

THE DETERMINATION OF THE ACUTE TOXICITY,
UPTAKE AND ELIMINATION RATES FOR KEPONE
BY Crangon septemspinosus SAY

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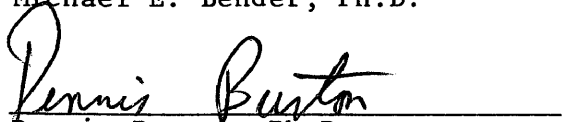

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ABSTRACT

The acute toxicity of Kepone to sand shrimp, Crangon septemspinosa, was determined. The 96 hour lethal concentration to 50% of the population (LC₅₀) for Kepone (dissolved in acetone) was 263 ug/l. The 96 hour LC₅₀ for Kepone dissolved in 0.5 M NaOH at pH 7.8 and 17°C could not be determined by the data generated in this study. Sand shrimp concentrated Kepone at a rate of 202 days⁻¹ after 12 days of exposure and depurated Kepone at a rate of 0.018 days during 15 days in Kepone-free water, yielding an estimated equilibrium bioconcentration factor for sand shrimp of 11,000. Sand shrimp concentrated Kepone to levels 2100 times that in seawater after 12 days of exposure. This bioconcentration factor is not an equilibrium value. The bioconcentration factor is affected slightly by exposure concentration. Minimal uptake occurred during 64 hours of exposure to 0.41 ug/l at 17°C and pH 8.3 or 18°C and pH 7.8.

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INTRODUCTION

Kepone is the trade-name for the organochlorine pesticide, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta (cd) petalene-2-one, which was used to control potato bugs, banana pests, and roaches (U.S. EPA, 1975; Saleh and Lee, 1978). Kepone was manufactured at the Allied Chemical Semi-works plant from 1966-1974 and by Life Science Products Co. plant in Hopewell, Va. from 1974 until the factory was closed in 1975 (Dawson, 1978; Bender et al., 1977). During the period 1966 to 1975, quantities of Kepone were released into the environment via atmospheric emissions, disposal of off-specification batches and waste-water discharges (Dawson, 1978). Kepone-laden effluents from the factory entered the sewage treatment facility at Hopewell and were subsequently discharged into the upper tidal James River at Bailey's Creek (Bender et al., 1977).

The U.S. EPA in 1975 reported large quantities of Kepone in air, soil, and water around Hopewell. Aqueous samples contained concentrations ranging from <50 ng/l at Skiffes Creek to 4.0 ug/l in Bailey's Creek (U.S. EPA, 1975). Though more soluble than related organochlorine pesticides, Kepone is still relatively hydrophobic (Saleh and Lee, 1978; Hodgson et al., 1978; Oswald and Moseman, 1976). Kepone tends to adhere to organic substrates in water masses and is largely associated with sediments, suspended particulates, and biota in the James River estuary. In 1975, the Kepone content of sediments in the James River and tributaries ranged from non-detectable in the Appomatox River to 4 ug/g dry weight in the Bailey's Creek area.

Finfish and shellfish samples taken from the James River in 1975 contained 0.01 to 2.0 ug/g dry weight Kepone (U.S. EPA, 1975). The concentrations in edible animal tissues were above those allowable by health standards (Hansen et al., 1976). The James River was therefore closed to certain fish, shellfish and crab fisheries in 1975 (Dawson, 1978).

In the summer of 1979, water samples from the James River bridge to the western tip of Jamestown Island contained an average of 6.7 ng/l Kepone (Strobel et al., unpublished manuscript). Kepone residues in sediments appear to have declined during the interval 1977 to 1979, based on comparisons of data from Trotman and Nichols (1978) and Croonenberghs (personal communication, 1980). Since Kepone is a very stable pesticide (Dawson, 1978), the apparent reduction in Kepone concentration has probably resulted from alluvial sediment deposition diluting and covering contaminated sediments and fauna accumulating and translocating the Kepone.

KEPONE CHEMISTRY

Kepone, a cyclodiene insecticide related to Mirex, Aldrin, Dieldrin, and Heptachlor, is synthesized from hexachlorocyclopentadiene (Dawson, 1978). Kepone is slightly polar due to the presence of the carbonyl group. This property accounts for its relatively high water solubility. The solubility of Kepone in water is a function of pH. In basic solutions, aqueous solubility increases (Dawson, 1978).

Mirex is a related pesticide used widely to control fire ants. This compound photodegrades to Kepone (Carlson et al., 1976). Kepone constitutes up to 10% of the Mirex recovered from sites of aerial application (Kaiser, 1974).

KEPONE TOXICITY

Organochlorine pesticides are toxic to a variety of species (Gleason et al., 1963; Van Valin et al., 1967; Lowe et al., 1971; Leffler, 1975; Tagatz et al., 1975; Walker et al., 1979). Following the Hopewell incident the toxicity of Kepone to estuarine organisms was shown to vary widely among species. Invertebrates are generally more resistant than fish species when exposed to Kepone in solution.

Sublethal levels of pesticides may disrupt physiological processes, which may result in decreased survival for exposed populations (de la Cruz and Lue, 1978; Leffler, 1975). Organisms exposed to sublethal quantities of organochlorine pesticides exhibit varied responses. Mirex and DDT increase the metabolic rate of bluecrabs (Callinectes sapidus) (Leffler, 1975). Mirex alters carapace thickness in blue crabs (Leffler, 1975) and decreases the growth of bluegill (Lepomis macrochirus) (Van Valin et al., 1967; Carlson et al., 1976). Mirex at levels as low as 0.01-1.0 ng/l affects both the development and growth of larval Rhithropanopeus harrissii and Menippe mercenaria (Bookout et al., 1972). Kelthane decreases food consumption, rate of ecdysis, and cannibalism in Crangon franciscorum (Sharp et al., 1979).

Pesticides, such as Mirex and Kepone, induce behavioral changes such as loss of equilibrium and coordination, which may lead to paralysis (Lowe et al., 1971; Leffler, 1975; Tagatz et al., 1975; Hansen et al., 1977b; de la Cruz and Lue, 1978). Twenty-day exposure of mysids (Mysidopsis bahia) to Kepone at 0.02 ug/l resulted in reduced growth and survival (Nimmo et al., 1977). Sheepshead minnow juveniles (Cyprinodon variegatus) exposed to 0.08 ug/l were stunted and scoliotic (Hansen et al., 1977a). Oyster larvae exposed to Kepone concentrations of 56 ug/l developed shells, but the shell diameters were less than those of control animals (Hansen et al., 1977b). The symptoms of acute poisoning in fishes, including: scoliosis, increasing body pigmentation, hemorrhaging, edema, finrot, and uncoordinated and lethargic behavior, seem to be a function of Kepone concentration and exposure time (Hansen et al., 1977a; Bender et al., 1977; Roberts and Bendl, 1980; Roberts et al., 1980).

Organochlorine pesticides often reduce the reproductive success of organisms. The decline of eagles and other birds of prey which feed on fish, has been attributed directly to the disruption of eggshell formation by DDT, DDE, and PCB's (Livingston, 1977; Bitman et al., 1970). Likewise, avian species which prey on fish and invertebrates from Mirex treated areas have exhibited decreased reproductive success (Kaiser, 1978). Kepone affects the reproductive capabilities of mice (Jaeger, 1976), rats (Gellert, 1978), the Japanese quail (Coturnix coturnix japonica) (Eroschenko, 1979), and man as exhibited by the increase in functional sterility in some

employees at Life Science Products, Inc. (Cohn et al., 1978). Substantial residues of Kepone have been found in dead eagles and ospreys (Dawson, 1978). No data are available on the impact of these accumulations on the reproductive success of these raptorial species. Organochlorine pesticides may interact to alter embryonic survival in exposed finfish species (Reinert and Bergman, 1974; Koenig, 1977). Studies indicate that Kepone causes a decrease in fecundity and an increase in teratogenicity of mysids and sheepshead minnows (Nimmo et al., 1977; Schimmel and Wilson, 1977; Hansen et al., 1977a).

BIOCONCENTRATION THEORY

Bioconcentration of organochlorine pesticides from water and food may be the most serious problem resulting from pesticide pollution (Metcalf, 1977). Bioconcentration is the process by which organisms or a population of individuals accumulate a chemical compound directly from aqueous solution (Hamelink et al., 1971; Metcalf, 1977; Dawson, 1978; Macek et al., 1979; Veith et al., 1979). Absorption of chemicals from water generally occurs through the gill membrane (Macek et al., 1979; Veith et al., 1979).

The bioconcentration of pesticides by organisms follows a first order kinetic model (Pentreath, 1973; Branson et al., 1975; Blanchard et al., 1977; Hamelink, 1977; Krzeminski et al., 1977; Veith et al., 1979). The increase in concentration of the pesticide in animal tissues is proportional to the quantity of chemical in the external medium. When exposed to an organic compound in aqueous solution, an

organism will absorb the compound from water at a constant rate, termed the uptake rate (k_1) (Hamelink, 1977; Hamelink et al., 1971). The uptake rate is affected by the length of exposure and the volume of water passed over the gills or exterior cuticle (Hamelink et al., 1977; Metcalf, 1977). The change in the concentration of chemical in an organism is given by the equation:

$$\frac{dC_a}{dt} = k_1 C_w - k_2 C_a \quad (1)$$

where: C_a = concentration of the substance in the animal (ug/l)

C_w = concentration of the substance in water (ug/l)

t = time of exposure (days or hours)

k_1 = the specific rate constant for the movement of the chemical into the animal (1/time), and

k_2 = the specific rate constant for the movement of the chemical out of the organism (1/time).

Assuming C_w is constant over time, then equation (1) can be integrated to yield:

$$C_a = (k_1/k_2) C_w (1 - e^{-k_2 t}) \quad (2)$$

Upon initial exposure to a chemical, the rate of elimination (k_2) is negligible. Since the rate of absorption is constant, the concentration in the organism will increase over time. The rate of elimination increases over time and uptake continues until the rate of uptake equals the rate of depuration when the concentration in water is held constant (Hamelink et al., 1971; Hamelink et al., 1977). When

the rate of concentration equals the rate of elimination, the tissue residue level of the pesticide is neither increasing or decreasing. This steady state is termed equilibrium and the concentration of pesticide in the organisms is termed the maximum body burden (Hamelink, 1977; Metcalf, 1977). When $\frac{dC_a}{dt} = 0$, $(1 - e^{-k_2t})$ equals unity. Subsequently,

$$C_a/C_w = k_1/k_2 = BCF \quad (3)$$

The maximum body burden in animals held in uncontaminated water is not maintained at the equilibrium concentration, since there is no uptake. While being held in uncontaminated water, organisms will excrete the accumulated compound at a specific elimination rate such that the body residues decline. This decrease over time is termed the depuration rate (Hamelink, 1977; Hamelink et al., 1977; Metcalf, 1977). The mathematical expression for this loss is:

$$-\ln C_a = k_2t + \text{constant} \quad (4)$$

This equation is derived by integrating equation (1), when $C_w = 0$.

The bioconcentration factor (BCF) is the theoretical limit of an accumulation process (Hamelink, 1977). The ratio of the maximum body burden to exposure concentration at equilibrium is termed the bioconcentration factor. This non-dimensional quantity can be calculated in two ways: 1) the equilibrium concentration in the animals divided by the concentration in the external milieu, or 2) the uptake rate divided by the depuration rate. Determination of the

bioconcentration factor is a method of assessing a sublethal impact of pesticides on organisms. It is a useful basis for comparing various pollutants and their effects on animals (Hamelink, 1977).

KEPONE BIOCONCENTRATION

Bioconcentration of organochlorine pesticides by aquatic animals is well documented (Macek and Korn, 1970; Collins et al., 1973; Livingston, 1977; Harding and Vass, 1979). Various species have been shown to accumulate Kepone (Jaeger, 1976; Bahner et al., 1977; Bender et al., 1977; Dawson, 1978). Aquatic organisms have been shown to bioconcentrate Kepone up to 20,000 times the amount present in surrounding waters (Hansen et al., 1976). Four phytoplankton species were shown to bioconcentrate Kepone from aqueous solution (Walsh et al., 1977). Algal growth rates in laboratory studies were higher than those in the field (U.S. EPA, 1975), suggesting that Kepone may not be acutely toxic to certain algae at the concentrations tested. Nevertheless, algal bioconcentration may lead to food chain magnification (Walsh et al., 1977).

The Kepone residues in field samples from the James River differ among species. Shad (Alosa sapidissima) and menhaden (Brevoortia tyrannus), which are short term residents of the estuary, contained relatively low Kepone levels (0.1 ug/g), whereas long term residents, such as spot (Leiostomus xanthurus) and Atlantic croaker (Micropogon undulatus), had residues as high as 0.75 and 0.81 ug/g, respectively (Bender et al., 1977). Kepone accumulations in crustaceans ranged

from 0.27 ug/g dry weight in xanthid crabs to 2.0 ug/g dry weight in Crangon septemspinosus. In laboratory experiments, Bahner et al (1977) determined that blue crabs bioconcentrate low quantities of Kepone, grass shrimp and mysids intermediate amounts, and sheepshead minnows and spot concentrate relatively large quantities. Although blue crabs do not concentrate Kepone from water to any great degree, they do accumulate it from food (Schimmel et al., 1979; Fisher, 1980), as do mysids, grass shrimp, and spot (Bahner et al., 1977). This knowledge is especially significant since these species are important components in the estuarine food web and because spot and blue crabs are commercially important to man.

