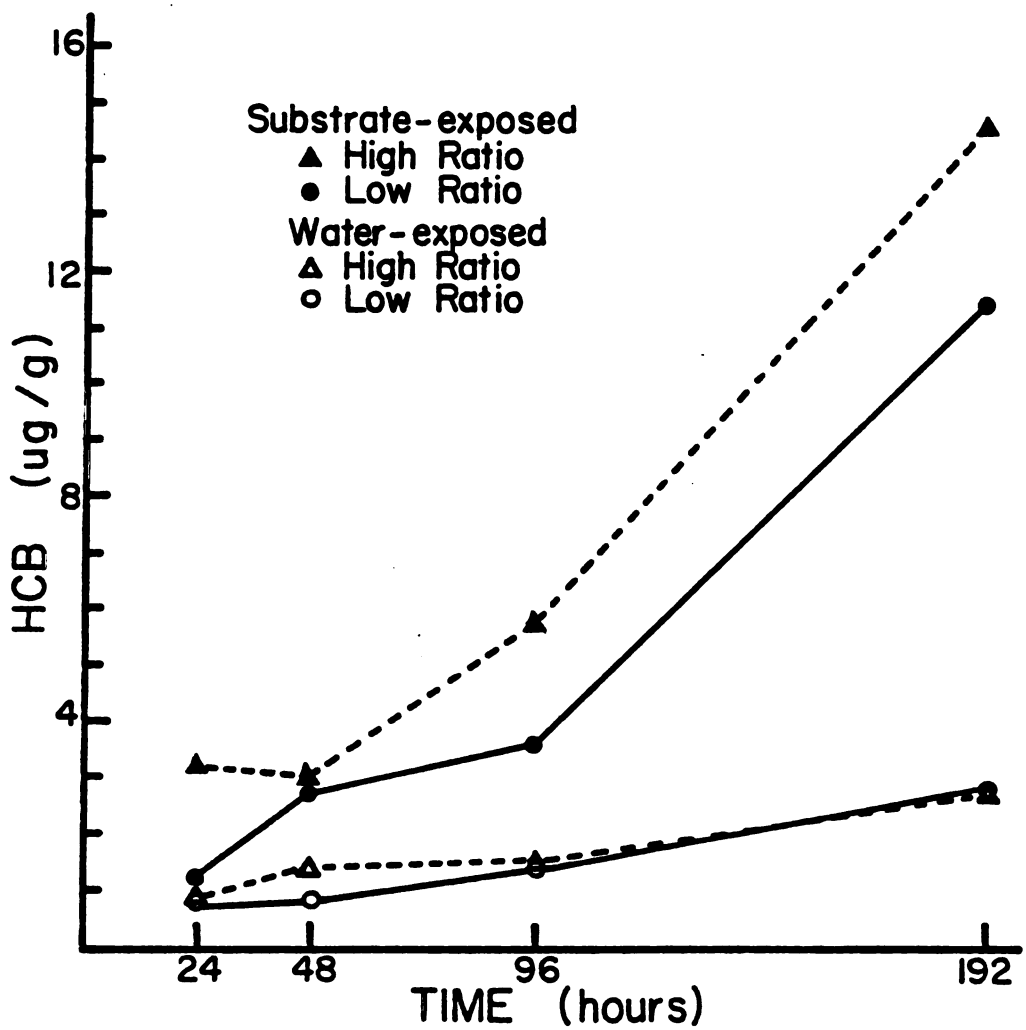

Figure 13. Accumulation of HCB residues by amphipods which were exposed to Cheny Creek substrates at high (11.25:1.00) and low (1.00:100) water to substrate ratios (grams of H_2O:grams of substrate). Substrate-exposed amphipods were subject to uptake from water and substrates while uptake by water-exposed amphipods was from contaminated water only.

96 and 192 hours than did substrate-exposed organisms from the low ratio tanks (1:1). There was no difference between amphipods exposed to the overflow water from either set of aquaria. The longer residence time and larger volume of water in the high ratio aquaria may have allowed a greater quantity of HCB to desorb from the substrates; the HCB would also remain available for uptake by the amphipods for a longer period of time.

