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Comparative Bioavailability of Sediment-Sorbed Hexachlorobiphenyl

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To the Graduate Council:

I am submitting herewith a dissertation written by C. Michael Swindoll entitled "Comparative Bioavailability of Sediment-Sorbed Hexachlorobiphenyl." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

M. C. Whiteside, Major Professor

We have read this dissertation and recommend its acceptance:

Gary Sayler, Fred Applehans, Richard Strange, Dewey Bunting

Accepted for the Council:

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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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COMPARATIVE BIOAVAILABILITY OF SEDIMENT-SORBED HEXACHLOROBIPHENYL TO ORGANISMS AT THREE DIFFERENT TROPHIC LEVELS

A Dissertation

Presented for the

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Degree

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C. Michael Swindoll

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ABSTRACT

The bioavailability of sediment-sorbed hexachlorobiphenyl (HCB) to bacteria, midge larvae (Chironomus tentans), and mosquitofish (Gambusia affinis) was examined in order to elucidate the influence of abiotic and biotic factors on the bioaccumulation process. Factors examined included sediment type, sediment organic matter, HCB concentration, temperature, and biological viability. Additional experiments were conducted to evaluate the relative role of sediment and dietary HCB sources to the accumulation of HCB in mosquitofish.

In general, the bioavailability of sediment-sorbed HCB was inversely related to the surface area and the organic content of the sediment. The organic content of the sediment was deemed important due to its large surface area and its hydrophobic intermatrix, which accounts for the affinity of organic matter for hydrophobic organic compounds. The bioaccumulation of HCB was proportional to the sediment HCB concentration. Changes in temperature did not alter the steady-state HCB concentrations in midge. HCB accumulation was similar in live and dead bacteria; however, live midge had greater HCB accumulation than dead midge. Mosquitofish HCB accumulation from dietary and sediment sources was additive and approximately equal.

This investigation indicates that the physical process of equilibrium partitioning is responsible for the bioaccumulaiton of sediment-sorbed HCB. However, the process of equilibrium partitioning can be facilitated by physiological activity.

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1.0 INTRODUCTION

The proliferation of chemical usage in our modern society has led to the widespread distribution of potentially toxic chemical residues in the environment, which poses a threat of exposure and subsequent effects to the health of humans as well as to other organisms. It has been estimated that as many as 70,000 chemical compounds are in use (Shaikh and Nichols, 1984) and hundreds of additional synthetic chemicals are introduced each year. The occurance of high levels of contamination in areas far removed from discharge sources has raised concern about our ability to control the dispersal of new and potentially hazardous chemicals. Compounds of greatest concern are those which are lipophilic, and resistent to environmental and biological degradation.

Although stable organic pollutants have probably been present in the environment since the beginning of the Industrial Revolution, most of those compounds considered to be potentially hazardous have been synthesized and commercially produced within the last fifty years. In the production and use of a chemical, it is nearly impossible to avoid some release of the chemical into the environment. Some compounds, such as the organochlorine pesticides, have been intentionally introduced into the environment because of the chemical stability and desired toxicities, while others, such as the PCBs, were accidently introduced and were not detected for many years. Once released into the environment, the chemical may be transported by water or air, and result in exposure to man and other organisms in the environment (Freed, Choiu and Haque, 1977).

The principle sink for many persistent anthropogenic compounds is the aquatic environment (Halter and Johnson, 1977). Sources of aquatic contamination include: (1) direct waste effluent discharge, (2) terrestrial runoff, (3) atmospheric deposition, and (4) recharge from contaminated aquifers. Once introduced into the aquatic environment, the contaminant's fate is governed by the chemical and physical characteristics of the compound. Water soluble compounds will tend to remain in solution, while hydrophobic compounds will sorb onto suspended particles and eventually be deposited in bottom sediments.

Sediment can serve either as a sink for chemicals, if these compounds are deposited and buried by further sedimentation, or as a reservior for chemicals which desorb into the surrounding water and become available to biota (Sodergren and Larsson, 1982). The continued occurrence of compounds, such as the polychlorinated biphenyls, in the tissues of aquatic organisms suggests that contaminated sediments continue to be a source of organic compounds long after the initial source of contamination has been alleviated. As the water concentration of organochlorine compounds decrease due to pollution abatement and dilution, sediments may become an increasingly important source of contamination (Shea et al., 1980).

To predict the biological significance of environmental contamination, it is necessary to quantitatively understand the mechanisms of exposure to biota within ecosystems. Knowledge of the behavior of chemical contaminants in aqueous systems is needed for predictions of the fate of these compounds and assessments of the potential for exposure and

detrimental effects on organisms and ecosystems. Important aspects of a chemical's environmental behavior include the interactions of the compound with abiotic materials and with living biomass. Knowledge concerning the sorption of organic chemicals to physical substrates is necessary to more accurately predict the bioavailability of these compounds.

The bioavailability of substrate-sorbed organic compounds is of concern because it determines the extent to which the biota can accumulate these contaminants. A high bioavailability may endanger the benthic fauna, and in turn, these organisms may be a source for accumulation of residues by their predators. The availability of sediment-sorbed chemicals will depend upon factors such as the chemical-physical properties of the compound, substrate composition, the chemical nature of the sorption, and the mechanism and length of exposure to the compound.

Polychlorinated biphenyls (PCBs) are excellent model compounds for the study of bioavailability of sediment-adsorbed chemicals. The PCBs have a ubiquitous distribution, and are extremely stable, being resistent to both environmental and biological degradation (Kalmaz and Kalmaz, 1979). The chemical-physical properties of PCBs, as well as their toxicological effects on a wide range of organisms, have been extensively studied and characterized. Therefore, information concerning the bioavailability of PCBs can be interfaced with existing information, and the potential biological impact of environmental contamination with PCBs more accurately assessed.

2.0 BACKGROUND

2.1 Polychlorinated Biphenyls

Production

The polychlorinated biphenyls (PCBs) are a class of aromatic, chemically inert, chlorinated hydrocarbons first synthesized in 1881, and commercially produced since 1929 (Anonymous, 1976a). The biphenyl molecule has a total of ten carbon-hydrogen bonds at which chlorine substitution is possible. There are 209 possible combinations of chlorine substitution on the biphenyl; however, in actual practices, only about 100 formulations have been used (Swain, 1983). PCBs have been produced by most industrialized countries (Kalmaz and Kalmaz, 1979) and marketed under different tradenames, such as Aroclor (U.S.A.), Clophen (Germany), Kanechlor (Japan), and Phenoclor and Pyralene (France). Although little information is available on worldwide production of PCB (Hutzinger et al., 1974), it has been estimated that more than 2×10^9 kg of PCB have been commercially produced worldwide (Eisenreich et al., 1983). The estimated U.S. production of PCB between 1930 and 1975 was 570×10^6 kg, and an additional 1.4×10^6 kg was imported (Buckley, 1982). Details of the production and uses of PCBs in the USA have been summarized by Nisbet and Sarofim (1972).

PCBs are manufactured by the batch chlorination of biphenyl with anhydrous chlorine using iron filings or ferric chloride as catalyst (Hutzinger et al., 1974; Hammond, et al., 1972). The different commercial

products are complex mixtures of biphenyls chlorinated to varying degrees. Aroclors® (Monsanto) exist as mixtures of isomers and congeners with chlorine content of 21, 32, 42, 48, 56, 60 and 62% by weight (Kalmaz and Kalmaz, 1979). The Aroclor® mixtures are identified by a 4-digit number, the first two of which are 12, designate the molecular skeleton of the biphenyl. The second pair of numbers indicates the percentage of the total weight of the Aroclor® that is contributed by the chlorine (Kalmaz and Kalmaz, 1979). Commercial PCB mixtures range from liquids to crystalline materials or hard resins (Edwards, 1971). The physical-chemical properties of PCBs which make useful industrial compounds, i.e. low vapor pressure, low aqueous solubility, and chemical inertness to water, acid, alkali, and heat, (Hutzinger et al., 1974) also make them extremely persistent environmental contaminants. The complexity of the PCB mixtures has complicated the determination of environmental levels, fate, and their impact on the biota (Tucker et al., 1975).

Properties

The PCBs are inert, stable chemicals with low water solubilities and low vapor pressures at ambient temperatures. Due to their low water solubility, an exact determination of aqueous solubility of PCBs is very difficult (Haque and Schmedding, 1975). Published water solubility values vary a great deal, ranging from 0.953 to 8.8 µg l^{-1} for 2,2',4,4',5,5'-hexachlorobiphenyl and 637 to 1900 µg l^{-1} for 2, 4'-dichlorobiphenyl (Wallnofer et al., 1973; Haque and Schmedding, 1975). Water solubility decreases with increasing chlorine content of the PCB

(Haque and Schmedding, 1975). Due to their low water solubilities, PCBs are highly adsorbed by surfaces, such as glass, particulates and sediment (Hammond, et al., 1972; Freed, et al., 1977).

Chemical and physical properties of PCBs are given in Hutzinger et al. (1974). Vapor pressure and vaporization rate are relatively low; however, they are much higher in the less chlorinated PCBs than in the more chlorinated PCBs. This is important to the long term fate of PCB mixtures, because the lesser chlorinated PCBs can evaporate into the atmosphere where they may be destroyed by photodecomposition, which will result in a greater percentage of environmental contaminants being the higher chlorinated PCBs.

Use

Due to their physical and chemical stability, PCBs have had numerous usages such as dielectic fluids, industrial fluids, heat transfer applications, and fire retardants (Hutzinger et al., 1974; Brown and Jones, 1981). By far, the greatest use of PCBs produced in the U.S. has been as a liquid insulating material for electrical capacitors and transformers (Brown and Jones, 1981). PCBs have also been used in the formulation of lubricating and cutting oils, and as plasticizers in paints, carbonless copying paper, adhesives, sealants, and plastics. PCBs had been recommended as extenders of the effective life of insecticides (Tsao et al., 1953; Duda, 1957); however, they were apparently never used on a large scale for this purpose (Hutzinger et al.,1974).

Legal Status in USA

Due to the widespread recognition of the distribution and bioaccumulation of PCBs, the Monsanto Industrial Chemicals Company, the sole U.S. producer of PCBs, voluntarily limited its sales in 1971 to those PCBs used in closed systems, i.e., capacitors and transformers, and soon terminated its production of the most highly chlorinated Aroclors (Hutzinger et al., 1974; Anonymous, 1976a). During 1972 and 1973, the Food and Drug Administration placed limitations on PCBs concentrations for dietary items and packaging materials used for foods. These restrictions reinforced the elimination of PCB usage in the U.S. except for closed systems. The Toxic Substances Control Act of 1976 placed limits on the manufacture and use of PCB, and stipulated that all production of PCBs in the U.S. end January 1, 1979, and that all U.S. sales and distribution of PCB end July 1, 1979 (Brown and Jones, 1981). In 1977, the FDA placed the limit of PCB in edible fish tissue at 5 ppm , and 2 ppm in the spring of 1979. However, due to the adverse impact on the fishing industry, the 2 ppm level in fish tissue was soon rescinded, and the 5 ppm level remained in effect until August, 1984, at which time the 2 ppm level was once again reinstated (Anonymous, 1984).

Although production and use of PCBs have been banned in the U.S., they continue to be of concern due to: (1) their persistence and relatively long biological half-lives; (2) their widespread geographic occurance; (3) the demonstrated long range atmospheric transport of these substances; (4) the ability to accumulate in food chains leading to man;

(5) their continued use in other countries; and (6) continued release from products in use (Greichus et al., 1978; Sayler et al., 1982).

Environmental History of PCB

Although PCBs have been in industrial use since 1929, the distribution of PCBs in the environment was not recognized until Jensen, in 1964, began an investigation to ascertain the origins of unknown peaks observed during gas chromatographic separation of organochlorine pesticides from wildlife samples. After Jensen's reported discovery (Jensen, 1966), an onslaught of environmental analyses followed and the ubiquitous distribution of PCBs became apparent. Prior to this time, PCBs went unreported either because the analytical procedures for PCBs had not been refined, or often, the residues were wrongly identified as other organochlorine compounds, such as DDT. PCBs have been identified in air (Harvey and Steinhauer, 1974; Atlas and Giam, 1981), soil (Carey and Gowen, 1976), and water (Duke et al., 1970) from almost every conceivable environment.

Transport Processes

Of the total world production of PCB, more than one-half is believed to be in waste dumps and landfills (Anonymous, 1976b). The movement of PCB from dump sites should be limited by their low solubility in water and by their tendency to sorb to soil surfaces. This conclusion has been supported by the monitoring of surface and groundwaters around landfills, which have found little or no contamination by PCBs (Lidgett and Vodden, 1970; Robertson and Li, 1976; Hesse, 1971; Moon et al., 1976). Much of

the remaining PCBs are dispersed in the environment principally through industrial use, manufacturing, production, accidental spills, and deliberate discharge (Sayler et al., 1982). In 1972, the rate of loss of PCBs was estimated to be on the order of $1.35 - 2.25 \times 10^3$ metric tons per year into the atmosphere, $3.6 - 4.5 \times 10^3$ metric tons per year into fresh and coastal waters and 1.62×10^4 metric tons per year into dump sites (Hammond, et al., 1972). However, due to the reduction in production and usage, present environment inputs would be expected to be much less.

Atmospheric contamination with PCBs occurs during the incineration of industrial and municipal wastes and from volatilization from spills, soils and sewage sludge (Anonymous, 1976b). Atmospheric transport is believed to be the major route of dissemination of PCB and other chlorinated hydrocarbons throughout the environment (Mackay and Wolkoff, 1973; Harvey and Steinhauer, 1976; Nisbet and Sarofim, 1972; Risebrough et al., 1968), and has been implicated as a primary input pathway to freshwater and marine environments (Eisenreich et al., 1981).

Numerous reports confirm the ubiquitous atmospheric distribution of PCBs transported with the prevailing winds, and deposited by particle sedimentation or rain-out (Hammond, et al., 1972; Risebrough and DeLappe, 1972; Kalmaz and Kalmaz, 1979). Since the vapor pressures of PCB congeners decrease with increasing chlorine content, the lower chlorinated congeners would be expected to disperse to a greater degree than the more highly chlorinated PCBs (Hammond, et al., 1972).

Aquatic ecosystems serve as the principle reservoirs of PCB that enter the environment (Anonymous, 1978). The entry of PCBs into water

occurs mainly at the points of discharge of industrial and urban waste into waters (Anonymous, 1976b). Other localized sources of PCB contamination are leakage and waste disposal from ships (Tatsukawa, 1976). Due to their low water solubility and high specific gravity, most PCBs discharged into aquatic environments will be adsorbed to particulates and sediments (Hammond, et al., 1972; West and Hatcher, 1980) Transport in aquatic systems will be primarily by means of waterborne particles (Hammond, et al., 1972; Kalmaz and Kalmaz, 1979), additional transport is accomplished by the uptake of the PCBs by biota (Young et al., 1977).

Biological Distribution of PCB

The biological distribution of PCBs has been reviewed by Hammond et al. (1972) and Kalmaz and Kalmaz (1979). Nisbit and Sarofim (1972) estimated that less than 900 metric tons of PCBs are located in living organisms throughout the world; therefore, biota would appear to have little impact on the worldwide fate and distribution of PCBs. However, the presence of PCBs in living organisms is of great ecotoxicological significance (Anonymous, 1976b). The biological accumulation of PCBs or of any compound becomes important when the acute toxicity of the compound is low. Usually the physiological effects go unnoticed until there are chronic effects due to increasing chemical accumulation (Neely et al., 1974; Bruggeman et al., 1981). By the time chronic effects are noticed, corrective action may be too late to alleviate the situation before irreparable damage has been done to the organism, population, and ecosystem.

Low levels of PCBs are so widespread that it is virtually impossible to find aquatic organisms without some residues (Hansen et al., 1976). The accumulation of PCBs in aquatic organisms may be explained by PCBs high affinity for lipids and the inability of the organisms to entirely metabolize them (Gruger et al., 1975; Hutzinger et al., 1972; Melancon and Lech, 1976). The actual PCB levels in aquatic organisms vary with the species, age, body weight, tissue types, lipid content, and appear to show seasonal fluctuations (Tatsukawa, 1976). Tissues of high lipid content tend to have high PCB levels, and the more stable highly chlorinated PCBs tend to accumulate more readily than low chlorine PCBs (Hammond et al., 1972; Kalmaz and Kalmaz, 1979).

At environmentally encountered levels, PCBs are generally thought not to be acutely toxic (Hutzinger et al., 1974), although in laboratory experiments, PCBs have been shown to affect survival (Halter and Johnson, 1974; Mauck et al., 1978; Verma et al., 1978; Weis and Weis, 1982), growth (Hansen et al., 1976; Mauck et al., 1978), reproduction (Freeman and Idler, 1975; Halter and Johnson, 1974; Nebeker et al., 1974), and behavior (Camp et al., 1974; Folmar and Hodgins, 1982; Hansen et al., 1974) of various aquatic organisms. The effects of PCBs are species specific, and there appears to be little relationship between taxonomic level and response to PCB. The toxicological characterization of PCBs is confounded by the fact that many PCB congeners have different chemical and physical properties (Hammond et al., 1972; Hansen et al., 1976).

Analysis of Environmental Samples for PCBs

The analytical determination of PCBs in environmental samples differs little from that of chlorinated hydrocarbon pesticides (Hutzinger et al., 1974). The analytical procedures for PCB analysis include; sampling, extraction, cleanup, separation from interfering compounds, and quantitation. Since PCBs in environmental samples are usually present in minute quantities (ng l^{-1} to mg l^{-1}), care must be taken throughout the analytical procedures to avoid laboratory contamination. To avoid contamination, all materials, glassware, and reagents should be chemically clean.

A review of procedures for PCBs determination in environmental samples is provided by Hutzinger et al. (1974). This discussion will be limited to PCB determination in water, sediment and biological samples.

PCBs can be easily recovered from water samples by extracting with non-polar solvents. Hexane is the solvent of choice for extracting PCBs from water (Hutzinger et al., 1974). More elaborate extraction procedures have been used (Ahling and Jensen, 1970; Gesser et al., 1971) which involve the use of non-polar chemicals coated on supports for reverse liquid-liquid partitioning between water and the non-polar chemical. The adsorbant is then extracted with a non-polar solvent, such as hexane or petroleum ether.

Solvent mixtures are usually used, often with a soxhlet apparatus, to extract PCBs from sediment and biological samples. Popular mixtures include varying hexane-acetone (Goerlitz and Law, 1974), hexane-ether

(Schmidt et al., 1971), hexane-isopropanol (Holden, 1970),and hexane-ethanol (Wakimoto et al., 1971).

A major problem with the extraction of PCBs from environmental samples is the coextraction of other compounds, particularly non-polar compounds such as lipids, chlorinated hydrocarbons, and polynuclear aromatics (Hutzinger et al., 1974). Lipids interfere with gas chromatographic (GC) quantitation of PCBs by contaminating the GC column and detector. The removal of lipids from extracts is accomplished through cleanup procedures. Lipids are commonly removed from extracts by using procedures involving chemical decomposition of the lipids, solvent partitioning or column chromatography.

Cleanup procedures using the chemical decomposition of lipids are based on the fact that PCBs are stable in the presence of concentrated sulfuric acids and alkaline media, whereas lipids are destroyed (Hutzinger et al., 1974). This cleanup procedure has been widely used (Murphy, 1972); however, does have the disadvantage of destroying some chlorinated and organophosphate pesticides. Separation of lipids and PCBs by liquid partitioning is less widely used and is based on the preference of PCBs for acetonitrile, dimethylformamide or dimethylsulfoxide, while lipids remain in the hexane extract (Hutzinger et al., 1974). Column chromatography is commonly used for extraction cleanup. Alumina and Florisil® are used as the column packing and retain lipids while allowing PCBs and other chlorinated hydrocarbons to be collected in the eluate. Solvents and solvent mixtures reported for column chromatographic cleanup include methylene chloride-hexane, acetonitile-

water, petroleum ether, and hexane-methanol (Horwitz, 1970; Mills et al., 1972; Porter and Burke, 1973).

If chlorinated hydrocarbons are extracted from the sample along with PCBs, it may be necessary to separate these compounds in order to quantitate the PCBs. In addition to column chromatography on Florisil®, silica gel is frequently used as an adsorbant for the separation of PCB and chlorinated pesticides (Hutzinger et al., 1974).

PCBs are most often quantitated by gas chromatography - electron capture detection, and more recently with gas chromatography coupled with mass spectrometry.

2.2 Biological Accumulation

The biological accumulation of persistent pollutants, such as DDT and mercury, first gained public attention in the 1960's with the widespread discovery of residues in fish and wildlife (Spacie and Hamelink, 1985). The terms bioconcentration, bioaccumulation, and biomagnification are sometimes used to denote the source of the accumulated residues in animals. The inconsistent use of these terms in the literature has sometime resulted in confusion. For the purpose of this discussion, these terms will be used as defined by Brungs and Mount (1978). Bioconcentration refers to an increase in residue concentration in an organism by direct uptake from water; bioaccumulation indicates the combined intake from water as well as from diet; and biomagnification refers to the process by which tissue concentrations of bioaccumulated residues increase as the materials pass up the food chain.

The direct uptake of PCBs and other persistent organochlorine compounds has been experimentally demonstrated for many aquatic organisms including phytoplankton (Biggs et al., 1980), arthropods (Lynch and Johnson, 1982), mollusks (Courtney and Denton, 1976), and fish (Frederick, 1975; Gruger et al., 1975). Uptake is defined here as the transfer of a chemical into (absorption) or onto (adsorption) an organism. Although a variety of mechanisms may be involved in the uptake, the most significant are diffusion, active and facilitated transport, and adsorption (Spacie and Hamelink, 1985).

Passive diffusion can occur whenever there exists a concentration gradient, and surfaces which are permeable to the chemical. Most experimental evidence indicates that the uptake of chemicals by aquatic organisms occurs most often by passive diffusion, which usually occurs through semipermeable surfaces such as the gills, lining of the mouth, or gastrointestinal tract (Spacie and Hamelink, 1985). Diffusion is also known to occur through the cuticle of arthropods (Derr and Zabik, 1974; Wilkes and Weiss, 1971; Crosby and Tucker, 1971; Wildish and Zitko, 1971). The higher concentrations of xenobiotics in smaller organisms compared to larger organisms (Nisbet and Sarofim, 1972), would indicate that direct absorption is the primary route of accumulation.

Active and facilitated transport processes involve the complexing of the chemical with a carrier molecule in the cellular membrane. Although these transportation mechanisms are an important means of metal accumulation in organisms, (Spacie and Hamelink, 1985) they would not be expected to significantly contribute to the accumulation of chemicals, such

as PCBs and organochlorine pesticides, which are readily soluble in lipids of cellular membranes. Adsorption refers to the binding of a chemical to a surface by chemical or physical forces. Although adsorption contributes to the total body burden, it generally does not contribute to the toxic effect level within the organisms (Spacie and Hamelink, 1985).

Bioconcentration of residues from water has been correlated with hydrophobicity of the chemical (Neely et al., 1974; Branson et al., 1975; Chiou et al., 1977; Kurihara et al., 1973; Veith et al., 1979; Tulp and Hutzinger, 1978; Oliver and Niimi, 1983), which is often expressed as the partitioning coefficient (K). The partitioning coefficient expresses the equilibrium concentration ratio of an organic chemical partitioned between an organic liquid and water (Choiu et al., 1977). Octanol is the most widely used organic phase, because of its similarity in polarity with biological membranes (Freed et al., 1977). The partitioning coefficients indicate the propensity for uptake and storage of chemicals in biological systems (Freed et al., 1977). Therefore, the octanol-water partitioning coefficient (K_{ow}) seems to be a useful indicator of bioaccumulation potential in many instances (Veith et al., 1979), with chemicals with high K_{ow} values having the highest bioaccumulation potential.

Bioaccumulation of organic compounds by aquatic organisms occurs through at least three pathways: direct partitioning from the aqueous phase via the gills, integumental sorption and diet (Swartz and Lee, 1980). Water is the probable medium of exchange for all three pathways (Rubinstein et al., 1984). Bioaccumulation can occur only if the rate of uptake of a chemical by an organism is greater than the rate of its

elimination (Spacie and Hamelink, 1985). Factors known to affect bioaccumulation include: concentration in the surrounding environment, duration of exposure, solubility of the chemical, and lipid content of the organisms (Gooch and Hamdy, 1983; Hansen et al., 1974).

Until recent years, biomagnification has been a dominant theory explaining the accumulation of persistant chemicals in fish and wildlife. The theory was supported indirectly by the observation that the highest concentrations of PCBs and other persistent organic compounds were found in the higher trophic levels, and appeared to increase in a stepwise fashion from one trophic level to the next (Woodwell et al., 1967; Greichus et al., 1978). An understanding of the contribution of dietary exposure to the total accumulation of residues by organisms is needed, because currently, water quality standards are based on water concentration alone.

The biomagnification process can occur because non-degradable, lipophilic compounds accumulate in fatty tissues, and effective transformation and excretion mechanisms do not exist for the consumed xenobiotics therefore, the consumed residues continue to accumulate throughout the life of the consumer (Bruggeman et al., 1981; Spacie and Hamelink 1985). The widespread distribution of DDT has become a classic example of this phenomena (Neely et al., 1974).

Accumulation of xenobiotics from food will be dependent on the uptake efficiency and on the clearance rate of the compound (Bruggeman et al., 1981). A low clearance rate has been determined to be the main factor responsible for a high bioconcentration factor of chemicals in fish (Neely

et al., 1974; Konemann 1980). It has been suggested that the correlation between amount of a lipophilic compound and trophic level is due to the increase in lipid content with trophic level. Although this is a logical and sometimes emperically supported explanation, there are reports of increase in xenobiotics with trophic level without a concurrent increase in lipids (Greichus et al., 1978). It is now clear that the route of uptake and accumulation of residues in aquatic organisms depends upon the specific food chain, on the availability and persistence of the contaminant in water, and especially on the physical-chemical properties of the compound (Spacie and Hamelink, 1985).

Although biomagnification is still accepted as the primary means of accumulation of chemical residues in terrestrial fauna (Bruggeman et al., 1981) its validity as a means of explaining accumulation in higher trophic levels within aquatic environments is a matter of controversy. Dietary compounds, especially extremely hydrophobic compounds, have been reported to be a major source of accumulated organic compounds in organisms at or near the top of the trophic structure (Macek and Korn, 1970; Harrison et al., 1970; Johnson et al., 1971; Eberhart et al., 1971; Jarvinen et al., 1977; Bruggemann et al., 1981; Jensen et al., 1982; Pizza and O'Connor, 1983; Thomann and Connolly, 1984). However, Moriarty (1972) concluded that for aquatic organisms, direct intake of the chemical from the physical environment is the most important source of accumulation, and that any pollutant ingested with the food is of minor significance. This conclusion has been supported by other researchers (Hamelink et al., 1971; Clayton et al., 1977; Jarvivnen et al., 1977; Williams and Giesy, 1978;

Norstrom et al., 1976). Macek et al., (1979) concluded that food chain transfer was insignificant compared to uptake from water by fish for 6 of 7 compounds examined. DDT was the one exception, with the diet increasing the accumulation in the fish by 30 - 60% over that of uptake from the water alone. Adding confusion to the controversy is the study of Pizza and O'Connor (1982) which reported that the introduction of PCB contaminated algae (Chroococcus) resulted in a decrease in PCB uptake by its amphipod predator Gammarus daiberi, as compared with PCB accumulation resulting from exposure in water only.

2.3 Adsorptive Fate

Extremely hydrophobic compounds, such as PCBs, have a strong affinity for particulate materials (Rubenstein et al., 1984). Consequently, in aquatic systems these compounds are commonly associated with bottom sediments (Sodergren, 1973; Rubenstein et al., 1984). Remobilization of the compounds can occur through slow desorption into overlying waters, resuspension and redistribution during periods of pertubations, and translocation through biological activity (Halter and Johnson, 1977). Due to their physical and chemical properties, Sodergren and Larsson (1982) concluded that once PCBs adsorb to sediment they are unlikely to desorb into water.

PCB concentrations in sediment vary greatly (Fowler et al., 1978); reported values range from $\mu g\ kg^{-1}$ (Hom et al., 1974) to hundreds of $mg\ kg^{-1}$ (Duke et al., 1970). In general, reported amounts of PCBs are higher in sediment than in overlying water (Courtney and Langston, 1978). Nimmo

et al. (1975) reported PCB levels in sediments of Escambia Bay, Florida at 2.3 $\mu g\ g^{-1}$ and seawater at < 0.1 $\mu g\ l^{-1}$. Martell et al., (1975) reported the ratio of PCB in water to sediments of 1:500 for a watershed in Vancouver. These examples demonstrate the tendency for sediments to contain more PCB residue than the overlying water, which is explained by PCB's insolubility.

The bioavailability of substrate-sorbed chemical which have low water solubilities and/or are strongly sorbed to the substrate is of special interest because the accumulation of chemicals by aquatic organisms is assumed to be controlled by the truly dissolved concentration of the chemicals in the water (Hamelink and Spacie 1977; Macek et al., 1979; Hamelink, 1977). Extremely hydrophobic chemicals might be expected to demonstrate low bioaccumulation, since organisms would be exposed only to minute quantities in ambient water. However, extremely hydrophobic chemicals have demonstrated the greatest bioaccumulation potential (Chiou et al., 1977), which is probably due to the inability of the organism to degrade and eliminate the compounds.

The extent to which organic compounds partition between a substrate and the surrounding solution is determined by physical and chemical properties of both the chemical and the substrate (Freed et al., 1977). A chemical is said to be adsorbed to a substrate if the concentration of the chemical is higher in a boundary region, (i.e., substrate's surface) than in an adjacent medium (Tinsly, 1979). The degree of a chemical's adsorption onto a substrate is important because it will affect the

chemical's mobility, bioavailability and toxicity (Pionke and Chesters, 1973; Lyman, 1982; Strek and Weber, 1982).

The importance of organic matter to the sorption of organic chemicals to substrates has been recognized for over 40 years (Chisholm and Koblitsky, 1943), and has since been emphasized in several reviews of soil sorption of organic chemicals (Bailey and White, 1964; Goring, 1967; Hamaker and Thompson, 1972; Kenaga and Goring, 1980). Sorption interactions responsible for the binding of organic chemicals to substrates can be extremely complex and include van der Waal's interactions, hydrophobic bonding, hydrogen bonding, ion exchange, dipole-dipole interactions and chemisorption (Tinsley, 1979; Hamaker and Thompson, 1972). Despite the complexity of the sorption process, in most cases, it is possible to describe the adsorption of a chemical to a substrate in terms of the parameter K_{oc} (partitioning coefficient for organic carbon), which is the amount of chemical adsorbed per unit weight of organic carbon in the substrate divided by the concentration of the chemical in solution (Lyman, 1982; Kenaga and Goring, 1980). Expressing sorption of chemicals using K_{oc} instead of the total substrate eliminates much of the variation in sorption coefficients ordinarily encountered among different substrates (Kenaga and Goring, 1980).

Characterization of Substrate Adsorbants

Sediment represents a heterogeneous mixture of various constituents, namely organic matter, sand, clays, and inorganic salts (Freed et al.,

1977). The nature of the interaction between sediment and the chemical is of great importance in determining the fate and impact of the compound on the environment. To predict the behavior of a particular chlorinated hydrocarbon over a range of sediments, the fundamental reasons for the differences in the behavior must be known.

Most non-ionic organic molecules follow a physical type sorption, with the amount of chemical sorbed being inversely related to its solubility (Lambert, 1967; Freed et al., 1977). The specific surface area of the substrate is very important and will determine the availability of adsorption sites (Hiraizumi et al., 1979; Tinsley, 1979; Morrill et al., 1982). The number and distribution of hydrophobic adsorptive sites are also important (Tinsley, 1979). Strek and Weber (1982) concluded that the type of surface is as important as the amount of surface for PCB adsorption.