The residue level of pesticide in an organism's body is a function of both the rates of uptake and excretion for that substance. Some fish attain an equilibrium concentration of Kepone within 8-17 days, while concentrations in oysters (Crassostrea virginica) reach a steady state in even less time (Bahner et al., 1977). The rate of Kepone depuration by species is variable. Oysters in Kepone-free water depurate 90% of their body burden within four days (Bender et al., 1977). Anguilla rostrata, Micropogon undulatus, Leiostomus xanthurus, and Cyprinodon variegatus, on the other hand, take three weeks or longer to lose 30-50% of their Kepone content (Hansen et al., 1976; Hansen et al., 1977b; Hedgepeth and Stehlik, 1979; Hedgepeth et

al., 1979). Kepone levels are, therefore, somewhat higher in finfish species than in oysters.

EFFECT OF TEMPERATURE AND PH ON BIOCONCENTRATION

Accumulations of organochlorine pesticides are dependent on the relationship between a number of factors including; solubility characteristics of the pesticide, organisms' size and lipid content, and water quality (Johnson 1968). Temperature and oxygen concentration are critical in determining depuration rates (Bender et al., 1977; Hedgepeth et al., 1979). Atlantic croaker, for example, will not depurate Kepone at a temperature below 15°C (Doyle et al., 1978). Finfish from warmer waters concentrate higher residues of DDT than those organisms from cooler waters (Reinert, 1972). pH directly affects the toxicity of Endrin, Sevin, and some herbicides (Henderson et al., 1959; Lipscheutz and Cooper, 1961; Burdick et al., 1960). Hydrogen ion potential may also affect pesticide toxicity indirectly by altering the water solubility of the toxicant (Hamelink et al., 1971). In general, as the water solubility increases, bioconcentration decreases (Johnson, 1968; Hamelink et al., 1971; Metcalf, 1977; Goerke et al., 1979).

Chlorinated hydrocarbon pesticides tend to be lipophilic and have low water solubility (Holden, 1962). The extent to which an organic pesticide bioconcentrates depends upon water solubility and lipid solubility (Dawson, 1978). Chlorinated hydrocarbons tend to accumulate most readily in blood lipids, adipose tissue, and

lipid-rich tissues such as the liver and spleen (Van Valin et al., 1967; Hamelink et al., 1971; Lowe et al., 1971; Cahn et al., 1977; Piltz, 1979). Therefore, a high lipid content in a given organism will tend to increase the animal's ability to store pesticides (Bridges, 1963; Goerke et al., 1979).

Residue levels of several organochlorine pesticides, such as DDT and Mirex, vary as a function of organism size for a given fish species (Lowe et al., 1971; Murphy and Murphy, 1971; Reinert and Bergman, 1974). In general, size is positively correlated with pesticide concentration (Murphy and Murphy, 1971), possibly due to a relative increase in adipose tissue with increasing size. Kepone accumulation, however, does not seem to vary with size for spot, Atlantic croaker, hogchoker, and bluefish (Bender et al., 1977; Hedgepeth et al., 1979).

In order to reopen the James River to fishing, the tissue residue levels of Kepone in organisms must decrease to levels compatible with health standards. Routes of uptake and elimination need to be studied to determine the levels of contamination in water and sediments which will not result in residue levels in edible animal tissues beyond FDA limitations. This study was designed to determine: 1) The rate of uptake of Kepone by sand shrimp at two aqueous exposure concentrations, 2) The rate of clearance of Kepone by sand shrimp, and 3) The equilibrium body burden accumulated by sand shrimp as a result of each exposure concentration. Preliminary studies were conducted to determine 24, 48, 72, and 96 hour LC₅₀ values for sand shrimp exposed to Kepone in aqueous solution.

MATERIALS AND METHODS

ANIMAL COLLECTION AND MAINTENANCE

The sand shrimp, Crangon septemspinosa, was chosen as the experimental animal because sand shrimp samples from the James River contained the highest quantity of Kepone of the crustaceans collected (Bender et al., 1977) and because the species is important in the James River food web (Haefner, 1979). Test specimens of both sexes ranging in length from approximately 2.0-6.5 cm and weighing 0.1 to 1.5 grams wet weight were used. Only males and non-ovigerous females were used in the long term uptake and depuration study. Berried and non-berried individuals do not weigh the same in relation to length, adding a source of variation (Haefner, 1973). In other experiments a minimum number of ovigerous females were used only when a critically low number of "acclimated" individuals was available for experimentation.

Shrimp were collected in the York River during the colder months of 1978-1980. During the summer months shrimp were captured in the Chesapeake Bay near Cape Charles City. Test specimens were captured using a 30 foot otter trawl. The trawling period lasted an average of ten minutes at a ship speed of 5 knots. Shrimp from the Bay were rinsed in seawater after capture and then transferred to a 91.4 by 137.2 cm diameter fiberglass tank, containing standing seawater. For the remainder of the return trip to the Virginia Institute of Marine Science, fresh seawater was pumped intermittently into the tank.

Shrimp caught in the York River were held in seawater in styrofoam coolers while on shipboard during the 1978 and early 1979 cruises, while those shrimp collected in the York River in the fall of 1979 and winter of 1980 were held in the fiberglass tank.

Acclimation Period

During the quarantine period for the first acute test, the animals were held in 37.5 liter experimental tanks. In all subsequent experiments, sand shrimp were held and quarantined in flowing seawater in a shallow rectangular fiberglass tank located outside for a minimum of 10 days. Sublittoral sand from the York River served as a substrate in which the shrimp could hide in the shallow tank. This bottom cover helped decrease the amount of cannibalism experienced in early experiments.

Dilution Water

York River water served as the diluent for all experiments. In some tests it was filtered to 10 μ m and in other tests it was not. Dissolved oxygen, temperature, and pH were measured daily from the outflow and inflow of the outside holding tank. Salinity of a water sample from the reservoir was measured daily in those experiments in which the shrimp were held inside and from the outflow of the outside tank. Dissolved oxygen and temperature in the 37.5 liter aquaria were monitored daily near the outflow. Dissolved oxygen and temperature were measured using a YSI model 51A oxygen meter. pH was measured

with a Perkin-Elmer Metrion IV pH meter, while salinity was measured with a Beckman Induction Salinometer RS-7A.

Diet

Crangon septemspinosa are omnivorous (Price, 1962; Wilcox and Jeffries, 1974). Since most available suspended and particulate matter was removed from the incoming seawater, shrimp were fed during the quarantine and experimental periods when the diluent was filtered to 10 μ m. During all other acclimation periods, the diet of the shrimp was not supplemented.

During the acclimation period of the first of six experiments, shrimp were fed Tetra-min fish food flakes. During all subsequent acclimation periods and the uptake studies, shrimp were fed chopped squid. Fresh squid was donated by Mr. Charlie Amory (Fass Bros., Inc., Hampton, Va.), filleted, frozen, and cut into small pieces (approximately 1 cm long by 0.5 cm wide). The shrimp were fed during the early evening hours, since Wilcox and Jeffries (1974) noted that activity level and feeding rates are highest at night. Unconsumed food was removed daily.

KEPONE ANALYSIS

Water

Water samples were siphoned from tanks with glass tubes and stored in 300 ml glass B.O.D. bottles under refrigeration. Samples of stock solutions were also analyzed. A 50 ml aliquot of a water sample

was poured into a separatory funnel and shaken with 5 ml of benzene for two minutes. Following phase separation, the aqueous layer was drained into a graduated cylinder and the benzene layer percolated through anhydrous sodium sulfate into a graduated centrifuge tube. The aqueous layer was re-extracted with another 5 ml of benzene, which was combined with the first extract. Clean-up on an activated flourisil column was necessary on occasion (Moseman et al., 1977). The extract was analyzed by electron capture gas chromatography (Thompson, 1977).

Percent recovery of Kepone in stock samples prepared with 0.5 M NaOH was determined through standard addition analysis.

Shrimp

Shrimp were killed by submerging them head first into boiling water, measured, and weighed. This method of killing was used because it is rapid, prevents the shrimp from jumping out of the container of water, and rinses loosely adsorbed Kepone from the exterior cuticle of the shrimp. Shrimp were preserved by wrapping in aluminum foil, and stored frozen until assayed. Shrimp were weighed, thawed, chopped, and dessicated with anhydrous sodium sulfate plus Quso G-30 (precipitated silica, Philadelphia Quartz Co.). Samples were refrozen for several hours to aid cell disruption, thawed, ground to a powder, and weighed into a cellulose thimble for soxhlet extraction. The sample was extracted with 1:1 diethyl ether-petroleum ether for 16 hours. Extracts were evaporated with gaseous nitrogen to decrease

their volume. PCB's and related organochlorine pesticides were removed by passage through an activated flourisil column, as described by Thompson (1977). A chromatographic column was filled with activated flourisil and anhydrous sodium sulfate. The column was moistened with petroleum ether. The concentrated extract was passed through the column. A series of two solvents was passed through the column. Solvent I (2:4 methanol benzene in hexane) absorbed and eluted organochlorine pesticides other than Kepone. Solvent II (1:2:4:93 methanol/acelenitrile/benzene/hexane) eluted the Kepone. This eluate was collected and analyzed by gas chromatography.

A few shrimp were analyzed for Kepone at the beginning of the 1978-1980 sampling period. A sample of shrimp was analyzed for Kepone content prior to initiating the toxicant flow in each experiment. Kepone was not detectable in these animals.

DILUTER SYSTEM

York River water was pumped from a reservoir to a plexiglass header tank. Glass siphons carried the diluent to the glass mixer funnels. Stock solutions were contained in glass flasks fitted with neoprene stoppers. Toxicant was delivered via glass tubing inserted into the neoprene stoppers and silastic tubing through a Harvard peristaltic pump to the mixer funnels. Glass siphons carried the mixture to splitter tanks. The diluent flowed via glass siphons into the glass test aquaria (Figure 1). The volume of the test aquaria for the acute bioassays was 37.5 liters each. Glass test aquaria used for

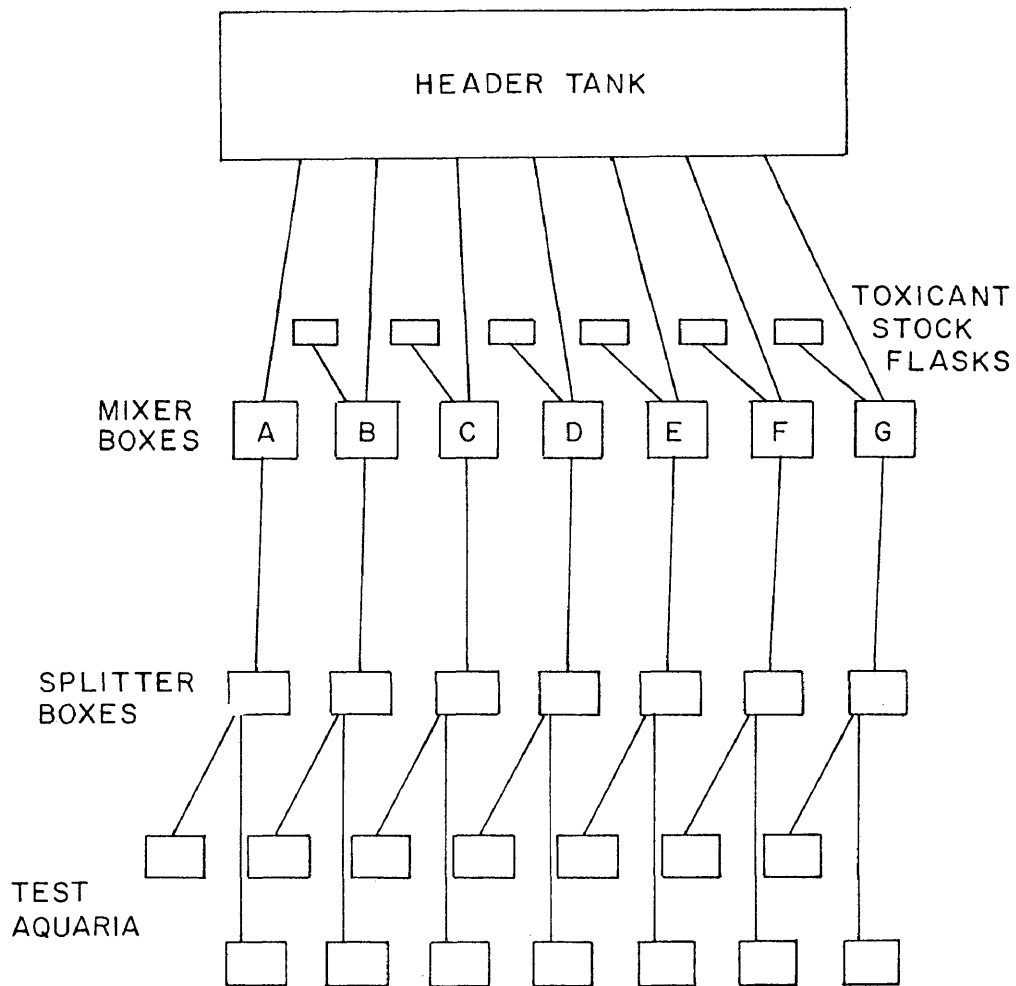


Figure 1. Diluter system for the experiments.
(The number of test aquaria differed depending on the experimental design).

the uptake and depuration studies were 109 cm long, 17.5 cm wide, and 8.5 cm deep. This 162 liter volume was divided into three sections. The head section received the test solution via glass siphons from the mixing funnels. When the header filled to capacity, the test solution overflowed through weirs cut into a piece of fiberglass mounted on a glass partition into the test area. The central or test section of each tank was divided into 70 chambers (7 columns and 10 rows) by fiberglass sheets. The bottom section of each tank contained the overflow standpipe.

Stock and diluent flow rates were measured and adjusted daily. A float and microswitch system on the header tank turned off the toxicant and diluent pumps in the event of a decrease in diluent flow into the head tank. This fail-safe system prevents large quantities of undiluted toxicant from being pumped into the test chambers.