Bioaccumulation factors at 192 hours ranged from 1.21-1.28 x 10^6 for substrate-exposed amphipods while bioconcentration factors ranged from 2.78 - 3.19 x 10^5 for water-exposed organisms. The values for the two exposure conditions (substrate vs. water) differed by a factor of about four which could be assumed to represent the maximum contribution to HCB accumulation from contaminated substrates via direct contact and ingestion as opposed to uptake from water alone.

Partition coefficients for substrate-exposed amphipods (concentration in amphipods/concentration in the substrates) at 192 hours ranged from 19.2 to 22.8 which would drop to 14.4-18.5 when compensated for direct uptake from water. These values are in general agreement with similar data on Cheny Creek substrates from Trial 3.

Substrate partition coefficients (concentration in the substrate/concentration in the water) ranged from 53,300 at the high water-to-substrate ratio to 66,700 at the low water-to-substrate ratio. These values are slightly higher than

values obtained in Trial 3 which was not unexpected since the substrates in the present experiment had natural levels of organic material (0.41%) whereas those in Trial 3 consisted of inorganic materials only. The presence of organic material would reduce the availability of the residues to the water which, in turn, would result in a higher partition coefficient.

Sieved Substrates. Of 169 water samples taken during the experiments, only 11.8% had HCB residues above the limit of detection (0.0059 ug/liter) and, of these, the mean values were 0.012 and 0.009 ug/liter for the Cheny Creek and Arboretum Pond systems respectively.

The residue levels of HCB which were accumulated by amphipods after 192 hours of exposure to each particle size fraction of the different substrates is presented in Table 26.

Amphipods, which were exposed to the contaminated size fractions of unaltered Cheny Creek substrate (Trial 1), accumulated the least residues when exposed to particles less than 0.125 mm. Concentrations in amphipods exposed to particles less than 0.063 mm were only about 5% of those in amphipods exposed to the particles larger than 0.25 mm. Residue levels in amphipods were inversely correlated ($r = -0.94$, $P < 0.05$) with the organic matter content of the particle size fractions.

Table 26. Hexachlorobiphenyl concentrations (ug/g) in amphipods and organic matter content (%) of substrate particle size fractions on day 8 of each experiment. During Trials 1 and 3 the substrate particle size fractions had naturally occurring levels of organic matter. Prior to Trials 2 and 4 the substrates were combusted to remove natural organics.

	Particle Size Range (mm)				
	1.0-0.5	0.5-0.25	0.25-0.125	0.125-0.063	0.063
Cheny Creek					
Trial 1					
organic matter	0.51	0.26	2.96	28.39	37.47
HCB conc.	1.91	2.07	1.15	0.24	0.11
Trial 3					
HCB conc.	3.34	4.48	7.16	0.69	0.47
Arboretum Pond					
Trial 2					
organic matter	6.02	2.71	3.83	5.42	5.63
HCB conc.	1.05	3.15	2.07	0.99	0.83
Trial 4					
HCB conc.	1.68	2.20	1.97	1.57	1.05
Maple Leaves					
Trial 5					
organic matter	72.8	74.5	73.2	70.6	66.7
HCB conc.	0.40	0.21	0.16	0.19	0.19

Amphipods which were exposed to oxidized Cheny Creek substrates (Trial 3) accumulated higher residue levels from each particle size fraction compared to residue uptake during Trial 1. Organisms which were exposed to particles less than 0.125 mm accumulated the least HCB.

Accumulation of HCB by amphipods which were exposed to the various particle size fractions of unaltered Arboretum Pond substrate (Trial 2) was inversely correlated with the level of organic matter in the size fractions ($r = -0.97$, $P < 0.01$). There was no statistically significant relationship between HCB uptake by amphipods and the particle size of the substrates but the lowest residues were found in those amphipods exposed to the smallest particles.

Compared to the results of Trial 2, a slight increase in amphipod residue levels was observed at the two smallest size fractions of oxidized Arboretum Pond substrates (Trial 4). However, lowest residues in organisms were found in those exposed to the finest particles.