The two major fractions of sediment, the organic fraction and the mineral fraction, influence the adsorption process (Tinsley, 1979). The mineral fraction consists of clays and sand. Clay minerals are composed of silicates and metal hydroxides, each of which form layers which interact in various combinations to the the characteristic layered structure of clays. A detailed discussion of the properties of clays is provided by Morrill et al. (1982). Kaolinite is the most common and simple type of clay mineral, being composed of one aluminum hydroxide sheet and one silicon tetrahedral sheet. Montmorillonite clays are composed of two silica sheets per alumina sheet.

The adsorptive capacity of the montmorillonite group of clays is quite large due to adsorption sites on both the outer surface and an expandable interlayer (Huang and Liao, 1970; Morrill et al., 1982). adsorption produces relatively strong organo-montmorillonite complexes and allows for very little desorption of organic compounds from the clay (Lambert, 1975). The affinity of PCBs to montmorillionite clay is reported strong enough to reduce the toxicity to plants (Strek and Weber, 1982).

The two layers forming the lattice structure of kaolinite carry opposite charges at their planes of contact, as a result, kaolinite has a limited capacity for interlayer sorption (Morrill et al., 1982). Adsorption is limited, for the most part, to the external surface of kaolinite minerals; hence organo-clay complexes involving kaolinite are less stable than those with montmorillonite.

Sand has a relatively simple structure when compared to the clays. There is no internal lattice structure which would affect adsorption; therefore, all bonds must form on the external surface. In pesticide adsorption studies using sand as a substrate (Bouchler and Lee, 1972; King et al., 1969), sand had a very low adsorption capacity when compared to clays. This may be due to its low cation exchange capacity and/or the relatively small quantity of surface area (Lambert, 1975).

The organic matter in sediment consists of decomposition products of plants and animals. They form very complex molecular structures and are significant to the sorptive behavior of chemicals because of their very large surfaces, including many interstitial spaces and a very high

cation exchange capacity (Weber and Weed, 1974; Morrill et al., 1982). The organic fraction is hydrophobic and organophilic, which is significant to the sorption of non-ionic organic compounds (Tinsley, 1979), such as PCBs.

Sediment organic matter can be broadly classified into humic and non-humic substances. Non-humic substances consist of unaltered remains of organisms such as cellulose, starch, proteins, chitin, and fats. Humic substances are biological components which have been altered by microbial and/or chemical processes (Morrill et al., 1982). Humic materials are structurally quite heterogeneous; however, they can be operationally defined as organic materials extractable in mildly basic solutions. When the basic extract is acidified, the humic acids are precipitated (Ballard, 1971; Schnitzer, 1978). It has been suggested that humic substances may be important to the movement of organic compounds because soluble humic acids may carry sorbed compounds into solution (Ballard, 1971).

The importance of organic matter to the bioavailability of low water-soluble, non-ionic compounds is recognized (Weber and Weed, 1974) and appears to be the most important component in soil influencing PCB sorption (Haque et al., 1974; Haque and Schmedding, 1976; Lee et al., 1979). Strek and Weber (1982b) found organic matter to be more important than montmorillonite clay at decreasing the bioavailability and toxicity of Aroclor® 1254 to plants.

3.0 OBJECTIVES

Although a great deal of research has been conducted in recent years on the environmental distribution and toxicological characterization of PCBs and other organochlorine compounds, relatively few studies have examined the bioavailabiliy of sediment-sorbed PCB to aquatic organisms. Any meaningful evaluation of the significance of biological exposure to these compounds must consider the abiotic and biotic factors which govern the bioavailability of sediment-sorbed residues. With this in mind, the principle objectives of this investigation were to investigate the relative importance of abiotic and biotic factors on the bioavailability of sediment-sorbed hexachlorobiphenyl (HCB), and to characterize the relative role of environmental and dietary exposure to the bioaccumulation of HCB. In order to achieve these goals it was necessary to develop reproducible procedures for the quantitation of bioaccumulation of sediment-sorbed HCB.

4.0 MATERIAL AND METHODS

4.1 Experimental Design

As discussed in the introduction, several factors may influence the flux of HCB in aquatic systems. Experiments were designed to elucidate the contribution of abiotic and biotic factors to the acccumulation of sediment-sorbed HCB by aquatic organisms. Among the factors examined were sediment type, organic content of sediment, HCB concentration of sediment, and the influence of biological processes on accumulation. Superimposed on all processes was exposure time.

Sediment HCB concentrations were chosen to expose experimental test organisms to environmentally realistic concentrations of HCB. Aquatic organisms used in these studies were mixed sediment bacteria, midge larvae (Chironomus tentans), and mosquitofish (Gambusia affinis). These organisms represent three trophic levels and are indicative of the diversity of biota in aquatic ecosystems potential exposed to PCBs. These organisms were examined independently to determine the sediment bioavailability of HCB at each trophic level.

Principle research tasks included:

1) Developing a quantitative procedure to separate bacteria from sediment.

2) Determining the time course bioaccumulation of HCB for each biological population.

3) Determining the steady-state kinetics of bioaccumulation and partitioning of HCB by each laboratory biological population exposed to HCB contaminated substrates.

4.2 Reagents and Chemicals

Hexane, acetonitrile, acetone, methanol, and isooctane used for the extraction and analysis of samples were all Fisher pesticide grade solvents (Fisher Scientific, Norcross, Ga.), and were used without additional purification. Formaldehyde (37% w/w) was obtained from Aldrich (Aldrich Chemical Co., Milwaukee, Wi.). Mercuric chloride, used as bacteriocide, was obtained from Fisher, as was the Florisil® and anhydrous sodium sulfate used in cleanup procedures.

^{14}C-2,2',4,4',5,5'-hexachlorobiphenyl (^{14}C-HCB) was purchased from Pathfinder Laboratories, Inc.(St. Louis, Mo.) ^{14}C-HCB was uniformly labelled, with a specific activity of 14.09 mCi mmol^{-1}. D-[1-^{3}H] glucose, used to radiolabel bacteria, was obtained from Amersham Corp. (Arlington Heights, Il.), and had a specific activity of 12.7 mCi mmol^{-1}. Ready-solv HP/b scintillation cocktail was purchased from Beckman (Beckman, Instruments, Inc. Fullerton, Ca.). 2,2',4,4',5,5'-hexachlorobiphenyl (HCB) was purchased from Ultra Scientific, Inc. (Hope, R.I.), and had a reported purity of 99%; it was used without further purification.

4.3 Media

Yeast extract polypeptone glucose agar (YEPGA) (Sayler, et al., 1979) was used to culture sediment bacteria. The formulation (in g l^{-1})

was : purified agar (Fisher), 18; dextrose (Fisher), 1.0; polypeptone (BBL), 2.0; yeast extract (BBL), 0.2; NH_4NO_3, 0.2. The minimal salts medium used for culturing bacteria contained (in g l^{-1}): $NaNO_3$, 4.0; KH_2PO_4, 1.5; Na_2HPO_4, 0.5; $FeSO_4$-$7H_2O$, 0.0011; $MgSO_4$-$7H_2O$, 0.2; $CaCl_2$-$2H_2O$, 0.01; final pH 7.0.

4.4 Water

Distilled water was double distilled and had a pH of ~ 6.3. Deionized water was obtained from tap from the Hesler Biology Building (University of Tennessee) and had a pH of ~ 6.2. Reservoir water was collected from Center Hill Reservior, near Crossville, Tennessee. This reservoir is pristine in nature and essentially free of PCB contamination (Sayler et al., 1979). Reservior water was filtered in the laboratory, using a stainless steel millipore apparatus (Millipore Corp., Bedford, Ma.) with Nucleopore polycarbonate filters (0.2 μ, 90 mm diameter) and Millipore type AW prefilter. The filtered reservoir water was stored in a sterile Nalgene carboy (15 l). The pH of the filter reservoir water was ~ 7.5.

4.5 Physical Substrates for Sorbed HCB

The substrates used in this investigation were kaolinite, montmorillonite, sand, natural reservoir sediment, oxidized reservoir sediment, and humic acids. Kaolinite was obtained from Fisher Scientific. Montmorillonite (No. 25, Upton, Wy.) was obtained from the reference collection of the Department of Plant and Soils at the University of Tennessee. Silica sand, previously acid washed, was seived between 0.5

and 0.25 mm seives and washed with deionized water. Humic acids were obtained from Aldrich. Natural sediment was collected from the Little River embayment of Fort Loudoun Reservior, Knoxville, Tn., adjacent to the Tennessee Valley Authority's Singleton Plant (Kong and Sayler, 1983).

Little River sediment, collected with an Ekman dredge, had a loose consistency and was free of obvious detritial material (i.e., leaves and sticks). Sediment was transferred to the laboratory in 4-l glass jars, where it was spread over aluminum foil and allowed to air dry at room temperature (20 - 22°C) for several months. The dried sediment was ground to a fine powder with a mortar and pestle and stored in 4-l glass jars.

The sediment consisted of 49% silt, 34% clay and 17% sand, as determined by a hydrometer method (Day, 1965). Combustable carbon content was 4.5% (SD = 0.08) by dry weight, as determined with a carbon deteriminator (Model CR12, Leco Corp.). A differential scanning calorimeter (Model 910, R-90 Thermal Analyzer, Dupont) operated at 20°C min^{-1} from 255 - 585°C, showed that most activity was due to organics. A qualitative analysis using Norelco X-ray Diffraction Analyzer (Philips Electronics), showed that the sediment contained small quanties of mica, kaolinite, vermiculite and/or chlorite, and a large amount of quartz; these determinations were provided as a courtesy by Dr. David Lietzke.

Sediment free of organic matter (oxidized sediment) was obtained by combusting a sample of dry natural sediment in an oven at 550°C for one hour (Dean, 1974). Analysis of oxidized sediment with a carbon determinator (Model CR12, Leco Corp.) showed that it contained < 0.1% combustable carbon.

4.6 Preparation of HCB Contaminated Sediment

Sediment experimentally contaminated with HCB was prepared separately for each group of experiments. ^{14}C-HCB or HCB, dissolved in acetone, was added with microsyringe (Hamilton Co., Reno, Nv.) to the surface of a weighed aliquot of dry sediment. The acetone was allowed to evaporate before further processing. The preparation of sediment for each group of experiments are described below.

Preparation of Sediment for Bacterial Exposures

Sediments contaminated with ^{14}C-HCB were prepared prior to each experiment. Although the quantity of sediment prepared depended upon the size of the individual experiment, all batches contained sediment (dry weight) to water ratio of 3.7 mg ml^{-1}. Natural sediment and oxidized sediment were used in bacteria HCB accumulation experiments.

An aliquot of dry sediment (usually 67.5 mg) was weighed and transferred to a 25-ml, Teflon®-lined capped, glass bottle. ^{14}C-HCB, dissolved in acetone, was dispersed with microsyringe (Hamilton) onto the sediment. Amounts of ^{14}C-HCB used in the preparations ranged from 54 to 320 µg HCB g^{-1} sediment, in volumes of acetone ranging from 25 to 100 µl. The acetone was allowed to evaporate, then 18.75 ml of distilled water was added. The contents of the bottle were then mixed by sonication (Sonic 300 dismembrator, Artex Systems Corp., Farmingdale, N.Y.) for approximatly three minutes. The bottle and HCB-sediment mixture were then placed on a mechanical shaker and gently agitated for at least 48 hours prior to being used in an experiment.

Preparation of Sediment for Midge Exposures

Sediments used in midge HCB accumulation experiments were: natural sediment, oxidized sediment, kaolinite, montmorillonite, sand, humic acid, and mixtures of natural sediment with humic acid.

^{14}C-HCB contaminated sediments used in midge experiments were prepared in a similar fashion as those for bacteria experiments. An aliquot of air-dried sediment (4.0 g) was weighed and transferred to a 250-ml glass beaker. The ^{14}C-HCB, dissolved in acetone, was added to the sediment using a microsyringe. Volumes of acetone added ranged from 25 to 100 µl. Once the acetone had evaporated, distilled water (100 ml) was added and the beaker's contents mixed for approximately three minutes with sonication. The mixture (sediment/water, 1:25) was allowed to settle for at least 72 hours prior to being used. Sediment HCB concentrations ranged from 1 to 6 µg g^{-1}. Duplicate beakers were prepared for each experimental treatment.

^{14}C-HCB sediment, used to contaminate midge larvae to be used as fish prey, was obtained in a similar manner. An aliquot of sediment (50.0 g) was transferred to a round, flat bottom bowl (20 x 7 cm) and 50 µg ^{14}C-HCB dissolved in acetone was added. After the acetone had evaporated, 750 ml distilled water was added. The mixture was then sonicated for approximately three minutes, and allowed to settle for 24 hours before midge were added.

Preparation of Sediment for Fish Exposures

Sediments used in mosquitofish HCB accumulation studies were: natural sediment, oxidized sediment, sand, and kaolinite. Each batch of HCB-contaminated sediment was obtained by transferring an aliquot of dry sediment into a 4-l glass jar and adding either HCB, or ^{14}C-HCB dissolved in acetone. Once the acetone evaporated, the jar's contents were shaken by hand for approximately 30 minutes. Experimental sediment HCB concentrations ranged from 0.03 to 10.03 µg HCB g^{-1}.

4.7 Collection, Culture and Preparation of Organisms

Bacteria

Mixed bacteria strains were taken from benthic sediment samples collected from the Little River embayment of Fort Loudoun Reservior. Many aquatic bacteria are known to be sensitive to PCBs in low concentrations (Gooch and Hamby, 1983); therefore, these bacteria were chosen because they occur at a site known to be contaminated with PCBs. Bacteria were cultured to a high density (OD > 1.0 at 660 nm) in 500-ml flasks containing sterilized reservoir water supplemented with minimal salt medium (10% v/v) and glucose (2% w/w). The culture was then filtered through glass fiber filters (Type A-E, 25 mm, Gelman Sciences, Inc.) in order to remove cell aggregates and cells too large to pass through the filter. Cells in the filtrate were then pelleted by centrifugation (4060 x g for 10 minutes; Sorvall RC2-B, Dupont) and washed two times with reservoir

water. The final volume of suspended bacteria was adjusted to the desired dilution with additions of reservoir water.

Optical density of the suspended bacteria cells was determined with a spectrophometer (Spectronic 70, Bausch and Lomb); dry weight determinations were made from 5 ml aliquots pipetted into pre-dried tared 6 cm diameter aluminum pans, and heated at 105°C until a constant weight was obtained. Enumerations of viable bacteria were determined using serial dilutions on agar plates (Sayler et al., 1979). The carbon and nitrogen content of the mixed bacteria was determined with an elemental analyzer (Perkin Elmer, Model 240B) to be 50.7% (SD = 3.1) and 10.7% (SD = 0.5) by weight, respectively (n = 4).

The relationship between optical density (OD) at 660 nm and dry weight of serial dilutions of cultured bacteria (initial density = 1.27×10^9 colony forming units ml^{-1}) is adequately described ($r^2 = .99$) with the linear regression model:

$$Y = 1.7 \times 10^{-5} + (6.7 \times 10^{-4} X)$$

where: Y = bacteria dry weight (mg ml^{-1})

and X = OD of bacterial dilution at 660 nm.

This model was used to determine the biomass of bacteria used in the experiments from the OD measurements taken at the initiation of each experiment.

Midge

Midge (*Chironomus tentans*) egg masses were obtained from a population maintained at Oak Ridge National Laboratories (Oak Ridge, Tn.)

courtesy of Paul Franco. Midge were cultured in 35-l aquaria, filled to a depth of approximately 5 cm with deionized tap water. Shredded paper towels, rinsed for at least 24 hours with deionized water, served as substrate for the larvae. Initially, filtered pond water (~ 1 l) was added to each tank in order to establish a microbial population. Midge cultures were fed 0.5 g Tetramin® fish food (Tetre Weke, F.R.G.) mixed in 10 ml deionized water twice per week. About 10 g Cerophyl (powdered grass leaves; Cerophyl Laboratories, Inc., Kansas City, Mo.) was added as a food supplement approximately every two months.

Third and fourth instar midge were used for HCB accumulation experiments. Wet weight of indiviual midge ranged from 5.6 to 17.2 mg; dry weight was 8.73% (SD = 1.11) of the wet weight. Water was maintained at room temperature (20 - 22°C) and continuously aerated. The culture did not require any special lighting.

Mosquitofish

Mosquitofish (Gambusia affinis) were collected with dip net from two abandoned hatchery ponds at the Rhea Springs Fish Hatchery, Fountain City, Tennessee. To reduce variability fish were separated by sex, and only male mosquitofish of approximately the same size were kept. The mosquitofish used in this study weighed 0.09 to 0.18 g (wet weight). The fish were maintained at room temperature (20 - 22°C) in an aerated, filtered 30-gallon aquaria. Fish were fed Tetramin® flake fish food daily. Analysis of mosquitofish and fish food by solvent extraction and electron capture gas chromatography showed both to be free of HCB residues.

4.8 Bacteria and Sediment Separation

To determine the fate of HCB in experiments involving the exposure of bacteria to HCB-contaminated sediments, it was necessary to devise a procedure which would separate bacteria and sediment. A filtration procedure was adopted which provided a reproducible, quantifiable separation, which allowed for the determination of HCB in the bacteria and sediment fractions.

The filtration apparatus consisted of a vacuum filter holder (K25, Micro Filtration Systems, Dublin, Ca.) mounted through a rubber stopper onto an aspirator flask (500-ml). Filters were glass fiber filters (Type A-E, Gelman). Aspirator pressure was 450 psi. A plastic scintillation vial (20-ml) was taped to the exit port of the filtration apparatus to collect the filtrate.

The recovery of bacteria using the filtration procedure was determined with ^{3}H-labelled bacteria. A high density culture of bacteria was diluted with distilled water to 100 ml in a 250-ml flask. ^{3}H-glucose (Amersham) was added to the culture and the flask and contents were placed on a shaker for 18 hours. The ^{3}H-labelled bacteria were centrifuged (4060 x g for 5 minutes) and the supernatant decanted, and the cells were washed with distilled water three times. Washed bacteria were brought to a final volume of 75 ml with distilled water. Four bacterial concentrations were obtained by diluting the 75 ml bacteria culture with additions of distilled water at culture to water ratios of 20:0, 7:1, 3:1, and 5:3. Aliquots of each dilution were taken for dry weight and optical density

determinations. Bacteria (1 ml) were then transferred with pipet into 4-ml vials, which contained either 0.5 ml of water and 1.83 mg sediment, or only 0.5 ml water. The contents of the vial were then separated using the filtration procedure. Recoveries of radio-labelled bacteria in each fraction were determined using liquid scintillation spectrometry.

The recoveries of ^{14}C-HCB labelled sediment using the filtration procedure were determined for each experiment from controls (without bacteria). The same experimental procedures were followed; however, distilled water instead of diluted bacteria, was mixed in the 4-ml vial with the contaminated sediment. The contents of the vials were then filtered and the HCB recoveries determined using liquid scintillation spectrometry.

4.9 HCB Accumulation by Organisms

Bacteria Accumulation of HCB

The following procedure was followed for all experiments involving bacteria. A schematic of the experimental procedure is shown in Figure 1. An aliquot of suspended ^{14}C-HCB contaminated sediment (0.5 ml), containing 1.83 mg sediment, was transferred with a Corning 1 ml borosilicate glass pipet (Fisher) to a 4-ml glass vial (Fisher). To reduce variability in the HCB content among replicate samples, the pipet was repeatedly filled and emptied prior to the first transfer in order to saturate the surface of the pipet with HCB. In addition, the sediment was suspended by sonication before each transfer.

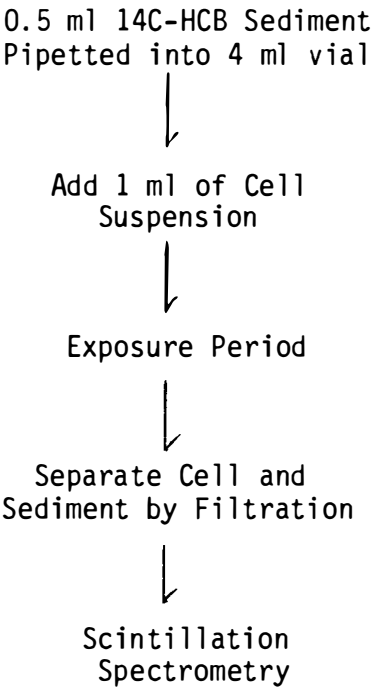

Figure 1. Schematic of the experimental procedure for the exposure and separation of bacteria to 14C-HCB contaminated sediment.

One ml of the bacterial sample was transferred with a second pipet to each vial containing HCB-sediment (sediment/water, 1:818). The vials were then sealed with Teflon®-lined caps, vortexed (~ 3 seconds), and placed in a test tube rack for the duration of the exposure period.

At the end of each exposure period, vials containing each experimental treatment and control were randomly chosen for filtration. The vial's contents were mixed with a vortex, and transferred with a clean Pastuer pipet to the surface of the filter. Care was taken to avoid getting any of the mixture on the walls of the filtration apparatus. The filtrate was collected in a plastic scintillation vial, which was then removed and replaced with a second scintillation vial. The 4-ml exposure vial was then rinsed with 1.5 ml distilled water, which was transferred to the filter with pipet. The second scintillation vial with filtrate was then replaced with a third scintillation vial. The exposure vial was rinsed for a second time with 1.5 ml distilled water, which was transferred to the filter and collected as the third filtrate. The third scintillation vial with filtrate was then removed.

The filter was removed with forceps from the apparatus and placed in a fourth scintillation vial. The filtration apparatus was then rinsed with 10 ml scintillation cocktail (Beckman Ready-solv HP/B), which was collected in the fifth scintillation vial. The 4-ml exposure vial is then placed into a sixth scintillation vial. The filtration apparatus was then rinsed with tap water, blotted dry with paper towel and reassembled for the next sample filtration. The quantity of ^{14}C-HCB in each of the six

scintillation vials was determined using liquid scintillation spectrometry.

Bacteria and sediment HCB concentrations were calculated based on the assumption, that once the HCB recoveries were corrected for the filtration separation efficiency, the ^{14}C activity of the filtrate and the filter represented HCB sorbed to bacteria and sediment, respectively. HCB concentrations of bacteria and sediment were based on dry weight. Concentrations factors were calculated as the HCB concentration of the bacteria divided by the sediment HCB concentration. Steady-state HCB concentrations in bacteria were assumed when there was no significant difference ($\alpha = 0.05$), as determined with linear regression, in mean HCB concentrations of bacteria samples pooled from each experimental treatment.

Midge Accumulation of HCB

Glass beakers (250-ml) were used as exposure containers for midge HCB accumulation experiments. Each beaker contained 4 g of ^{14}C-HCB sediment and 100 ml deionized water. Immediately prior to beginning an experiment, 3rd and 4th instar midge were collected from rearing tanks, rinsed in a beaker of distilled water and transferred to the experimental beakers. Approximately 15 midge were added to each beaker. Two beakers were used for each experimental treatment. Midge were not fed during the course of the experiments. Water level was maintained at 100 ml throughout each experiment. Durations of exposures ranged from 3 hours to 8 days.

At the end of each exposure period, 3 - 5 midge were collected with forceps from each replicate beaker. Each midge was rinsed for approximately 10 seconds in a beaker of deionized water, then blotted with paper towel. The wet weight of the blotted midge was measured with a Roller-Smith balance (BioLar Corp., North Grafton, Ma.). The weighed midge was then placed in a 20-ml scintillation vial, and later analyzed for ^{14}C-HCB.

Midge HCB concentrations were determined on a wet-weight basis. Concentration factors were calculated as HCB concentration of midge (wet weight) divided by HCB concentration in sediment (dry weight) (McLeese et al., 1980). Steady-state midge HCB concentrations were determined using linear regression as described with bacteria. Dead or pupating midge were removed and not used in the analyses.

HCB Contaminated Midge as Prey for Fish

Midge (3rd and 4th instar), contaminated with ^{14}C-HCB, were used as prey for fish to determine the contribution of diet to the fish HCB body burden. Approximately 200 midge were collected from the rearing tanks and transferred to the bowl containing 50 g of ^{14}C-HCB natural sediment. The bowl was continually aerated, and the water level was maintained at 750 ml. Midge were fed 0.5 g Tetramin® fish food mixed in 10 ml deionized water on the 3rd or 4th day of exposure. At the end of eight days exposure, midge were transferred to a bowl containing 750 ml water and 50 g of HCB-free natural sediment. After 24 hours, midge were collected with forceps, rinsed in a beaker of deionized water, then blotted with a paper

towel. Midge were placed individually in the wells of a multiwell tissue culture plate (Falcon Corp., Oxnard, Ca.) and frozen at -5°C.

Mosquitofish Accumulation of HCB

Mosquitofish HCB accumulation experiments were conducted in 4-l glass jars, containing 20 g of HCB contaminated sediment and 3 l of deionized water (sediment/water, 1:150). Experimental containers were prepared and allowed to equilibrate for at least one week before fish were added. Fish were randomly collected with a dip net from the 30-gallon tank, and one fish was placed in each jar. Food was sprinkled onto the water's surface, and was readily consumed by the fish throughout the experiment. Daily food rations of Tetramin® were estimated at 5% of body weight. Water level was maintained at 3 l throughout each experiment.

After each exposure period, five jars were randomly chosen from each experimental treatment. The fish were collected with a dip net, rinsed in the net with deionized water, and sacrificed. The fish were then individually wrapped in aluminum foil and frozen at -5°C until analyzed. Results were determined on a whole-body, wet-weight basis. Concentration factors and steady-state HCB concentrations were determined as with midge. Fish that died during exposures were not included in the analysis.

4.10 Depuration and Biological Half-Life of HCB

The depuration rate constants, K_d, were calculated from HCB contaminated organisms that had been transferred to uncontaminated water using the equation:

$$\ln C_a = \ln C_o - K_d t$$

where: C_o = [HCB] of the organism at time zero

C_a = [HCB] of the organism after depuration

and t = length of the depuration phase.

The biological half-life ($t_{1/2}$) of HCB in an organism was determined as:

$$t_{1/2} = \frac{\ln 0.5}{K_d}$$

where: $t_{1/2}$ = half-life of elimination

and K_d = depuration rate constant.

Depuration of HCB from Bacteria

Depuration of HCB from bacteria was determined using the same culture and experimental procedures as were used in the accumulation experiments. Bacteria were cultured for four days in a flask contaminated with ^{14}C-HCB. The bacteria were then washed and diluted. Mean bacteria ^{14}C-HCB concentration at the beginning of the depuration phase was 220.8 µg g^{-1}. Depuration experiments were conducted using natural sediment and oxidized sediment. Depurations were measured over a 48 hour period.

Depuration of HCB from Midge

The depuration of HCB from midge was determined following 96 hours exposures to natural sediment contaminated with 1 µg ^{14}C-HCB g^{-1}. Midge contaminated with ^{14}C-HCB were transferred to 250-ml beakers containing either 100 ml deionized water and natural or oxidized sediment (4 g), or

only 100 ml deionized water. Midge HCB concentrations were then determined at 0, 24, 48 and 72 hours.

Depuration of HCB from Mosquitofish

The clearance of HCB from mosquitofish was determined using fish exposed for 46 days to natural sediment containing 1.03 µg HCB g^{-1}. After the uptake phase, each fish was transferred with net to a 4-l jar containing 3 l of deionized water. At 0, 5, 10, 20, and 30 days, fish were randomly selected, sacrificed, individually wrapped in aluminum foil, and frozen at -5°C until analyzed for HCB.

4.11 Mosquitofish HCB Accumulation from Diet versus Sediment

Three experimental treatments were used to determine the relative importance of dietary and sediment HCB exposures to mosquitofish accumulation of HCB. Mosquitofish HCB concentrations were determined for: (a) exposure to HCB contaminated sediment, (b) dietary exposure to ^{14}C-HCB, and (c) both sediment and dietary HCB exposures. Accumulation resulting from diet and sediment sources were distinguished by using ^{14}C-HCB to contaminate midge prey and non-radiolabelled HCB to contaminate sediment. The contaminated sediment was obtained by spiking natural sediment with HCB to a final concentration of 1.03 µg g^{-1}. Contaminated prey were obtained by rearing midge (3rd and 4th instar) for eight days in natural sediment contaminated with ^{14}C-HCB at a concentration of 1 µg g^{-1}. During the experiment, fish were fed either uncontaminated or contaminated midge each day, alternating between one and two midge per day for the exposure

period, which was equivalent to a food ration of approximately 8% of the fish body weight day^{-1} and a mean dosage of 5.1 ng ^{14}C-HCB day^{-1}.

To determine accumulation of HCB residues in fish, five fish were removed from each experimental treatment at 12, 23, 35, and 46 days, and each fish was analyzed for HCB and ^{14}C-HCB, using gas chromatography and liquid scintillation techniques. The amount of HCB measured with liquid scintillation spectrometry estimated the result of dietary exposure; the difference in the amounts of HCB determined by gas chromatography and liquid scintillation techniques was assumed to be the result of sediment exposure. Results were determined on a whole-body, wet-weight basis.

The diet concentration factor (DCF) was calculated as:

$$DCF = \frac{C_t}{TD}$$

where: C_t = [HCB] in fish at time t

TD = total HCB in the diet.

Mosquitofish Dietary HCB Accumulation Efficiency

To calculate the assimulation of HCB from midge prey, fish were fed ^{14}C-HCB contaminated prey and the fecal sclera (eaten midge) were recovered and analyzed for ^{14}C-HCB content. Since midge larvae were swallowed whole by the fish and the fecal sclera remained intact, it was possible to collect the waste products with forceps. During the experiment, each fish was fed one midge, and the fecal sclera were collected 24 hours later. Five fish were used, each fed one midge on three non-consecutive days, for a total of 15 samples.

Control (not eaten) ^{14}C-HCB contaminated midge (n = 15) and the fecal sclera (n = 15) were analyzed for ^{14}C-HCB using liquid scintillation procedures. The extraction efficiency was calculated as:

$$1 - \frac{\text{HCB fecal sclera}}{\text{HCB non-prey}}$$

4.12 HCB Extraction and Sample Preparation

Water

Water samples (5 ml) from midge experiments were analyzed for ^{14}C-HCB using liquid scintillation spectrometry. Each water sample was taken with a 5-ml volumetric pipet and transferred to a 20-ml scintillation vial. The pipet was then rinsed with hexane, which was collected in the same scintillation vial. The contents of the vial was concentrated in an oven at 80°C until approximately 1 ml remained. Scintillation cocktail (Beckman Ready-solv HP/b) was then added to each vial and activity determined with scintillation spectrometry.

Water samples from bacteria and mosquitofish experiments were extracted with hexane (Fisher) using a procedure adapted from Konemann and van Leeuwen (1980). To obtain water samples from bacterial HCB accumulation experiments, exposures were conducted in 1.5 ml plastic centrifuge tubes. Following sample centrifugation (12,500 x g for 30 seconds, Eppendorf Centrifuge 5414, Brinkman Instruments, Inc., Westbury, N.Y.) 1 ml water samples were taken with pipet and each transferred to a 10-ml glass vial. The pipet was then rinsed three times with hexane, which was

collected with the water sample. The water and hexane were vigorously shaken and then allowed to separate. The organic phase was transferred to a concentrator tube, and the extraction of the water was repeated.

Water samples from mosquitofish experiments were removed from the experimental containers with a 20-ml volumetric pipet, and transferred to a 250-ml separatory funnel. The pipet was then rinsed three times with hexane (10 ml), with the rinse being collected in the separatory funnel. The separatory funnel was shaken vigorously for approximately one minute, then allowed to stand quiescent for complete separation of the phases. The aqueous layer was collected in a glass beaker and the organic phase in a round bottom flask (125-ml). The water was returned to the original separatory funnel, and the beaker was rinsed twice with 10 ml hexane, which was also transferred to the separatory funnel. The mixing and separation of the phases was repeated, with the organic phase being added to 125-ml flask containing the first separated phase.