The entire study consisted of a series of six experiments. The first three were 96 hour acute tests designed to determine the concentration of Kepone which would kill 50% of a sand shrimp population. The fourth experiment was designed to determine the uptake and elimination rates of Kepone by sand shrimp. The final two studies were designed to determine the uptake rate of Kepone by sand shrimp within a 64 hour period.

PROTOCOL FOR 96 HOUR LC₅₀'SExperiment 1

The objective of the first flow-through bioassay was to determine the concentration of Kepone (introduced in acetone) which would kill 50% of a sand shrimp population within 96 hours (LC₅₀).

The shrimp were quarantined in filtered York River water for 15 days in 37.5 liter test tanks. Water temperature was increased with Vycor heaters from ambient to 20°C during the period, at a rate not greater than 3°C per day.

The test was not conducted in duplicate as recommended by Stephan (1975) because of high mortality as a result of cannibalism during the quarantine period.

Two controls were used: 1) filtered York River water and 2) filtered York River water plus reagent grade acetone. The five nominal test tank concentrations were 6.4, 20, 64, 200, and 640 ug/l. A nominal concentration is the desired test concentration. The stock solutions were prepared by diluting a master stock containing 6 g/l Kepone (dissolved in acetone).

Initially, the seven test chambers were filled with 30 liters of filtered seawater and 5 liters of the respective stock solution. Ten shrimp were distributed randomly to each of the seven test aquaria. The toxicant pump was turned on and all siphons started.

Temperature, dissolved oxygen, pH, and salinity were measured daily. Water samples were collected on days 0 and 4 from each experimental tank for Kepone analysis. In addition a water sample was collected on day 4 from the splitter box of the highest concentration (Figure 1). An acetone rinse was also collected from the test tank at this concentration to determine if there was significant absorption of Kepone to glass.

Test animals were observed at 0, 1, 2, 4, 8, 24, 48, 72 and 96 hours. Each animal was poked with a glass rod to check reflexes and activity levels. Dead shrimp and exuvia were removed at these times. After 96 hours the remaining live shrimp were killed and preserved for Kepone analysis.

Experiment 2

Mortalities in the first study suggested that the acetone solvent may have affected survival in the acetone control. Since Kepone is readily soluble in basic solutions (Dawson, 1978), the carrier was changed to 0.5 M NaOH and a second 96 hour bioassay conducted.

Test organisms were quarantined for 13 days in the outside tank in unfiltered York River water. The diluent water was not filtered during the test, since the level of suspended particulates in the incoming river water was very low.

Each shrimp was maintained in an individual test chamber. Fiberglass sheets with holes to allow water exchange were fitted

together, egg crate fashion, so that each 37.5 liter tank was divided into 20 chambers. Ten shrimp were placed at random in each experimental tank.

Dividers were available for only 12 tanks. Therefore, only four nominal test tank concentrations were used: 5.0, 14.0, 41.0, and 120.0 ug/l Kepone. Each Kepone concentration and the two controls (York River water and York River water and 0.5 M NaOH) were tested in duplicate. Stock solutions were made from a master stock of 1 g/l Kepone (dissolved in 0.5 M NaOH).

Initially, the shrimp were held inside in a cooler until the difference in temperature between the test tanks and cooler did not exceed 2-3°C. Test specimens were, then, placed at random in the experimental chambers filled with flowing seawater and toxicant. The test temperature was gradually increased from ambient (13°C) to 18°C over the first 6 hours of the experiment.

Dissolved oxygen, temperature, pH, and salinity were measured daily. Water samples were collected from all experimental chambers at approximately hour 2 and again on day 4 for Kepone analysis. Behavior and mortality were observed at hours 0, 1, 2, 4, 8, 24, 48, 72, and 96. Dead shrimp were removed, pooled by tank and preserved for Kepone analysis at these times. All remaining live shrimp were killed and preserved for analysis at hour 96.

Experiment 3

Comparatively high pH readings (pH 8.3 as compared with 7.8 for ambient seawater) in the experimental aquaria of Experiment 2 may have affected the response of the sand shrimp to the Kepone. A third 96 hour LC₅₀ was, subsequently conducted after reducing the experimental pH to approximately that of ambient seawater.

Test organisms were quarantined for 17 days in the shallow outside tank in ambient unfiltered York River water. The test shrimp were moved inside, placed at random in 37.5 liter test aquaria and held in filtered flowing seawater for another 4 days. During this period, the water temperature was increased at a rate of 3°C per day from 8°C to 18°C, as recommended by Stephan (1975).

The experimental system was identical to that used in the previous acute tests, except that the toxicant was delivered through Grad-co tubing instead of silastic tubing. Filtered York River water served as the diluent. Test tanks were divided into 20 chambers each. Since there were not enough dividers for 14 tanks, a replicate filtered seawater control was not used. The five nominal test tank concentrations were 3.2, 10.0, 32.0, 100.0, and 320.0 ug/l Kepone. The stocks were prepared from a master stock of 1 g/l Kepone (dissolved in 0.5 M NaOH). The pH of all stock solutions was 13.6. Hydrochloric acid (0.1 N) was added to these stocks until the pH decreased to 10.5. After dilution of 0.2 ml of adjusted stock with

500 ml of seawater, the pH of the test solutions approximated the pH of ambient seawater.

Initially, the tank volumes were reduced to one third and toxicant flow was begun. Water and shrimp was sampled on the schedule described previously and preserved for Kepone analysis. Water quality was monitored daily.

UPTAKE AND DEPURATION PROTOCOL

The two experimental approaches used to determine uptake and depuration rates and bioconcentration factors are the extrapolation method and the plateau method (ASTM Committee E35; Hamelink, 1977). The latter approach requires that the exposure of the test organisms continues until the rate of increase of the test material in the organisms equals zero or nearly so. Twenty-eight days is usually sufficient for this purpose (Hamelink, 1977). In the extrapolation method the equilibrium bioconcentration factor is estimated from uptake and elimination rates. The uptake rate is calculated following exposure of organisms to a compound for a period of time insufficient for a steady state body burden to be reached (ASTM Committee E35). The maximum tissue residue levels observed during an extrapolation bioconcentration test are not equivalent to the equilibrium body burden. Consequently, two distinct bioconcentration factors can be calculated from the extrapolation method: 1) a non-equilibrium bioconcentration factor computed as C_a/C_w over the duration of the

test and 2) an estimated equilibrium bioconcentration factor calculated as the uptake rate divided by the rate of elimination.

A 12-day extrapolation bioconcentration test was conducted to determine the rate of uptake and non-equilibrium bioconcentration factor. A 15-day depuration study followed from which the rate of elimination was ascertained. The data from both of these tests were used to estimate an equilibrium bioconcentration factor.

Experiment 4 (Phase 1: Uptake)

Seventy-four sand shrimp, Crangon septemspinosus, were exposed to 1) 0.5 M NaOH and filtered York River water and 2) to uncontaminated filtered York River water (controls). An additional 74 shrimp were exposed to each toxicant concentration (0.04 and 0.42 ug/l).

The shrimp were dosed for 12 days with toxicant. The uptake phase of the test was terminated after 12 days because of high mortalities. Each chamber was "vacuumed" with a glass siphon daily to remove feces and unconsumed food prior to feeding. Exuvia and dead shrimp were also removed at this time. Dead shrimp were pooled by tank and preserved for Kepone analysis. Four shrimp per treatment were killed and preserved for Kepone analysis on days 0, 2, 7, and 12.

Temperature, dissolved oxygen, pH, and salinity were measured daily. Toxicant and diluent flow rates were measured and adjusted daily. The rates of diluent flow were not recorded. Water samples were taken from a fixed location in the exposure chambers throughout

the tests. On days 0, 2, 4, 7, and 12, one water sample from each tank was collected and preserved for Kepone analysis.

Experiment 4 (Phase 2: Depuration)

After sampling on day 12, the experimental tanks were emptied to one third volume and refilled with filtered Kepone-free water. The toxicant pump was shut off and a 15-day depuration study begun. The test did not last longer because of high mortalities. Four live shrimp per treatment were sampled on days 0, 2, 4, 8, and 15 of the depuration period. Each chamber was "vacuumed" daily to remove leftover food and feces. Dead shrimp and exuvia were also removed at this time. Dead shrimp were preserved for Kepone analysis.

Water samples were collected and analyzed daily for temperature, dissolved oxygen, pH, and salinity. Additional water samples were collected on days 0, 1, and 15 for Kepone analysis.

64-HOUR UPTAKE STUDY (Experiment 5 and 6)

Initial analyses of the results from the 12-day uptake study suggested that the shrimp might have reached an equilibrium level of Kepone within 2 days. Since the data were not available for an interval of two days, a short term uptake study was conducted to determine the length of time it takes for sand shrimp to reach an "equilibrium" level of Kepone. Sand shrimp were exposed to one of four treatments: 1) flowing seawater; 2) seawater and 0.5 M NaOH; 3) 0.04 ug/l Kepone; or 4) 0.41 ug/l Kepone.

Experiment 5

The animals were quarantined in the outside tank in unfiltered seawater for 14 days.

Initially, the test shrimp were placed at random in each experimental tank. The test temperature was increased from 13°C (ambient) to 18°C over the first 6 hours of the experiment. The diluent water was not filtered, since the level of suspended organics in the incoming seawater was very low.

The frequency of sampling was designed to be in a geometric progression (hours 0, 1, 2, 4, 8, 16, 32, and 64). The 32-hour sample was actually collected at hour 38. The number of animals sampled from each tank per time period varied, fewer shrimp being taken from the two control tanks each time than from the experimental tanks (Table 7). Dead shrimp and exuvia were removed daily.

Temperature, oxygen, pH, and salinity were measured daily. Water samples were preserved for Kepone analysis at hours 8, 32, and 56.

Experiment 6

Comparatively high pH values in the experimental chambers as compared with the pH in the diluent control may have affected the outcome of Experiment 5. Therefore, a second 64-hour study was conducted with the experimental pH values controlled to approximately that of ambient York River water.

The experimental animals were quarantined in the outside tank in unfiltered ambient seawater for 27 days. Shrimp were placed at random in the experimental chambers (49 per tank) and acclimated for another 9 days. Over this period the water temperature was increased with Vycor heaters from 4° to 18°C. Test chambers were "vacuumed" daily to remove unconsumed food, exuvia, and dead shrimp.

The flow-through system was identical to that described previously, except that the toxicant was delivered through Grad-co tubing. Shrimp were sampled at 0, 1, 2, 4, 8, 16, 24, 32, and 64 hours, pooled by treatment and preserved for analysis. Water samples were collected from each tank on hours 8, 25, 32, and 64 for Kepone analysis. Temperature, pH, dissolved oxygen, and salinity were measured daily.

The pH of the stock solutions was decreased by addition of 0.1 N HCl. After diluting 0.2 ml of adjusted stock solution with 1000 ml of diluent, the pH approximated that of ambient seawater.

Data Analysis

Three distinct aqueous concentrations are referred to in these studies; nominal concentration, applied dose, and measured tank concentration. The nominal concentration is the desired experimental concentration, whereas the measured concentration is that determined by chemical analysis of water samples (Schimmel et al., 1979). The technical grade Kepone used to prepare the stocks contained only 70% active ingredient. The stock solution values for the nominal stocks

were adjusted for this proportion prior to computations. The applied dose is the concentration calculated to be in seawater based on the measured concentration of Kepone in the stock solutions and measured stock and diluent flow rates. The measured concentration of Kepone in the stock solutions was corrected for percent recovery prior to computation of the applied doses.

The Litchfield and Wilcoxin (1949) log-probit method was the first analysis performed on the data from the 96 hour acute bioassays. In the two tests with replicates, an analysis of variance was conducted to determine if the mean survivorship of the replicates were significantly different. Since they were not, the data were pooled. Following Abbott's correction (Finney, 1971), the percent mortality data from the 96 hour acute test with acetone were plotted on log-probit paper as a function of log dose and a line fit by eye. The 96 hour LC₅₀ value (in measured applied dose) was read from the dose-response curves. Since this method of determining an LC₅₀ is invalid in the event that several data points represent 0 or 100% mortality (Litchfield and Wilcoxin, 1949), these data were also analyzed by least squares regression of $\sin^{-1} q$ on log-dose (where q equals the number dead/number tested).

Bioconcentration of a chemical by aquatic organisms is assumed to follow the first order kinetic model (Pentreath, 1973; Branson et al., 1975; Branson et al., 1977; Hamelink, 1977; Krzeminski et al., 1977), described by equation (1). During the initial uptake phase of a bioconcentration test when the clearance rate (k_2) is very small,

equation (1) reduces to:

$$dC_a/dt = k_1 C_w \quad (5)$$

Integration of equation (5) yields:

$$C_a = k_1 C_w t + \text{constant} \quad (6)$$

by which k_1 can be determined by least squares regression. When C_w is held constant at zero, the clearance phase is described by the equation:

$$dC_a/dt = -k_2 C_a \quad (7)$$

Integration of equation (7) yields equation (4) by which k_2 can be estimated by least squares regression.

Since the processes of uptake and elimination occur simultaneously, it is advantageous to use a model for bioconcentration which utilizes all data points over the time interval when $k_2 C_a$ is increasing from zero to infinity (Roberts, 1980). Equation (1) can be integrated to yield equation (2). Since this equation is non-linear, determination of k_1 and k_2 as linear functions is difficult. The data were analyzed by an approach used by Blanchard et al. (1977) and expanded by Roberts (1980). Briefly, this method involves 1) determination of k_2 by least squares regression using equation (4), 2) plotting uptake data to ascertain the portion of the curve over which $k_2 C_a$ seems to have a negligible effect, 3) estimation of k_1 using two or more pairs of points selected from that portion of the curve, 4)

calculation of predicted values for each k_1 and the k_2 determined previously, and 5) selection of the k_1 value with the least residual sum of squares of the difference between predicted and observed C_a values. The uptake and clearance rates were determined by this approach. A student t-test was used to test the hypothesis that k_2 was not significantly different from zero (Sokal and Rohlf, 1969).