The maple leaf substrate had an organic matter content which averaged 66.7-74.5% for the different particle size fractions. Residue accumulation by amphipods exposed to particles greater than 0.125 mm was markedly reduced when compared to the results of Trials 1 - 4. Hexachlorobiphenyl concentrations in amphipods exposed to particles less than 0.125 mm were comparable to those accumulated by amphipods from the same particle sizes in Trial 1. It will be recalled that these latter two fractions had high organic matter

contents. Organic matter in the form of crushed maple leaves apparently reduced the availability of HCB to the amphipods exposed to the medium and coarse particle sizes and behaved in a similar manner to naturally occurring organic matter in the fine particle sizes.

The accumulation of HCB by amphipods in the simple laboratory systems appears to result primarily from direct uptake from water. Direct contact with, or ingestion of, contaminated substrates is also significant but of secondary importance. Furthermore, amphipods are capable of accumulating measurable residue levels in their body tissues from extremely dilute solutions within a relatively short time. The residue levels in water result from desorption from the substrates according to the properties of adsorption-desorption equilibrium reactions and may be below the level of detection in flowing water systems.

Literature data appears to confirm that direct uptake from water is the dominant mechanism of accumulation of hydrophobic organics by aquatic organisms. Direct uptake from water has been reported to be the dominant uptake mechanism for Daphnia exposed to DDT (Crosby and Tucker 1971); for various freshwater invertebrates exposed to DDT and aldrin (Johnson et al. 1971), or to polychlorinated biphenyls (Mayer et al. 1977); for bacteria exposed to DDT and methoxychlor (Johnson and Kennedy 1973); for marine amphipods exposed to PCB's (Wildish and Zitko 1971); and by a

simple food chain exposed to dieldrin (Reinert 1972).

The present study also indicates that body burdens of hydrophobic contaminants can be enhanced by contact with, or ingestion of, contaminated substrates. Derr and Zabik (1974) suggested that exposure of chironomid larvae to DDT contaminated substrates may have resulted in the higher bioconcentration factors in their data compared to the results of Wilkes and Weiss (1971) for dragonfly nymphs exposed to DDT in water only. Isensee and Jones (1975) discounted the importance of TCDD uptake from contaminated hydrosoils but observed that concentrations in organisms were within an order of magnitude of TCDD concentrations in the soils. Residue levels of PCB's in fish which were exposed to contaminated sediments were about six times higher than when fish were denied direct access to the substrates (Halter and Johnson 1977). These authors believed that uptake from water, which followed desorption from the substrates, was the mechanism of accumulation. Kobylinski and Livingston (1975) attributed a portion of the mirex burden of the hogchoker (Trinectes maculatus, a carnivorous, bottom dwelling fish) to be from direct contact with contaminated substrates.

Direct uptake from water of hydrophobic organic compounds by aquatic organisms and sediments has been most often attributed to chemical partitioning based on exchange equilibria between two or more media (Hartung and Klingler 1970; Hamelink et al. 1971; Lenon 1968; Clayton et al. 1977;

Haque et al. 1974; Branson et al. 1975; Herbes 1977; Steen et al. 1978; Paris et al. 1978; Karickhoff et al. 1979). Strong inverse correlations exist between water solubility and octanol/water partition coefficients (Chiou et al. 1977) and between aqueous solubility and bioconcentration factors (Chiou et al. 1977; Metcalf et al. 1973, 1975). A strong positive correlation between bioconcentration potential and octanol/water partition coefficients was indicated by Metcalf et al. (1975) and Branson et al. (1975).

The results of the substrate experiments demonstrate that substrate characteristics control the availability of HCB to the overlying water and in turn to aquatic organisms. The most important control factor appears to be the level of organic matter while substrate particle size is of less importance. The data clearly indicate that the uptake of HCB was increased in both substrate-exposed and water-exposed organisms when the organic content of the substrates was reduced by either artificial (combustion) or natural factors (increase in particle size). Organic materials represent a medium that enhances partitioning into substrates thereby reducing HCB equilibrium concentrations in the overlying water. When organic materials are reduced or eliminated the exchange equilibria reactions allow greater quantities of HCB to desorb into the water i.e., organic materials are simply a better "solvent" for hydrophobic compounds than are inert materials.