The organic phase from bacterial and mosquitofish water extractions were then concentrated to approximately 0.5 ml using a rotovap (Brinkman) and heat bath (40°C). The extract was then transferred with Pasteur pipet to a 10-ml volumetric concentrator tube. The 125-ml flask was rinsed three times with hexane, and the rinsate was transferred with the pipet to the concentrator tube containing the extract. Cleanup of the extract was not required. The extracts were stored at -5°C until analyzed by gas chromatography.

Sediments

Sediment HCB concentrations for the bacterial accumulation experiments were determined on a dry weight basis using the same procedures as for bacterial HCB determinations with liquid scintillation spectrometry.

Sediment HCB concentrations for the midge accumulation experiments were determined on a dry weight basis by subtracting the quantity of HCB accumulated by midge from the initial sediment HCB concentration. This mass balance approach assumed that all HCB not accumulated by midge was sorbed to the sediment.

Sediment in the mosquitofish accumulation experiments was extracted using procedures adapted from those of Goerlitz and Law (1974) and Watts (1980). Sediment was allowed to air dry at room temperature before 2 g subsamples were taken for extraction. Each sample was transferred with spatula to 50-ml test tubes with Teflon®-lined caps. The sample was moistened with 1 ml distilled water, followed with the addition of 20 ml hexane-acetone (1:1). The test tube's contents were mixed for 24 hours using a wrist-action shaker. The phases were allowed to separate, then the organic phase was transferred with Pastuer pipet to a 125-ml separatory funnel. The extraction procedure was then repeated two additional times with 10 ml hexane-acetone (1:1) and a 30 minutes mixing period. The organic phases were transferred to the separatory funnel, and mixed with 50 ml of hexane-extracted distilled water. The layers were allowed to separate, then the aqueous phase was drained into a second

separatory funnel (250-ml). The organic phase in the first separatory funnel was then washed two more times, by mixing with 50 ml distilled water, each time with the aqueous phase being transferred to the second separatory funnel.

The contents of the second separatory funnel were then mixed with approximately 20 ml hexane. The phases were allowed to separate and the aqueous layer was discarded. The organic layer was drained into the first separatory funnel. The second separatory funnel was then rinsed twice with hexane, with the rinse being collected to the first separatory funnel. The organic extract was drained into a 125-ml round bottom flask containing approximately 1 g anhydrous sodium sulfate (Fisher). The separatory funnel was then rinsed twice with hexane.

The extract was concentrated to approximately 0.5 ml using a rotovap (Brinkman) and heat bath (40 D°C), then transferred with pipet to a 10-ml volumetric concentrator tube. The flask was then rinsed three times with hexane (Fisher). The desired final volume was obtained by the addition of hexane to the concentrator tube.

Extraction efficiencies were calculated for HCB-spiked sediment by dividing the sediment HCB recovery ($\mu g\ g^{-1}$) by the amount of HCB added to sediment ($\mu g\ g^{-1}$). Measured sediment HCB concentrations were corrected for extraction efficiencies.

Bacteria

The six scintillation vials collected during the initial filtration procedure contained the filtrate, two rinse filtrates, the filter, the

4-ml glass exposure vial, and the 10 ml scintillation cocktail used to rinse the filtration apparatus. Ready-solv HP/b scintillation cocktail (10 ml) was added to each of the vials, except the vial which already contained cocktail. The vials and contents were then placed on a mechanical shaker for 24 hours. The samples did not receive any further treatment.

Midge

One ml of tissue solubilizer (Protosol®, New England Nuclear, Boston, Ma.) was pipetted into each scintillation vial containing midge. The vials were then placed on a mechanical shaker for approximately 72 hours. This procedure provided sufficient aggitation and time to fully digest the midge tissue. Liquid scintillation cocktail (10 ml; Beckman Ready-solv HP/b) was then added, and the vials mixed on the mechanical shaker for 24 hours.

Mosquitofish

The procedure used for the extraction and cleanup of HCB from fish tissue was one recommended by Watts (1980) for micro quantity samples. Each frozen fish was thawed, blotted with paper towel and the wet weight determined using a Mettler balance. The fish was then extracted with acetonitrile (Fisher) in a 10-ml glass tissue grinder. The residues were removed from the acetonitrile with hexane, which was then concentrated with rotovap (Brinkman). The concentrated extract was cleaned by eluting through a Florisil® packed glass column. The hexane eluate was then con-

centrated with ropovap and transferred to a 10-ml concentrator tube. No further treatment was necessary for gas chromatographic analysis.

Extracts from fish exposed to ^{14}C-HCB were collected in 20-ml scintillation vials. The extracts were concentrated to approximately 1 ml under a stream of nitrogen gas. Liquid scintillation cocktail (10 ml; Beckman Ready-solv HP/b) was added to each vial, and the vials were placed on a mechanical shaker for 24 hours.

4.13 HCB Quantitation

Quantitation of ^{14}C-HCB was done by liquid scintillation spectrometry using a Tracer Analytic Model 6892 liquid scintillation spectrometer (Elk Grove Village, Il.). Counting time was set at 2 minutes or until 10,000 counts were reached. Ten ml aliquots of scintillation cocktail (Beckman Ready-solv HP/b) were used for all samples. Counts per minute (CPM) were corrected for background, and converted to disintergrations per minute (DPM) by dividing the corrected CPM by the counting efficiency. Mean background levels for untreated test samples were determined to be 50 cpm for water, 50 cpm for bacteria samples, 50 cpm for sediment, and 65 cpm for midge. Counting efficiencies, determined using ^{3}H and ^{14}C standards, were always greater than 96%.

Quantitation of the non-^{14}C labelled HCB used in this study was accomplished using gas chromatographic (GC) techniques. The GC used in this study was a Hewlett-Packard 5840A gas chromatograph equipped with a ^{63}Ni electron capture detector. The glass column (1.8 m x 2 mm ID) was packed with 80-100 mesh Gas-Chrom Q coated with 4% SE-30/6% OV-210. The

injector, column and detector temperatures were 225, 190, and 275°C, respectively. Argon-methane gas (95:5) served as the carrier gas at a flow rate of 20 ml min^{-1}.

After the extraction and cleanup of the sample, the final volume of the solvent was recorded. An unknown quantity of HCB was introduced into the GC by 5 µl injections with a microsyringe (Hamilton). Under the previously described GC conditions, it took approximately 11 minutes for HCB to be eluted from the column. Temperature programming was used as a means of increasing the rate of elution of residues which were retained on the column after HCB. For temperature programming, the column temperature was increased at 13 minutes from 190°C to 225°C, at a rate of 5°C min^{-1}. This procedure resulted in the elution of all residues from the column within 35 minutes of sample injection.

Known concentrations of HCB in isooctane (Fisher) were prepared and served as standards. The quantities of HCB in samples were determined by comparing the area of the samples' chromatograms to the area of a known quantity of the HCB standard.

4.14 Statistical Analysis

Data were subjected to statistical analysis using a Dec 10, IBM 3081 computer system. Statistical analysis subprograms for linear regression and t-test were derived from SAS (1982).

^{14}C-HCB concentrations in samples were calculated after correcting for scintillation counting efficiency, background, and recovery. Steady-state HCB concentrations for each experiment was determined from pooled

samples using linear regression; mean concentrations not significantly different were considered at apparent steady-state. Significance limits were set at the 0.05 level.

5.0 RESULTS

5.1 Filtration Recovery of Bacteria and Sediment

The recovery efficiencies of bacteria and sediment were used as correction factors for filtration efficiency in bacterial accumulation experiments. The percent recovery of ^{3}H-labelled bacteria in the presence of 1.83 mg sediment using filtration for the separation of bacteria and sediment are given in Table 1. The mean percent recoveries for four different concentrations of bacteria were 75.22% (SD = 2.16), 24.59% (SD = 2.09) and 0.19% (SD = 0.08) for the filtrate, filter and exposure vial, respectively.

The percent recovery of ^{14}C-HCB labelled sediments using the filtration procedure is given in Table 2. Mean percent recoveries of sediments for the filtrate, filter and exposure vial, respectively were: 5.91% (SD = 1.42), 91.10% (SD = 3.24), 2.99% (SD = 2.93) for natural sediment; 14.16% (SD = 3.06), 83.38% (SD = 2.69), 2.46% (SD = 0.87) for oxidized sediment.

5.2 Water HCB Concentration

Water HCB concentrations using the bacterial experimental design are given in Appendix, Table A-1. The mean water HCB concentrations over five exposure periods were variable, but averaged 0.64 $\mu g\ l^{-1}$ (SD = 0.34) and 1.35 $\mu g\ l^{-1}$ (SD = 0.85) for exposures with bacteria and without bacteria (controls), respectively.

Table 1. Mean percent recovery of 3H-labelled bacteria in the filtrate, filter and the exposure vial, using the filtration procedure for the separation of bacteria and sediment.

Cell mass[a] (mg/ml)	n	Filtrate	Filter	Vial
0.4840	5	71.52 (2.08)[b]	28.16 (2.00)	0.32 (0.23)
0.4356	5	76.98 (1.62)	22.87 (1.63)	0.15 (0.11)
0.3872	5	76.08 (2.25)	23.74 (2.25)	0.18 (0.17)
0.2738	5	76.32 (1.90)	23.59 (1.89)	0.10 (0.16)
		$\bar{X}$ = 75.22 (2.16)	$\bar{X}$ = 24.59 (2.09)	$\bar{X}$ = 0.19 (0.08)

[a] dry weight of cells dried at 105°C

[b] Standard deviation

Table 2. Mean percent recovery of 14C-labelled natural and oxidized sediment in the filtrate, filter, and the exposure vial, using the filtration procedure for the separation of bacteria and sediment.

Sediment	n	Filtrate	Filter	Exposure Vial
Natural	36	5.91 (1.42)[a]	91.10 (3.24)	2.99 (2.93)
Oxidized	6	14.16 (3.06)	83.38 (2.69)	2.46 (0.87)

[a] Standard deviation

The HCB concentrations in water using the midge experimental design resulting from exposures to four different HCB-contaminated sediment, each contaminated with 1 µg ^{14}C-HCB g^{-1}, are given in Table A-2. Measured HCB concentrations with midge present in the sediment were 2 - 8 times greater than concentrations measured without midge present.

Water HCB concentrations during mosquitofish exposures to natural sediment contaminated with 0.03, 0.13, 1.03, and 10.03 µg HCB g^{-1}, and oxidized sediment contaminated with 1.03 µg HCB g^{-1} are given in Table A-3.

5.3 Influence of Sediment on Bioavailabilty

Bacteria HCB Accumulation from Different Sediments

The HCB concentrations of bacteria exposed to contaminated sediments are given in Table 3, and shown in Figure 2. The accumulation of HCB by bacteria exposed to contaminated natural and oxidized sediment was characterized by a rapid initial period of accumulation followed by a second slower phase of accumulation (Figure 2). During the initial 720 minutes exposure, mean rates of HCB accumulation by bacteria exposed to the natural and oxidized sediment were determined (t-test) significantly different and resulted in bacterial HCB accumulation rates of 401 and 614 µg g^{-1} min^{-1}, respectively (Table 3). Mean rates of accumulation during the secondary phase (1440 - 2880 minutes) were not significantly different for bacteria exposed to the two sediments (Table 3). Regression analysis of HCB concentrations in bacteria show that apparent steady-state con-

Table 3. Mean bacteria HCB concentrations, bacteria HCB accumulation rates, sediment HCB concentrations, and concentration factors resulting from exposure of bacteria to 14C-HCB contaminated natural and oxidized sediment.

Sediment	n	Exposure period (min)	Bacteria[a] HCB (µg/g)	Bacterial accumulation rate (ng/g/min)	Sediment HCB (µg/g)	Concentration[b] factor
Natural sediment	3	720	288.48 (6.60)[c]	400.66 (9.16)	190.08 (2.29)	1.52
	3	1440	407.77 (10.22)	168.65 (14.19)	146.04 (3.75)	2.79
	3	2880	485.65 (26.36)	49.11 (18.31)	117.21 (10.32)	4.14
Oxidized sediment	3	720	442.20 (17.63)	614.16 (24.49)	131.20 (6.67)	3.37
	3	1440	475.81 (32.03)	46.16 (44.49) *[d]	119.09 (9.74)	4.00
	3	2880	535.57 (40.32)	39.07 (28.00) *	98.85 (14.14)	5.42

[a] Data normalized to an initial sediment HCB concentration of 298 ug/g.

[b] Concentration factor calculated as bacteria HCB concentration (dry weight) divided by sediment HCB concentration (dry weight).

[c] Standard deviation

[d] Means followed by * were determined not significantly different ($\alpha = 0.05$) with linear regression.

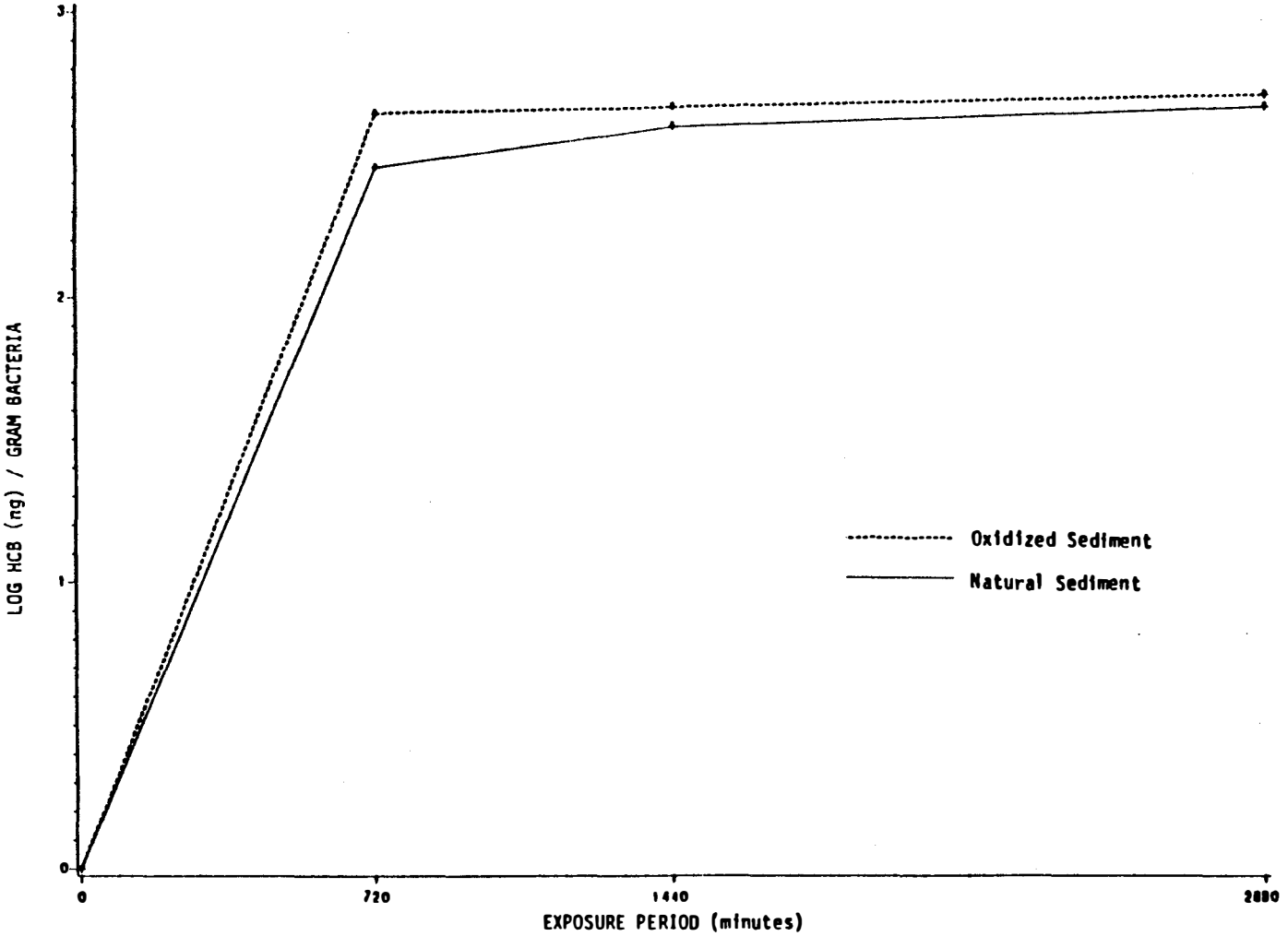

Figure 2. Mean bacteria HCB concentrations resulting from exposure to natural and oxidized sediment, each initially contaminated with 298 µg 14C-HCB/g.

centrations were reached within 1440 minutes in bacteria exposed to oxidized sediment; however, steady-state concentrations were not discernable in bacteria exposed to natural sediment (Table 3). Mean bacterial HCB concentrations resulting from 2880 minutes exposure to contaminated natural and oxidized sediment were not significantly different ($t = 1.79$; $df = 4$).

Midge HCB Accumulation from Different Sediments

Mean midge concentrations of HCB resulting from exposures to ^{14}C-HCB contaminated natural sediment, oxidized sediment, sand, kaolinite and montmorillonite are shown in Figure 3. The mean midge weight, midge HCB concentration, substrate HCB concentration, and rate of midge HCB accumulation for each substrate exposure at each exposure period are given in Table 4.

The rates of HCB accumulation by midge during the initial 24 hours exposure period were determined significantly different (t-test) for all sediments, except sand and montmorillonite (Table A-4). Bioavailability of sediment-sorbed HCB to midge, as indicated by midge HCB concentrations after 120 hours exposure, followed a sequence: natural sediment < oxidized sediment < sand < montmorillonite < kaolinite (Table 4, Figure 3). They were determined by statistical comparison (t-test) to be significantly different for all substrates, except oxidized sediment and montmorillonite (Table A-5).

Regression analysis of HCB concentrations of midge exposed to each sediment indicate that apparent steady-states were reached by 72 hours

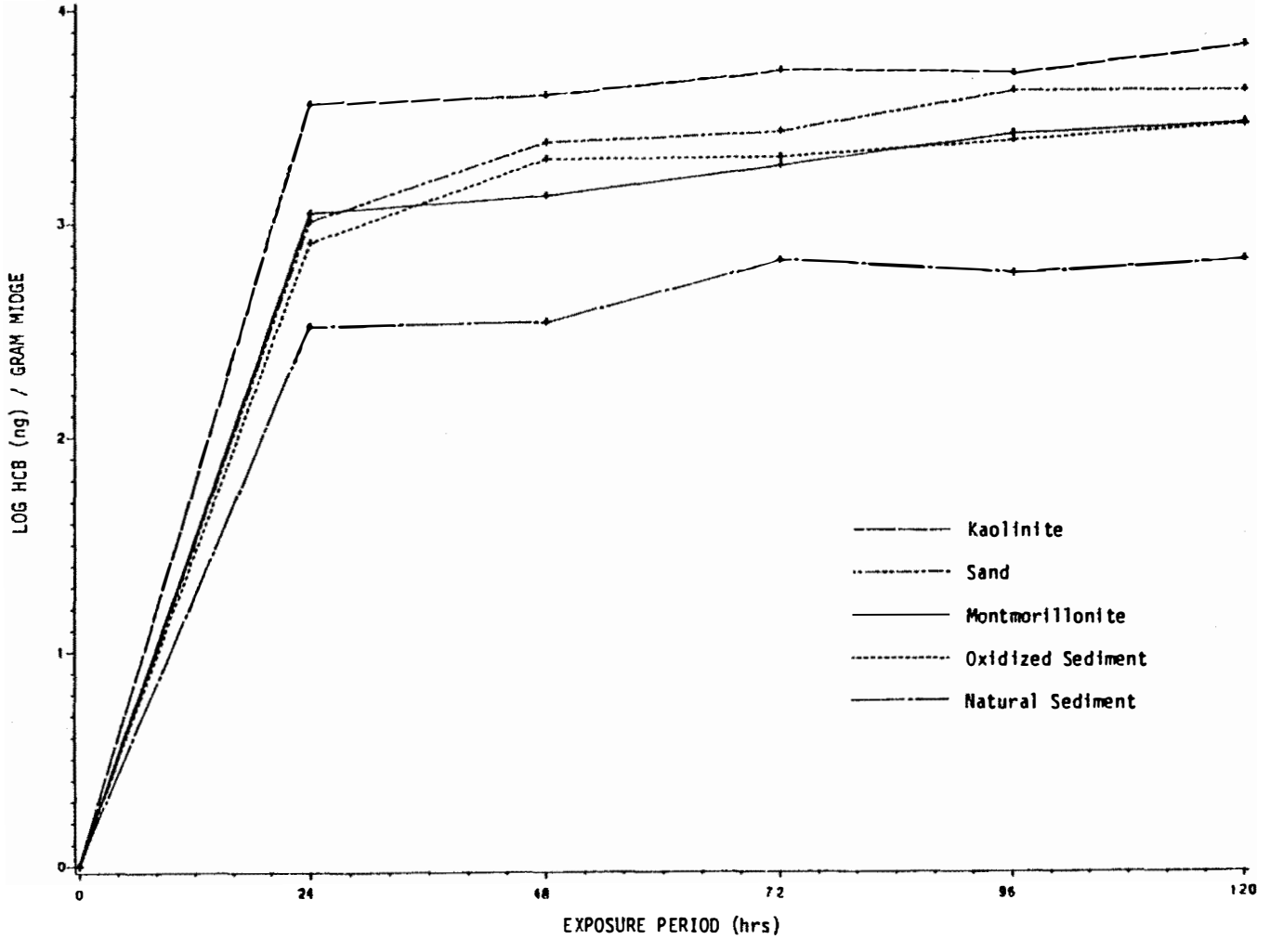

Figure 3. Mean midge HCB concentrations resulting from exposure to natural sediment, oxidized sediment, sand, kaolinite, and montmorillonite, each initially contaminated with 1 µg 14C-HCB/g.

Table 4. Mean midge HCB concentrations, midge HCB accumulation rates, midge weights, sediment HCB concentrations, and concentration factors resulting from midge exposure to natural sediment, oxidized sediment, sand, kaolinite, and montmorillonite, each initially contaminated with 1 ug 14C-HCB/g.

Substrate	n	Exposure period (hrs)	Midge wet weight (mg)	Midge HCB (ng/g)	Midge HCB Accumulation rate (ng/g/hr)	Sediment HCB (ng/g)	Concentration[a] factor
Natural sediment	5	24	8.68 (0.89)[b]	339.02 (77.80)	14.13 (3.24)	995.86	0.34
	7	48	9.06 (3.02)	352.91 (59.21)	0.58 (2.47)	991.96	0.36
	6	72	8.52 (0.79)	689.85 (136.00) *[c]	14.04 (5.67)	984.16	0.70
	7	96	11.93 (1.70)	602.49 (119.58) *	-3.64 (4.98)	977.01	0.62
	5	120	7.70 (1.97)	699.09 (132.40) *	4.02 (5.52)	970.48	0.72
Oxidized sediment	5	24	8.36 (2.40)	807.41 (66.86)	33.64 (2.79)	995.40	0.81
	7	48	8.90 (1.37)	2039.29 (282.34)	51.32 (11.76)	978.60	2.08
	5	72	10.10 (2.71)	2058.31 (315.11)	-1.29 (13.13)	955.09	2.16
	9	96	11.00 (3.92)	2515.45 (643.96) *	19.06 (26.83)	924.95	2.72
	7	120	9.43 (1.68)	2985.81 (395.08) *	19.60 (16.46)	889.08	3.36
Sand	6	24	6.62 (0.68)	1038.48 (101.12)	43.27 (4.21)	992.19	1.05
	7	48	8.77 (1.21)	2514.88 (908.98)	61.52 (37.87)	964.42	2.61
	5	72	7.84 (0.81)	2717.79 (297.44)	8.45 (12.39)	948.98	2.86
	11	96	11.12 (3.44)	4186.57 (442.49) *	61.20 (18.44)	884.09	4.74
	4	120	6.58 (0.94)	4369.77 (813.66) *	7.63 (33.90)	867.70	5.04
Kaolinite	5	24	8.98 (0.99)	3656.42 (662.02)	192.35 (27.58)	970.09	3.77
	8	48	11.46 (3.08)	3865.17 (906.27)	8.70 (37.76)	926.28	4.17
	5	72	10.08 (0.92)	5248.24 (488.94)	57.63 (20.37)	872.69	6.01
	7	96	8.64 (1.26)	5230.26 (894.86)	-0.75 (37.29)	815.22	6.42
	6	120	8.45 (0.71)	7050.49 (722.32)	75.84 (30.10)	761.70	9.26
Montmorillonite	7	24	7.69 (3.05)	1134.91 (216.60)	47.29 (9.02)	990.79	1.15
	7	48	7.91 (2.45)	1396.05 (377.84)	10.88 (15.74)	977.92	1.43
	7	72	7.00 (1.38)	1854.07 (214.76)	19.08 (8.95)	964.23	1.92
	6	96	6.60 (1.17)	2635.63 (305.62)	32.57 (12.73)	948.01	2.78
	6	120	7.30 (1.32)	2996.54 (168.98)	15.04 (7.04)	925.25	3.24

[a] Concentration factor calculated as midge HCB concentration (wet weight) divided by sediment HCB concentration (dry weight).

[b] Standard deviation

[c] Mean midge HCB concentrations for each substrate exposure which are followed by * are not significantly different ($\alpha = 0.05$) as determined with linear regression.

for midge exposed to natural sediment, and 96 hours for oxidized sediment and sand exposures; however, apparent steady-states were not reached during the 120 hours exposures to kaolinite and montmorillonite clays (Table 4).

Midge HCB Accumulation from Sediments Varying in Organic Content

The mean HCB concentrations of midge resulting from exposures to five ^{14}C-HCB contaminated sediments, each varying in organic matter content, are shown in Figure 4, and the data given in Table 5. The accumulation of sediment-sorbed HCB by midge was inversely proportional to organic content of the sediment and followed the sequence: humic acid < sediment/humic (1:3) < sediment/humic (1:1) < sediment/humic (3:1) < sediment (Table 5, Figure 4). The rates of midge HCB accumulation during the initial 24 hours exposure period were significantly different (t-test) for all sediments, except for natural sediment/humic (1:1) and natural sediment/humic (1:3) (Table A-6).

Regression analysis of HCB concentrations of midge exposed to the five sediments suggests that apparent steady-states were reached by 48 hours for midge exposed to natural sediment, and by 24 hours for midge exposed to the four sediments containing humic materials (Table 5). T-test comparisons of mean midge HCB concentrations resulting from 96 hours exposure to the five different sediments shows that accumulation from natural sediment was significantly greater than accumulation from sediment which contained humic acid, and that accumulation from sediments

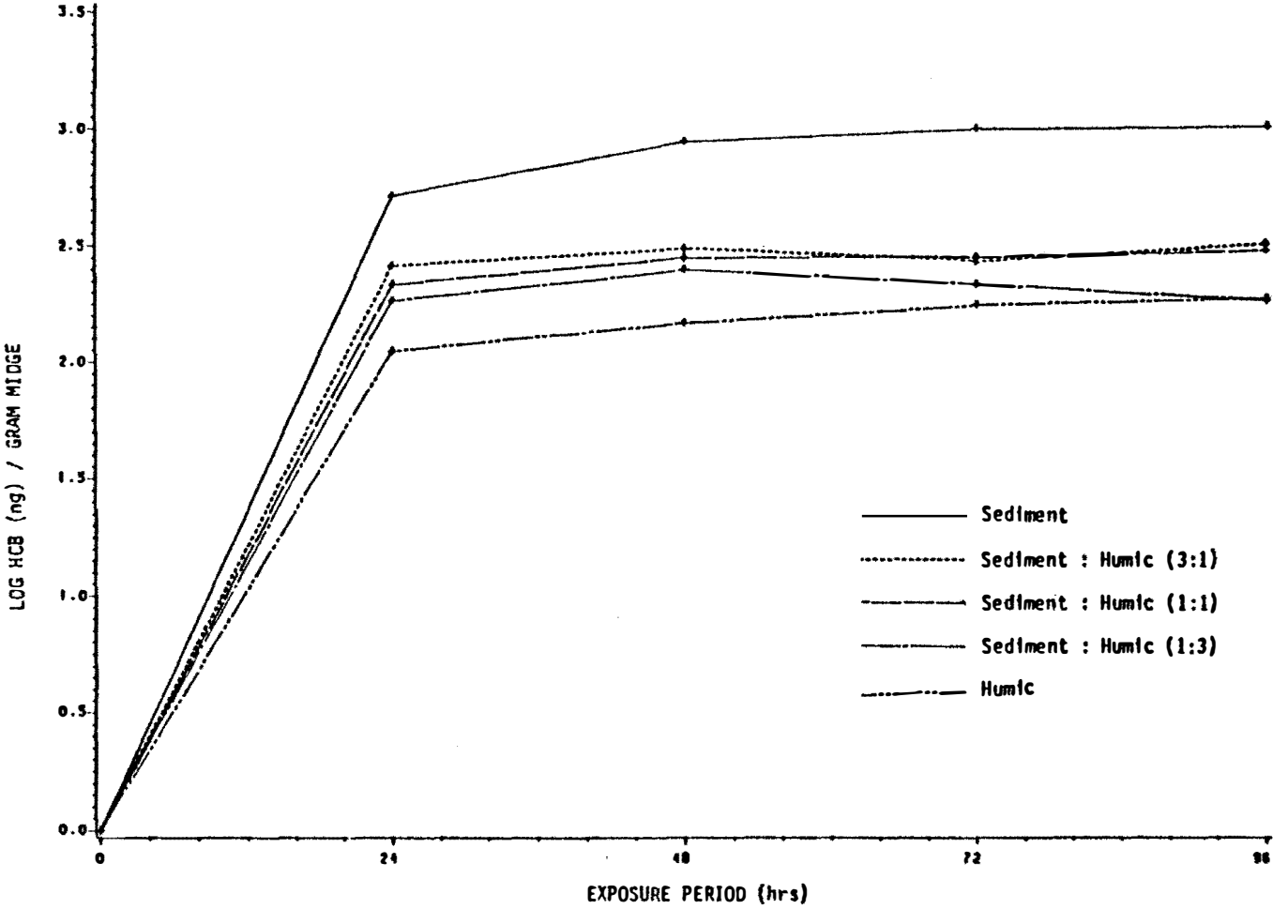

Figure 4. Mean midge HCB concentrations resulting from exposure to five sediments varying in organic content, each initially contaminated with 2 µg 14C-HCB/g.

Table 5. Mean midge HCB concentrations, midge HCB accumulation rates, midge weights, sediment HCB concentrations, and concentration factors resulting from midge exposure to five substrates varying in organic matter content, each initially contaminated with 1 µg 14C-HCB/g.

Substrate	n	Exposure period (hrs)	Midge wet weight (mg)		Midge HCB (ng/g)			Midge HCB accumulation rate (ng/g/hr)		Sediment HCB (ng/g)	Concentration[a] factor
Natural sediment	5	24	6.38	(0.97)[b]	528.26	(61.78)		22.01	(2.57)	1991.7	0.27
	5	48	14.52	(5.21)	943.16	(229.44)	*[c]	17.29	(9.56)	1959.3	0.48
	4	72	8.68	(0.72)	1076.80	(218.06)	*	5.57	(9.09)	1941.2	0.55
	4	96	11.73	(2.26)	1114.03	(179.02)	*	1.55	(7.46)	1920.1	0.58
Natural sediment-humic (3:1)	5	24	8.96	(3.49)	267.85	(36.96)	*	11.16	(1.54)	1993.9	0.13
	5	48	8.78	(1.62)	325.08	(74.89)	*	2.38	(3.12)	1985.1	0.16
	4	72	12.08	(2.60)	323.65	(16.85)	*	-0.06	(6.99)	1976.1	0.16
	4	96	11.30	(4.65)	355.47	(78.73)	*	1.33	(3.28)	1967.4	0.18
Natural sediment-humic (1:1)	5	24	7.64	(1.20)	218.47	(21.88)	*	9.10	(0.91)	1993.9	0.11
	5	24	9.15	(3.97)	287.08	(18.24)	*	2.86	(0.76)	1987.1	0.14
	5	72	6.70	(2.06)	322.29	(171.46)	*	1.47	(7.14)	1981.2	0.16
	4	96	10.85	(2.61)	343.21	(133.98)	*	0.87	(5.58)	1975.2	0.17
Natural sediment-humic (1:3)	5	24	9.86	(2.01)	192.66	(55.73)	*	8.03	(2.32)	1997.2	0.10
	5	48	10.98	(3.40)	261.02	(29.83)	*	2.85	(1.24)	1992.7	0.13
	4	72	10.40	(1.36)	229.67	(58.01)	*	-1.31	(2.42)	1989.9	0.12
	5	96	7.64	(2.30)	200.31	(59.70)	*	-1.22	(2.49)	1987.9	0.10
Humic	5	24	10.30	(2.42)	121.61	(54.99)	*	5.07	(2.29)	1995.9	0.06
	4	48	13.38	(6.65)	163.72	(72.34)	*	1.75	(3.01)	1989.9	0.08
	5	72	6.82	(1.26)	192.07	(69.92)	*	1.18	(2.91)	1985.6	0.10
	5	96	7.10	(1.05)	207.30	(57.04)	*	0.63	(2.38)	1982.1	0.10

[a] Concentration factor calculated as midge HCB concentration (wet weight) divided by sediment HCB concentration (dry weight).