Non-equilibrium bioconcentration factors were determined for the 64-hour, 96-hour, and 12-day uptake tests by dividing the maximum tissue residue levels reached during the respective time period by the applied dose. An equilibrium bioconcentration factor was estimated from the data generated by the 27-day uptake and clearance test by dividing the rate of uptake by the rate of elimination. To make this calculation, the clearance rate was accepted as a discrete number, different from zero. Approximation of the equilibrium bioconcentration factor provided a value to compare with other species and pesticides and allowed calculation of the potential maximum body burden of Kepone in sand shrimp at equilibrium.

RESULTS

SHRIMP COLLECTION

Sand shrimp, Crangon septemspinosa, were caught in large numbers in the York River channels from October, 1978 to May, 1979 and again from October, 1979 to January, 1980. Water temperature ranged from 5 to 19°C at a mean salinity of 18‰ (range 14 to 21‰). Attempts to capture specimens from both shoal and channel waters of the York River in the summer of 1979 were unsuccessful. During the summer, sand shrimp were caught in large numbers in water 24 to 37 meters deep over Old Plantation Flats in the Chesapeake Bay. Water temperature was approximately 25°C and the salinity was 27‰. Shrimp were abundantly available only during maximum flood current. During ebb tide, large numbers of sea trout (Cynoscion spp.), spot (Leiostomus xanthurus), and Atlantic croaker (Micropogon undulatus), but only one to ten sand shrimp were captured per trawl.

LABORATORY WATER QUALITY CONDITIONS

Water quality values varied seasonally over the course of this study; temperature ranged from 7.4°C to 24°C, dissolved oxygen ranged from 7.4 to 10.1 mg/l, pH from 7.48 to 9.56, and salinity ranged from 11‰ to 21‰ (Tables 1 and 2). Water quality values were within the tolerance range for this species (Haefner, 1976).

TABLE 1
MEAN WATER QUALITY VALUES DURING
THE QUARANTINE PERIODS

Experiment	Origin of Sample (Tank and Location in Tank)	Temperature (°C)	Dissolved oxygen (mg/l)	pH	Salinity (‰)
		mean (\pm S.D.)	mean (\pm S.D.)	mean (\pm S.D.)	mean (\pm S.D.)
1		17.2(\pm 2.5)	9.1(\pm 0.04)	7.7(\pm 0.1)	21.1(\pm 0.07)
	outside inflow	15.1(\pm 2.2)	9.0(\pm 0.4)	7.7(\pm 0.1)	
	outside outflow	15.1(\pm 2.2)	9.0(\pm 0.4)	7.8(\pm 0.07)	16.4(\pm 0.5)
	outside inflow outside	8.0(\pm 1.0)	9.9(\pm 1.0)	7.6(\pm 0.2)	
	outflow inside	7.9(\pm 1.0) 18.0(\pm 1.6)	10.1(\pm 1.0) 9.3(\pm 0.2)	7.6(\pm 0.2) 7.8(\pm 0.03)	17.1(\pm 0.8) 13.9(\pm 2.2)
	outside inflow	23.9(\pm 1.8)	7.4(\pm 0.6)	7.5(\pm 0.2)	
	outside outflow	23.9(\pm 1.6)	7.4(\pm 0.6)	7.5(\pm 0.2)	16.6(\pm 7.5)
	outside inflow	14.9(\pm 2.2)	9.0(\pm 0.4)	7.8(\pm 0.08)	
	outside outflow	14.9(\pm 2.3)	9.0(\pm 0.4)	7.8(\pm 0.06)	16.4(\pm 0.5)
	outside inflow	7.5(\pm 1.3)	9.8(\pm 0.8)	7.7(\pm 0.2)	
	outside outflow inside	7.4(\pm 1.3) 11.3(\pm 0.1)	9.9(\pm 0.8) 9.7(0.04)	7.7(\pm 0.1) 7.7(\pm 0.02)	15.8(\pm 1.9) 16.3(\pm 1.5)

TABLE 2

MEAN WATER QUALITY VALUES DURING THE EXPERIMENTS

Experiment	Tank No.	Temperature (°C)	Dissolved oxygen (mg/l)		pH	Salinity (‰)
			mean (± S.D.)	mean (± S.D.)		
1	A-G	18.6(+0.6)	8.4(+0.2)	7.8(+0.1)	20.0(+0.4)	
2	A	18.3(+0.07)	9.4(+0.07)	7.9(+0.01)	15.9(+2.4)	
	B-F	18.3(+0.07)	9.4(+0.07)	8.4(+0.06)	15.9(+2.4)	
3		19.0(+1.6)	9.2(+0.6)	7.8(0.03)	14.2(+0.8)	
4	I-IV	23.1(+1.0)	7.7(+0.1)	7.7(+0.2)	15.4(+0.6)	
	I-IV	19.9(+0.2)	8.6(+0.02)	7.6(+0.03)	14.6(+1.1)	
6	I	17.6(+0.5)	9.6(+0.2)	7.8(+0.9)	15.7(+0.3)	
	II	17.2(+0.3)	9.5(+0.2)	8.2(+0.03)		
	III	17.3(+0.3)	9.5(+0.2)	8.3(+0.01)		
	IV	17.2(+0.3)	9.5(+0.2)	8.2(+0.03)		
	I-IV	18.4(+0.1)	9.2(+0.2)	7.9(+0.07)	17.5(+0.4)	

KEPONE RECOVERY

Kepone recovery from spiked samples of 0.5 M NaOH stocks was nearly 100% (98.2 ± 3.76).

The concentration of Kepone measured in experimental water samples was consistently lower than the applied dose based on measured stock solutions and flow rates. Acetone rinses of the component parts of the flow-through systems contained non-detectable quantities of Kepone. The quantity of Kepone concentrated by the test animals does not account for the lower measured values in the water samples. Applied dosage was used for all calculations and comparisons of these experimental data.

CANNIBALISM

Sand shrimp are highly cannibalistic. Individuals of this species prey on smaller, weaker individuals, especially those which have recently molted. Mortality in the filtered water control of the acute test using acetone was 20%. Mortality as a result of cannibalism was observed frequently in experiments in which the shrimp were not separated and in the outside holding tank. To reduce this problem, a sand substrate was provided in the outside holding tank prior to the long term uptake and depuration study and during all subsequent acclimation periods. To reduce the possibility of cannibalism in the experimental aquaria, shrimp were held in individual chambers within the test tanks in Experiments 2-6.

Sand shrimp exhibited a 96 hour LC₅₀ of 263 ug/l (260 ug/l to 266 ug/l) applied Kepone dose (dissolved in acetone) calculated by least squares regression of arc sin q on log dose (Table 3, Figure 2). The 96 hour LC₅₀ value for sand shrimp determined by the Litchfield and Wilcoxin (1949) log-probit method for the same data (Figure 3) is 140 ug/l (41.5 to 471.8 ug/l). All survival data were corrected for mortality in the controls by Abbott's correction prior to analysis (Finney, 1971). The 72 hour LC₅₀ value for sand shrimp exposed to Kepone in acetone is 440 ug/l estimate by the arc sin q method (Figure 3). Confidence limits could not be calculated since only one data point differed from zero. Following correction of these data by Abbott's correction, mortality was not significantly different from zero at four of the concentrations. The log-probit method is, therefore invalid and could not be applied to the 72 hour survival data. The 24 and 48 hour LC₅₀ values exceeded 504 ug/l Kepone (dissolved in acetone). The mean pH in this study for all toxicant concentrations and the two controls was 7.8 ± 0.1 . The mean temperature was 18.6°C.

The 96 hour LC₅₀ exceeded the maximum applied dose (86.5 ug/l) for data from Experiment 2 and therefore cannot be calculated. At a mean pH of 8.4 ± 0.6 and a mean temperature of $18.3 \pm 0.07^\circ\text{C}$ in the test aquaria, mortality in all treatments did not differ greatly from zero (Table 4). The pH of the diluent control averaged 7.9 ± 0.01 .

TABLE 3

EXPERIMENT 1
 PERCENT SURVIVAL OF SAND SHRIMP
 EXPOSED TO DIFFERENT LEVELS OF KEPONE FOR 96 HOURS

Mean Temperature 18.6°C Mean pH 7.8

Tank	Measured Tank Concentration (ug/l)		Applied Dose (ug/l)	No. of Animals	Percent Survival					
	0 Hours	96 Hours			0	24	48	72	96	
Sea Water Control	0	0	0	10	100	80	80	80	80	
Acetone Control	0	0	0	10	100	100	100	100	80	70
C	8.8	5.6	5.0	10	100	100	90	80	70	
D	8.5	15.0	15.4	10	100	100	80	80	80	
E	40.0	31.0	49.6	10	100	90	90	70	50	
F	63.0	88.0	154	10	100	100	80	70	60	
G	200.0	200.0	504	10	100	80	80	30	20	

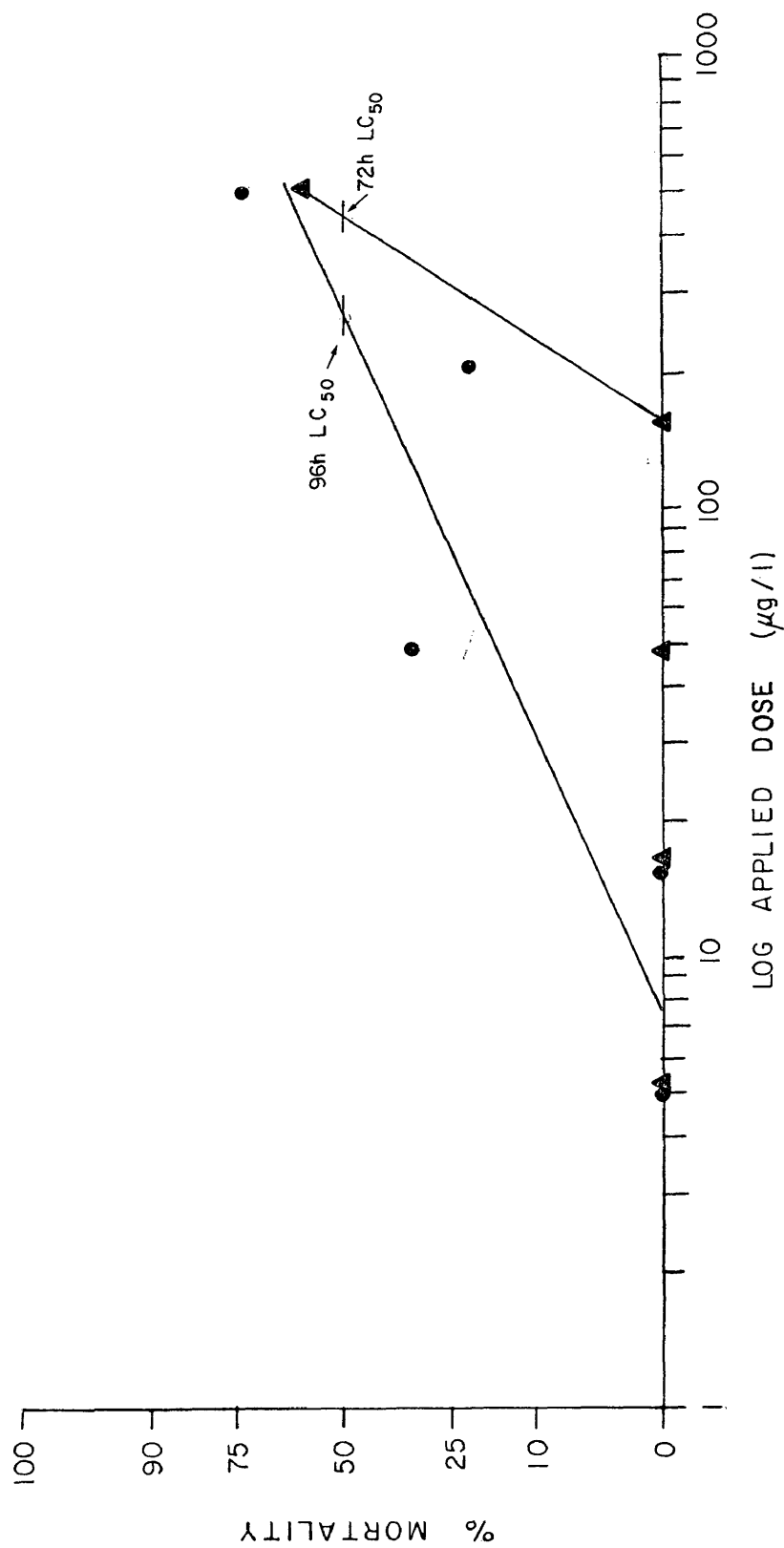


Figure 2. Experiment 1. Percent mortality of sand shrimp exposed to Kepone (dissolved in acetone). (72 hour LC50 calculated by the arc sin transformation x---x; 96 hour LC50 calculated by the arc sin transformation o---o).

TABLE 4

EXPERIMENT 2
 PERCENT OF SAND SHRIMP EXPOSED TO KEPONE FOR
 96 HOURS

Mean Temperature 18.3°C Mean pH (Experimental aquaria) 8.4

Tank	Measured Tank Concentration (ug/l)		Applied Dose (ug/l)	No. of Animals	Percent Survival		
	0 Hours	96 Hours			0	24	48
Sea Water Control	0.18	0.52	0	20	95	95	95
0.5 M NaOH Control	0.38	0.33	0.02	20	100	100	95
C	7.0	7.0	2.9	20	100	100	100
D	6.4	6.9	13.1	20	100	100	95
E	11.8	11.8	18.3	20	100	95	95
F	46.5	52.5	86.5	20	100	100	100

There was 25% mortality after 96 hours of exposure in the highest concentration (applied dose = 125.1 ug/l) of Experiment 3 (Table 5). Since only one point differed from zero, an LC₅₀ cannot be determined using the Litchfield and Wilcoxin (1949) method. These data were plotted on log-probit paper (Figure 4). In contrast to Experiment 2, the mean pH of these test solutions was 7.8 (\pm 0.03) with a range of 7.75 to 7.83, approximately that of ambient estuarine water and the mean water temperature was 19 C. One additional shrimp was near death at 96 hours. This individual exhibited scathognathite activity, although several of its posterior somites exhibited a color change similar to that observed in dead animals (color change from translucent to an opaque white). This animal was not counted as dead.