The particle size of the substrate also affects the availability of HCB to the water and organisms. Amphipods which were exposed to the silt and clay fraction (< 0.063 mm) had consistently lower levels of HCB than did those exposed to larger particle sizes. This result was observed in both the presence and absence of organic materials but was less influential than organic materials in determining HCB availability to the amphipods.

The role of substrate characteristics in the sorption of organic compounds by aquatic sediments has been investigated in detail only recently and most studies have dealt with the adsorption side of the exchange reaction. However, any process or characteristic that favors adsorption will inhibit desorption and thereby lower equilibrium concentrations in the water which would result in reduced uptake by organisms.

Haque et al. (1974) concluded that reduction of PCB levels in water depends on the area, organic content, nature and pH of contacted surfaces. Organic matter is often the prime determinant of pesticide adsorption by soils (Browman and Chesters 1977) and organic sediments adsorb many pesticides more strongly than do mineral sediments (Pionke and Chesters 1973). Lotse et al. (1968) found a direct relationship between the organic content of lake sediments and the amount of lindane they adsorbed while Haque et al. (1974) attributed the high adsorbing capacity of Woodburn

soil to its high organic content. Differences in the sorption of hydrophobic pollutants within the silt and clay fractions of several natural sediments was also related to differences in the organic carbon content (Karickhoff et al. 1979).

In a study with aldrin, a high organic matter soil sample adsorbed 44% more of the pesticide than did oxidized samples of the same soil and after leaching, the organic soil retained 67% more aldrin than did the oxidized soil (Yaron et al. 1967). In a similar manner, DDT concentrations in water increased when organic matter was removed by oxidation with H_2O_2 (Richardson and Epstein 1971) while ashing reduced the uptake of DDT by substrates in another study (Hargrave and Phillips 1974).

In a more natural setting, toxaphene detoxification occurs more rapidly in eutrophic lakes than in oligotrophic lakes (Terriere et al. 1966) and is due at least partially to sorption onto sediments and suspended particles (Veith and Lee 1971b; Johnson et al. 1966).

As shown in the present study a decrease in the particle size of substrates is often associated with higher organic contents which complicates analysis of partitioning behavior. Sorption is usually strongest in the fine particle sizes which often results in toxicant enrichment in these fractions while sand fractions which often comprise the bulk of substrate mass usually adsorb only small quantities of hydrophobic compounds (Baughman and Lassiter 1978;

Browman and Chester 1977; Karickhoff and Brown 1978; Hetling et al. 1978; Haque and Schmedding 1976).

Haque and Schmedding (1976) found that of a number of compounds tested, adsorption by sand fractions was significant only for hexachlorobiphenyl which may help to explain the results obtained in the present study where sand fractions were observed to contain the bulk of adsorbed residues.

The interaction of fine particle size and elevated organic contents is complex especially if one considers that in soils with organic carbon contents greater than five to eight percent only organic surfaces appear to be available for bonding (Browman and Chesters 1977). Thus, in order to interpret and predict partitioning behavior one must understand the respective roles of both organic matter and particle size (Steen et al. 1978).

SUMMARY

In the present study the residue dynamics and metabolism of hexachlorobiphenyl (HCB) were determined in a series of semi-natural laboratory model streams. Laboratory experiments were also conducted to determine the relative importance of residue accumulation by amphipods from water, from contaminated food, and from direct contact with contaminated substrates. The bioavailability of substrate-bound residues as a function of substrate type, organic matter content, and particle size distribution was also investigated.

The present study indicates that HCB is rapidly accumulated by the substrates and biota of laboratory systems to concentrations that range from about 3-6 orders of magnitude higher than concentrations in the water. These bioaccumulation values would be expected to increase as there was no evidence that equilibrium was approached even after thirty days of exposure to the chemical. The compound was persistent in the substrates and biota of the model streams and there was no evidence that the compound was metabolized in either the substrates or the biota of the laboratory streams.