[b] Standard deviation

[c] Mean midge HCB concentrations for each substrate exposure which are followed by * are not significantly different ($\alpha = 0.05$) as determined with linear regression.

containing 50% or more humic acid was not significantly different (Table A-7).

Mosquitofish HCB Accumulation from Different Sediments

The mean HCB concentrations of mosquitofish resulting from exposure to natural sediment, oxidized sediment, sand, and kaolinite, each initially contaminated with 1 μg ^{14}C-HCB g^{-1}, are shown in Figure 5, data are given in Table 6.

The HCB concentrations of mosquitofish resulting from five-day exposure to four contaminated substrates were less with natural sediment than the other sediment types, and concentrations were not significantly different among the other sediment types (Table A-8). However, after 15 days exposure, mosquitofish HCB concentrations were significantly different for all substrate exposures, except oxidized sediment and sand (Table A-9).

The bioavailability of sediment-sorbed HCB to mosquitofish after 15 days followed the sequence: natural sediment < sand < oxidized sediment < kaolinite. Regression analysis of mosquitofish HCB concentrations show that apparent steady-state concentrations were not reached during the 15 days exposures to the four different HCB contaminated substrates (Table 6).

Organic Matter Influence on HCB Availibility to Mosquitofish

The influence of sediment organic matter (OM) on the bioavailability of HCB was assessed by exposing mosquitofish to natural sediment (OM =

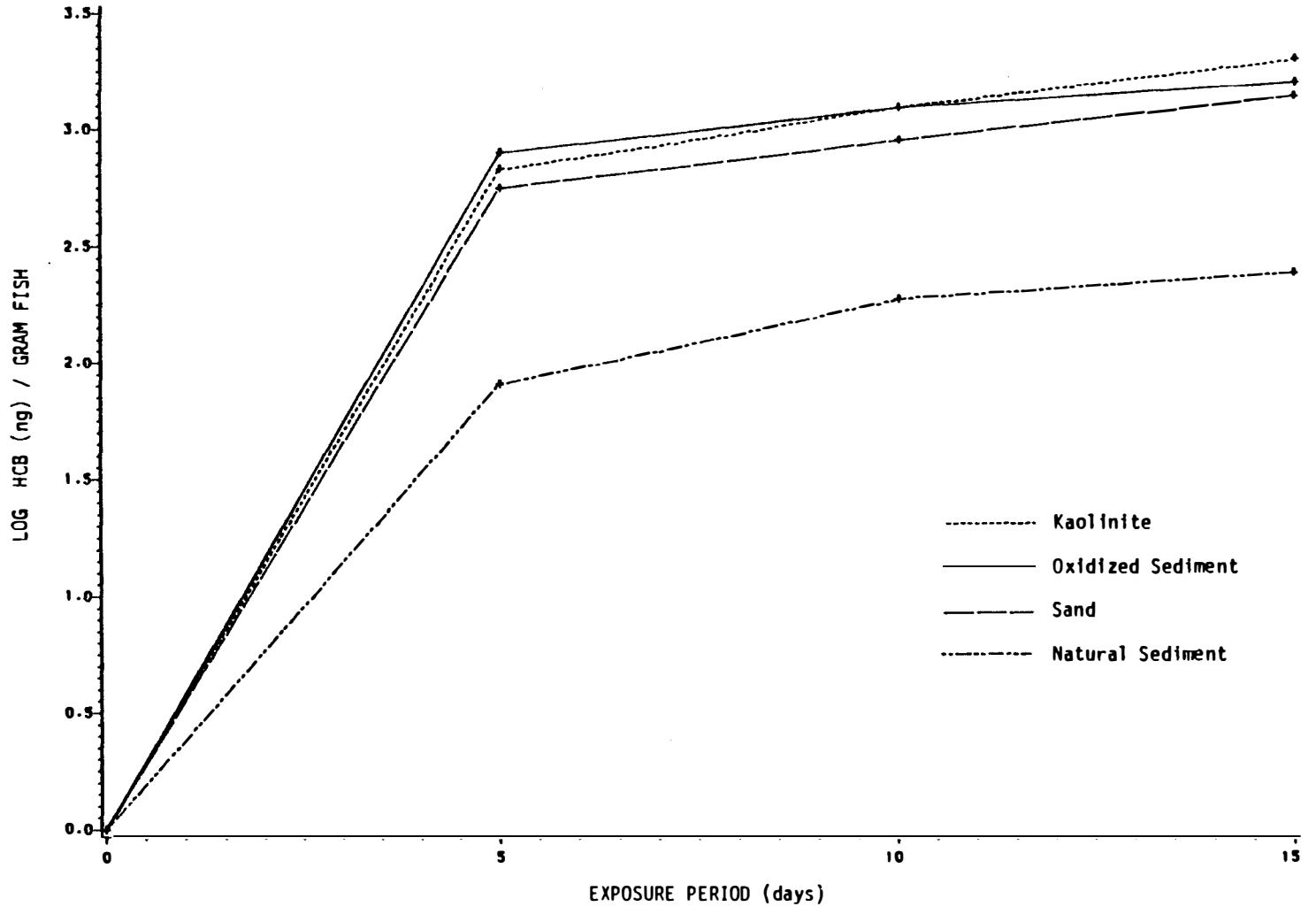

Figure 5. Mean mosquitofish HCB concentrations resulting from exposure to natural sediment, oxidized sediment, sand, and kaolinite, each initially contaminated with 1 µg 14C-HCB/g.

Table 6. Mean mosquitofish HCB concentrations, HCB accumulation rates, and weights of fish exposed to natural sediment, oxidized sediment, sand and kaolinite, each initially contaminated with 1 μg 14C-HCB/g.

Substrate	n	Exposure period (days)	Fish wet weight (g)		Fish HCB (ng/g)	
Natural sediment	6	5	0.101	(0.020)[a]	81.80	(11.26)
	6	10	0.116	(0.011)	195.83	(38.59)
	6	15	0.125	(0.016)	250.60	(26.25)
Oxidized sediment	6	5	0.122	(0.004)	823.41	(233.52)
	6	10	0.123	(0.004)	1290.17	(268.31)
	6	15	0.125	(0.006)	1686.27	(344.75)
Sand	6	5	0.117	(0.010)	612.62	(241.52)
	6	10	0.124	(0.009)	925.37	(137.50)
	6	15	0.132	(0.009)	1462.02	(277.06)
Kaolinite	6	5	0.128	(0.005)	675.06	(60.95)
	6	10	0.130	(0.005)	1294.06	(327.38)
	6	15	0.123	(0.007)	2109.00	(257.74)

[a] Standard deviation

4.5%) and oxidized sediment (OM < 0.1%), each initially contaminated with 1.03 µg HCB g^{-1}, for exposure periods of up to 57 days (Table 7). Semi-logarithmic plots of the mean HCB concentrations in fish versus the exposure periods gave curves which showed an initial period of rapid uptake followed by a much slower phase of uptake characterized by linear slopes which approach horizontal (Figure 6).

Statistical comparisons (t-test) of the initial mean accumulation rates ($t = 12.74$; $df = 8$) and the mean accumulation rates during the secondary phase ($t = 14.07$; $df = 7$) show that the accumulation rates resulting from exposure to the natural sediment or oxidized sediment, each initially contaminated with 1.03 µg HCB/g were significantly different. Mosquitofish HCB concentrations after a 57 days exposure period were significantly different for exposure to natural and oxidized sediment ($t = 22.03$; $df = 3.4$; unequal variances). HCB concentrations range from 5 to 7.7 times greater in fish exposed to oxidized sediment than those exposed to natural sediment. Concentrations of HCB in fish exposed to natural sediment reached an apparent steady-state by 46 days of exposure; however, fish exposed to oxidized sediment did not reach a discernable equilibrium within the 57 days exposure period (Table 7, Figure 6).

5.4 Influence of Sediment HCB Concentration on Bioaccumulation

Effect of Sediment HCB Concentration on Accumulation by Bacteria

The role of sediment HCB concentration in the accumulation of HCB by bacteria was examined by exposing bacteria to natural sediment con-

Table 7. Mean mosquitofish HCB concentrations, fish HCB accumulation rates, fish weights, sediment HCB concentrations, and concentration factors resulting from fish exposure to natural sediment contaminated with 0.03, 0.13, 1.03, and 10.03 μg HCB/g, and oxidized sediment contaminated with 1.03 μg HCB/g.

Sediment	Initial sediment HCB (μg/g)	n	Exposure period (days)	Fish wet weight (g)	Fish HCB (ng/g)	Fish HCB accumulation rate (ng/g/day)	Sediment HCB (ng/g)	Concentration[a] factor
Natural sediment	0.03	5	12	0.136 (0.027)[b]	59.98 (7.53)	5.00 (0.63)	32.7 (4.8)	1.83
		4	23	0.115 (0.012)	107.50 (51.39) *[c]	4.32 (4.67)	30.7 (1.5)	3.50
		5	35	0.108 (0.014)	105.29 (13.65) *	-0.18 (1.14)	35.6 (2.6)	2.96
		5	46	0.115 (0.017)	161.20 (42.80) *	5.08 (3.89)	32.2 (0.8)	5.01
		5	57	0.136 (0.024)	135.57 (19.88) *	-2.33 (1.81)	33.0 (0.5)	4.11
Natural sediment	0.13	5	12	0.122 (0.023)	58.62 (20.69)	4.89 (1.72)	120.4 (7.2)	0.49
		5	23	0.136 (0.051)	89.10 (30.44)	2.77 (2.77)	135.6 (7.1)	0.66
		4	35	0.117 (0.015)	115.15 (19.75) *	2.17 (1.65)	135.0 (17.9)	0.85
		4	46	0.123 (0.026)	169.19 (53.76) *	4.91 (4.89)	124.3 (8.0)	1.36
		5	57	0.134 (0.028)	156.92 (33.85) *	-1.12 (3.08)	112.4 (9.1)	1.40
Natural sediment	1.03	5	12	0.136 (0.053)	239.95 (73.18)	20.00 (6.10)	1037.9 (162.7)	0.23
		6	23	0.124 (0.087)	282.83 (71.42)	3.98 (6.49)	1018.7 (21.5)	0.27
		4	35	0.143 (0.010)	470.26 (21.96)	15.62 (1.83)	1079.8 (10.2)	0.44
		5	46	0.145 (0.037)	559.24 (67.91) *	8.08 (6.17)	1001.3 (49.4)	0.56
		5	57	0.172 (0.026)	615.60 (89.49) *	5.12 (8.14)	922.8 (106.9)	0.67
Natural sediment	10.03	5	12	0.159 (0.031)	804.73 (225.20)	67.06 (18.77)	9847.2 (5296.8)	0.08
		6	23	0.135 (0.038)	1520.01 (395.03)	65.03 (35.91)	8800.9 (53.9)	0.17
		5	35	0.124 (0.012)	3492.47 (436.18)	164.37 (36.35)	10947.0 (1314.1)	0.32
		5	46	0.170 (0.016)	4546.64 (543.08) *	95.83 (49.37)	9748.1 (150.2)	0.47
		5	57	0.144 (0.037)	4492.54 (382.18) *	-4.91 (34.74)	9245.9 (623.8)	0.49
Oxidized sediment	1.03	5	12	0.117 (0.008)	1811.55 (265.91)	150.96 (22.16)	739.6 (75.5)	2.45
		5	23	0.126 (0.037)	2170.45 (264.83)	32.68 (24.08)	691.1 (78.2)	3.14
		5	35	0.112 (0.030)	2524.68 (234.91)	29.52 (19.58)	652.4 (132.1)	3.87
		5	46	0.157 (0.030)	3489.46 (462.71)	87.71 (42.06)	700.2 (121.9)	4.98
		5	57	0.140 (0.038)	4285.10 (323.41)	72.33 (29.40)	738.2 (93.3)	5.80

[a] Concentration factor calculated as mosquitofish HCB concentration (wet weight) divided by sediment HCB concentration (dry weight).

[b] Standard deviation

[c] Mean mosquitofish HCB concentrations for each sediment exposure which are followed by * are not significantly different ($\alpha = 0.05$) as determined with linear regression.

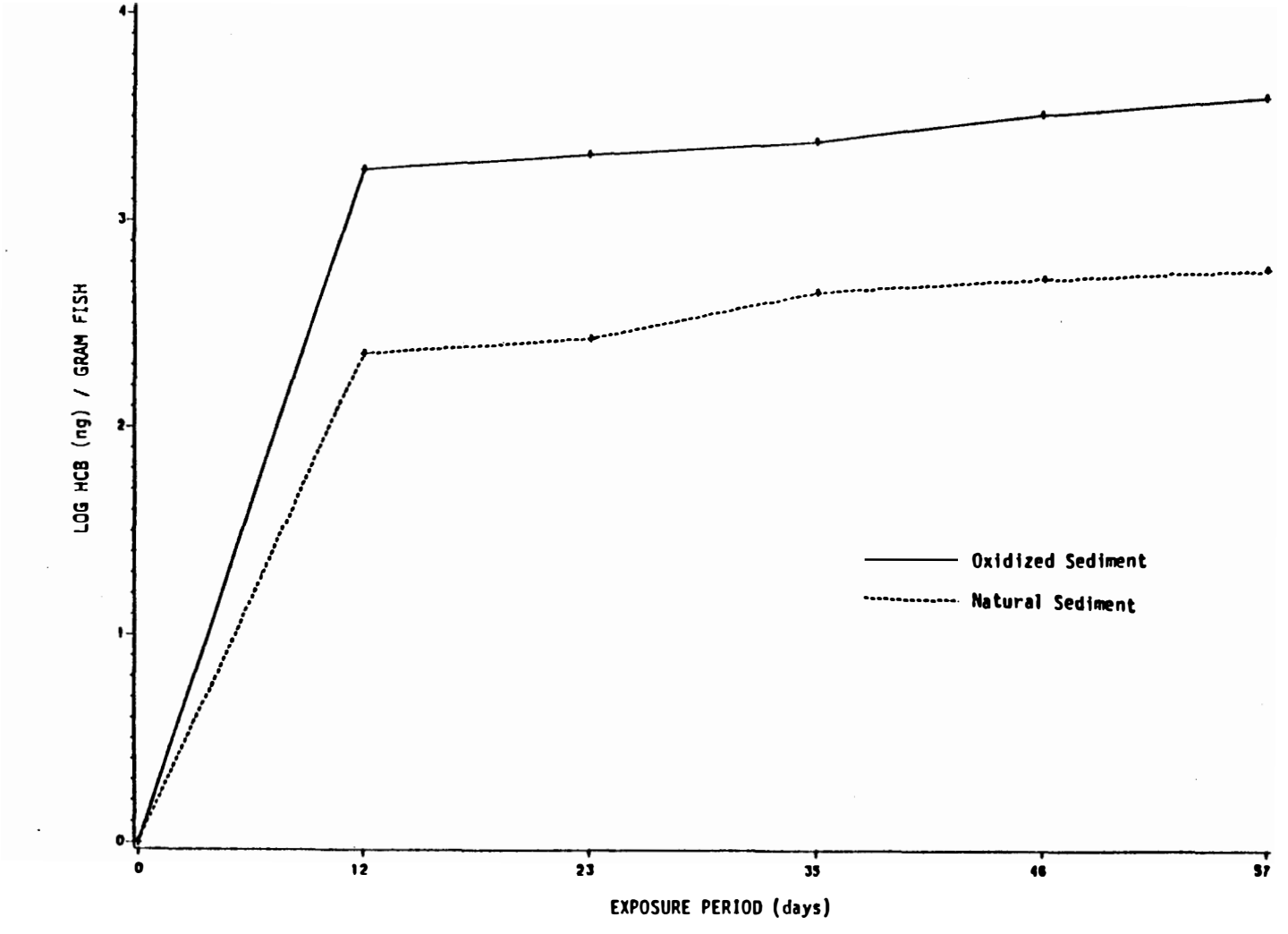

Figure 6. Mean mosquitofish HCB concentrations resulting from exposure to natural and oxidized sediment, each initially contaminated with 1 µg HCB/g.

taminated with 77.7, 127.6 and 273.7 μg ^{14}C-HCB g^{-1}. The results of this exposure are given in Table 8 and are shown in Figure 7. Accumulation by bacteria was proportional to the sediment HCB concentration and can be described (r^2 = .99; n = 9) by the linear model:

log [HCB] bacteria = 0.90 + 0.89 log [HCB] sediment.

This relationship compares favorably with the relationship between bacteria and natural sediment HCB concentrations of samples pooled from nine separate experiments (Figure 8), involving both live and dead bacteria, which can be described by the linear model (r^2 = .69; n = 80):

log [HCB] bacteria = 0.88 + 0.87 log [HCB] sediment.

Effect of Sediment HCB Concentration on Accumulation by Midge

Midge exposed to natural sediment and oxidized sediment, each initially contaminated with ^{14}C-HCB concentrations of 1, 3, and 6 μg g^{-1}, accumulated HCB in amounts proportional to the sediment HCB concentration (Table 9, Figure 9). The bioavailabilities of HCB to midge during the initial 24 hours exposure period were significantly different (t-test) for most sediment HCB concentrations (Table A-10). Mean midge HCB concentrations resulting from 192 hours exposure showed that concentrations were significantly different for all sediment HCB concentrations, and for two sediments with the same HCB concentrations (Table A-11).

Regression analyses show that apparent steady-state HCB concentrations in midge were obtained with exposures to natural sediment contaminated with 1, 3 and 6 μg HCB g^{-1} by 96, 72 and 72 hours, respectively (Table 9). Steady-state HCB concentrations in midge exposed to contam-

Table 8. Mean bacteria HCB concentrations, sediment HCB concentrations, and concentration factors resulting from bacteria exposure for 24 hours to natural sediment contaminated with 77.7, 127.6, and 273.7 µg 14C-HCB/g.

Initial sediment HCB (µg/g)	n	Bacteria[a] HCB (µg/g)	Sediment HCB (µg/g)	Concentration[b] factor
77.7	3	106.16 (3.84)[c]	46.59 (2.86)	2.28
127.6	3	169.07 (6.31)	75.76 (7.08)	2.23
273.7	3	353.22 (14.90)	179.60 (6.85)	1.97

[a] Bacteria dry weight = 0.47 mg/ml

[b] Concentration factor calculated as bacteria HCB concentration (dry weight) divided by sediment HCB concentration (dry weight)

[c] Standard deviation

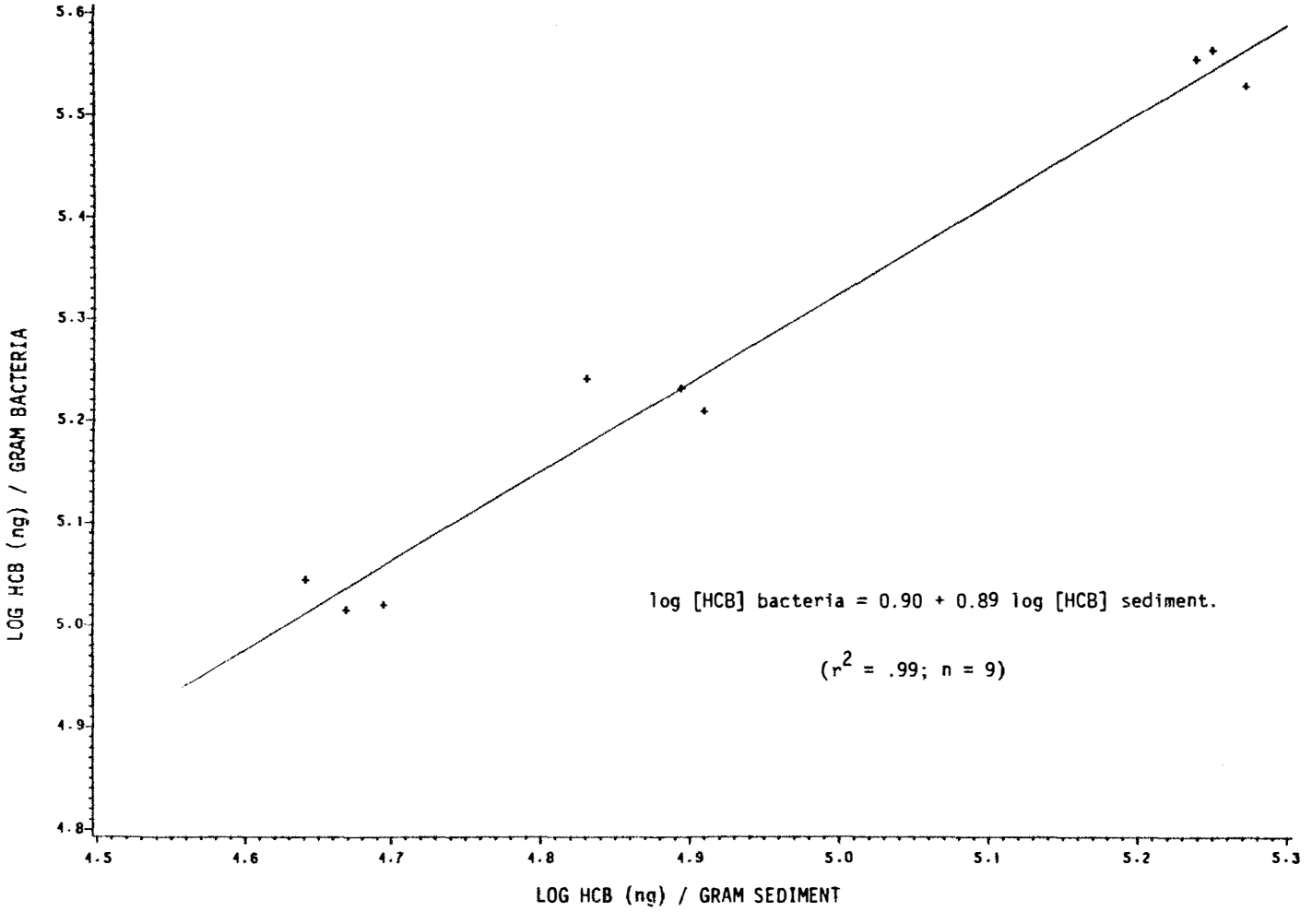

Figure 7. Mean bacteria HCB concentrations after 2880 minutes exposure to natural sediment initially contaminated with 77.7, 127.6, and 273.7 µg 14C-HCB/g.

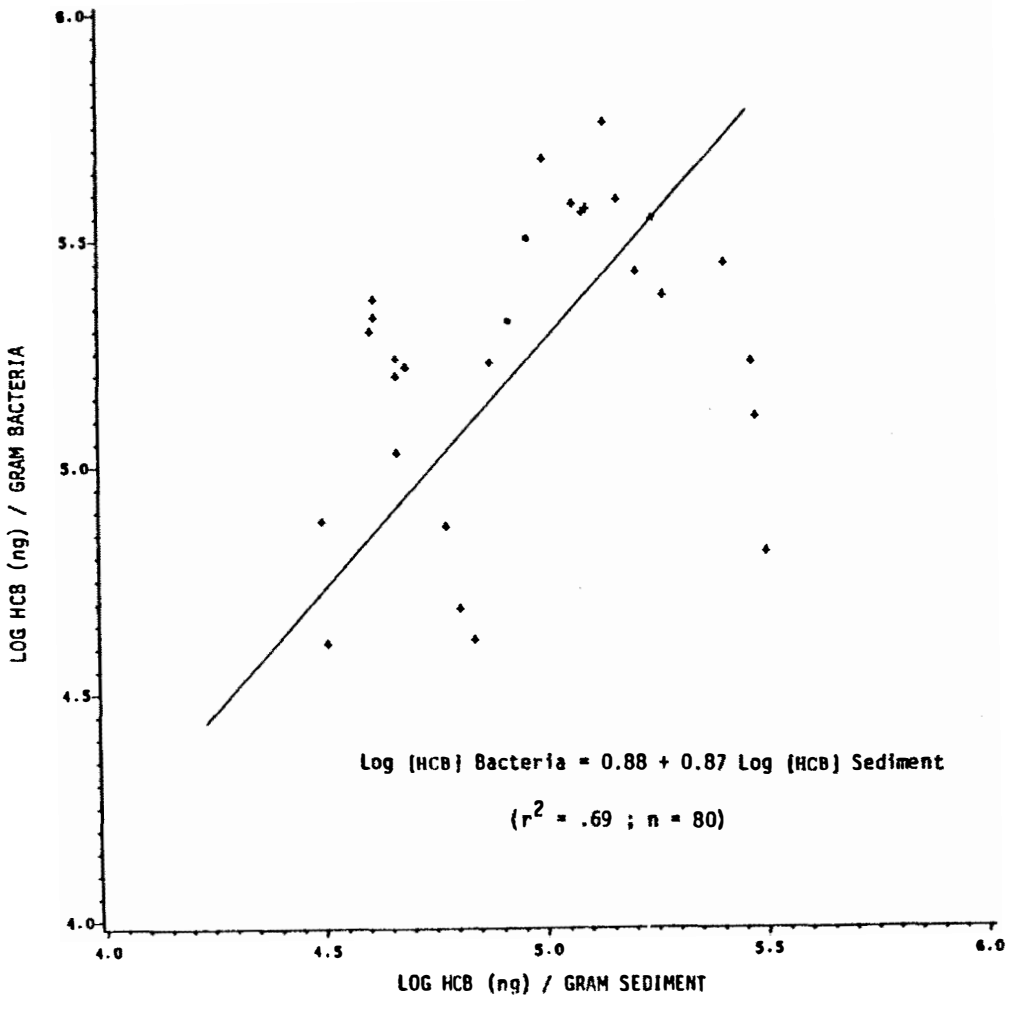

Figure 8. Relationship between HCB concentrations in bacteria and natural sediment at apparent steady-state.

Table 9. Mean midge HCB concentrations, midge HCB accumulation rates, midge weights, sediment HCB concentrations, and concentration factors resulting from midge exposure to natural and oxidized sediment, each initially contaminated with 1, 3, and 6 ug 14C-HCB/g.

Substrate	Initial sediment HCB (ug/g)	n	Exposure period (hrs)	Midge wet weight (mg)	Midge HCB (ng/g)		Midge HCB accumulation rate (ng/g/hr)		Sediment HCB (ng/g)	Concentration[a] factor
					[b]	[c]				
Oxidized sediment	1	5	24	8.74 (2.99)	444.18	(139.70)	18.51	(5.82)	995.66	0.45
		5	48	6.30 (1.43)	658.98	(112.55)	8.95	(4.69)	991.35	0.66
		4	72	6.00 (1.82)	818.23	(193.15)	6.63	(8.05)	985.65	0.83
		5	96	8.76 (1.05)	1454.57	(111.94)	26.52	(4.66)	973.98	1.49
		6	120	7.50 (0.90)	2704.46	(337.96) a	52.08	(14.08)	958.36	2.82
		3	192	6.87 (2.74)	3439.91	(794.70) *	10.21	(11.04)	940.77	3.66
Oxidized sediment	3	5	24	7.36 (1.17)	1587.89	(409.46)	66.16	(17.06)	992.13	1.60
		5	48	6.68 (1.25)	2281.34	(503.50)	28.89	(20.98)	976.79	2.33
		4	72	7.30 (2.23)	3135.10	(565.90)	35.58	(23.58)	955.70	3.28
		5	96	7.98 (1.11)	3889.44	(163.92)	31.43	(6.83)	933.61	4.17
		5	120	7.20 (0.58)	6892.43	(389.58)	125.13	(16.23)	889.41	7.75
		7	192	6.34 (1.05)	9592.69	(136.80)	37.50	(15.79)	835.44	11.48
Oxidized sediment	6	5	24	7.20 (1.42)	2793.63	(1149.23)	116.40	(47.88)	970.00	2.85
		5	48	6.86 (0.74)	6191.36	(1466.46)	141.57	(61.10)	941.24	6.58
		5	72	7.40 (1.10)	5792.97	(1491.61)	-16.60	(62.15)	891.45	6.50
		4	96	6.55 (1.16)	10226.02	(2530.29)	184.71	(105.43)	857.17	11.93
		5	120	7.74 (0.91)	13011.06	(1015.01) *	116.04	(42.29)	768.83	16.92
		3	192	7.87 (0.15)	14882.43	(1530.46) *	25.99	(21.26)	694.60	21.43
Natural sediment	1	5	24	7.86 (1.54)	467.20	(180.92)	19.47	(7.54)	997.12	0.47
		5	48	7.18 (1.11)	718.46	(78.43)	10.47	(3.27)	991.87	0.72
		5	72	5.88 (0.50)	719.70	(306.08) *	0.05	(12.75)	988.71	0.73
		4	96	5.85 (1.09)	899.66	(151.83) *	7.50	(6.33)	982.58	0.92
		7	120	7.16 (2.04)	698.88	(149.02) *	-8.37	(6.21)	977.13	0.72
		7	192	8.21 (2.21)	933.58	(56.00) *	3.26	(0.78)	969.83	0.96
Natural sediment	3	5	24	9.92 (2.86)	1551.44	(197.59)	64.64	(8.23)	982.59	1.58
		5	48	7.04 (1.00)	1795.67	(452.08)	10.18	(18.84)	958.39	1.87
		5	72	5.86 (1.08)	1966.91	(986.94) *	7.13	(41.12)	932.40	2.11
		4	96	7.33 (1.47)	2306.69	(199.63) *	14.13	(8.32)	904.19	2.55
		5	120	8.08 (1.13)	2200.93	(229.71) *	-4.41	(9.57)	859.14	2.56
		9	192	7.77 (2.57)	2646.76	(416.45) *	6.19	(5.78)	827.32	3.20
Natural sediment	6	5	24	9.14 (2.60)	2969.92	(326.78)	123.75	(13.62)	982.66	3.02
		5	48	6.28 (1.03)	3572.76	(1392.56)	25.12	(58.02)	958.46	3.73
		5	72	8.80 (0.79)	3916.51	(901.68)	14.32	(37.57)	932.47	4.20
		5	96	6.66 (0.60)	4678.56	(430.85) *	31.75	(17.95)	900.23	5.20
		6	120	7.88 (1.91)	5248.08	(850.43) *	23.73	(35.43)	869.26	6.04
		7	192	7.31 (2.13)	5622.92	(588.40) *	5.21	(8.17)	862.57	6.75

[a] Concentration factor calculated as midge HCB concentration (wet weight) divided by sediment HCB concentration (dry weight).

[b] Standard deviation

[c] Mean midge HCB concentrations for each sediment and concentration exposure which are followed by * are not significantly different ($\alpha = 0.05$) as determined with linear regression.