UPTAKE STUDIES

Experiment 4 (Phase 1)

The quantity of Kepone concentrated by the shrimp exposed to 0.04 ug/l was insufficient to calculate an uptake rate (Figure 5, Table 6). Test specimens exposed to 0.42 ug/l at a mean temperature of 23.1°C and pH 7.7 concentrated Kepone at a rate of 202 days⁻¹ (Table 6; Figure 6).

On the evening of day 7 the filter bag clogged decreasing the diluent flow. Toxicant flowed undiluted into the test tanks for an estimated 1 - 3 hours until the toxicant pump shut off. Toxicant and diluent flows were stopped for one to three hours. A similar problem occurred the following morning and again on day 12 for an estimated

TABLE 5

EXPERIMENT 3
PERCENT SURVIVAL OF SAND SHRIMP EXPOSED
TO KEPONE FOR 96 HOURS

Mean Temperature 19°C Mean pH 7.8

Tank	Measured Tank Concentration (ug/l)		Applied Dose (ug/l)	No. of Animals	Percent Survival				
	0 Hours	96 Hours			0	24	48	72	96
Sea Water Control	0	0.0	0.0	10	100	100	100	100	100
0.5 M NaOH Control	0.2	0.0	0.0	20	100	100	100	100	100
C	1.13	1.1	1.38	20	100	100	95	95	95
D	3.3	3.0	4.4	20	100	100	100	100	100
E	13.5	9.5	19.7	20	100	95	95	95	95
F	31.0	25	46.8	20	100	100	100	100	100
G	77	58	125	20	100	100	100	100	75

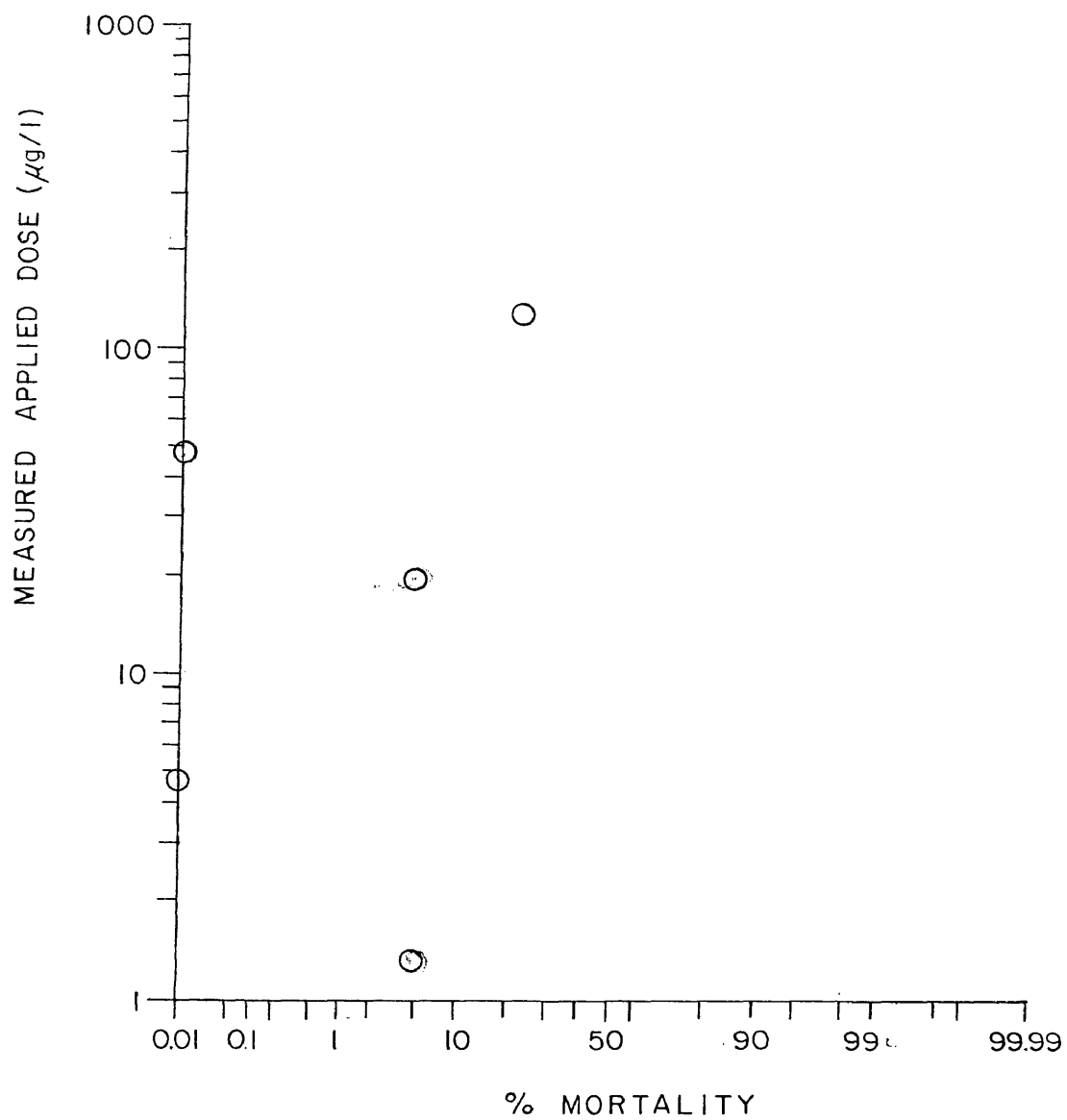


Figure 4. Experiment 3: Percent mortality of sand shrimp exposed to Kepone (dissolved in 0.5 M NaOH) for 96 hours.

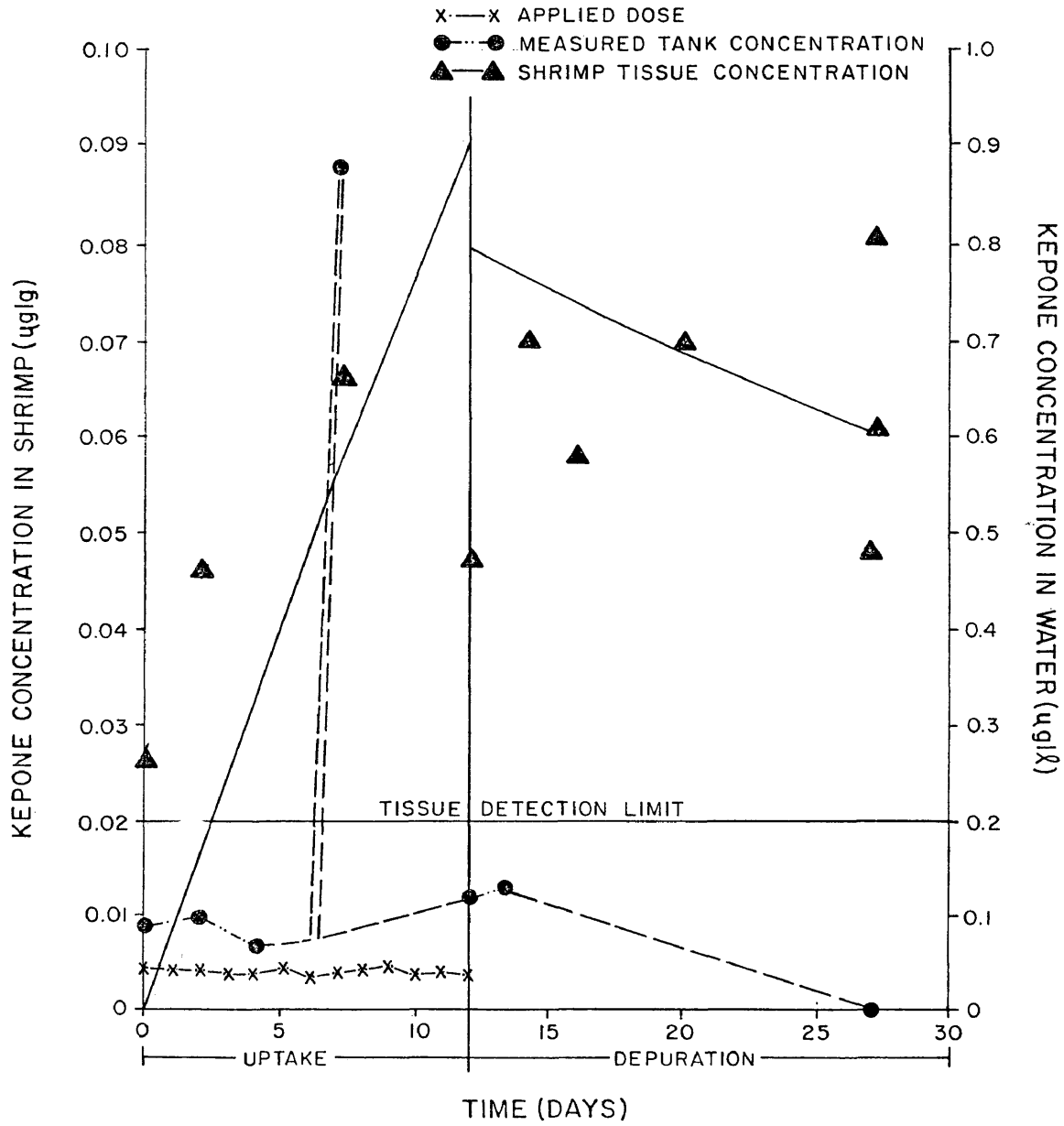


Figure 5. Tissue residue levels of Kepone in sand shrimp after exposure to 0.04 µg/l for 12 days and 15 days in Kepone-free water.

TABLE 6

EXPERIMENT 4

TISSUE RESIDUE LEVELS OF KEPONE IN SAND SHRIMP
DURING 12 DAYS OF UPTAKE AND 15 DAYS OF DEPURATION

Day	No. of Animals Sampled per time period	Nominal Tank Concentration (ug/l)											
		0.0			0.03			0.04			0.41		
		Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	Shrimp Tissue Conc. (ug/g)	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	Shrimp Tissue Conc. (ug/g)	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	Shrimp Tissue Conc. (ug/g)	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	Shrimp Tissue Conc. (ug/g)
Phase 1: Uptake Study													
0	4	0.0	0.12	0.03	0.0	0.09	ND	0.04	0.09	0.03	0.42	0.46	0.02
1	4	0.0	-	-	0.0	-	-	0.04	-	-	0.42	-	-
2	4	0.0	0.10	0.02	0.0	0.10	0.03	0.04	0.10	0.05	0.42	0.35	0.47
4	4	0.0	ND	-	0.0	0.03	-	0.04	0.07	-	0.41	0.30	-
7	4	0.0	0.07	0.03	0.0	0.07	0.02	0.04	0.88	0.07	0.42	0.66	0.54
12	4	0.0	0.02	<0.02	0.0	0.03	0.02	0.04	0.12	0.05	0.43	0.64	0.86
Phase 2: Depuration Study													
0	4	0.0	0.02	<0.02	0.0	0.03	0.02	0.0	0.12	0.05	0.0	0.64	0.86
1	4	0.0	0.16		0.0	0.11		0.0	0.14		0.0	0.13	
2	4	0.0		<0.02	0.0		0.01	0.0		0.07	0.0		0.65
4	4	0.0		<0.02	0.0		0.01	0.0		0.06	0.0		0.89
8	4	0.0			0.0			0.0		0.07	0.0		0.62
15		0.0	0.07	<0.02	0.0	ND	<0.02	0.0	ND	0.05	0.0	ND	0.52
				<0.02			0.02			0.08			0.75
				0.02			0.02			0.06			0.57
				<0.02			<0.02						0.72