Accumulation of HCB by amphipods was primarily by direct uptake from water. However, concentrations in the

organisms after 192 hours of exposure could be increased from 2-14.4 times by either ingestion of contaminated food or by direct exposure to contaminated substrates.

Direct uptake from water was most rapid during the first 2-3 hours and was attributed to adsorption onto the substrates and external surfaces of the organisms; slower rates of uptake which were observed after the initial rapid accumulation were attributed to partitioning of the compound into the deeper tissues of the biota.

Residue levels in amphipods from the laboratory tests were dependent on exposure concentrations in the water. The availability of substrate-bound residues to the overlying water was controlled primarily by the concentration of organic matter in the substrates and to a lesser extent by the particle size of the substrate. In the laboratory studies where each substrate particle size fraction had the same nominal HCB concentration amphipods had lower concentrations of HCB when exposed to hydrosoils of high organic content and fine texture than did organisms exposed to other types of sediment. However, HCB concentrations and organic matter contents in unfractionated substrates were highest in the finest particle sizes when these sediments were sieved and analyzed. Literature data, previously cited,also indicate that the highest concentrations of chlorinated organic compounds in the sediments of natural systems occur in those of fine texture and high organic content. Thus, in natural

systems, organisms which are exposed to these types of sediment would also be exposed to the highest concentrations of hydrophobic organic compounds. At some point, exposure to these higher concentrations will result in increased body burdens in the benthic organisms. The relationships between sediment particle size, organic matter content, and sediment concentrations of hydrophobic organic compounds need further investigation with regard to accumulation of these compounds by benthic organisms before the full significance of bottom sediments as sinks or reservoirs of toxic materials is understood.

APPENDIX

Table A1. The daylength maintained in the laboratory during the experimental periods of the model streams experiment.

Experimental Period		Daylength (Hours)
Fall		
Start	August 11, 1976	14.50
End	November 9, 1976	10.75
Winter		
Start	November 10, 1976	10.25
End	February 7, 1977	11.00
Spring		
Start	February 9, 1977	11.00
End	May 9, 1977	14.70
Summer		
Start	May 11, 1977	14.70
End	June 8, 1977	15.30

Table A2. Temperature and water chemistry for the water used in the model streams study. Values are means for each month.

Month/ Year	Temperature °C Mean	Temperature °C Range	Dissolved Oxygen (mg/ liter)	pH Range	Hardness (mg/ liter $CaCo_3$)	Total Alkal-inity (mg/ liter)
9/75	12.1	10.0-12.8	9.9	7.65-7.8	210	192
10/75	10.0	7.8-10.5	9.4	7.75-7.95	209	191
12/75	4.8	2.8-5.0	10.8	7.85-7.85	203	191
2/76	4.0	2.8-3.9	11.2	7.9 -7.9	204	190
3/76	6.3	3.3-8.3	10.2	7.5 -7.7	182	177
4/76	9.5	5.5-12.2	9.9	7.7 -8.0	199	191
5/76	8 7	6.1-12.2	9.8	7.6 -8.0	192	192
6/76	12.3	8.9-12.8	9.0	7.8 -8.0	204	192
7/76	12.0	11.1-12.8	9.3	7.9 -7.9	202	190
8/76	11.4	8.3-13.3	9.8	7.8 -7.9	204	192
9/76	11.1	8.3-13.8	9.7	7.8 -7.9	203	191
10/76	7.8	6.7-9.4	9.8	7.6 -7.7	205	188
11/76	5.7	4.4-6.7	10.6	7.7 -7.8	206	188
12/76	3.7	0.5-5.0	11.3	7.6 -7.7	212	191
1/77	4.0	3.3-5.0	11.2	7.7 -7.8	217	190
2/77	3.3	1.1-7.2	11.4	7.6 -7.9	217	192
3/77	8.3	5.0-11.1	9.4	7.4 -7.7	187	164
4/77	11.0	7.8-13.8	9.7	7.6 -7.8	201	196
5/77	12.0	11.6-15.5	9.6	7.8 -7.8	202	196
6/77	10.5	9.4-11.7	9.7	7.8 -7.8	204	202

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