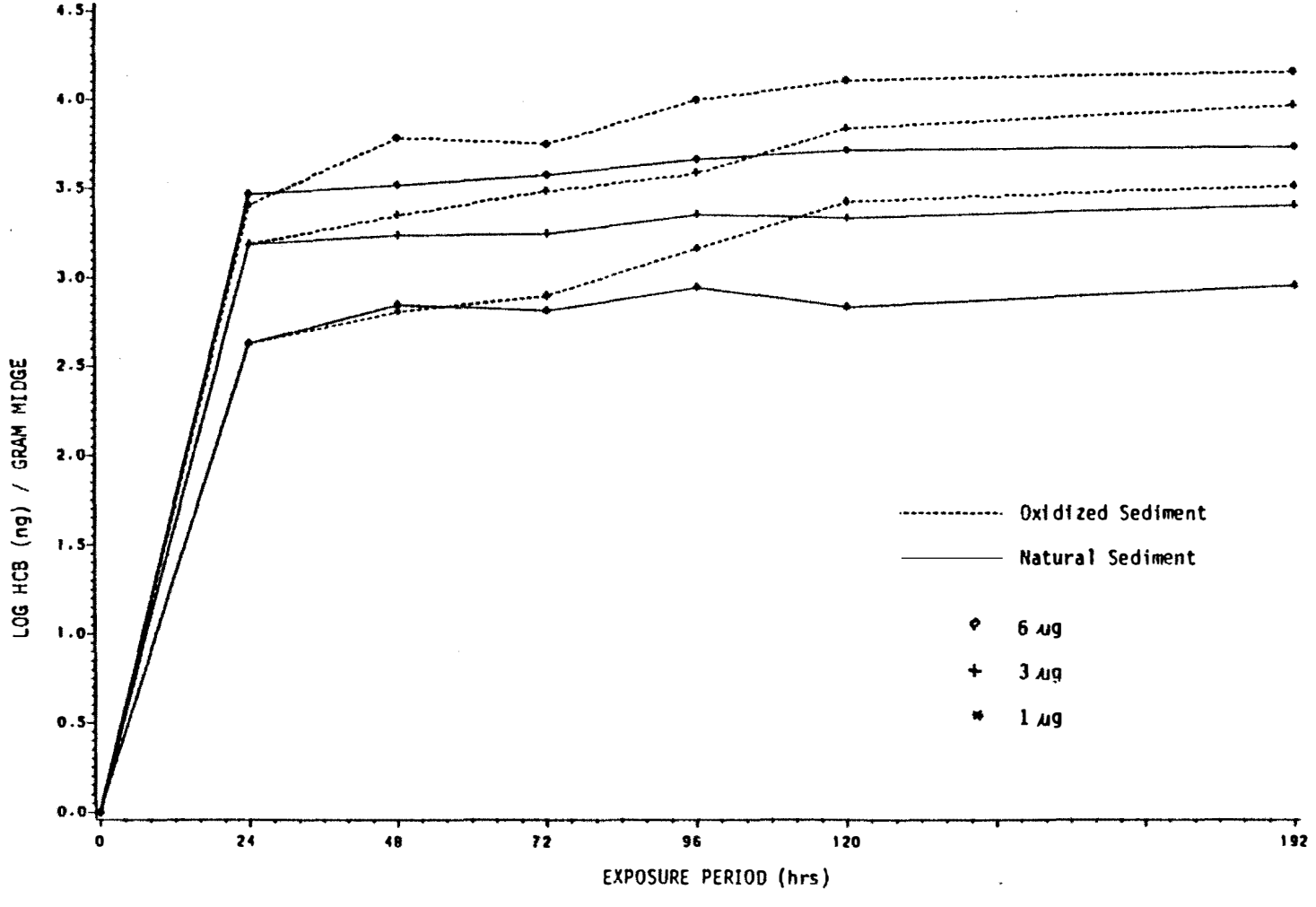

Figure 9. Mean midge HCB concentrations resulting from exposure to natural and oxidized sediment, each initially contaminated with 1, 3, and 6 ug 14C-HCB/g.

inated oxidized sediment were obtained by 120 hours for midge exposed to 1 and 6 µg HCB g^{-1}; however, steady-state HCB concentrations were not discernable during the experimental period for midge exposed to oxidized sediment with 3 µg HCB g^{-1} (Table 9).

The relationship at apparent steady-state between midge and natural sediment HCB concentrations from samples pooled from eight separate experiments is shown in Figure 10, and can be adequately described (r^2 = .93; n = 43) by the linear model:

$$\log [HCB]_{midge} = 1.10 \log [HCB]_{sediment} - 0.47.$$

Effect of Sediment HCB Concentration on Accumulation by Mosquitofish

Data on the HCB accumulation by mosquitofish exposed to natural sediment contaminated with 0.03, 0.13, 1.03, and 10.03 µg HCB g^{-1} is given in Table 7. The accumulation was characterized by an initial rapid period of accumulation followed by a second slower phase of accumulation (Figure 11). During the rapid uptake phase, mean HCB accumulation rates by mosquitofish were 5.0, 4.9, 20.0, and 98.8 ng g^{-1} d^{-1} for exposures to sediment contaminated with 0.03, 0.13, 1.03 and 10.03 µg g^{-1}, respectively. The period of rapid HCB accumulation lasted for 12 days for fish exposed to sediment contaminated with 0.03, 0.13, and 1.03 µg HCB g^{-1}, but continued for 46 days for fish exposed to 10.03 µg HCB g^{-1} (Table 7). After the initial uptake phase, the mean accumulation rates dropped to 1.7, 2.2, 8.3, and -4.9 ng g^{-1} d^{-1} for exposures to sediment contaminated with 0.03, 0.13, 1.03, and 10.03 µg g^{-1}, respectively. Regression ana-

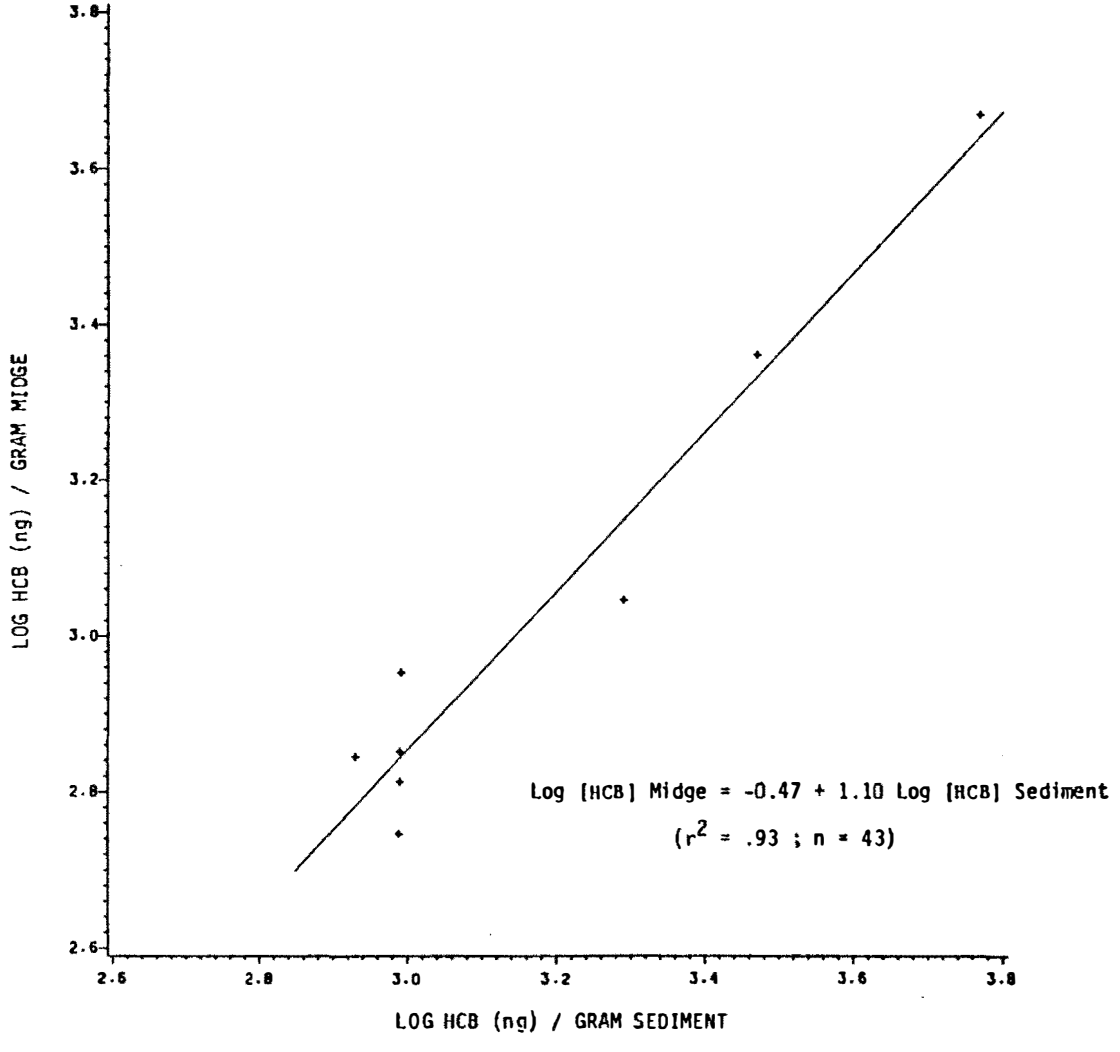

Figure 10. Relationship between HCB concentrations in midge and natural sediment at apparent steady-state.

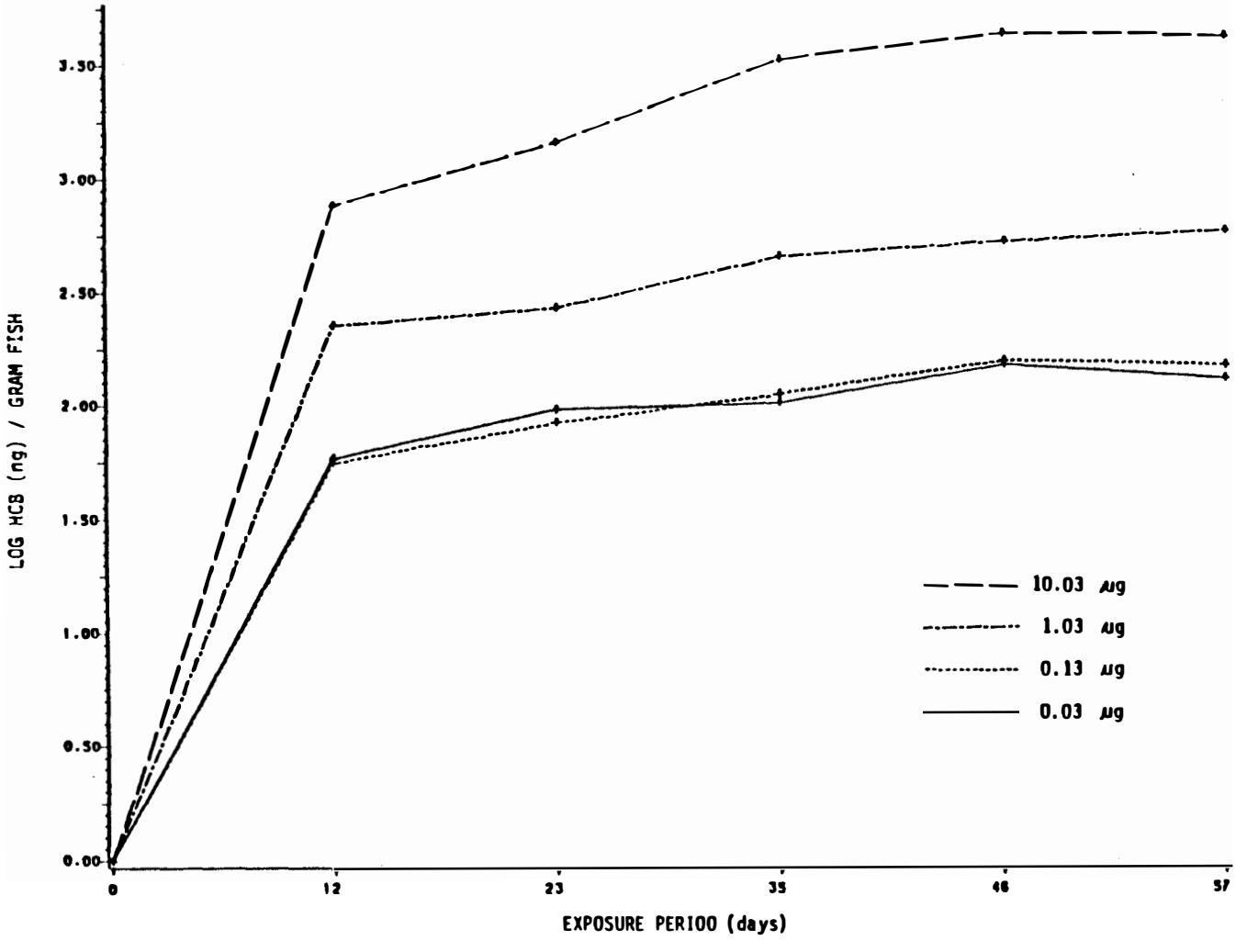

Figure 11. Mean mosquitofish HCB concentrations resulting from exposure to natural sediment initially contaminated with 0.03, 0.13, 1.03, and 10.03 µg HCB/g.

lyses show that apparent steady-state HCB concentrations in mosquitofish, resulting from exposure to sediment contaminated with 0.03, 0.13, 1.03 and 10.03, were obtained within 23, 35, 46 and 46 days, respectively (Table 7).

Mosquitofish HCB concentrations resulting from exposure to the four different sediment HCB concentrations were determined (t-test) to be significantly different for the initial 12 days exposure (Table A-12) as well as for the 57 days exposure (Table A-13), except for accumulation resulting from exposures to sediment contaminated with 0.03 and 0.13 μg HCB g^{-1}. The relationship at apparent steady-state between mosquitofish and natural sediment HCB concentrations is shown in Figure 12, and can be described (r^2 = .95; n = 123) by the linear model:

$$\log [HCB]_{fish} = 0.64 \log [HCB]_{sediment} + 0.99.$$

5.5 Effect of Bacterial Biomass on HCB Accumulation

Mean bacteria HCB concentration, weight of bacteria, sediment HCB concentration, and concentration factor for each bacterial dilution are given in Table 10. Increasing the bacterial biomass exposed to HCB contaminated sediment resulted in an linear increase in HCB sorbed to bacteria (Figure 13), but resulted in a decrease in the quantity of HCB sorbed per weight of bacteria (Figure 14). Mean bacteria HCB concentrations were significantly different for each bacterial dilution (Table A-14). HCB accumulation by different bacterial biomass can be adequately described (r^2 = .95; n = 12) with the linear regression model:

$$Y = 160.68 + 184.85\ X$$

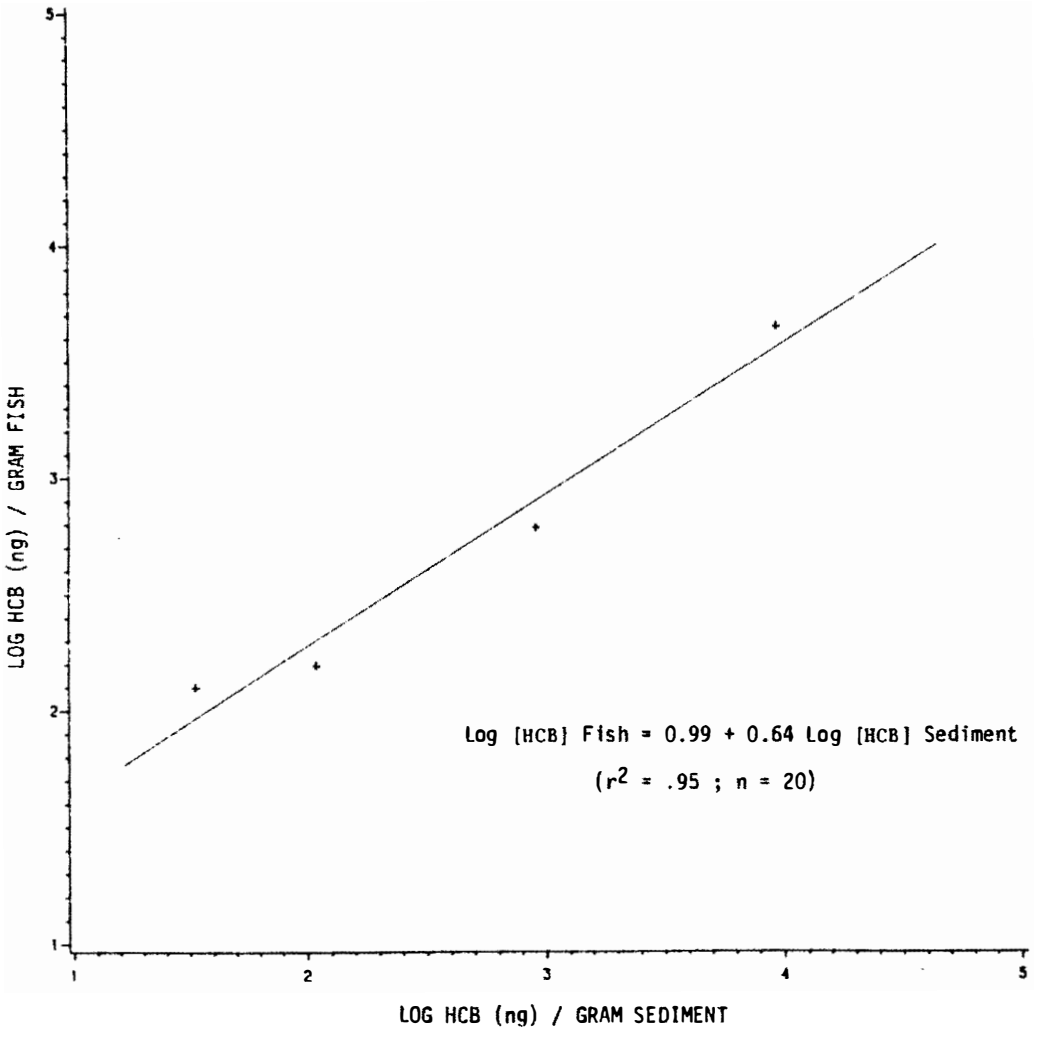

Figure 12. Relationship between HCB concentrations in mosquitofish and sediment at apparent steady-state.

Table 10. Mean bacteria HCB concentrations, sediment HCB concentrations, and concentration factors resulting from 48 hours exposure of four dilutions of bacteria to natural sediment initially contaminated with 320 µg 14C-HCB/g.

Bacteria dry weight (mg/ml)	n	Bacteria HCB (µg/g)		Sediment HCB (µg/g)		Concentration factor[a]
0.34	3	681.13	(33.63)[b]	187.34	(6.76)	3.6
0.44	3	559.25	(37.54)	180.85	(8.53)	3.1
0.67	3	447.57	(14.02)	167.53	(4.54)	2.7
0.82	3	377.27	(10.76)	146.52	(5.10)	2.6

[a] Concentration factor calculated as the bacteria HCB concentration (dry weight) divided by sediment HCB concentration (dry weight)

[b] Standard deviation

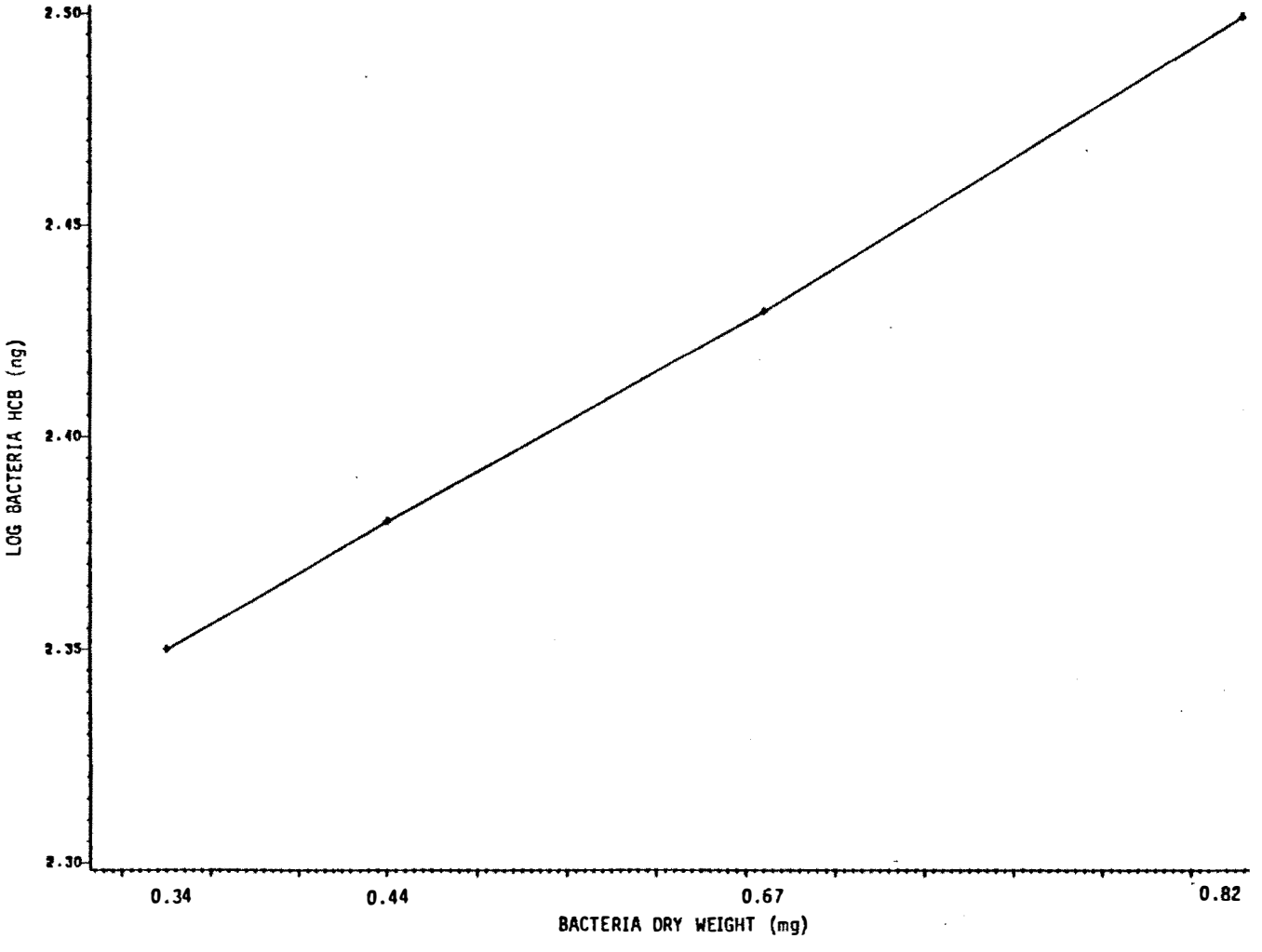

Figure 13. Mean bacteria HCB resulting from exposure of four bacterial dilutions to natural sediment initially contaminated with 320 µg 14C-HCB/g.

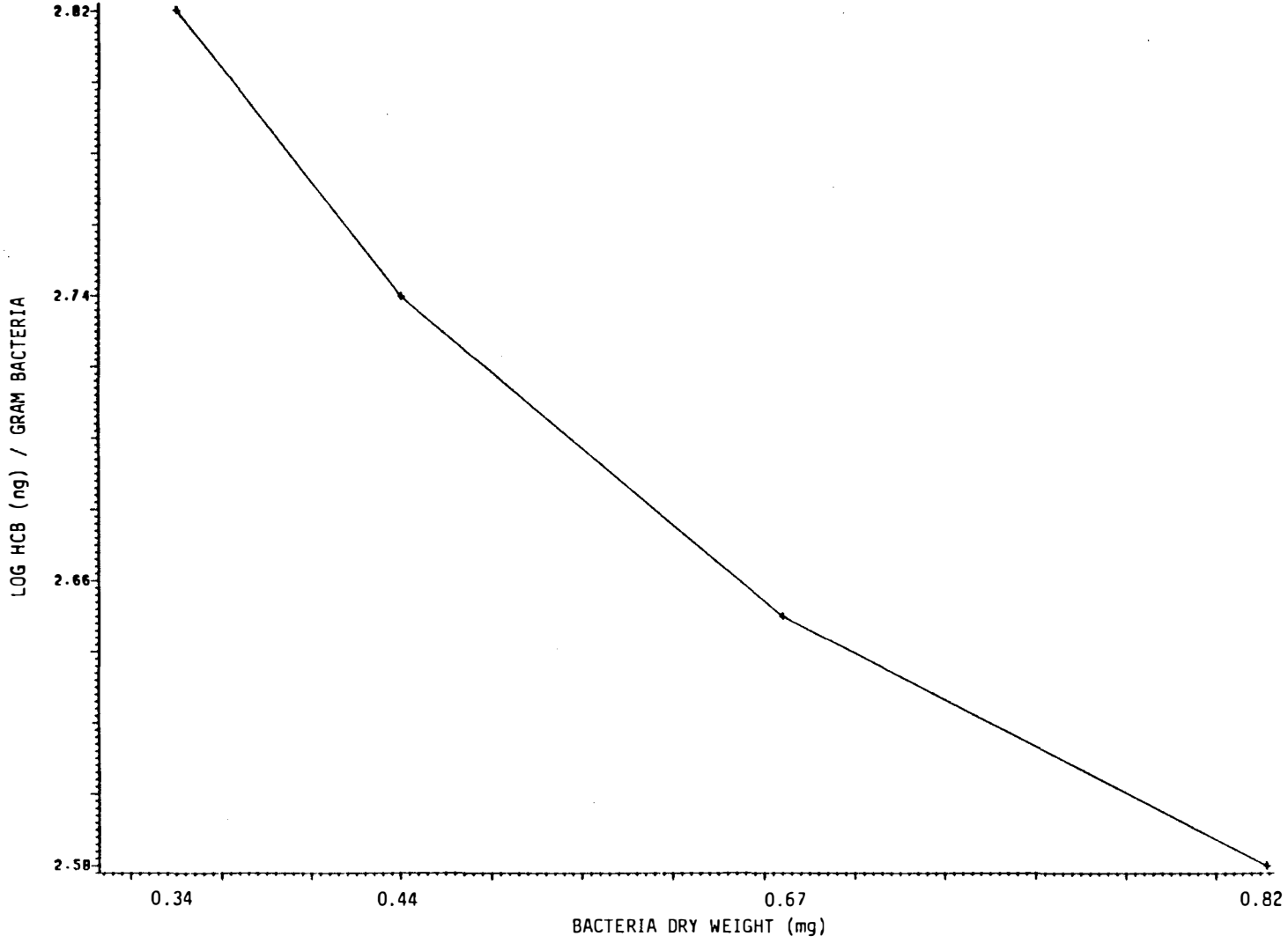

Figure 14. Mean bacteria HCB concentration resulting from exposure of four bacterial dilutions to natural sediment initially contaminated with 320 µg 14C-HCB/g.

where: Y = HCB sorbed to cells (ng)

and X = dry weight of bacteria (mg).

5.6 Effect of Biological Viability on HCB Accumulation

Accumulation by Live versus Dead Bacteria

Semi-logarithim plots of mean ^{14}C-HCB concentrations of live bacteria and bacteria killed with formaldehyde and mercuric chloride ($HgCl_2$) are shown in Figure 15. The mean bacteria and sediment HCB concentrations, and concentration factor at each exposure period for each group of bacteria are given in Table 11. HCB concentrations of live, formaldehyde-killed and $HgCl_2$-killed bacteria, resulting from 240 minutes exposure to HCB contaminated sediment, were not significantly different (Table A-15); however, the 2880 minute exposures resulted in significant differences for all three groups of bacteria (Table A-16).

Accumulation by Live versus Dead Midge

The mean loads of ^{14}C-HCB of live and formaldehyde-killed midge, each exposed to natural sediment contaminated with 1.0 µg ^{14}C-HCB g^{-1}, are shown in Figure 16, and the data summarized in Table 12. T-test comparison of the HCB accumulation rates during the first 3 hours of exposure showed no significant difference between live and dead midges ($t = 1.85$; $df = 6.3$; unequal variances). However, after three hours, the accumulation by the live midge was significantly greater than that of the dead midge ($t = 7.12$; $df = 8.8$; unequal variances), with live midges accumu-

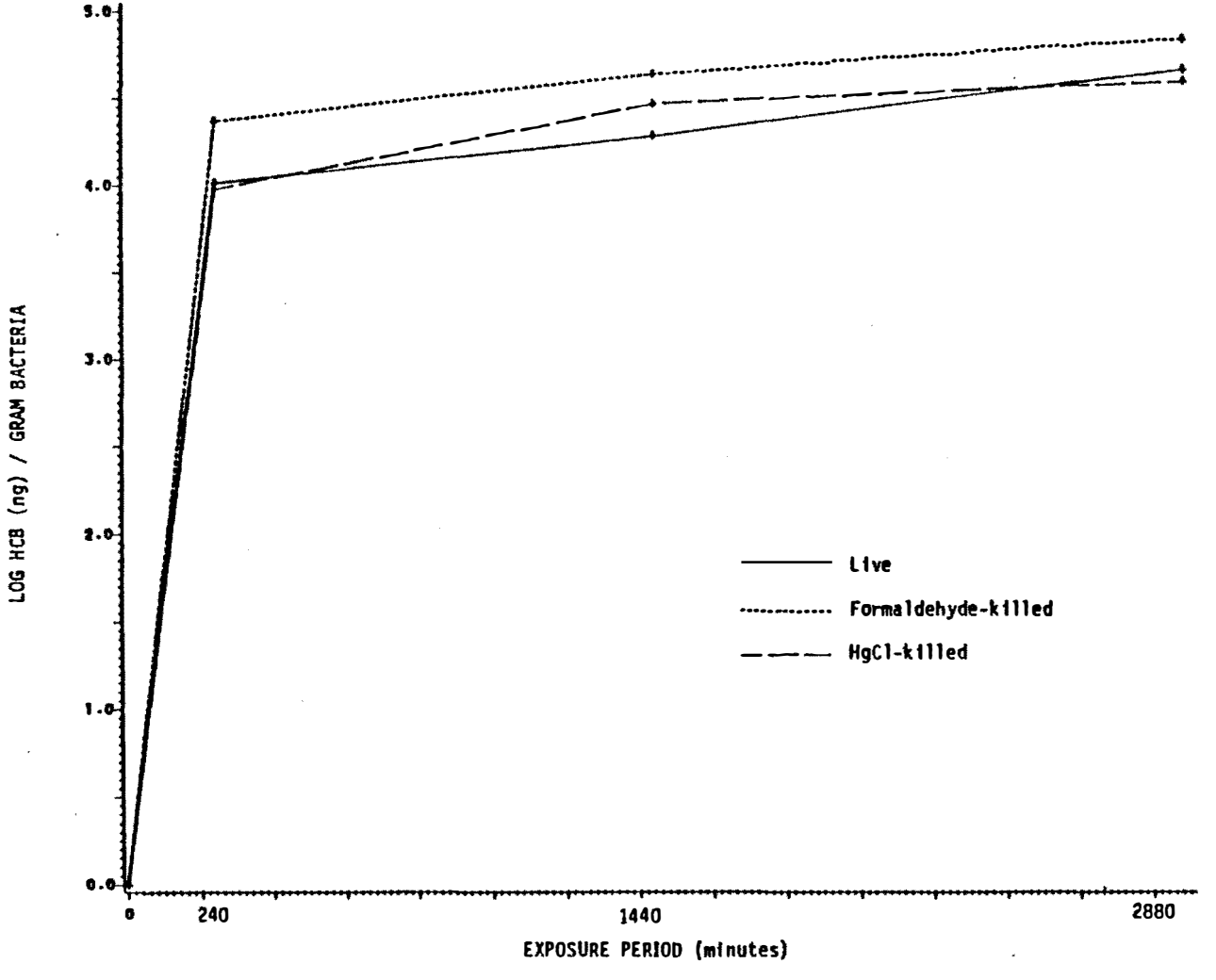

Figure 15. Mean bacteria HCB concentrations resulting from exposure of live, formaldehyde-killed, and HgCl-killed bacteria to natural sediment initially contaminated with 71 µg 14C-HCB/g.