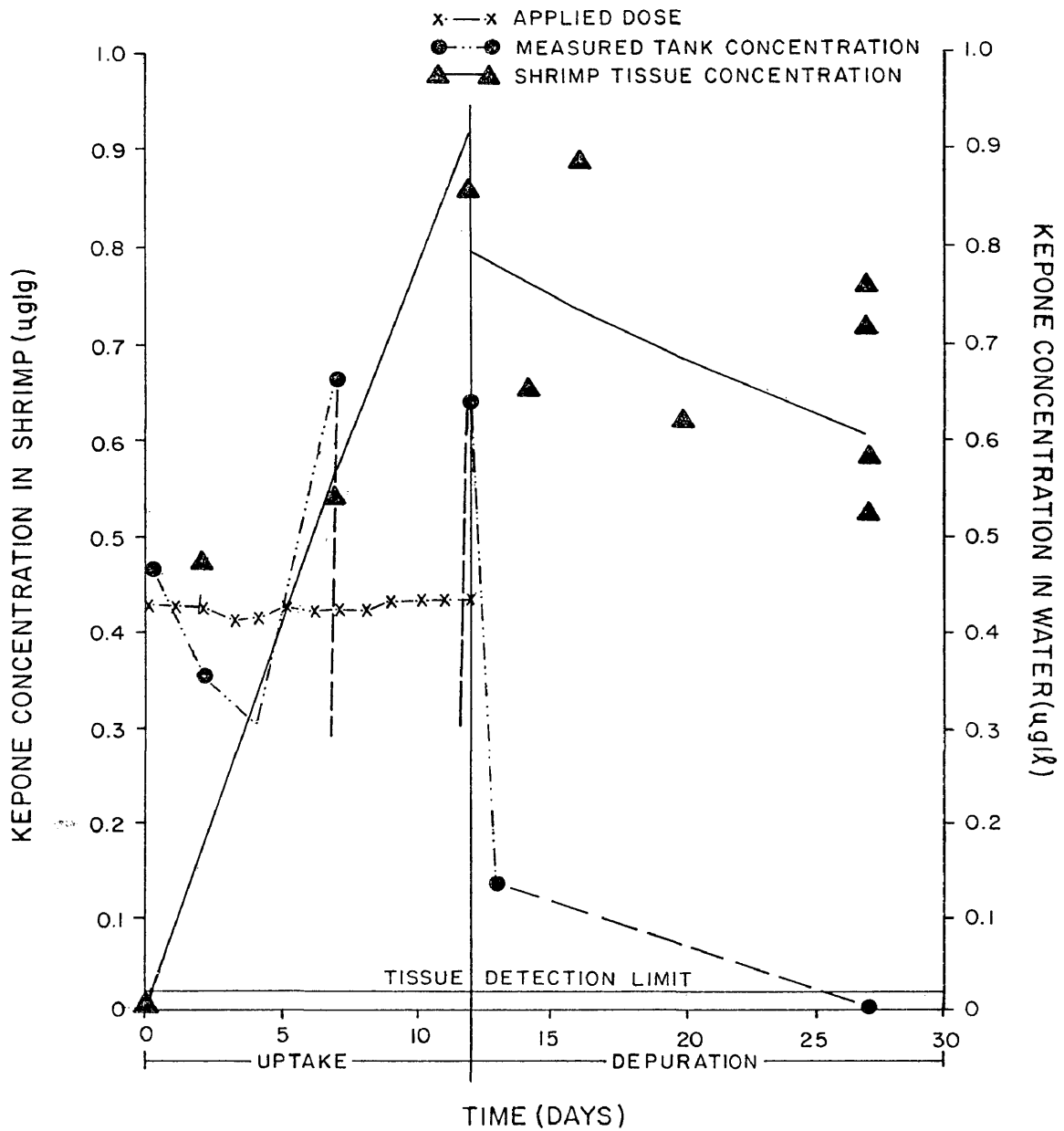


Figure 6. Tissue residue levels of Kepone in sand shrimp after exposure to 0.42 $\mu\text{g/l}$ for 12 days and 15 days in Kepone-free water.

1.5 hours each time. The influx of undiluted toxicant resulted in concentrations of aqueous kepone above the desired experimental concentrations for three short time intervals.

Experiment 5

The maximum body burdens reached by sand shrimp after exposure to nominal concentrations of 0.04 and 0.41 ug/l for 64 hours at a mean pH of 8.3 and temperature of 17.3°C were too low for an uptake rate to be calculated (Figures 7 and 8). The total tissue residue levels of most samples did not exceed the detection limit of 0.02 ug/g dry weight (Table 7). These concentrations are considerably below those observed in Experiment 4 after sand shrimp were exposed to similar concentrations for 48 hours.

Experiment 6

The total tissue residue levels from shrimp exposed to 0.04 and 0.41 ug/l Kepone at a mean pH of 7.8 and temperature of 18.3°C were too low to calculate an uptake rate (Table 8; Figures 7 and 8). These concentrations are considerably below those observed in Experiment 4 after sand shrimp were exposed to similar concentrations for 48 hours.

The mean rates of toxicant flow were 0.20 ml/min and 0.19 ml/min, except: 1) from hours 8 to 16 when no toxicant was pumped into the mixer funnels because of a reversal in direction of pump peristalsis; 2) from hours 32 to 51 during which all 500 ml of stock for the low dose siphoned into the test chamber. (Average flow rate was 0.45

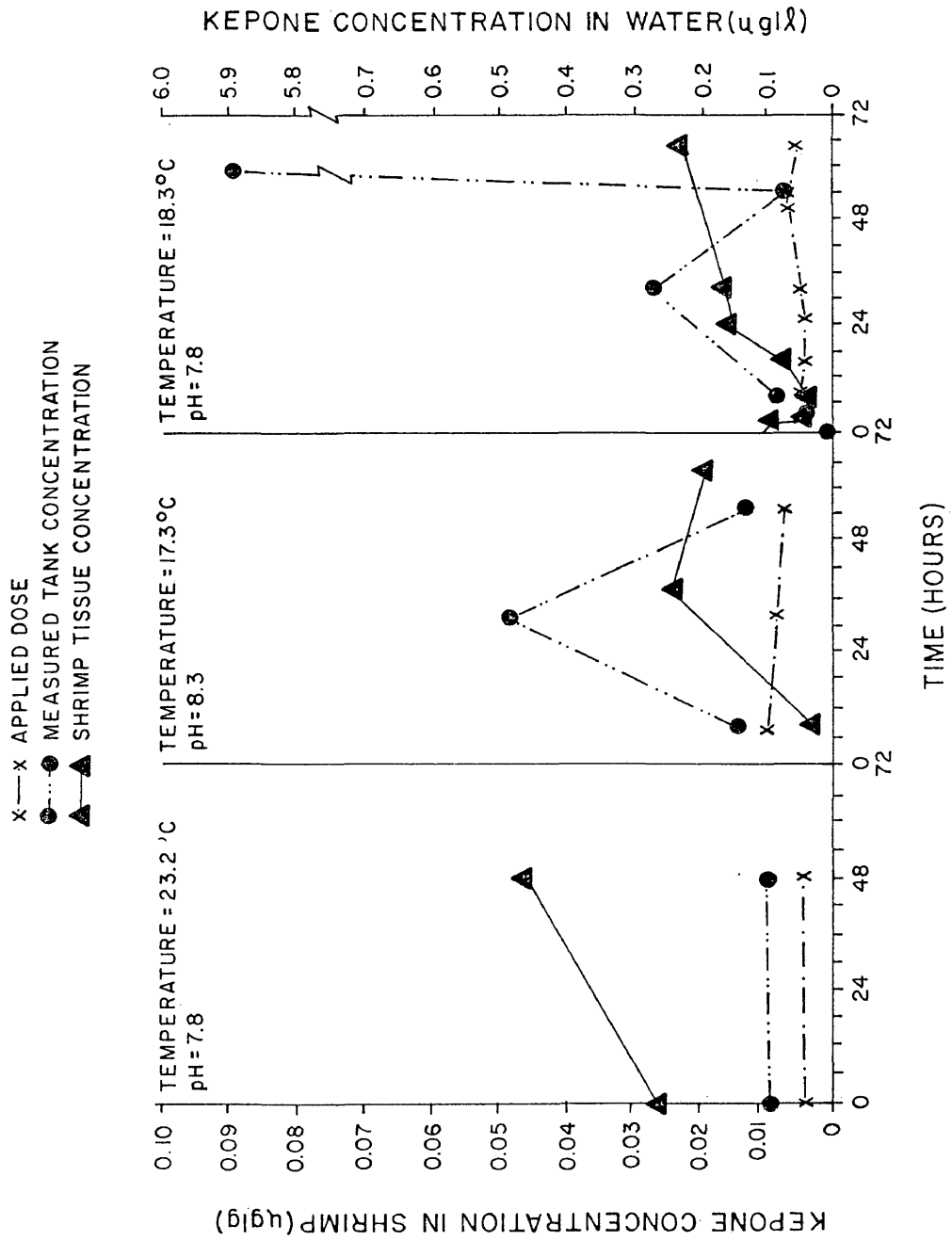


Figure 7. Tissue residue levels of Kepone in sand shrimp after exposure to 0.04 ug/l for 64 hours at different pH and temperature levels.

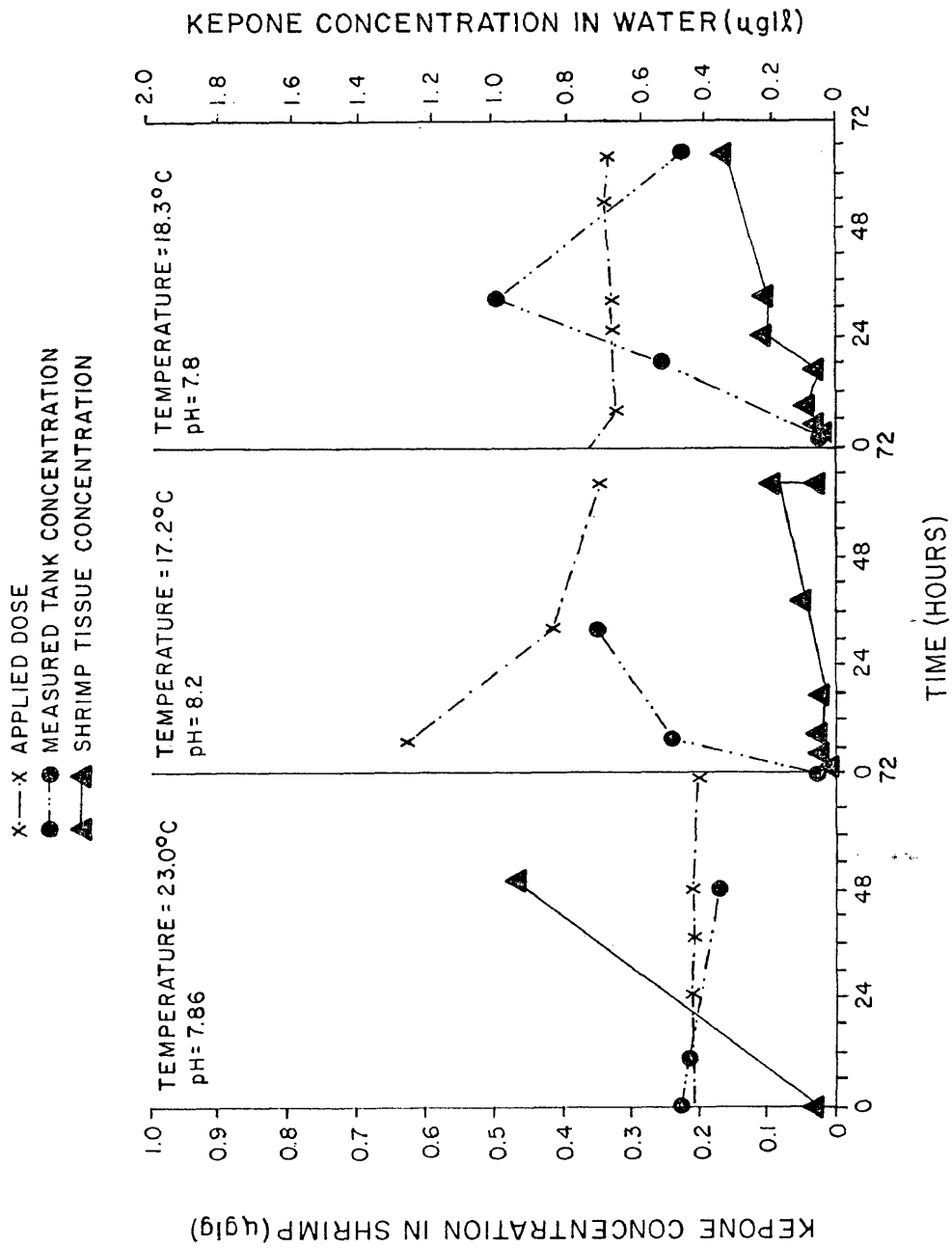


Figure 8. Tissue residue levels of Kepone in sand shrimp after exposure to 0.41 ug/l for 64 hours at different pH and temperature levels.

TABLE 7
EXPERIMENT 5
TISSUE RESIDUE LEVELS OF KEPONE IN SAND SHRIMP
DURING 64 HOURS OF EXPOSURE
Mean Temperature 17.2°C Mean pH 8.2

Hour	0.0						0.04						0.41					
	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	No. of Animals Sampled	Shrimp Tissue Conc. (ug/g)	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	No. of Animals Sampled	Shrimp Tissue Conc. (ug/g)	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	No. of Animals Sampled	Shrimp Tissue Conc. (ug/g)	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	No. of Animals Sampled	Shrimp Tissue Conc. (ug/g)		
0	0.0	<0.02	2	<0.02	0.0	0.0	2	<0.02	0.0	0.0	2	<0.02	0.0	0.0	2	<0.02		
1	0.0	<0.02	2	<0.02	0.0	0.0	2	0.05	0.0	0.0	2	0.02	0.0	0.0	2	0.02		
2	0.0	<0.02	2	<0.02	0.0	0.0	2	0.02	0.0	0.0	2	<0.02	0.0	0.0	2	<0.02		
4	0.0	0.02	2	0.02	0.0	0.0	2	<0.02	0.0	0.0	2	<0.02	0.0	0.0	2	0.02		
8	0.0	0.43	2	<0.02	0.0	0.32	2	<0.02	0.0	0.09	2	<0.02	1.3	0.5	3	0.02		
16	0.0	ND	2	ND	0.0	0.0	2	0.02	0.0	0.08	2	0.02	0.8	0.7	3	<0.02		
32	0.0	0.55	2	<0.02	0.0	0.74	2	0.02	0.0	0.08	2	0.02	0.8	0.7	3	0.04		
38	0.0	<0.02	2	<0.02	0.0	0.0	2	0.02	0.0	0.07	2	0.02	0.8	0.7	3	0.04		
56	0.0	<0.02	2	<0.02	0.0	0.0	2	0.02	0.0	0.07	2	0.02	0.8	0.7	3	0.04		
64	0.0	0.05	2	<0.02	0.0	0.4	2	0.02	0.0	0.1	3	0.02	0.7	0.4	4	0.09		
							3	0.04			4	0.02			2	0.02		
								0.03								0.06		

TABLE 8
EXPERIMENT 6
TISSUE RESIDUE LEVELS OF KEPONE IN SAND SHRIMP
DURING 64 HOURS OF EXPOSURE
Mean Temperature 18.3° Mean pH 7.8

Hour	0.0			0.0			0.04			0.41				
	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	No. of Shrimp Sampled	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	No. of Shrimp Sampled	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	No. of Animals Sampled	Shrimp Tissue Conc. (ug/g)	Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	No. of Animals Sampled	Shrimp Tissue Conc. (ug/g)
0	0.0	<0.02	5	0.0	<0.02	5	0.05	<0.02	5	<0.02	0.7	<0.02	5	<0.02
1	0.0	<0.02	5	0.0	<0.02	5		<0.02	5	<0.02		<0.02	5	<0.02
2	0.0	<0.02	5	0.0	<0.02	5		<0.02	5	<0.02		<0.02	5	<0.02
4	0.0	<0.02	5	0.0	<0.02	5		<0.02	5	<0.02		<0.02	5	0.02
8	0.0	<0.02	5	0.0	0.09	5	0.04	<0.02	5	<0.02	0.6	0.5	5	0.04
16	0.0	<0.02	3	0.0	<0.02	3		0.02	3	<0.02		<0.02	3	0.02
24	0.0	<0.02	3	0.0	<0.02	3		<0.02	3	0.02		<0.02	3	0.09
25	0.0			0.0			0.04				0.06			
32	0.0	<0.02	5	0.0	0.4	5	0.04	<0.02	5	0.02	0.7	1.0	5	0.09
51	0.0			0.0			0.06				0.7			
54	0.0			0.0			0.05				0.7			
64	0.0	0.3	10	0.0	4.0	5	0.05	<0.02	8	0.02	0.7	0.4	11	0.2

ml/min over this period); and 3) between 62 and 63 hours during which all three toxicant flows were terminated when the water level in the reservoir dropped and activated the float switch, turning off the toxicant pump.