Table 11. Mean bacteria HCB concentrations, sediment HCB concentrations, and concentration factors resulting from exposure of live bacteria, formaldehyde-killed bacteria, and mercuric chloride-killed bacteria to natural sediment initially contaminated with 71 µg 14C-HCB/g.

Bacteria	n	Exposure period (min)	Bacteria[a] HCB (µg/g)		Sediment HCB (µg/g)		Concentration[b] factor
Live	3	240	9.99	(2.60)[c]	68.57	(11.48)	0.15
	3	1440	21.13	(3.95)	66.85	(14.64)	0.32
	3	2880	47.47	(2.96)	62.22	(3.39)	0.76
Formaldehyde killed	3	240	24.68	(8.46)	66.13	(7.26)	0.37
	3	1440	44.40	(5.53)	62.89	(5.19)	0.71
	3	2880	72.17	(10.25)	58.10	(8.43)	1.24
$HgCl_2$ killed	3	240	10.53	(3.89)	68.41	(7.64)	0.15
	3	1440	31.48	(8.15)	64.86	(6.23)	0.49
	3	2880	38.33	(2.58)	63.60	(3.61)	0.60

[a] Bacteria dry weight = 0.31 mg/ml

[b] Concentration factor calculated as the bacteria HCB concentration (dry weight) divided by sediment HCB concentration (dry weight)

[c] Standard deviation

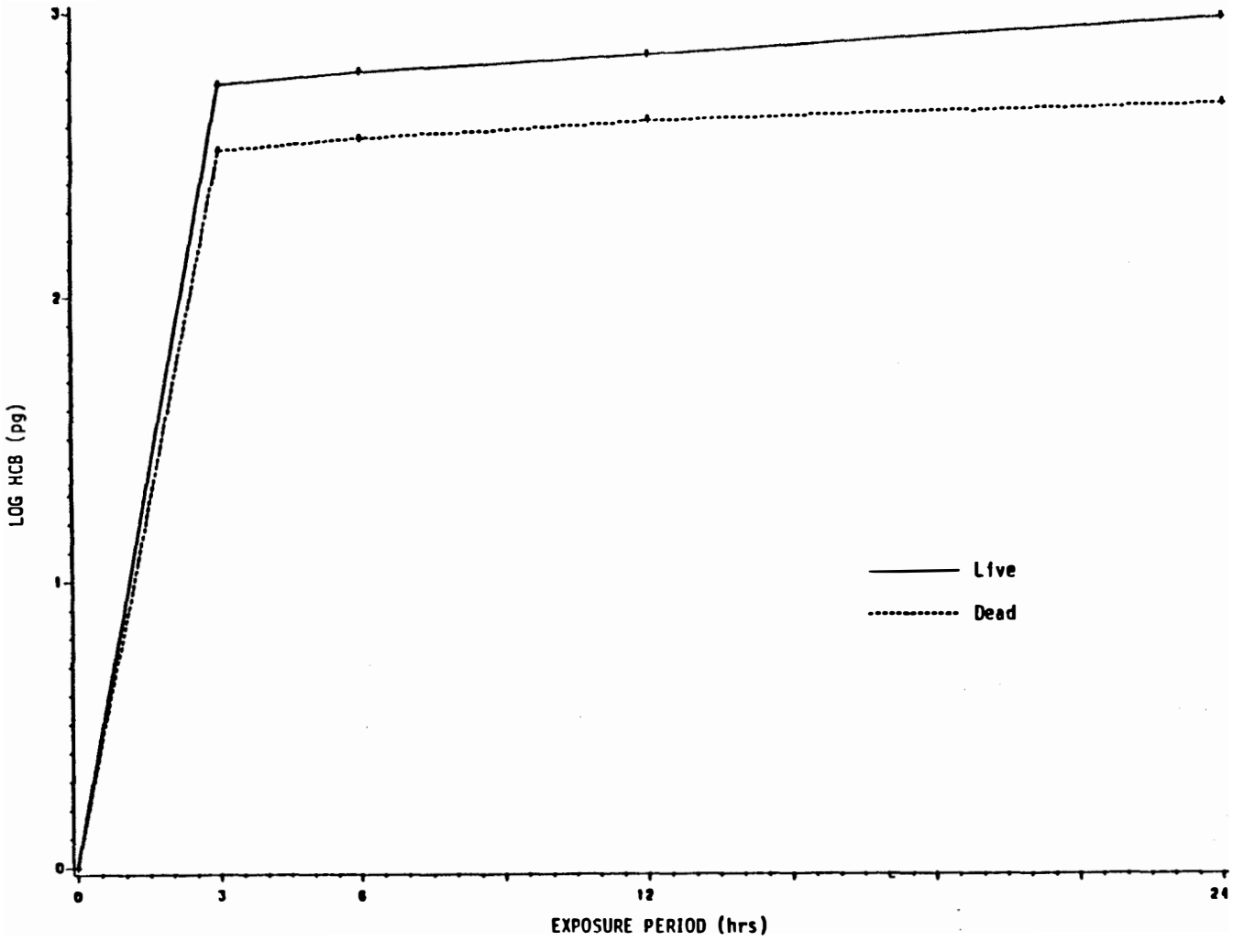

Figure 16. Mean midge HCB load resulting from exposure of live and formaldehyde-killed midge larvae to natural sediment initially contaminated with 1 µg 14C-HCB/g.

Table 12. Mean HCB loads and HCB accumulation rates of live and formaldehyde-killed midge resulting from exposure to natural sediment initially contaminated with 1 μg 14C-HCB/g.

Midge	Exposure period (hrs)	n	Midge HCB (ng)		Midge HCB accumulation rate (ng/hr)	
Live	3	7	558.07	(39.26)[a]	22.61	(15.49)
	6	8	622.38	(63.66)	21.61	(21.22)
	12	8	735.31	(97.81)	18.82	(16.30)
	24	8	1077.48	(146.54)	28.50	(5.05)
Dead	3	9	330.78	(29.15)	11.66	(2.61)
	6	9	365.44	(50.54)	11.66	(16.85)
	12	8	433.88	(71.52)	11.41	(11.92)
	24	11	502.86	(60.64)	5.75	(12.21)

[a]Standard deviation

lating approximately twice that of dead midge after a 24 hours exposure (Table 12).

5.7 Effect of Temperature on Midge HCB Accumulation

The mean midge HCB concentrations, weights, and HCB accumulation rates, as well as sediment HCB concentrations and concentration factors at three exposure temperatures are given in Table 13. The mean ^{14}C-HCB concentrations of midge exposed to sediment initially contaminated with 1 µg ^{14}C-HCB g^{-1} increased with exposure temperature (Figure 17). During the first 24 hours, midge accumulated HCB at the mean rates of 9.7, 14.1, and 20.7 ng g^{-1} hr^{-1} at exposure temperatures of 13, 20, and 27° C, respectively (Table 13). There were no significant differences between midge HCB concentrations at 13 and 20°C at 24 hours; however, HCB concentrations at 20 and 27°C, and at 13 and 27°C were significantly different (Table A-17). Mean midge HCB concentrations after 120 hours exposures were not significantly different for the three exposure temperatures (Table A-18). Regression analyses show that midge steady-state HCB concentrations were reached within 72 hours at all three exposure temperatures (Table 13).

5.8 Depuration of HCB from Organisms

The bacteria HCB concentration, K_d, and $t_{½}$ for each period of the depuration phase are given in Table 14, and the data is plotted in Figure 18. Mean bacterial HCB depuration rate constants (K_d), determined in the

Table 13. Mean midge HCB concentrations, midge HCB accumulation rates, midge weights, sediment HCB concentrations, and concentration factors resulting from exposure at three temperatures (13, 20, and 27°C) to natural sediment initially contaminated with 1 µg 14C-HCB/g.

Temperature (°C)	n	Exposure period (hrs)	Midge wet weight (mg)		Midge HCB (ng/g)		Midge HCB accumulation rate (ng/g/hr)		Sediment HCB (ng/g)	Concentration[a] factor
13	6	24	7.10	(1.03)[b]	231.57	(1.03)	9.65	(3.49)	997.50	0.23
	7	72	7.70	(1.76)	505.32	(1.76)*[c]	5.70	(2.36)	992.25	0.51
	3	120	7.83	(1.58)	556.24	(1.58)*	1.06	(1.18)	983.31	0.51
20	5	24	8.68	(0.89)	339.34	(0.89)	14.14	(3.22)	996.17	0.34
	6	72	8.52	(0.79)	699.93	(0.79)*	7.51	(3.21)	988.42	0.71
	5	120	7.70	(1.97)	709.87	(1.97)*	0.21	(2.73)	979.50	0.72
27	6	24	8.43	(1.38)	496.52	(1.38)	20.69	(3.08)	994.95	0.50
	8	72	6.60	(0.83)	742.32	(0.83)*	5.12	(1.65)	985.58	0.75
	9	120	7.78	(2.06)	649.14	(2.06)*	-1.94	(3.16)	978.85	0.66

[a] Concentration factor calculated as midge HCB concentration (wet weight) divided by sediment HCB concentration (dry weight)

[b] Standard deviation

[c] Mean midge HCB concentrations for each exposure temperature which are followed by * are not significantly different ($\alpha = 0.05$) as determined with linear regression.

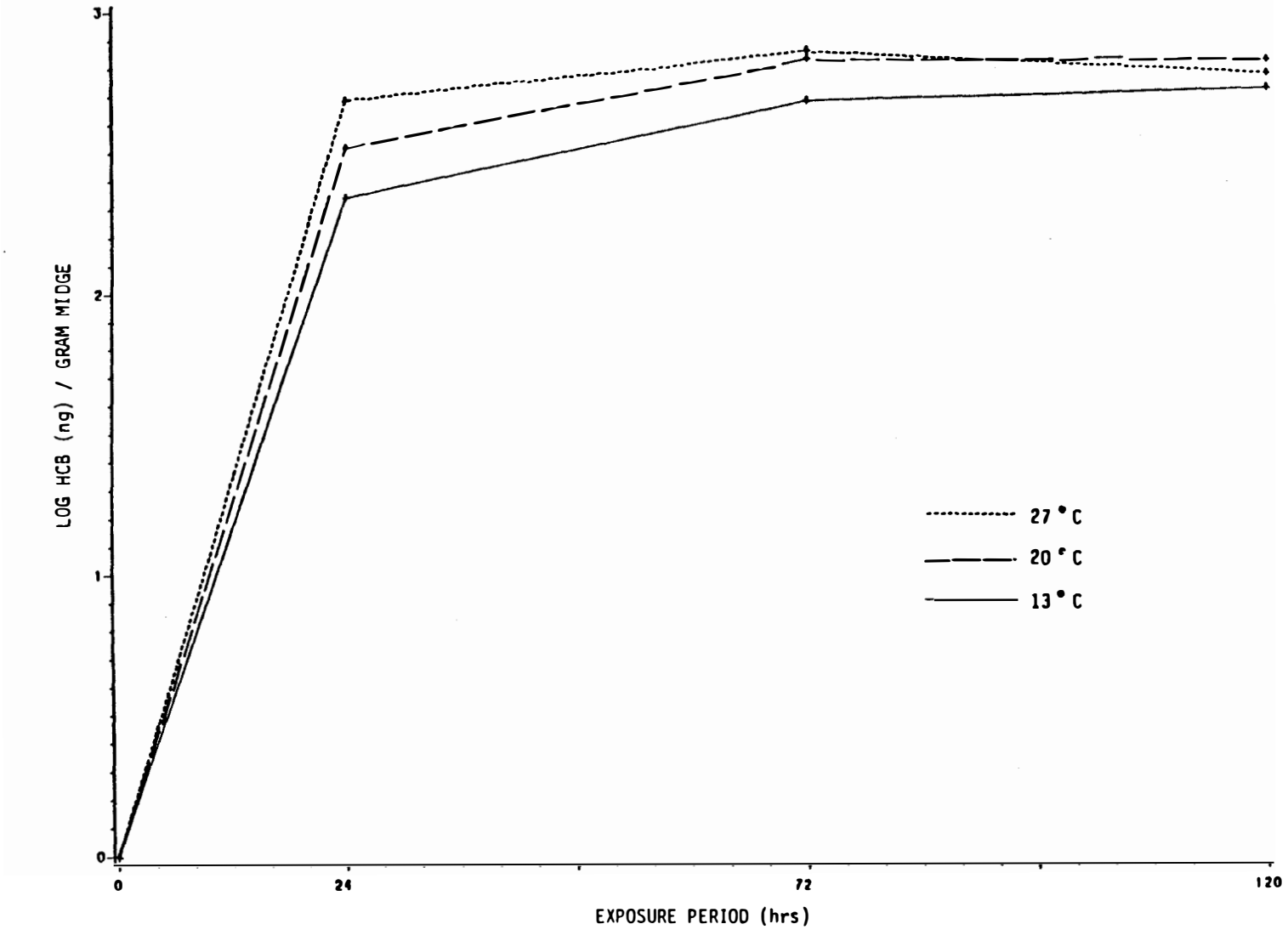

Figure 17. Mean HCB concentrations resulting from exposure at 13, 20, and 27 °C to natural sediment initially contaminated with 1 µg 14C-HCB/g.

Table 14. Mean bacteria HCB concentrations, depuration rate constants (Kd), and HCB biological half-life ($t_{½}$) during depuration in the presence of natural and oxidized sediment.

Substrate	n	Depuration period (min)	Bacteria[a] HCB (µg/g)	K_d (min)	$t_{½}$ (min)
Oxidized sediment	3	0	220.8 (0.4)[b]	--	--
	3	1	196.1 (1.3)	0.1187	5.8
	3	15	194.8 (0.5)	0.0005	1386
	3	120	193.3 (0.4)	0.0001	6930
	3	1440	187.1 (0.7)	0.00002	34650
	3	2880	183.3 (0.8)	0.00001	69300
				$\bar{X}$ = 0.0239	$\bar{X}$ = 22454
Natural sediment	3	0	220.8 (0.4)	--	--
	3	1	193.6 (0.7)	0.1317	--
	3	15	196.4 (0.3)	--	--
	3	120	194.9 (0.9)	0.00007	9900
	3	1440	188.4 (1.1)	0.00003	23100
	3	2880	183.2 (0.7)	0.00002	34650
				$\bar{X}$ = 0.033	$\bar{X}$ =16913

[a] Bacteria dry weight = 0.52 mg/ml

[b] Standard deviation

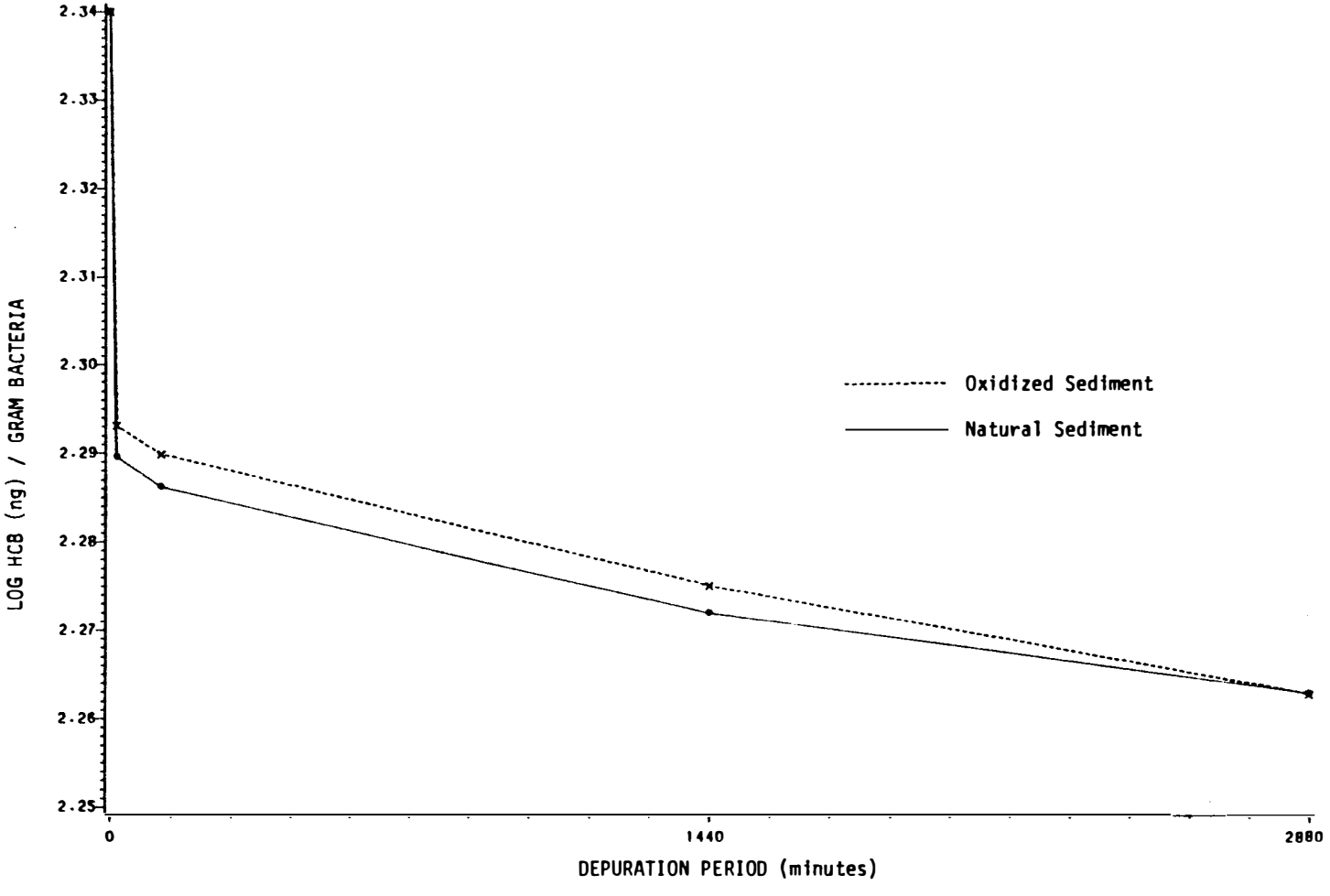

Figure 18. Mean bacteria HCB concentrations during depuration in the presence of natural and oxidized sediment.

presence of oxidized sediment and natural sediment, were 0.024 and 0.033 min^{-1}, respectively (Table 14). Mean HCB biological half-life ($t_{½}$) in bacteria was 22,454 and 16,913 minutes in the presence of oxidized and natural sediment, respectively (Table 14). T-test comparison showed no significant difference between bacterial K_d after 2880 minutes in the presence of natural sediment and oxidized sediments ($t = 0.43$; $df = 4$).

Midge HCB depuration rate constants (K_d) were determined following a 96 hours exposure to natural sediment initially contaminated with 1 µg ^{14}C-HCB g^{-1}. Mean midge HCB concentration, K_d, and $t_{½}$ for each period of the depuration phase are given in Table 15, and shown in Figure 19. Midge K_d for the 72 hours clearance phase in the presence of natural sediment and oxidized sediment, and in the absence of sediment were 0.014, 0.006, and 0.005 hr^{-1}, respectively (Table 15). Mean HCB biological half-life ($t_{½}$) was 50, 116, and 139 hours in the presence of natural sediment, oxidized sediment, and without sediment, (Table 15). There were no significant differences in depuration rates in the presence of natural sediment, oxidized sediment, or without sediment during the initial 24 hours depuration phase (Table A-19), or during the last 24 hours of the 72 hours depuration phase (Table A-20).

The mean concentrations of HCB in mosquitofish during the depuration phase, following 46 days exposure to natural sediment initially contaminated with 1.03 µg HCB g^{-1}, are given in Table 16, and shown in Figure 20. The average K_d for the 30 day depuration phase was 0.00147 day^{-1}, and the mean $t_{½}$ was 591 days.

Table 15. Mean midge HCB concentrations, midge weights, depuration rate constants (Kd), and HCB biological half-life ($t_{½}$) during depuration in the presence of natural or oxidized sediment, or without sediment, following a 96 hours exposure to natural sediment initially contaminated with 1 µg 14C-HCB/g.

Substrate	n	Depuration period (hrs)	Midge wet weight (mg)	Midge HCB (ng/g)	K_d (hr^{-1})	$t_{½}$ (hrs)
Natural sediment	7	0	7.87 (2.24)[a]	767.07 (153.59)	--	--
	6	24	7.70 (1.65)	533.56 (180.42)	0.016	43
	5	48	6.78 (0.94)	398.06 (87.81)	0.011	63
	5	72	6.56 (2.20)	283.42 (73.43)	0.014	50
					$\bar{X}$ = 0.014	$\bar{X}$ = 50
Oxidized sediment	7	0	7.87 (2.24)	767.07 (153.59)	--	--
	6	24	9.33 (1.83)	577.35 (111.23)	0.012	58
	5	48	6.42 (1.15)	543.14 (43.44)	0.002	346
	6	72	7.12 (1.46)	489.06 (74.14)	0.005	139
					$\bar{X}$ = 0.006	$\bar{X}$ = 116
Without sediment	7	0	7.87 (2.24)	767.07 (153.59)	--	--
	6	24	8.23 (2.84)	625.91 (46.48)	0.008	87
	5	48	8.72 (2.26)	574.38 (35.96)	0.004	173
	5	72	6.14 (1.25)	520.53 (27.74)	0.005	173
					$\bar{X}$ = 0.005	$\bar{X}$ = 139

[a] Standard deviation

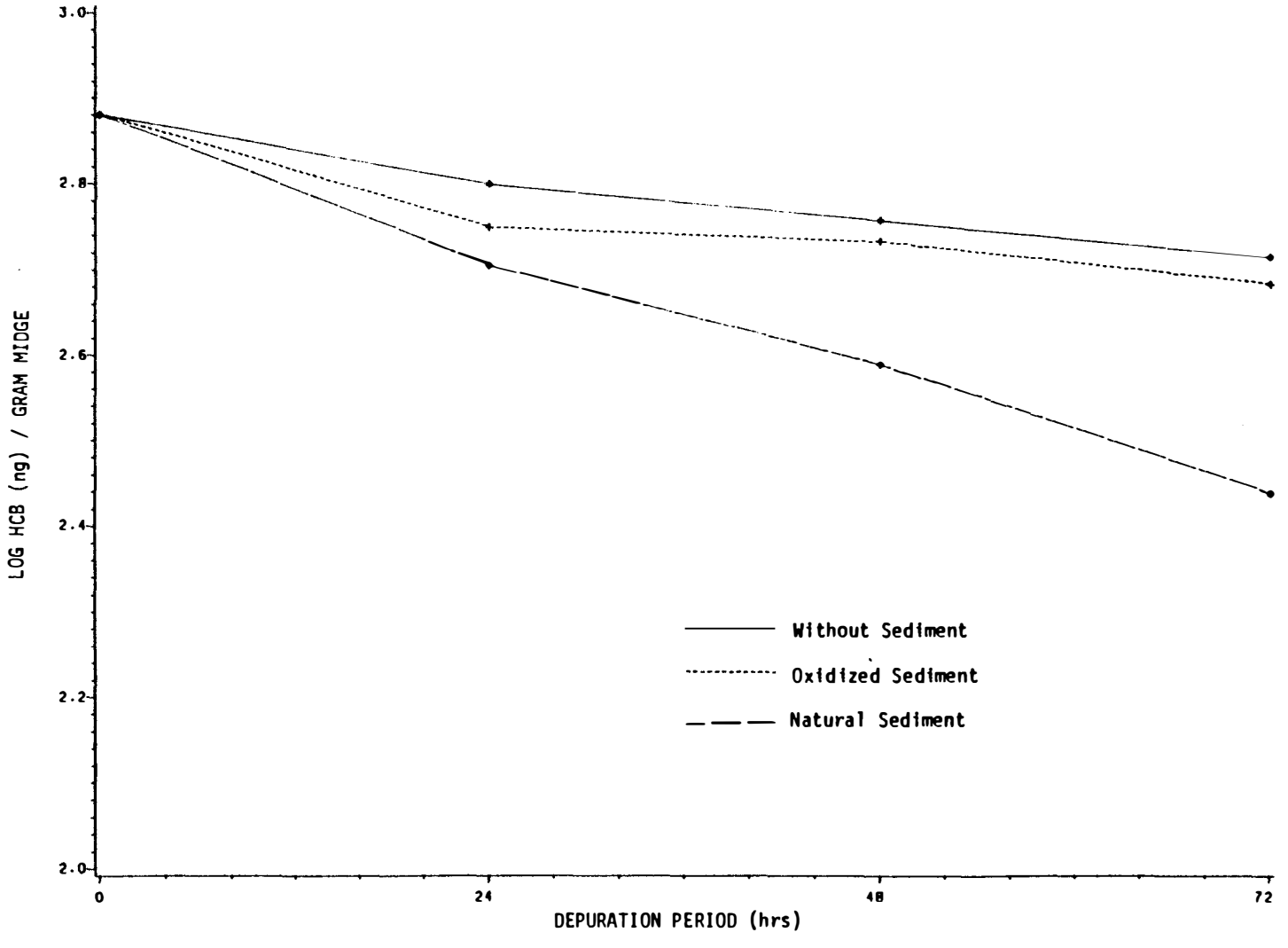

Figure 19. Mean midge HCB concentrations during depuration in the presence of natural and oxidized sediment, and without sediment.

Table 16. Mean mosquitofish HCB concentrations, fish weights, depuration rate constants (K_d), and HCB biological half-life ($t_{½}$) during depuration following a 46 days exposure to natural sediment initially contaminated with 1.03 µg HCB/g.

Depuration period (days)	n	Mosquitofish wet weight (g)	Mosquitofish HCB (ng/g)	K_d (day^{-1})	$t_{½}$ (days)
0	4	0.148 (0.042)	569.6 (73.7)[a]	--	--
5	6	0.140 (0.011)	566.1 (52.0)	-0.0017[b]	--
10	5	0.138 (0.014)	555.6 (24.3)	0.0007	990
20	4	0.123 (0.004)	544.6 (52.1)	0.0014	495
30	4	0.137 (0.008)	521.1 (28.6)	0.0024	289
				$\bar{X}$ = 0.0015	$\bar{X}$ = 591

[a] Standard deviation

[b] not used to calculate the mean k_d or $t_{½}$

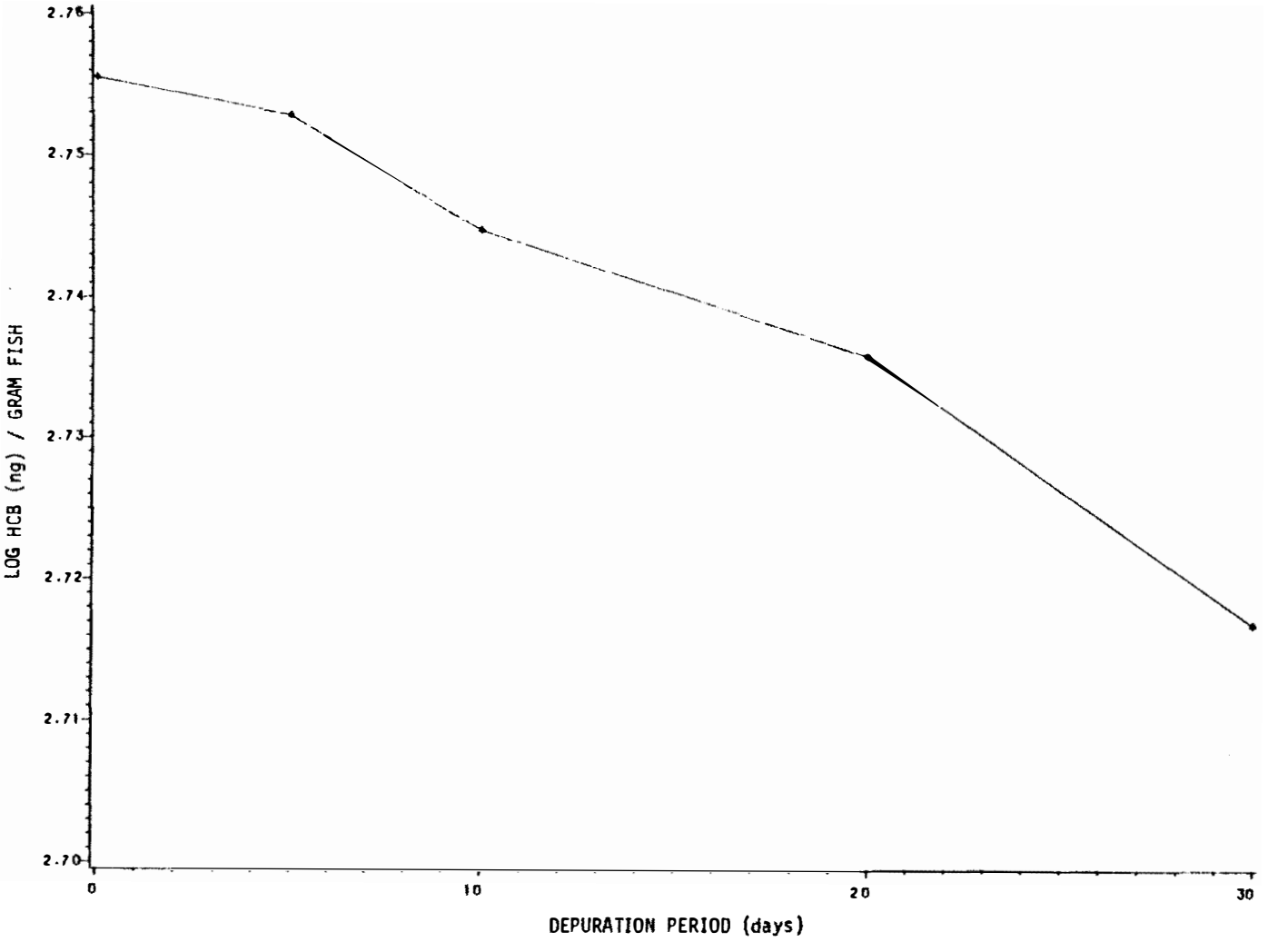

Figure 20. Mean mosquitofish HCB concentrations during depuration following a 96 hours exposure to natural sediment initially contaminated with 1 µg HCB/g.

5.9 Fish HCB Accumulation from Diet versus Sediment

The mean mosquitofish HCB concentrations resulting from exposures to either: (1) ^{14}C-HCB contaminated diet, (2) HCB contaminated sediment, or (3) contaminated diet and sediment are given in Table 17 and shown in Figure 21. HCB accumulation from the two sources was additive, with fish HCB concentrations resulting from exposure to both HCB sources being approximately twice of those concentrations resulting from exposure to either source alone (Table 17). For fish exposed to both HCB sources for 46 days, diet and sediment exposures accounted for 46 and 54% of the mean HCB concentration, respectively.

T-test comparisons of the HCB concentrations in fish after a 12 days exposure period reveal no significant differences between sources of HCB, except between concentrations resulting from contamined diet (alone) and contaminated diet in the presence of contaminated sediment, and between contaminated diet (alone) and contaminated sediment in the presence of contaminated diet (Table A-21). However, after 46 days exposure, t-test comparisons revealed that HCB concentrations resulting from exposure to sediment (alone) were significantly different from concentrations resulting from diet (alone) and diet in the presence of contaminated sediment; all other concentrations were not significantly different (Table A-22). Regression analyses of mosquitofish HCB concentrations show that mean steady-state concentrations were obtained within 35 days as the result of dietary HCB exposure (alone). The mean ^{14}C-HCB content of midge which served as mosquitofish prey was 3.84 ng (SD = 0.64; n = 15) and the

Table 17. Mean mosquitofish HCB concentrations, fish HCB accumulation rates, and weights of fish exposed to natural sediment initially contaminated with 1.03 µg HCB/g or to a diet of midge larvae contaminated with 3.84 ng 14C-HCB/midge, or exposed to both HCB contaminated sediment and 14C-HCB contaminated midge.

Source of HCB exposure	Source of[a] accumulated HCB	n	Exposure period (days)	Fish wet weight (g)		Fish HCB (ng/g)		Fish HCB accumulation rate (ng/g/day)	
Sediment	Sediment	5	12	0.1358	(0.0532)[b]	239.95	(73.18)	19.99	(6.10)
		6	23	0.1243	(0.0876)	282.83	(71.42)	3.98	(6.49)
		4	35	0.1432	(0.1000)	470.26	(21.96)	15.62	(1.83)
		5	46	0.1454	(0.0372)	559.24	(67.91)	8.09	(6.17)
Diet	Diet	5	12	0.1121	(0.0114)	294.32	(18.47)	24.53	(1.53)
		5	23	0.1272	(0.0156)	363.98	(60.44)	6.33	(5.49)
		5	35	0.1326	(0.0111)	434.87	(45.91) *[c]	5.91	(3.83)
		5	46	0.1370	(0.0190)	466.83	(20.21) *	2.91	(1.84)
Sediment and diet	Both	5	12	0.1492	(0.0160)	497.79	(33.77)	41.48	(2.81)
	sediment					258.03	(25.96)	21.50	(2.16)
	diet					239.76	(46.93)	19.98	(2.81)
Sediment and diet	Both	5	23	0.141	(0.0316)	687.98	(75.67)	18.92	(6.88)
	sediment					355.06	(86.09)	8.82	(7.83)
	diet					332.93	(46.93)	8.47	(6.88)
Sediment and diet	Both	5	35	0.1545	(0.0077)	941.48	(63.34)	21.12	(5.28)
	sediment					531.91	(55.80)	14.74	(4.65)
	diet					409.57	(29.90)	6.39	(2.49)
Sediment and diet	Both	5	46	0.1359	(0.0233)	1090.97	(189.52)	13.59	(17.23)
	sediment					622.51	(160.15)	8.24	(14.56)
	diet					468.46	(44.36)	5.35	(4.03)

[a] 14C-HCB was attributed to diet source and non-radiolabelled HCB was attributed to sediment source. HCB from both diet and sediment is denoted as "both".

[b] Standard deviation

[c] Means followed by * were determined not significantly different ($\alpha = 0.05$) with linear regression

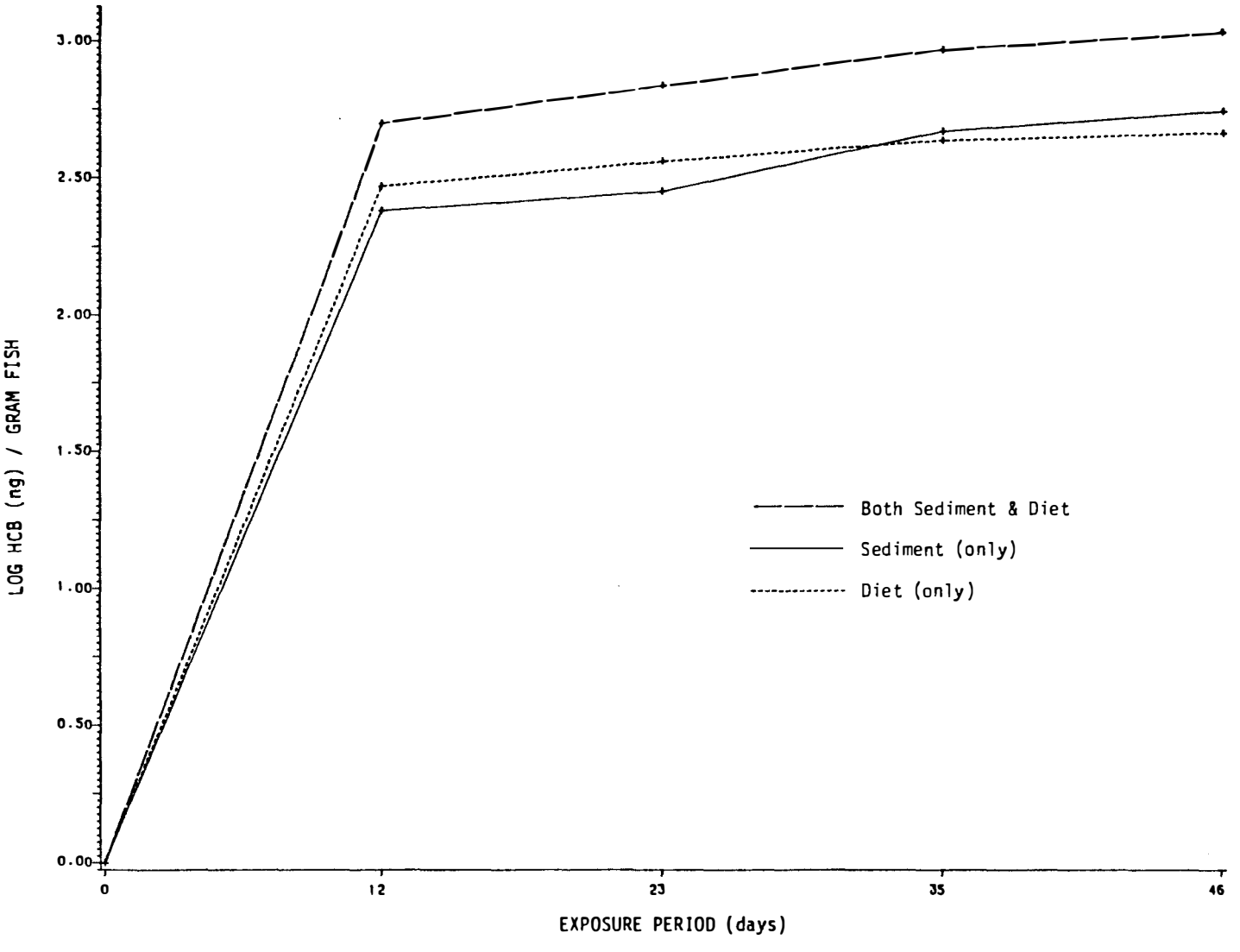

Figure 21. Mean mosquitofish HCB concentrations resulting from exposure to HCB contaminated natural sediment, 14C-HCB contaminated midge diet, or both contaminated sediment and diet.

mean ^{14}C-HCB load of fecal sclera (consumed midge) was 0.33 ng (SD = 0.66). Mean extraction efficiency, determined as the percent decrease in ^{14}C-HCB content of consumed midge, was 91.5%.

Based on the extraction efficiency and the number of midge consumed, the potential HCB load of fish attributable to diet was 56.2, 108.9, 165.1, and 214.3 ng after 12, 23, 35 and 46 days exposures. The mosquitofish dietary concentration factors were inversely related to exposure period and ranged from 59.9% to 27.3% after 12 and 46 days (Table 18).

Table 18. Mean dietary HCB exposure, dietary HCB accumulation and dietary concentration factor of mosquitofish fed a diet of 14C-HCB contaminated midge.

HCB[a] exposure	Exposure period (days)	n	Midge consumed	Dietary[b] HCB exposure (ng)	Fish dietary HCB accumulation (ng)	Dietary[c] concentration factor
Diet	12	5	16	61.44	32.92	0.54
Diet and sediment	12	5	16	61.44	36.82	0.60
Diet	23	5	31	119.04	46.39	0.39
Diet and sediment	23	5	47	119.04	47.54	0.40
Diet	35	5	47	180.48	57.53	0.32
Diet and sediment	35	5	47	180.48	63.37	0.36
Diet	46	5	61	234.24	63.91	0.27
Diet and sediment	46	5	61	234.24	63.86	0.27
						$\bar{X}$ = 0.39

[a] Fish were exposed to 14C-HCB contaminated midge either with non-contaminated natural sediment (labelled as "diet") or with HCB contaminated natural sediment (labelled as "diet and sediment").

[b] Calculated as 3.84 ng 14C-HCB/midge times the number of midge consumed.

[c] Calculated as dietary HCB accumulation divided by dietary HCB exposure

6.0 DISCUSSION

6.1 Bioaccumulation of HCB

An objective of this investigation was to characterize the accumulation of HCB by aquatic organisms and to elucidate the importance of abiotic and biotic factors to the bioaccumulation process. Knowledge of the bioavailability and accumulation of chemicals by organisms is needed to assess the potential for exposure and detrimental effects on natural communities. In this study, the bioaccumulation of HCB resulting from exposure to contaminated substrates was characterized by an initial phase of rapid HCB uptake, followed by a progressively slower second uptake phase (Figures 2-6, 9, 11, 15, 16, and 17). The rates and durations of the first and second accumulation phases varied with the different organisms, substrate HCB concentrations, and with the type of substrate.

The initial phase of rapid bioaccumulation is a phenomenum reported for a wide range of chemicals and organisms (Sodergen, 1968; Paris, et al., 1977; Roberts and Meier, 1982; Pinkney et al., 1985). Bioaccumulation of chemical residues during the initial exposure period is primarily the result of sorption of the residues onto the surface of the organism (Spacie and Hamelink, 1985). The rapidity of HCB bioaccumulation is the result of the concentration gradient which exists when the organisms are initially exposed to the contaminant. Also contributing to the rapidity of bioaccumulation is the fact that an inverse relationship generally exists between the rate of chemical adsorption and the solubility of the chemical (Lambert, 1975). The aqueous solubility

of HCB is extremely low (< 1 µg l^{-1}; Haque and Schmedding, 1975) which, due to thermodynamic considerations, promotes the sorption of HCB onto surfaces.

Since adsorption is a surface phenomena, it is proportional to the surface area of the sorbant (Weber, 1972). Thus, the rate of HCB bioaccumulation during the initial exposure period was related to size (bacteria > midge > mosquitofish; Tables 4, 5 and 7). This is expected, since the surface-to-volume ratio decreases with increasing size of the organism (Prosser and Brown, 1961), thus decreasing the area per volume available for HCB sorption.

If bioaccumulation were solely the result of sorption of HCB onto the surface of an organism, then the accumulation process should cease when the surface becomes saturated with HCB. However, HCB accumulation by the bacteria (Table 3), midge (Table 4), and mosquitofish (Table 6) continued, although at a progressively slower rate, throughout the period of exposure. The second, slower phase of bioaccumulation was probably the result of sorbed residues moving across the surface membranes into the organisms (Sugiura et al., 1975). Absorption of HCB and other non-polar organic compounds by organisms is possible because of the semi-permeable nature of biological membranes, which readily allows passive diffusion of these non-polar, lipophilic compounds (Spacie and Hamelink, 1985). It is probable that as surface-sorbed HCB diffused into the organism, surface sites became available for additional HCB sorption, thus allowing for continued HCB bioaccumulation. The continued accumulation of HCB by dead organisms over the entire duration of exposure (Table 11 and 12) discounts

the necessity of biological transport processes for the absorption of HCB, and appears to support the hypothesis that physical diffusion is responsible for this second phase of HCB bioaccumulation.

6.2 Apparent Steady-State

Steady-state concentrations of HCB in organisms were considered to have been reached when there was no significant difference ($\alpha = 0.05$) in mean HCB concentrations with exposure time. Steady-state HCB concentrations were obtained in bacteria within 1 to 2 days (Table 3), midge within 4 to 5 days (Tables 4, 5, 9, and 13), and mosquitofish within 46 to 57 days (Tables 6 and 17).

Baughman and Paris (1981) concluded in their review of microbial accumulation of chemicals, that steady-state concentrations are reached within a matter of minutes to a few hours. The only noted exception to this was that of Reinert (1972), who observed equilibrium times of 24 to 36 hours for the accumulation of dieldrin by the alga Scenedesmus obliquus. However, in this study, bacterial HCB concentrations increased throughout the periods of exposure, with steady-state concentrations not reached until 24 to 48 hours (Table 4). There are two apparent explanations for the discrepency in the length of time required for bacteria to reach steady-state which was observed in this study and those reported by Baughman and Paris (1981). First, the time required to reach steady-state concentrations in biota is inversely related to the solubility of the compound. This conclusion is drawn from several studies which compared microbial accumulation of several organochlorine compounds (Paris et al.,

1975; Neudorf and Khan, 1975; Chanko and Lockwood, 1967; Rice and Sikka, 1973). Since HCB has a lower water solubility than the organic compounds included in Baughman and Paris' review, HCB would be expected to require a longer period to obtain steady-state concentrations than those compounds. However, solubility differences alone cannot account for the difference in equilibrium time.

A second, and probably more important reason for the discrepancy in equilibrium times observed in this study and those reported by others concerns the method used to expose the organisms to the chemicals. Microbial bioaccumulation studies are typically conducted using chemicals in solution (Johnson and Kennedy, 1973; Grimes and Morrison, 1975; Paris and Lewis, 1976; Paris, et al., 1977) and not, as in this study, with sediment-sorbed chemicals. The sediment used in this study provided many sorption sites for HCB, which in a sense, competed with bacteria for HCB in the ambient water. The sediment HCB sorption-desorption processes would be expected to increase the time required for the biota to reach steady-state HCB concentrations.

Midge obtained steady-state concentrations of HCB in 4 to 5 days (Tables 4, 9, and 13), which would appear to be a remarkably short period when compared to the time period required by the much smaller bacteria. However, these results are consistent with those reported of midge with other chemicals (Muir et al, 1983; Muir et al., 1985; Gerould, et al., 1983), and those reported for PCBs and other benthic invertebrates (Roberts and Meier, 1982). The proximity of midge to the contaminated sediment and to interstitial water, probably contributed to the rapidity

of the equilibrium process. Another probable contributing factor, noted by Nimmo et al. (1971), is that benthic organisms ingest sediment. Consumption of HCB contaminated sediment by midge would increase the organism's surface of exposure, as well as decrease the distance into tissue that HCB must diffuse; therefore, sediment consumption would expedite the obtainment of steady-state HCB concentrations.

The 46 to 57 days exposure required for mosquitofish to reach steady-state HCB concentrations when exposed to contaminated natural sediment (Table 7) is consistent with other studies, which reported that steady-state concentrations of PCBs were obtained in fish within 40 days (Hansen et al., 1971; McLeese et al., 1980; Rubinstein et al., 1983; 1984). Mosquitofish would be expected to require longer periods of exposure to reach equilibrium HCB concentrations than would midge, both due to differences in the surface-to-volume ratios of the organisms, and because of differences in habitats. However, on a weight basis, steady-states were obtained within approximately the same time period for both mosquitofish and midge. Even if surface-to-volume ratios were equal for midge and mosquitofish, midge would still be expected to reach equilibrium HCB concentrations earlier than fish due to midge's greater exposure to HCB resulting from consumption of contaminated sediment. The relatively short period required by mosquitofish to obtain steady-state HCB concentrations is probably the result of the highly efficient respiratory system of fish, which contains a counter-current circulatory system. This type of circulatory system maximizes the chemical concentration gradient be-

tween water and blood, thus facilitating the passive diffusion of chemical residues into the organism.

6.3 Influence of Sediment Organic Matter on Bioavailability

The accumulation of HCB by bacteria (Table 3), midge (Tables 4 and 9) and mosquitofish (Table 7) was inversely related to the organic content of the sediment. Organic matter's high affinity for organic compounds and its influence on bioaccumulation of sorbed compounds is well documented (Goring, 1967; Hamaker and Thompson, 1972; Kenaga and Goring, 1980). Two properties of organic matter appear significant in explaining the lower bioavailability of HCB in the presence of organic matter. First, the irregular structure of organic matter provides large surfaces for the sorption of hydrophobic compounds (Tinsley, 1979; Morrill et al., 1982). The importance of surface area to the sorption of PCBs and other non-ionic organic compounds is well recognized (Pionke and Chesters, 1973; Choi and Chen, 1976; Steen et al, 1978; Haque et al., 1974; Hamaker and Thompson, 1972; Tinsley, 1979; Morrill et al., 1982), as it provides a means of avoiding the thermodynamically unfavored restructuring of water molecules that occurs when non-polar compounds are in aqueous solution (Hamaker and Thompson, 1972).

A second property of organic matter, which is important to the sorption of HCB and the bioavailability of sorbed compounds, is that organic matter contains both hydrophobic and hydrophilic components. When in water, the hydrophobic surfaces of organic matter associate with each other, which results in the formation of a hydrophobic matrix within the

organic molecule (Pierce et al., 1974). The hydrophilic components contribute to organic matter's aqueous solubility, which in turn, increases organic matter's exposure to chemical residues in the ambient water (Pierce et al., 1974). The sorption of non-polar compounds to organic matter occurs first at the surface of the organic molecule, then is followed with the diffusion of the compound into the hydrophobic matrix (Goring, 1967). The desorption of chemical residues from organic matter is slowed by diffusion from this hydrophobic intermatrix (Khan, 1980); therefore, the rate of bioaccumulation of HCB is lower in the presence of organic matter than with inorganic substrates.

The influence that organic matter (OM) can have on the rate of bioaccumulation of organic compounds is apparent when HCB concentrations in biota resulting from exposure to natural sediment (OM ≈ 4.5%) are compared to those concentrations resulting from exposure to oxidized sediment (OM < 0.1%). Rates of HCB bioaccumulation during the initial exposure period were significantly less for organisms exposed to contaminated natural sediment than those resulting from exposure to contaminated oxidized sediment (Tables 3, A-4, and A-8). These results indicate that the rates of HCB bioaccumulation were limited by the rate at which HCB diffused from the hydrophobic intermatrix of sediment organic matter.

Steady-state HCB concentrations found in midge and mosquitofish are consistent with this explanation. However, bacteria HCB concentrations resulting from exposure to natural versus oxidized sediments is unexpected (Table 3), and suggests that organic matter had little influence on equilibrium concentrations of HCB in bacteria. It is not clear whether

this apparent anomaly is the result of the high sediment HCB concentration used in this experiment (~ 300 μg g^{-1}), which possibly saturated the bacteria with HCB, or whether the close proximity of bacteria to sediment facilitated bioaccumulation to the point of nullifying organic matter's impeding affect on bioaccumulation. Midge accumulated a relatively greater amount of HCB from exposure to natural sediment, as compared to oxidized sediment, than did mosquitofish, suggesting that proximity to sediment may be of some importance to bioaccumulation.

As would be expected, if one increases the organic content of natural sediment with additions of humic material, it decreases the rate of accumulation of sediment-sorbed HCB by midge (Table 5). Increasing the organic content of sediment signficantly reduced the bioaccumulation of HCB because the rate HCB desorption from organic matter is much slower than from inorganic surfaces. However, once the organic content of sediment reached 50%, further additions of organic matter did not significantly reduce the bioaccumulation of HCB by midge (Table A-7). It is probable that once the organic content reached 50% all of the HCB was sorbed, and thus further additions of organic matter was of little consequence to the fate of HCB. The rate at which an organism accumulates chemical residues could be of greater consequence to the organism than the steady-state concentrations of the chemical in the organism. Situations of this sort could easily occur if the organism's exposure to the contaminant was of a shorter duration than the time required to achieve equilibrium. Any factor which impedes the rate of residue bioaccumulation reduces the likelihood that the biota will be exposed to chronic or acute

concentrations of the chemical, and thus reduces the ecological impact of the contamination.

6.4 Bioaccumulation from Different Substrates

HCB accumulation in midge resulting from exposures to different contaminated substrates followed the sequence: natural sediment < oxidized sediment ≈ montmorillonite < sand < kaolinite (Table 4). This sequence is approximately the inverse of that of the surface area of the substrates: organic matter > montmorillonite > kaolinite > sand (Bailey and White, 1964, Lambe and Whitman, 1969); and except for the bioaccumulation resulting from exposure to sand, suggests that the bioavailability of HCB to midge is inversely related to the surface area of the substrate.

Midge HCB concentrations resulting from exposure to contaminated kaolinite were greater than concentrations resulting from exposure to sand. Because sand has less surface area than kaolinite, this observation appears inconsistent with the proposed hypothesis that equilibrium partitioning is responsible for the bioaccumulation of hydrophobic organic compounds (Hamelink et al., 1971; Pavlou and Dexter, 1979). This could occur if midge preferentially consumed kaolinite over sand. The importance of sediment consumption to the accumulation of organic contaminants by benthic invertebrates is well recognized (Fowler et al., 1978; Langston, 1978; Meir and Rediske, 1979; 1984; Roberts and Meir, 1982). Consumption of contaminated sediment increases the surface area of the organism which is exposed to the contaminant, thus facilitates residue

bioaccumulation. The preferential consumption of kaolinite over sand could occur if midge were unable to consume sand or were limited to smaller sand grains. This seems a reasonable explanation of the midge HCB concentrations since the sand used in this study (250 - 500 μ diameter) was much larger than the kaolinite (< 4 μ). These data illustrate a potential problem in the development of bioaccumulation models based on the assumption that partitioning processes alone are responsible for residue bioaccumulation. Biological activity, such as consumption of contaminated sediment, may significantly increase the amount of residue that would be expected to bioaccumulate based on the influence of abiotic factors alone.

Mosquitofish HCB concentrations resulting from exposure to contaminated substrates followed the sequence: natural sediment < sand < oxidized sediment < kaolinite (Table 6). Since the uptake of hydrophobic compounds by fish occurs primarily at the gill surfaces (Spacie and Hamelink, 1985), the HCB concentrations of mosquitofish probably reflect the amount of HCB in the water column, either sorbed to suspended particulates or in true solution. Mosquitofish HCB concentrations, resulting from exposure to each of the three inorganic substrates, were similar in magnitude, while HCB concentrations resulting from exposure to natural sediment were approximately 6 - 8 times less than the concentrations resulting from exposure to the inorganic substrates (Table 6). These data indicate that the bioavailability of sediment-sorbed HCB was more influenced by the organic content of the sediment than by the amount or nature of the surface area of the sediment. That mosquitofish exposed for 57 days to contaminated oxidized sediment (OM < 0.1%) accumulated

seven times more HCB than did fish exposed to natural sediment (OM ≈ 4.5%) (Table 7) further demonstrates the dominating influence that organic matter can have on the bioavailability of sediment-sorbed hydrophobic compounds.

In addition to the influence of the organic content of sediment, the amount of particulates suspended in the water column may have contributed to the difference in mosquitofish HCB concentrations that resulted from exposure to the four different substrates (Table 6). Suspended particulates are known to sorb PCBs and other organochlorine compounds (Pierce et al., 1974) and would be expected to facilitate fish HCB accumulation by increasing the amount of HCB to which the fish is exposed to at the gill surfaces. A t-test comparison of water HCB concentrations in the presence of mosquitofish to those without fish (Table A-3) shows that the mean HCB concentrations were significantly different ($t = 2.57$; $df = 22$); thus, the movement of the fish was sufficient to increase, via suspended particulates, the HCB concentration in the water column. Due to density differences, kaolinite is more easily suspended and remains suspended in solution for longer periods than does sand; therefore, the mosquitofish were probably exposed to greater amounts of HCB contaminated kaolinite than sand.

6.5 Concentration Factors

The concentration factor (biota HCB concentration/sediment HCB concentration) can be viewed as an index of the efficiency by which

organisms accumulate sediment-sorbed contaminants. Concentration factors are usually measured at steady-state (Spacie and Hamelink, 1985) which allows for comparisons of data among different studies. Results of this study show that concentration factors were influenced by a variety of factors including sediment HCB concentration, sediment type, and biomass. At assumed steady-state, concentration factors ranged from 0.21 to 5.42 for bacteria (Tables 3, 8, 10, and 11), 0.06 to 21.43 for midge (Tables 4, 5, 9, and 13) and 0.67 to 5.7 for mosquitofish (Table 8). These concentration factors are in good agreement with those reported for PCBs in benthic organisms (Fowler et al., 1978; Courtney and Langston, 1978; Elder et al., 1979; McLeese et al., 1980; Oliver, 1984).

Sediment organic matter had a profound affect on bioaccumulation, with concentration factors being inversely related to the amount of organic matter in sediment (Tables 3, 4, 7, and 9). A similar relationship was reported for Glycera dibranchiata, a polychaete worm, and Crangon septoemspinosa, a shrimp, by McLeese et al. (1980). Concentration factors for bacteria, midge, and mosquitofish exposed to HCB contaminated oxidized sediment (OM < 0.1%) were all greater than concentration factors resulting from exposure to natural sediment (OM ≈ 4.5%). Except in the case of the bacteria, these data clearly show that the presence of organic matter reduced the efficiency of HCB uptake by the biota.

Concentration factors for midge were approximately proportional to the sediment HCB concentrations (Table 9). The same relationship between sediment PCB concentration and concentration factors has been previously reported (McLeese et al., 1980; Fowler et al., 1978; Oliver, 1984).

However, concentration factors for bacteria (Table 8) and mosquitofish (Table 7) were inversely related to sediment HCB concentrations. Wheatly and Hardman (1968) reported an inverse relationship between concentration factors in earthworms exposed to soil-sorbed organochlorine pesticides, but did not speculate on the cause of this phenomenum. A possible explanation for this inverse relationship is that at higher sediment HCB concentrations, a greater percentage of the total HCB is sorbed to the hydrophobic intermatrix of organic matter than at the lower HCB concentrations, and the bioaccumulated HCBs are primarily those HCB molecules which desorbed from the outer portion of the organic matter. Therefore, a greater percentage of the total sediment HCB concentration desorbs at lower concentrations than at higher concentrations.

6.6 Influence of Substrate HCB Concentration on Bioaccumulation

Steady-state HCB concentrations of bacteria (Table 8), midge (Table 9) and mosquitofish (Table 7) were approximately proportional to the sediment HCB concentrations, which is consistent with results reported from other PCB bioaccumulation studies (Hansen et al., 1974; Harding and Phillips, 1978; Wyman and O'Conners, 1980; Biggs et al., 1980). The dose-dependent uptake observed in this study supports the hypothesis that equilibrium partitioning is responsible for bioaccumulation. In this study, the steady-state relationship between

HCB concentrations of biota and natural sediment, determined by linear regression from pooled data, is adequately described (r^2 = .93) by the model:

$$\log \text{biota [HCB]} = 1.08 \log \text{sediment [HCB]} - 0.3.$$

This relationship, shown in Figure 22, suggests that a fundamental relationship exists between the sediment and the bioavailability of sediment-sorbed compounds. If so, knowledge of the sediment concentration of hydrophobic organic compounds should allow for reasonable predictions of the bioaccumulation potential of these chemicals at contaminated aquatic sites. However, this relationship was determined for only one sediment, and as already discussed, other factors such as organic content and surface area of the sediment, influence bioaccumulation. Therefore, more data on the bioavailability of compounds sorbed to a wide variety of sediments is needed before predictive models can conveniently be constructed.

6.7 Influence of Bacterial Biomass on HCB Accumulation

The concentration of HCB in bacteria varied inversely with the amount of biomass (Figure 13), although the total amount of HCB accumulated by the bacteria community increased with biomass (Figure 14). These results are in agreement with those of other researchers (Rice and Sikka, 1973; Neudorf and Khan, 1975; Lederman and Rhee, 1982). The logical explanation for the observed relationship between bioaccumulation and bacterial biomass, as Rice and Sikka (1973) postulated, is that increasing the cell density resulted in a decrease in the amount of HCB around each cell,

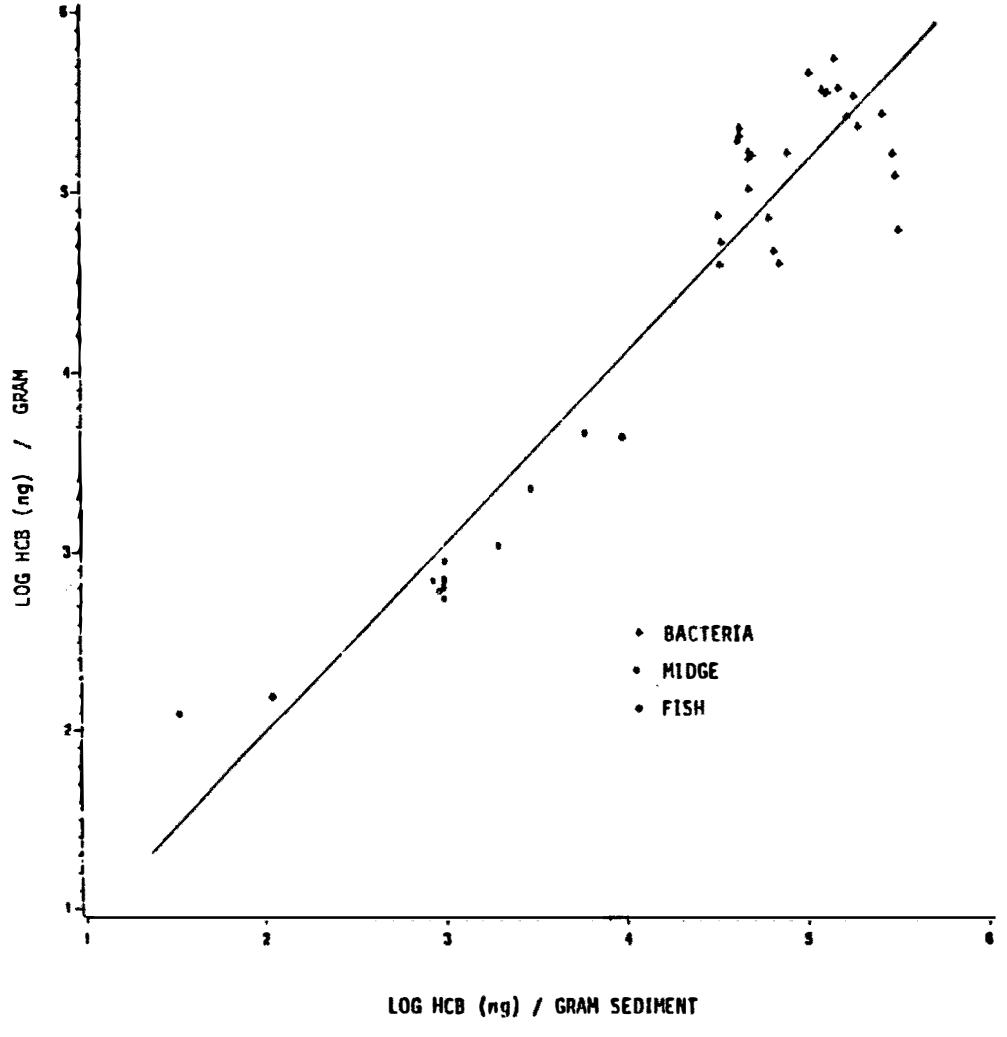

Figure 22. Relationship between HCB concentrations in natural sediment and bacteria, midge and mosquitofish at apparent steady-state.

thus, smaller amounts of HCB were available for accumulation by individual cells. Additionally, at higher bacterial densities, crowding of cells may have resulted in less surface area per cell exposed to contaminated water and sediment than at lower bacterial densities. These results demonstrate that laboratory determined bioaccumulation of hydrophobic chemicals will be influenced by organismal density. If the experimental densities are greater than natural densities, as they were in this study, laboratory determined bioaccumulation may be underestimations of the true accumulation potential of a chemical. Ecologically, these findings suggest that temporal and spacial differences in population densities could result in varying amounts of chemicals being mobilized via the biota, which could be significant to the ultimate fate of the compound if bioaccumulation were a major vehicle to its environmental distribution.

6.8 Influence of Biological Viability on HCB Accumulation

Comparison of HCB accumulation by live and dead organisms should provide information on the means of residue bioaccumulation. If equilibrium partitioning is solely responsible for the bioaccumulation of residues, then the viability of the organism would not be expected to influence accumulation. However, if bioaccumulation is a biologically mediated process, then live organisms should accumulate greater amounts of residues than dead organisms.

HCB accumulation by live and dead bacteria was similar (Table 11) although formaldehyde-killed bacteria accumulated more HCB than did live bacteria. Other researchers have reported comparable residue accumu-

lation by live and dead organisms, and in some cases greater accumulation by dead organisms (Ko and Lockwood, 1968; Sodergren, 1968; Chanko and Lockwood, 1967; Rice and Sikka, 1973; Johnson and Kennedy, 1973; Paris et al., 1975; Canton et al., 1975; Urey et al., 1976; Lederman and Rhee, 1982). Similar accumulation of HCB by live and dead bacteria supports the hypothesis that equilibrium partitioning is responsible for bioaccumulation in unicellular organisms. However, the greater HCB accumulation by formaldehyde-killed bacteria than that of live bacteria appears contrary to the concept of equilibrium partitioning, and suggests that viability reduced bioaccumulation. If bacteria were able to metabolize HCB, then viability could account for the lower accumulation. This explanation is unlikely due to the extreme stability and lipophilicity of the more highly chlorinated PCBs. A more probable explanation for this anomaly is that the chemical treatments used to kill bacteria disrupted the cellular integrety of the bacteria, which led to a greater surface area per cell for the sorption of HCB during the partitioning process. However, this possibility was discounted, because there was no significant difference in the HCB concentrations between chemically treated and non-treated sediment.