CLEARANCE PHASE

(Experiment 4: Phase 2)

Sand shrimp did not depurate large quantities of Kepone when held in uncontaminated seawater for 15 days (Figures 5 and 6). The rate of depuration could not be estimated from the data obtained from the lower test concentration (0.04 ug/l) since uptake was insignificant. The depuration rate (k_2) for shrimp exposed to 0.41 ug/l Kepone over 15 days was 0.018 days⁻¹. This value is not significantly different from zero (Student's $t = 1.65$). Since more than one sample was taken from each aquarium on day 15 of the depuration period, a mean tissue residue, adjusted for sample weight, was calculated for sand shrimp exposed to 0.42 ug/g Kepone. This value (0.63 ug/g dry weight) was used in determining the rate of depuration.

BIOCONCENTRATION FACTOR

Sand shrimp exposed to 0.42 ug/l Kepone for 12 days (Exp. 4: Phase 1) bioconcentrated the pesticide to a maximum tissue residue level of 0.86 ug/g dry weight. The mean non-equilibrium bioconcentration factor for sand shrimp under these exposure conditions (calculated as $BCF = C_a/C_w$) is 2100.

The extrapolated equilibrium bioconcentration factor, calculated as the rate of uptake (k_1) divided by the rate of clearance (k_2) for sand shrimp exposed for 12 days to 0.42 ug/l Kepone is 11,000. At this bioconcentration factor, the maximum equilibrium body burden of sand shrimp following exposure to 0.42 ug/l Kepone (dissolved in 0.5 M NaOH) would be 4.7 ug/g (calculated as $C_a = BCF \times C_w$).

The observed body burden of Kepone concentrated by sand shrimp is directly proportional to the exposure concentration and duration of exposure. Although the values are within the same order of magnitude for any given sampling time, the non-equilibrium bioconcentration factors for sand shrimp after 64, 96 hours and 12 days of exposure to Kepone increased directly with length of exposure. The bioconcentration factors were variable as a function of exposure concentration. No clear trend is exhibited by these data in relation to exposure concentration (Table 9 and 10). After 48 hours of exposure at 23°C and pH 7.8, the bioconcentration factor for sand shrimp was one to two orders of magnitude greater than that observed for sand shrimp exposed for 64 hours at 18.3°C and pH 7.8 or 17.2°C and pH 8.2. The 96 hour bioconcentration factors are within the same order of magnitude regardless of exposure temperature and pH values.

TABLE 9

RESIDUE CONCENTRATION OF KEPONE IN SAND SHRIMP
DURING 64 HOURS OR 12 DAYS OF EXPOSURE TO A NOMINAL
CONCENTRATION OF 0.04 ug/l AND 0.41 ug/l

		Experimental Temperature and pH	Applied Dose (ug/l)		Shrimp Tissue Conc. (ug/g)		BCF	
Tank	Days		III	IV	III	IV	III	IV
		Experiment 4 (Phase 1)						
	0	Mean Temperature	0.04	0.42	0.03	0.02		
	2	23°C	0.04	0.42	0.05	0.47	1200	1100
	7	Mean pH 7.8	0.04	0.41	0.07	0.54	1800	1300
	12		0.04	0.41	0.05	0.86	1200	2100
		Experiment 5						
	8	Mean Temperature	0.09	1.26	<0.02	0.02	<220	16
	38	17.2°C	0.08	0.83	0.02	0.04	250	48
	64	Mean pH 8.2	0.07	0.69	0.02	0.06	280	87
		Experiment 6						
	8	Mean Temperature	0.04	0.58	<0.02	0.04	<500	69
	24	18.3°C			<0.02	0.09		
	32	Mean pH 7.8	0.04	0.66	0.02	0.09	500	140
	64		0.05	0.66	0.02	0.16	400	240

TABLE 10
RESIDUE CONCENTRATION OF KEPONE IN SAND SHRIMP
AFTER 96 HOURS OF EXPOSURE

Species	Nominal Applied Dose (ug/l)	Measured Tank Conc. (ug/l)	Shrimp Tissue Conc. (ug/g)	BCF Applied Dose	BCF Measured Tank Conc.
	0.0	0.62	0.02		
Sand Shrimp Experiment 3 (Conducted at 17°C and pH 7.8)	0.0	0.47	0.02		
	1.4	1.92	0.42	300	220
	4.4	3.85	1.24	280	320
	19.7	7.6	3.9	200	510
	46.8	34.5	7.25	160	210
	125.1	66	18.6	150	280
	0.0	0.06	0.10		
Sand Shrimp Experiment 2 (Conducted at 18.3°C and pH 8.3)	0.0	0.15	0.06		
	2.9	7.0	0.97	330	140
	13.1	6.6	2.78	210	420
	18.3	11.8	9.18	500	780
	86.5	49.5	16.6	190	340
		0.0	0.0	0.2	
Sand Shrimp Experiment 1 (Conducted at 18.6 C and pH 7.8)	0.0	0.8	0.1		
	5.0	5.6	1.65	330	300
	15.4	15.0	3.06	200	200
	49.6	31.0	9.38	190	300
	154	88.0	62.81	410	710
	504	200.0	48.72	97	240

DISCUSSION

ACUTE TOXICITY

Organochlorine insecticides are prevalent in the marine environment. These pesticides are toxic to a number of aquatic species (Lowe et al., 1971; de la Cruze and Lue, 1978; Bookhout et al., 1972). Mirex, which may photodegrade to Kepone, is acutely toxic to blue crabs (Callinectes sapidus), pink shrimp (Penaeus duorarum), and crayfish (Procambrus blandings) at levels as low as 1 ug/l (Lowe et al., 1971; Tagatz et al., 1975). Kepone (dissolved in acetone) is acutely lethal to sand shrimp after 96 hours of exposure at a concentration of 263 ug/l.

The acute toxicity of Kepone to sand shrimp determined by least squares regression of arc sin q on log dose (96 hour LC₅₀ = 263 ug/l) is within the confidence limits for 96 hour LC₅₀ value (140 ug/l) determined by the log-probit method. The variation which occurs between the two methods may have been a consequence of human error in fitting the line by eye in the latter approach. The arc sin q method seems to be the more appropriate method of analysis when applied to these data. Although the method loses some power when several data points equal zero, the sin⁻¹ transformation does not become invalid, whereas the log-probit method does.

Since only 25% mortality occurred after 96 hours of exposure in the highest concentration (125 ug/l Kepone) of Experiment 3, an LC₅₀ value could not be determined. Any difference in toxicity that may

have occurred as a result of the solvent change from acetone to sodium hydroxide cannot be ascertained.

Sand shrimp are more resistant to Kepone poisoning than the brown shrimp, Penaeus aztecus, (LC₅₀ = 85 ug/l; Butler, 1963) and fish species, such as spot (LC₅₀ = 6.6 ug/l) and sheepshead minnows (LC₅₀ = 70 ug/l; Schimmel and Wilson, 1977) after 96 hours of exposure. The difference in sensitivity between the fish and shrimp is probably due to the protective covering that the crustacean exoskeleton provides or because of differences in gill structure. Palaemonetes pugio, the grass shrimp, with a 96 hour LC₅₀ of 120 ug/l (Schimmel and Wilson, 1977) may be somewhat more sensitive than sand shrimp. Blue crabs, C. sapidus, and a fiddler crab, Uca pugilator, are at least as resistant to Kepone poisoning as sand shrimp with 96 hour LC₅₀ values of > 210 ug/l and 1470 ug/l, respectively (Schimmel and Wilson, 1977; Dawson, 1978) (Table 11).

Temperature affects the acute toxicity of Kepone to certain species. Butler (1963) reported a 96 hour LC₅₀ for oysters (Crassostrea virginica) of 38 ug/l in water at 14°C and 11 ug/l in water at 31°C. The 96 hour LC₅₀ has also been documented to decrease with increasing temperature for redear sunfish, Lepomis microlophus (Bridges, 1963). In general, as temperature increases, the metabolic rate of poikilotherms increases (Prosser, 1973). This response leads to increased ventilation rate and, therefore, increased exposure to Kepone since absorption from an aqueous medium occurs primarily across the gill epithelia. Through this increase in uptake of Kepone, an

TABLE 11

ACUTE TOXICITY OF KEPONE TO
SEVERAL ESTUARINE SPECIES AFTER 96 HOURS OF EXPOSURE

Species	LC50 or EC50	Temperature	Reference
Spot (<u>Leiostomus xanthurus</u>)	6.6 ug/l	25°C	Schimmel and Wilson, 1977
Mysids (<u>Mysidopsis bahia</u>)	10.1 ug/l	18°C	Schimmel and Wilson, 1977
Oyster Shell Deposition (<u>Crassostrea virginica</u>)	11 ug/l	31°C	Butler, 1963
	66 ug/l	20°C	Hansen et al., 1976
	138 ug/l	14°C	Butler, 1963
Sheepshead Minnows (<u>Cyprinodon variegatus</u>)	70 ug/l	18°C	Schimmel and Wilson, 1977
Brown Shrimp <u>Panaeus aztecus</u>	85 ug/l		Butler, 1963
Grass Shrimp (<u>Palaemonetes pugio</u>)	120 ug/l	20°C	Schimmel and Wilson, 1977
Sand Shrimp (<u>Crangon septemspinosa</u>)	155 ug/l	19°C	Hixon, this study
Blue Crabs (<u>Callinectes sapidus</u>)	>210 ug/l	19°C	Schimmel and Wilson, 1977
<u>Uca pugilator</u>	1470 ug/l		Dawson, 1978

increase in temperature may affect toxicity. The 96 hour LC₅₀⁵⁹ values for C. *sapidus*, P. *pugio*, and M. *bahia*, (>210 ug/l, 120 ug/l, and 10 ug/l) decreased with increasing temperature (19°C, 20°C, and 26°C, respectively). Although temperature may have affected the toxicity of Kepone to sand shrimp, no clear trend was demonstrated by these experiments.

BIOCONCENTRATION

Aquatic organisms exposed to chlorinated hydrocarbons bioconcentrate these substances to levels several orders of magnitude greater than the concentration in the external milieu. Bioconcentration of lipid soluble organics is of primary importance since it can affect the safety of man's food supply (Metcalf, 1977). A wide variety of both mammals and fish contain levels of pesticides such as DDT, Dieldrin, PCB's, PBB's, and Kepone which are judged to be unacceptable because of risk to humans (Metcalf, 1977; U.S. EPA, 1975).

Uptake Rate

The rate of uptake of Kepone by sand shrimp follows first order kinetics. The quantity of pesticides penetrating the integument or cuticle of the gills per unit time is suggested to be exactly proportional to the concentration of exposure (Veith et al., 1979; Hamelink et al., 1971). The uptake rate (202 days⁻¹) by sand shrimp after exposure to 0.42 ug/l Kepone at 23°C and a mean pH of 7.9 is similar to that of other crustaceans. Mysidopsis *bahia* take up Kepone

from an aqueous solution of 0.41 ug/l at a rate of 433 days⁻¹. P. pugio exposed to 0.40 ug/l Kepone for 28 days concentrated the substance at a rate of 183 days⁻¹ (computed from Bahner et al., 1977).

Temperature may affect the ability of sand shrimp to bioconcentrate Kepone. Shrimp exposed to 0.41 ug/l Kepone at 17 and 18°C did not concentrate measurable quantities of toxicant. At a test temperature of 23°C, uptake was accelerated to a rate of 202 days⁻¹. The uptake rates of several species have been documented to be depressed at low winter temperatures (Dawson, 1978).

Elimination of Kepone

Sand shrimp eliminate Kepone at a rate of 0.018 days⁻¹ when held in uncontaminated water for 15 days (50% reduction in body burden in 38.5 days). The rate is similar to that observed in blue crabs fed contaminated oysters (Schimmel et al., 1979). Blue crabs did not deplete significant quantities of Kepone until held in uncontaminated seawater for 70 days (Schimmel et al., 1979). Spot deplete only 30 to 50 percent after 24 to 28 days in Kepone-free water (Bahner et al., 1977). The levels of Kepone decreased by 72% when James River spot were captured and held in Kepone-free water for 200 days (Hedgepeth et al., 1979). Atlantic croaker did not lose significant amounts of Kepone after 56 days (Doyle et al., 1978). Field contaminated oysters eliminate Kepone to non-detectable levels within 17-20 days (Bahner et al., 1977).