Disparity in accumulation of organochlorine compounds by live and dead invertebrates, as well as fish, has been reported (Harding and Vass, 1979; Crosby and Tucker, 1971; Cox, 1971; Ferguson et al., 1966; Murphy and Murphy, 1971). I found HCB accumulation by live midge was approximately twice that of dead midge following a 24 hours exposure to contaminated natural sediment (Table 13). At least three factors could have

contributed to this result. First, as already discussed, bioaccumulation of organochlorine compounds is a two fold process, with an initial adsorption onto the body surface followed by absorption into the organism (Addison, 1976). The difference in HCB accumlation by live and dead midge probably reflects a greater absorption into live midge as a result of the circulatory system facilitating the diffusion process. Secondly, the movement of live midge would continually renew their exposure to sediment and interstitial water with high HCB concentrations, which would facilitate the partitioning process by keeping the environmental-to-midge HCB ratios high. Thirdly, live midge consumption of HCB contaminated bacteria, detritus and sediment would increase the amount of surface exposed to HCB, which would facilitate accumulation.

6.9 Influence of Temperature on Bioaccumulation

Bailey and White (1964) speculated that the bioactivity of chemicals might be different at various temperatures. This seems a reasonable premise since increases in temperature are known to decrease sorption (Goring, 1967; Boucher and Lee, 1972) and increase the solubility and diffusion of organic compounds (Lambert, 1975; Khan, 1980). If temperature influences the bioavailability of sediment-sorbed compounds, then residue accumulation by aquatic organisms would be expected to vary with temperal and spacial differences in temperature. The affect temperature may have on bioaccumulation needs to be known in order to develop realistic models of the fate and ecological impact of environmental contamination.

In this study, midge were exposed to HCB contaminated sediment at 13, 20, and 27 °C, to determine if bioaccumulation varied with temperature. In general, HCB concentrations of midge increased with exposure temperature (Table 13). Midge HCB concentrations after 24 hours exposure to contaminated sediment were significantly greater at 27 °C than at 13 or 20 °C; midge HCB concentrations at 13 °C and 20 °C were not significantly different (Table A-17). Although temperature increased the rate of HCB accumulation, steady-state HCB concentrations were not significantly different at the different temperatures (Table A-18). These results are signficant because kinetic models describing the behavior of environmental contaminants are based on the assumption of steady-state (Spacie and Hamelink, 1985). Since temperature did not alter steady-state concentrations, there is no need to consider temperature as a variable in fate models.

The correlation between exposure temperature and the rate of bioaccumulation could be the result of a decrease in HCB sorption to sediment and an increase in diffusion accompanying an increase in temperature. However, activity of physiological processes of poikilotherm organisms (i.e., circulation) are also correlated with temperature (Prosser and Brown, 1961). Due to the narrow range of temperatures examined in this study, the varying rates of bioaccumulation probably were the result of changes in physiological activity of midge rather than changes in affinity of sediment for HCB. The importance that physiological processes can have on bioaccumulation was clearly demonstrated in experiments using live and dead midge, where after only 24 hours exposure to

contaminated sediment, live midge accumulated twice as much HCB as dead midge (Table 13).

These data suggest that temperature indirectly facilitated the partitioning of HCB in midge by increasing physioalogical activity, which in turn increased the rate of bioaccumulation. This can be of significant consequence to the organism, especially if the exposure is of shorter duration than the time required to achieve equilibrium.

6.10 Depuration of HCB

The rate at which aquatic organisms eliminate chemical residues from their bodies ultimately determines the extent of chemical accumulation in the organisms (Spacie and Hamelink, 1985); chemical residues will accumulate in biota only if the rate of uptake exceeds depuration. In this study, the elimination of HCB from the experimental organisms was much slower than its uptake. Comparisons of the HCB accumulation rates and the depurtaion rate constants of bacteria (Tables 3 and 14), midge (Tables 4 and 15), and mosquitofish (Tables 7 and 16) show that HCB accumulates in biota thousands of times faster than it is eliminated. This pattern of rapid accumulation and slow elimination is typical for organochlorine compounds and has been reported for aquatic organisms ranging from bacteria to fish (Sodergren 1968; Ware and Addison, 1973; Cox, 1971; Macek et al., 1970; Grzenda, et al., 1970; Gooch and Hamdy, 1982).

The slowness of HCB depuration from biota can be explained by the hydrophobic nature of HCB and the inability of organisms to degrade the compound into a more soluble metabolite. The partitioning coefficient of

HCB is extremely large (log K_{ow} > 6.7), and so, once sorbed to biota, HCB is probably sequestered in biological lipids. When HCB contaminated organisms were transferred to a non-contaminated environment the partitioning coefficient is so great that the depuration of organismal HCB was almost insignificant relative to the remaining body burden (Tables 14, 15, and 16).

The nature of the environment influenced the rate of HCB depuration from the biota. Depuration was greatest in the presence of sediment, due to the organic content of the natural sediment; the organic matter increased the affinity and the sorptive capacity of the sediment for HCB (Tables 14 and 15). These data support the conclusion that the depuration of HCB from aquatic organisms is the result of equilibrium partitioning. The addition of sediment increased depuration by providing sorptive surfaces for ambient water HCB, thus sediment helped maintain the HCB concentration gradient between the biota and water required for the depuration process.

However, even in the presence of organic matter, the biological half-life of HCB was almost as long or longer than the expected life-span of the organisms. Thus once an aquatic organism is contaminated with HCB, it is unlikely that the organism will ever be free of the contaminant. The slow depuration of HCB increases the likelihood that the organism will suffer from any chronic affects produced by the chemical, and increasing the likelihood that the organism will serve as a vector for the contamination of higher trophic levels.

6.11 HCB Accumulation from Diet versus Sediment

Two views have been advanced in the literature to explain the accumulation of organic compounds by aquatic organisms. One of these (Macek et al., 1970) argues that dietary inputs are responsible for an organism's residue body burden. The other view (Hamelink et al., 1971) is that residue accumulation by an organism is the result of the physical partitioning of the compound between the organism and its surroundings, and that the uptake of residues occurs primarily at the gill or body surfaces. The relative importance of dietary and environmental exposures to bioaccumulation is needed so that the ecological impact of contamination can be assessed and appropriate legal standards enacted (Jarvinen et al., 1977).

I found that mosquitofish accumulated HCB from exposure to contaminated sediment (Tables 7 and 17), as well as from a contaminated diet (Table 17). Steady-state HCB concentrations were reached in mosquitofish within 46-57 days exposure to contaminated sediment and within 35-46 days exposure to contaminated prey. However, when fish were simultaneously exposed to both contaminated sediment and diet, steady-state concentrations were not apparent after 57 days exposure; HCB accumulation from sediment and diet was additive and greater than the steady-state concentration resulting from exposure to either source alone. These data demonstrate that both bioconcentration (accumulation from water) and biomagnification (accumulation from prey) can be important to the accumulation of HCB in fish. In addition, these data show that the HCB accu-

mulation in fish was dose-dependent; increasing the HCB exposure increased bioaccumulation, irregardless of the source of exposure. Dose-dependent bioaccumulation indicates that equilibrium partitioning is responsible for the uptake of HCB from diet and sediment.

Mosquitofish HCB concentrations resulting from sediment and diet exposure were statistically indistinguishable for the initial 12 days exposure (Table A-21), as well as for the 46 days exposure (Table A-22), which indicates that bioconcentration and biomagnification processes could be of equal importance to bioaccumulation of hydrophobic organic compounds. These findings are similar to those reported for DDT and other PCBs (Macek and Korn, 1970; Jarvinen et al., 1977; Rubinstein et al., 1984); however, researchers have reported dietary contributions of other organic compounds to the fish's body burden of only 1-15% (Chadwich and Brocken, 1969; Reinert, 1967; Macek et al., 1979). Due to these reported differences, the relative importance of dietary and environmental exposures to the bioaccumulation of chemical residues has been a matter of controversy. However, these discrepencies in the dietary contribution of organic compounds to fish can be readily explained by invoking the concept of organic compounds to fish can be readily explained by invoking the concept of equilibrium partitioning (Hamelink et al., 1971). Compounds which have high partitioning coefficients, such as HCB and DDT, accumulate in fish as the result of the organisms inability to excrete these hydrophobic compounds; compounds with low partitioning coefficients have greater aqueous solubilities and are more easily eliminated from the organism.

A consideration of the HCB concentration in fish relative to the amount of HCB consumed by the fish is important to the understanding of how fish deal with ingested residues. A comparison of the amount of HCB in midge prey to that of the fecal sclera (consumed midge) showed a 91.5% decrease in the HCB load of midge during digestion. However, mosquitofish HCB dietary accumulation efficiency was only 27.3 to 59.9%; the accumulation efficiency decreased with time (Table 18). These data indicate that the mosquitofish easily digest the midge, and in the process released most of the midge HCB load. Yet the fish accumulated only one-third to one-half of these residues, which suggest that the HCB residues must have passed through the digestive tract of the fish faster than they could be accumulated. Due to HCB's lipophilicity it is probable that the HCB released from midge during digestion remained sorbed to fats and lipids, and that the fish accumulated HCB as the result of the partitioning between the digested material and the gastrointestinal tract of the fish. The decrease in dietary accumulation efficiency with time indicates that the mosquitofish HCB concentrations along the digestive tract were approaching equilibrium, which supports the conclusion that partitioning is responsible for dietary accumulation of HCB.

The results of this study indicate the sediment-sorbed organic compounds will accumulate in the biota and have the potential for biomagnification in aquatic ecosystems. Prey organisms can contribute to the rapid accumulation of residues in their predators and significantly increase the potential for any adverse impact these compounds may have on the organisms. Although HCB accumulation attributed to

biomagnification was less than 50% of the fish's total accumulation, it is probable that in natural environments where there are a greater number and array of prey, food chain transfer of chemical residues could be the primary means of bioaccumulation. This conclusion is supported by a study which estimated that 95% of the PCB residues in Great Lakes fish are the result of biomagnification (Weininger, 1978). As already discussed, the depuration of HCB from biota is very slow; therefore, contaminated prey would be expected to remain a source of HCB for predators long after the levels of the chemical in the environment had declined. This study suggests that it is unreasonable to try to explain the accumulation of hydrophobic organic compounds by fish as being the result of only sediment exposure or dietary exposure, and that both sources of residues need to be examined simultaneously in order to reasonably evaluate the bioaccumulation potential of a chemical.

7.0 SUMMARY

The bioavailability of sediment-sorbed hexachlorobiphenyl (HCB) to bacteria, midge, and mosquitofish was examined with the relative goal of determining the influence of abiotic and biotic factors on the bioaccumulation process. Factors examined included sediment type, the organic content of the sediment, HCB concentration, temperature, and biological viability. Additional experiments were conducted to determine the relative role of sediment and dietary HCB sources to the accumulation of HCB in mosquitofish.

Sediment type and organic content of the sediment had the greatest affect on the bioavailability of sediment-sorbed HCB. In general, the bioavailability of sediment-sorbed HCB was inversely related to the surface area and the organic content of the sediment. The greater the surface area of the sediment, the greater the chances are the HCB in water will come in contact with the sediment instead of the biota; thus, increases in sediment surface area reduce the bioaccumulation of HCB.

The inverse relationship between HCB bioavailability and sediment organic matter is probably the result of organic matter's extremely large surface area and its hydrophobic and hydrophilic components. The hydrophilic components of organic matter allowed it to remain suspended in solution, thereby increasing its exposure to HCB in solution. Once sorbed to sediment organic matter, HCB diffused into the hydrophobic intermatrix. The desorption of HCB from sediment organic matter was slowed

by diffusion from this hydrophobic intermatrix, thereby reducing the rate of bioaccumulation in the presence of organic matter.

The accumulation of HCB by bacteria, midge, and mosquitofish was approximately proportional to the sediment HCB concentration, which is consistent with the hypothesis that equilibruim partitioning is responsible for bioaccumulation. The linear model adequately describes the steady-state concentration of HCB in bacteria, midge, and mosquitofish resulting from exposure to contaminated natural sediment. This model indicates that knowledge of sediment composition and concentration of hydrophobic organic compounds would allow for reasonable predictions of the bioaccumulation potential for diverse aquatic organisms.

The amount of HCB accumulated by bacteria increased linearly with biomass; however, the bacterial HCB concentration decreased with increasing biomass. The correlation between total HCB accumulation and biomass supports the hypothesis that equilibrium partitioning is responsible for bioaccumulation of HCB. The observed decrease in bacterial HCB concentration with increased biomass was probably due to a reduction in bacterial exposure to HCB as the result of cell crowding.

HCB accumulation by live and dead bacteria was similar, which indicates that biological processes are not involved in accumulation and which supports the hypothesis that equilibrium partitioning is responsible for bioaccumulation of chemical residues. However, live midge accumulated HCB at a rate approximately twice that of dead midge. The disparity in HCB accumulation in live and dead midge is probably the result of biological activity (i.e., circulatory system), which facilitated the par-

titioning process by increasing midge exposure to HCB and maintaining a HCB concentration gradient between the midge and its surroundings.

Temperature did not have a significant influence on the steady-state HCB concentration in midge. However, at higher temperatures, the rate of HCB accumulation was greater than at lower temperatures. Differences in the rates of bioaccumulation with temperature were explained by the correlation of midge physiological processes and temperature, rather than the result of decreased HCB sorption to sediment. Elevation of physiological activity with increasing temperature facilitated equilibrium partitioning.

Depuration of HCB was a slow process and appears to be the result of equilibrium partitioning. Aquatic organisms were unable to excrete accumulated HCB due to the extreme lipophilicity of the compound and its stability which prevents biodegradation. The nature of the environment influenced the rate of HCB depuration; however, as evidenced by the long biologica half-life, HCB contaminated organisms will never be free of accumulated residues.

Both dietary and sediment sources of HCB exposure were important to the accumulation of HCB in mosquitofish; bioaccumulation from the two HCB sources was additive and approximately equal. The additive accumulation from dietary and sediment exposures indicates that bioaccumulation is dose-dependent and the result of equilibrium partitioning. Mosquitofish dietary accumulation efficiencies ranged from 27.3 to 59.9% over a 46 days exposure period. The decrease in dietary accumulation efficiency with time indicates that equilibrium HCB concentrations were being approached

and supports the conclusion that partitioning processes are responsible for the biomagnification of HCB.

8.0 CONCLUSIONS

The data obtained in this investigation provides important information on the bioavailability of sediment-sorbed hydrophobic organic compounds. This information contributes to a growing body of knowledge concerning the fate of environmental contaminants, and identifies the influence that abiotic and biotic factors bioaccumulation in natural environments.

The general conclusions that can be drawn from this investigation are:

(1) A wide range of aquatic organisms are involved in the mobilization of sediment-sorbed hexachlorobiphenyl (HCB).

(2) The physical process of equilibrium partitioning is responsible for bioaccumulation of HCB. However, equilibrium partitioning of HCB is facilitated by biological activity.

(3) The bioavailability of sediment-sorbed HCB is inversely related to the organic content and the surface area of the sediment.

(4) Bioaccumulation of HCB is proportional to the sediment concentration.

(5) Temperature may affect the rate of HCB uptake by biota; however, steady-state HCB concentrations are not affected by temperature.

(6) Depuration of HCB is the result of equilibrium partitioning, and is a slow process due to the extreme lipophilicity of

HCB. Once contaminated with HCB, aquatic organisms will retain HCB residues throughout their lives.

(7) HCB accumulation from dietary sources was additive to accumulation from environmental sources.

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APPENDIX

Table A-1. Mean water HCB concentrations during the experimental exposure of bacteria to natural sediment initially contaminated with 54.5 µg 14C-HCB/g.

Treatment	Exposure period (min)	n	Water HCB concentration (µg/l)	
Bacteria	1	4	0.39	(0.13)[a]
	15	3	0.60	(0.46)
	120	3	1.20	(1.38)
	1440	3	0.79	(0.25)
	2880	4	0.21	(0.07)
Control (no bacteria)	1	3	2.23	(1.04)
	15	2	1.93	(0.82)
	120	2	0.36	(0.04)
	1440	3	1.96	(1.64)
	2880	3	0.26	(0.31)

[a] Standard deviation

Table A-2. Mean water HCB concentrations during the experimental exposure of midge to natural sediment, oxidized sediment, kaolinite, and sand, each initially contaminated with 1 µg 14C-HCB/g.

Substrate	Midge present	Exposure period (days)	n	Water HCB concentration (µg/l)	
Natural sediment	yes	3	3	1.10	(0.42)[a]
	no	3	5	0.19	(0.09)
	no	6	5	0.14	(0.01)
	no	9	5	0.17	(0.01)
Oxidized sediment	yes	3	3	2.28	(0.39)
	no	3	5	0.66	(0.06)
	no	6	5	0.50	(0.02)
	no	9	5	0.67	(0.02)
Kaolinite	yes	3	3	1.80	(0.49)
	no	3	5	0.80	(0.04)
	no	6	5	0.81	(0.11)
	no	9	5	0.81	(0.10)
Sand	yes	3	3	1.71	(0.51)
	no	3	5	1.07	(0.03)
	no	6	5	1.21	(0.05)
	no	9	5	0.60	(0.05)

[a] Standard deviation

Table A-3. Mean water HCB concentrations during experimental exposure to natural sediment initially contaminated with 0.03, 0.13, 1.03, and 10.03 ug HCB/g, and oxidized sediment initially contaminated with 1.03 ug HCB/g.

Sediment	Sediment HCB (ug/g)	Fish present	Exposure period (days)	Water HCB concentration (ug/l)	
Natural	0.03	yes	12	0.16	(0.01)[a]
	0.03	yes	23	0.18	(0.01)
	0.03	yes	35	0.16	(0.01)
	0.03	yes	46	0.18	(0.01)
	0.03	yes	57	0.17	(0.01)
Natural	0.13	yes	12	0.20	(0.00)
	0.13	yes	23	0.18	(0.01)
	0.13	yes	35	0.14	(0.02)
	0.13	yes	46	0.17	(0.02)
	0.13	yes	57	0.20	(0.02)
Natural	1.03	yes	12	0.44	(0.11)
	1.03	yes	23	0.42	(0.02)
	1.03	yes	35	0.39	(0.01)
	1.03	yes	46	0.38	(0.02)
	1.03	yes	57	0.40	(0.01)
Natural	1.03	no	12	0.31	(0.10)
	1.03	no	23	0.39	(0.06)
	1.03	no	35	0.30	(0.18)
	1.03	no	46	0.33	(0.00)
	1.03	no	57	0.30	(0.14)
Natural	10.03	yes	12	2.62	(0.34)
	10.03	yes	23	2.48	(0.31)
	10.03	yes	35	2.09	(0.06)
	10.03	yes	46	3.14	(0.15)
	10.03	yes	57	3.75	(0.67)
Oxidized	1.03	yes	12	1.25	(0.02)
	1.03	yes	23	1.18	(0.03)
	1.03	yes	35	2.37	(0.02)
	1.03	yes	46	1.77	(0.39)
	1.03	yes	57	1.49	(0.13)

[a] Standard deviation

Table A-4. T-test comparisons of mean midge HCB accumulation rates during the initial 24 hours exposure to natural sediment, oxidized sediment, sand, kaolinite, and montmorillonite, each initially contaminated with 1 μg 14C-HCB/g.

Comparison	Variance	df	Calculated t-value
Natural sediment vs Oxidized sediment	equal	8.0	10.21 *
Natural sediment vs Sand	equal	9.0	12.63 *
Natural sediment vs Kaolinite	unequal	4.1	11.13 *
Natural sediment vs Montmorillonite	equal	10.0	7.77 *
Oxidized sediment vs Sand	equal	9.0	4.36 *
Oxidized sediment vs Kaolinite	unequal	4.1	9.57 *
Oxidized sediment vs Montmorillonite	unequal	7.5	3.76 *
Sand vs Kaolinite	unequal	4.2	8.76 *
Sand vs Montmorillonite	equal	11.0	1.00 NS
Kaolinite vs Montmorillonite	unequal	4.6	8.21 *

NS not significant at $\alpha = 0.05$

* significant

Table A-5. T-test comparisons of mean HCB concentrations of midge resulting from 120 hours exposure to natural sediment, oxidized sediment, sand, kaolinite, and montmorillonite, each initially contaminated with 1 µg 14C-HCB/g.

Comparison	Variance	df	Calculated t-value
Natural sediment vs Oxidized sediment	equal	10.0	12.31 *
Natural sediment vs Sand	unequal	3.1	8.93 *
Natural sediment vs Kaolinite	unequal	5.4	21.12 *
Natural sediment vs Montmorillonite	equal	9.0	24.67 *
Oxidized sediment vs Sand	equal	9.0	3.87 *
Oxidized sediment vs Kaolinite	equal	11.0	12.87 *
Oxidized sediment vs Montmorillonite	equal	11.0	0.06 NS
Sand vs Kaolinite	equal	8.0	5.48 *
Sand vs Montmorillonite	unequal	3.2	4.12 *
Kaolinite vs Montmorillonite	unequal	5.5	13.39 *

NS not significant at $\alpha = 0.05$

* significant

Table A-6. T-test comparisons of mean midge HCB accumulation rates during the initial 24 hours exposure to five sediments varying in organic content, each initially contaminated with 1 μg 14C-HCB/g (sediments were: (a) natural; (b) natural-humic (3:1); (c) natural-humic (1:1); (d) natural-humic (1:3); and (e) humic).

Comparison	Variance	df	Calculated t-value
a vs b	equal	8.0	8.09 *
a vs c	equal	8.0	10.57 *
a vs d	equal	8.0	9.10 *
a vs e	equal	8.0	10.99 *
b vs c	equal	8.0	2.57 *
b vs d	equal	8.0	2.51 *
b vs e	equal	8.0	4.94 *
c vs d	equal	8.0	0.96 NS
c vs e	equal	8.0	3.66 *
d vs e	equal	8.0	2.03 *

NS not significant at $\alpha = 0.05$

* significant

Table A-7. T-test comparisons of mean midge HCB concentrations resulting from 96 hours exposure to five sediments varying in organic content, each initially contaminated with 1 µg 14C-HCB/g (sediments were: (a) natural; (b) natural-humic (3:1); (c) natural-humic (1:1); (d) natural-humic (1:3); and (e) humic).

Comparison	Variance	df	Calculated t-value
a vs b	equal	6.0	7.76 *
a vs c	equal	6.0	6.89 *
a vs d	equal	7.0	10.85 *
a vs e	equal	6.0	9.65 *
b vs c	equal	6.0	0.16 NS
b vs d	equal	7.0	3.38 *
b vs e	equal	6.0	3.05 *
c vs d	equal	7.0	2.16 NS
c vs e	equal	6.0	1.87 NS
d vs e	equal	7.0	0.18 NS

NS not significant at $\alpha = 0.05$

* significant

Table A-8. T-test comparisons of mean mosquitofish HCB concentrations resulting from five days exposure to natural sediment, oxidized sediment, sand, and kaolinite, each initially contaminated with 1 µg 14C-HCB/g.

Comparison	Variances	df	Calculated t-value
Natural sediment vs Oxidized sediment	unequal	5.0	7.77 *
Natural sediment vs Sand	unequal	5.0	5.38 *
Natural sediment vs Kaolinite	unequal	5.3	23.44 *
Oxidized sediment vs Sand	equal	10.0	1.54 NS
Oxidized sediment vs Kaolinite	unequal	5.7	1.51 NS
Sand vs Kaolinite	unequal	5.6	0.61 NS

NS not significant at $\alpha = 0.05$

* significant

Table A-9. T-test comparisons of mean mosquitofish HCB concentrations resulting from 15 days exposure to natural sediment, oxidized sediment, kaolinite, and sand, each initially contaminated with 1 µg 14C-HCB/g.

Comparison	Variances	df	Calculated t-value
Natural sediment vs Oxidized sediment	unequal	5.1	10.17 *
Natural sediment vs Kaolinite	unequal	5.1	17.57 *
Natural sediment vs Sand	unequal	5.1	10.66 *
Oxidized sediment vs Kaolinite	equal	10.0	2.41 *
Oxidized sediment vs Sand	equal	10.0	1.24 NS
Kaolinite vs Sand	equal	10.0	4.19 *

NS not significant at $\alpha = 0.05$

* significant

Table A-10. T-test comparisons of mean midge HCB concentrations resulting from 24 hours exposure to natural and oxidized sediment, each initially contaminated with 1, 3, and 6 µg 14C-HCB/g.

Comparison	Variance	df	Calculated t-value
Natural sediment			
1µg vs 3µg	equal	8	9.05 *
3µg vs 6µg	equal	8	8.31 *
Oxidized sediment			
1µg vs 3µg	equal	8	5.91 *
3µg vs 6µg	equal	8	2.21 NS
Natural sediment vs Oxidized sediment			
1µg vs 1µg	equal	8	0.23 NS
3µg vs 3µg	equal	8	0.18 NS
6µg vs 6µg	equal	8	0.33 NS

NS not significant at $\alpha = 0.05$

* significant

Table A-11. T-test comparisons of mean midge HCB concentrations resulting from 192 hours exposure to natural and oxidized sediment, each initially contaminated with 1, 3, and 6 µg 14C-HCB/g.

Comparison	Variance	df	Calculated t-value
Natural sediment			
1µg vs 3µg	unequal	8.4	12.20 *
3µg vs 6µg	equal	14.0	11.87 *
Oxidized sediment			
1µg vs 3µg	equal	8.0	6.15 *
3µg vs 6µg	equal	8.0	8.40 *
Natural sediment vs Oxidized sediment			
1µg vs 1µg	equal	8.0	14.59 *
3µg vs 3µg	unequal	7.3	15.38 *
6µg vs 6µg	unequal	2.0	5.46 *

* significant at $\alpha = 0.05$

Table A-12. T-test comparisons of mean mosquitofish HCB concentrations resulting from 12 days exposure to natural sediment initially contaminated with 0.03, 0.13, 1.03, and 10.03 µg HCB/g.

Comparison	Variance	df	Calculated t-value
0.03 vs 0.13	equal	8.0	0.14 NS
0.13 vs 1.03	unequal	4.6	5.33 *
1.03 vs 10.03	equal	8.0	5.33 *

NS not significant at $\alpha = 0.05$

* significant

Table A-13. T-test comparisons of mean mosquitofish HCB concentrations resulting from 57 days exposure to natural sediment initially contaminated with 0.03, 0.13, 1.03, and 10.03 μg HCB/g.

Comparison	Variance	df	Calculated t-value
0.03 vs 0.13	equal	8.0	1.21 NS
0.13 vs 1.03	equal	8.0	10.72 *
1.03 vs 10.03	unequal	4.4	22.09 *

NS not significant at $\alpha = 0.05$

* significant

Table A-14. T-test comparisons of mean bacteria HCB concentrations resulting from 48 hours exposure of four bacterial dilutions to natural sediment initially contaminated with 320 µg 14C-HCB/g (dry weight of bacteria dilutions were: (a) 0.82; (b) 0.67; (c) 0.44; and (d) 0.34 mg/ml).

Comparison	Variance	df	Calculated t-value
a vs b	equal	4.0	6.63 *
a vs c	equal	4.0	9.33 *
a vs d	equal	4.0	17.60 *
b vs c	equal	4.0	6.46 *
b vs d	equal	4.0	15.66 *
c vs d	equal	4.0	5.57 *

* significant at $\alpha = 0.05$

Table A-15. T-test comparisons of mean bacteria HCB concentrations resulting from 2 hours exposure of live bacteria and bacteria killed with formaldehyde and mercuric chloride to natural sediment initially contaminated with 71 µg 14C-HCB/g.

Comparison	Variance	df	Calculated t-value
Formaldehyde vs Live	equal	4	2.40 NS
Formaldehyde vs $HgCl_2$	equal	4	2.68 NS
$HgCl_2$ vs Live	equal	4	0.44 NS

NS not significant at $\alpha = 0.05$

Table A-16. T-test comparisons of mean bacteria HCB concentrations resulting from 48 hours exposure of live bacteria and bacteria killed with formaldehyde and mercuric chloride to natural sediment initially contaminated with 71 μg 14C-HCB/g.

Comparison	Variance	df	Calculated t-value
Formaldehyde vs Live	equal	4	4.03 *
Formaldehyde vs $HgCl_2$	equal	4	5.17 *
$HgCl_2$ vs Live	equal	4	2.93 *

* significant at $\alpha = 0.05$

Table A-17. T-test comparisons of mean midge HCB concentrations resulting from 24 hours exposure at 13, 20, and 27°C, to natural sediment initially contaminated with 1 µg 14C-HCB/g.

Comparison	Variance	df	Calculated t-value
13°C vs 20°C	equal	9	2.20 NS
13°C vs 27°C	equal	10	5.81 *
20°C vs 27°C	equal	9	3.44 *

NS not significant at $\alpha = 0.05$

* significant

Table A-18. T-test comparisons of mean midge HCB concentrations resulting from 120 hours exposure at 13, 20, and 27°C, to natural sediment initially contaminated with 1 μg 14C-HCB/g.

Comparison	Variance	df	Calculated t-value
13°C vs 20°C	equal	6	1.88 NS
13°C vs 27°C	equal	10	1.01 NS
20°C vs 27°C	equal	12	0.75 NS

NS not significant at $\alpha = 0.05$

Table A-19. T-test comparisons of mean midge HCB depuration rates during the initial 24 hours clearance phase in the presence of natural or oxidized sediment, or without sediment.

Comparison	Variance	df	Calculated t-value
Natural sediment vs Oxidized sediment	equal	10.0	0.51 NS
Natural sediment vs Without sediment	unequal	5.7	1.21 NS
Oxidized sediment vs Without sediment	equal	10.0	0.99 NS

NS not significant at $\alpha = 0.05$

Table A-20. T-test comparisons of mean midge HCB depuration rates during the last 24 hours of a 72 hours clearance phase in the presence of natural or oxidized sediment or without sediment.

Comparison	Variances	df	Calculated t-value
Natural sediment vs Oxidized sediment	equal	9.0	1.88 NS
Natural sediment vs Without sediment	unequal	4.4	2.09 NS
Oxidized sediment vs Without sediment	equal	9.0	0.19 NS

NS not significant at $\alpha = 0.05$

Table A-21. T-test comparisons of mosquitofish HCB accumulation resulting from 12 days exposure to: (a) natural sediment contaminated with 1 µg HCB/g; (b) midge prey contaminated with 3.84 ng/g; (c) natural sediment when exposed to both contaminated sediment and midge; and (d) midge prey when exposed to both contaminated sediment and midge.

Comparison	Variance	df	Calculated t-value
a vs b	unequal	4.5	1.61 NS
a vs c	equal	8.0	0.52 NS
a vs d	equal	8.0	0.01 NS
b vs c	equal	8.0	2.55 *
b vs d	equal	8.0	3.82 *
c vs d	equal	8.0	1.11 NS

NS not significant at $\alpha = 0.05$

* significant

Table A-22. T-test comparisons of mosquitofish HCB accumulation resulting from 46 days exposure to: (a) natural sediment contaminated with 1 µg HCB/g; (b) midge prey contaminated with 3.84 ng/g; (c) natural sediment when exposed to both contaminated sediment and midge; and (d) midge prey when exposed to both contaminated sediment and midge.

Comparison	Variance	df	Calculated t-value
a vs b	unequal	4.7	2.92 *
a vs c	equal	8.0	0.81 NS
a vs d	equal	8.0	2.50 *
b vs c	unequal	4.1	2.16 NS
b vs d	equal	8.0	0.07 NS
c vs d	unequal	4.6	2.07 NS

NS not significant at $\alpha = 0.05$

* significant

VITA

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