The apparent tissue concentrations of Kepone in shrimp and related species fluctuate during the depuration period. The concentration of Kepone observed in sand shrimp tissue on day 0 of the depuration period was 0.86 ug/g. On day 2 the concentration had decreased to 0.65 ug/g, but increased again to 0.89 ug/g by day 4 (Figures 5 and 6). The measured concentrations of Kepone in blue crab tissue also fluctuated during the depuration period Schimmel et al., 1979). Since there was no detectable Kepone in the external milieu during these depuration periods, the apparent fluctuation in body burden cannot be attributed to uptake. The fluctuation in apparent tissue concentration is probably due to natural variation between individuals of the same species.

Tissue Residue Levels of Kepone

The mean tissue residue levels in laboratory exposed shrimp are significantly lower than the levels observed in sand shrimp from field samples (Table 12). At the higher exposure level (0.42 ug/l) shrimp attained a mean level of 0.47 ug/g Kepone after 12 days. Sand shrimp from the James River contained mean Kepone residues of 2.0 ug/g Kepone (Bender et al., 1977). The shrimp in the river were exposed for a considerably longer period of time, but to much lower concentrations. These data suggest that the laboratory exposed shrimp were not at equilibrium Kepone levels after 12 days.

The levels of Kepone in blue crabs captured from the James River (Table 12) also contained higher tissue residue levels than

TABLE 12

KEPONE RESIDUES ($\mu\text{g/g}$) IN ANIMALS FROM THE JAMES RIVER
(from Bender et al., 1977)

<u>Longterm Residents</u>	\bar{X}	N	Std. Error of \bar{X}
Spottail shiner (<u>Notropis hudsonius</u>)	0.08	6	0.02
Channel catfish (<u>Ictalurus punctatus</u>)	0.04	45	0.004
White catfish (<u>Ictalurus catus</u>)	0.25	14	0.03
American eel (<u>Anguilla rostrata</u>)	0.64	15	0.55
Black crappie (<u>Promoxis nigromaculatus</u>)	1.0	10	0.13
Largemouth bass (<u>Micropterus salmoides</u>)	2.4	14	0.54
White perch (<u>Roccus americanus</u>)	2.7	20	0.39
Bay anchovy (<u>Anchoa mitchilli</u>)	0.65	13	0.15
Atlantic silverside (<u>Menidia menidia</u>)	1.6	15	0.43
Hogchoker (<u>Trinectes maculatus</u>)	0.94	22	0.13
Grass shrimp (<u>Palaemonetes pugio</u>)	0.60	8	0.15
Sand shrimp (<u>Crangon septemspinosa</u>)	2.0	3	0.09
Xanthid crabs	0.27	3	0.03
Blue crab (<u>Callinectes sapidus</u>) female	0.19	180	0.02
Blue crab (<u>Callinectes sapidus</u>) male	0.81	43	0.07
Oyster (<u>Crassostrea virginica</u>)	0.16	140 ¹	0.01
Hard clam (<u>Mercenaria mercenaria</u>)	0.09	12 ¹	0.009
<u>Short-term Residents</u>			
American shad (<u>Alosa sapidissima</u>)	0.03	50	0.004
Atlantic menhaden (<u>Brevoortia tyrannus</u>)	0.05	8	0.02
Spot (<u>Leiostomus xanthurus</u>)	0.81	40	0.13
Croaker (<u>Micropogon undulatus</u>)	0.75	60	0.16
Bluefish (<u>Pomatomus saltatrix</u>)	0.29	30	0.20

¹Blends of 12 individuals

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laboratory-exposed specimens (mean level = 1.28 ug/g) (calculated from Schimmel et al., 1979). Blue crabs do not concentrate Kepone significantly from water, but do concentrate the pesticide from contaminated food (Schimmel et al., 1979; Fisher, 1980). Since laboratory-exposed shrimp contained low, non-equilibrium tissue residues following aqueous exposure, perhaps food is also a major route of uptake for sand shrimp.

Residue levels of pesticides in tissues of invertebrates are directly related to the concentration in the external milieu. At an exposure level of 0.04 ug/l the maximum body burden reached by the shrimp was 0.07 ug/g dry weight with a mean value of 0.05 ug/g after 12 days. Sand shrimp exposed to 0.42 ug/l contained levels as high as 0.86 ug/g dry weight (Table 9). Shrimp exposed to a range of concentrations for 96 hours exhibited a proportional increase in body burden with increasing exposure concentration (Table 10). The uptake of DDT by certain invertebrates is linearly related to the concentration of exposure (Hamelink et al., 1971), as is the quantity of DDE concentrated by zooplankters (Hamelink et al., 1977).

On three occasions (days 7, 8 and 12) during Exp. 4 the diluent flow was reduced because filters clogged. As a result, toxicant flowed undiluted into the experimental aquaria for a short period. Measured water samples reflected these increases. On day 7, the increase in Kepone content of water sampled from Tank III (0.04 ug/l) was proportionately higher than the increase in pesticide concentration in Tank IV (0.42 ug/l). The shrimp tissue concentration

from day 7 samples reflected these differences (Figures 5 and 6). On day 12, the measured water concentration of Kepone was proportionately higher in Tank IV than Tank III. Kepone content of shrimp tissue samples increased proportionately with the water concentration.

Tissue samples of control shrimp held in either uncontaminated filtered seawater, filtered seawater and acetone (Experiment 1), or filtered seawater and 0.5 M NaOH (Experiments 2-6), contained levels of a substance which behaved like Kepone on the gas chromatograph. Concentrations ranged from 0.0 to 0.2 ug/g. Kepone is non-detectable in sand shrimp tissue samples below levels of 0.02 ug/g. Water samples from the control aquaria contained up to 4.0 ug/l of this contaminant. Inconsistencies in the levels of measured Kepone in experimental aquaria suggest that these samples were also affected. Since the levels of Kepone in shrimp tissue samples were nowhere near this value, perhaps the water samples were contaminated during collection or analysis.

Consistent contamination as a result of dosing technique is unlikely. Acetone rinses of the experimental system were analyzed for Kepone content and none was detected. Analyses of acetone extracts of neoprene stoppers, freshly unwrapped from the factory, showed concentrations of a substance which behaved like Kepone on the gas chromatograph (Roberts, personal communication). In experiments in which acetone was used as the carrier for Kepone, the substance may have been leached out of the stoppers as the acetone in the stock solutions evaporated and recondensed, contaminating the stock

solutions in Experiment 1. While the stoppers were not extracted with 0.5 M NaOH, the possibility exists that sodium hydroxide may extract this substance also. This would explain the concentrations in water samples from the solvent test aquaria in Experiments 5 and 6.

The Bioconcentration Factor

The bioconcentration factor is calculated as the concentration of pesticide in an organism divided by the concentration in the external medium (Hamelink et al., 1971; Hamelink, 1977; Hamelink et al., 1977; Metcalf, 1977). This quantity is a non-equilibrium bioconcentration factor when the length of exposure is insufficient for a steady state body burden to be reached. At equilibrium, the uptake rate is equivalent to the rate of depuration, so that the concentration in the organism maintains a steady state (Hamelink et al., 1977; Krzeminski et al., 1977; Veith et al., 1979). When the tissue residue level is constant, an equilibrium bioconcentration factor can be calculated as either; 1) the uptake rate divided by the rate of clearance or; 2) the tissue residue level divided by the applied dose. Comparison of the bioconcentration factors for different species and pesticides indicates the relative abilities of pesticides to accumulate in organisms. However, caution must be used when comparing bioconcentration factors so that equilibrium values are not compared with non-equilibrium bioconcentration factors.

The process of bioconcentration is affected by temperature since temperature affects the processes which control the partitioning of

chemical substances between the aqueous medium and lipids in the gill tissues, blood and body of the organism (Veith et al., 1979; Fromm and Hunter, 1969; Dawson, 1978). An inverse relationship exists between the affinity of chlorinated organics for lipid-rich animal tissue and water (Metcalf, 1977; Hamelink et al., 1971; Dawson, 1978). The levels of Kepone in the experimental aquaria were well below saturation. Nevertheless, changes in the experimental pH may have affected the relationship between the solubility of Kepone in water and in shrimp tissues, thereby altering bioavailability and bioconcentration. Exposure to 0.42 ug/g Kepone for 48 hours at 23°C and pH 7.8 resulted in a bioconcentration factor (1100) 1 to 2 orders of magnitude greater than the values for shrimp exposed for 64 hours to 0.41 ug/l Kepone at either 17.2°C and pH 8.2 or 18.3°C and pH 7.8 (87 and 240, respectively). Since both pH and temperature differed in these studies no definitive correlation between increased Kepone content and increased temperature or decreased pH can be demonstrated. Either factor or both may have affected tissue residue levels and, subsequently, the bioconcentration factor.

The 96 hour non-equilibrium bioconcentration factor for sand shrimp, (280) is within the same order of magnitude as that observed for grass shrimp after 96 hours, 698 (Schimmel and Wilson, 1977). The 96 hour bioconcentration factor for blue crabs (8.1 ug/l Kepone; Schimmel and Wilson, 1977) is lower by two orders of magnitude than that for either grass shrimp or sand shrimp, since blue crabs do not concentrate Kepone from water significantly. The non-equilibrium

bioconcentration factor (2100) for sand shrimp after 12 days exposure at 23°C is within the same order of magnitude as that observed for grass shrimp (4500) after 9 days of exposure (calculated from Hansen et al., 1977b). The values observed for M. bahia were higher and depended on exposure concentration; 5000 at 0.02 ug/l and 15,000 at 0.41 ug/l after 11 days of exposure (Table 13).

The bioconcentration factors for mysids and grass shrimp increased to values of 12,000 and 11,000, respectively after 21 and 28 days of exposure, respectively. These data indicate that the 12 day bioconcentration factors for mysids and grass shrimp are not equilibrium values. The estimated equilibrium bioconcentration factor for sand shrimp calculated as the rate of uptake divided by the rate of elimination is 11,000. The estimated equilibrium body burden for sand shrimp is 4.7 g/kg Kepone. Since the maximum tissue residue levels in field samples was only 2.0 ug/l, the sand shrimp from the river were not at equilibrium.

TABLE 13
KEPONE RESIDUES IN ESTUARINE ORGANISMS
AFTER 12 DAYS OF EXPOSURE

Days	Species	III	IV	III	IV	III	IV	Reference
		Applied Dose (ug/l)	Applied Dose (ug/l)	Tissue Conc. (ug/g)	Tissue Conc. (ug/g)	BCF	BCF	
	Grass Shrimp	0.2	0.40					Hansen <i>et al.</i> , 1977b
2	(Conducted at 25.5 °C)			0.02	0.03	1000	75	
4								
7				0.04	0.47	2000	1200	
9				0.07	1.79	3500	4500	
	Mysids	0.03	0.41					Nimmo <i>et al.</i> , 1977
2								
4	(Conducted at 27 °C)			0.04	2.0	1300	4900	
7				0.16	3.3	5300	8000	
11				0.15	6.3	5000	15,000	
	Oysters	0.03	0.39					Hansen <i>et al.</i> , 1977b
1	(Conducted at 14.2 °C)			0.14	1.4	4700	3600	
4				0.11	1.3	3700	3300	
8				0.18	2.2	6000	5600	
12				0.21	2.4	7000	6200	
	Sand Shrimp Experiment 4 (phase 1) (Conducted at 23 °C, pH 7.7)	0.04	0.42	0.03	0.02			Hixon
2		0.04	0.42	0.05	0.47	1200	1100	
7		0.04	0.41	0.07	0.54	1800	1300	
12		0.04	0.41	0.05	0.86	1200	2100	
Hours								
	Sand Shrimp Experiment 5 (Conducted at 17.3 °C, pH 8.1)	0.09	1.26	<0.02	0.02	<220	16	Hixon
8		0.09	1.26	<0.02	0.02	<220	16	
38		0.08	0.83	0.02	0.04	250	48	
64		0.07	0.69	0.02	0.06	280	87	
	Sand Shrimp Experiment 6 (Conducted at 18 °C, pH 7.9)	0.04	0.58	<0.02	0.04	2500	69	Hixon
8		0.04	0.58	<0.02	0.04	2500	69	
24				0.02	0.09			
32		0.04	0.66	0.02	0.09	500	140	
64		0.05	0.66	0.02	0.16	400	240	

CONCLUSIONS

1. Kepone (dissolved in acetone) is acutely lethal to 50% of the sand shrimp population at 263 ug/l after 96 hours. The LC₅₀ after 72 hours of exposure to Kepone (dissolved in acetone) was 440 ug/l determined by the arc sin transformation. The LC₅₀ for Kepone dissolved in acetone is higher than the highest concentration (504 ug/l) after 24, and 48 hours of exposure.
2. The specific rate constant for uptake of Kepone by sand shrimp is 202 days⁻¹.
3. Sand shrimp do not reach an equilibrium concentration of Kepone after 12 days of exposure. The non-equilibrium bioconcentration factor for sand shrimp after 12 days exposure is 2100.
4. The estimated equilibrium bioconcentration factor for sand shrimp is 11,000. The maximum equilibrium body burden would be 4.7 ug/g for sand shrimp exposed to 0.42 ug/l Kepone.
5. Both equilibrium and non-equilibrium bioconcentration factors can be determined from data generated by uptake studies. Caution must be used when comparing bioconcentration factors so that equilibrium values are not compared with non-equilibrium values.
6. The rate of clearance of Kepone by sand shrimp was 0.018 days⁻¹. This rate was not significantly different from zero.

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