1980

Effects of ingestion of kepone contaminated food by juvenile blue crabs (*Callinectes sapidus* Rathbun)

Daniel J. Fisher

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<https://dx.doi.org/doi:10.25773/v5-hwkc-r742>

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EFFECTS OF INGESTION OF KEPONE CONTAMINATED FOOD
BY JUVENILE BLUE CRABS (Callinectes sapidus Rathbun)

A THESIS

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by

Daniel J. Fisher

1980

APPROVAL SHEET

This Thesis is Submitted in Partial Fulfillment of
the Requirements for the Degree of

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DEDICATION

This thesis is dedicated to Mom and Dad and the family they raised. Their guidance and love helped inspire my study of marine science and in particular this work.

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ACKNOWLEDGEMENTS

Thanks go to my major professor, Dr. Michael E. Bender, for his help in selection of this topic and his guidance throughout. Appreciation is also extended to my other committee members and especially to Dr. Morris H. Roberts, Jr.

Field collection could not have been completed without the help of Peter A. Van Veld. Special appreciation goes to Harold Slone and his staff who analyzed the many Kepone samples.

Special thanks goes to Elizabeth Anne Clark, whose love and support enabled this work to be completed.

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ABSTRACT

Two long term (65 day) laboratory experiments were conducted to determine lethal and sublethal effects of ingestion of Kepone contaminated food by juvenile blue crabs (Callinectes sapidus Rathbun). Food was contaminated at levels found in the James River in Virginia. The experimental temperature was 28°C during Experiment I and 21°C during Experiment II. Kepone levels in the food ranged from non-detectable levels (<0.02 ug/g) to 2.5 ug/g. In neither experiment were crab mortalities statistically different at any Kepone dose tested. This indicates a 65 day LD50 in excess of 0.5 ug/day, based on a feeding rate of 0.2 g/day.

The highest Kepone dose tested in both experiments caused significant increases in crab metabolic rates, as measured by oxygen consumption, and crab excitability during feeding. At the higher temperature of Experiment I there was an inverse relationship between carapace thickness and increasing Kepone dose. Biomagnification of Kepone by crabs to a tissue concentration greater than that in their food occurred at the highest treatment level in Experiment I. Kepone concentration in crabs did not reach equilibrium at any Kepone dose tested in either experiment. Crab molting frequency, autotomization ability, or overall growth were not statistically different at any Kepone treatment in either experiment.

EFFECTS OF INGESTION OF KEPONE CONTAMINATED FOOD
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INTRODUCTION

Kepone is an organochlorine pesticide (decachloroocthydro- 1, 3, 4, -metheno-2H-cyclobuta (cd) pentalene 2-one) used to control ants, cockroaches, and insect pests of potatoes and bananas. It was developed and produced by Allied Chemical Corporation at their Hopewell, Virginia plant from 1966 to 1973 (1.5 million pounds). In 1974, Life Science Products of Hopewell took over production. About 1.7 million pounds of the pesticide were produced by Life Science Products until their closure in July, 1975 when Kepone poisoning of workers was discovered. Some Kepone contamination of the James River occurred throughout the years of production by both Allied and Life Science Products. Increased levels of contamination occurred when effluent from the Life Science Products plant caused the digestors at the Hopewell Sewage Treatment Plant to fail, allowing discharge to the upper sections of the river. Run-off from a land disposal area also contributed to contamination of the river.

An initial report by the Environmental Protection Agency (1975) showed that Kepone was in the air, soil, and waters around Hopewell. A later report (Environmental Protection Agency, 1978) indicated that significant concentrations were present in various compartments of the estuarine system of the James River, including solution, suspension, sediment, and food chain. Average Kepone concentrations in estuarine

vertebrates and invertebrates were reported to range from 0.09 to 2.0 ug/g (Bender et al., 1977). Kepone concentrations in blue crab backfin muscle averaged 0.19 ug/g for females and 0.81 ug/g for males, with the difference initially attributed to the fact that males spend a greater part of their lives in the river system than females. Later studies (Roberts, 1980) indicated that the difference was actually due to partitioning of Kepone into the hepatopancreas and gonads in the female crabs. Top carnivores, such as bluefish (Pomatomus saltatrix), which enter the James virtually Kepone free, may accumulate the pesticide above the EPA-FDA action levels for fish of 0.3 ug/g in a matter of weeks.

Preliminary work on the physical/chemical behavior of Kepone once it enters the aquatic system showed some important trends (Environmental Protection Agency, 1978; Garnas et al., 1977). Kepone does not volatilize out of the aquatic ecosystem nor does it decompose under any of the conditions studied. It has a propensity to move into the sediment, especially the lighter suspendable, organically rich particulate matter, but does not bind irreversibly to these materials. The main sink for Kepone is therefore thought to be the James River bed sediments rather than the water column. The largest amounts of Kepone are present in the James River turbidity maximum zone which occurs between 38 and 52 miles from the river mouth, depending on river flow (Nichols and Trotman, 1977). Kepone levels commonly ranged between 10 and 200 ug/kg in this zone, in contrast to sediment concentrations of less than 30 ug/kg found near the river mouth. The same study indicated that Kepone extended to a depth of 80 cm into the sediment. Movement of Kepone out of the James

River by sediment transport is unlikely since most is trapped in the turbidity maximum. Nichols and Trotman (1977) also showed no appreciable decline in Kepone sediment concentrations through July, 1977. The evidence indicates that Kepone, like other organochlorine pesticides, is very persistent in the environment once introduced. The major transport routes from the James River system for Kepone appear to involve biological mechanisms following bioaccumulation from the environment.

There was little work on Kepone toxicity before the mid 1970s, when the extent of Kepone contamination was recognized. Butler (1963) showed that the pesticide was toxic to some estuarine organisms when applied as an aqueous solution. It acted in much the same way as Mirex, a chemically similar pesticide which may, in fact, photodegrade to Kepone (Ivie et al., 1974). Both pesticides reduced phytoplankton productivity and were toxic to shrimp (Penaeus sp.), juvenile blue crabs (Callinectes sapidus), oyster (Crassostrea virginica), clams (Mercenaria mercenaria), juvenile white mullet (Mugil curema), and longnose killfish (Fundulus similus). Butler also indicated that when applied in an aqueous solution both pesticides were relatively less toxic than DDT to juvenile blue crabs. The 48 hour EC₅₀ was 2.0 mg/l and 1.0 mg/l for Mirex and Kepone respectively, while that for DDT was 0.01 mg/l. EC₅₀s were reported because his endpoint was loss of equilibrium, which was always followed by death.

Kepone is toxic to birds and mammals, including man (Jaeger, 1976). Earliest data (Gleason et al., 1963) indicated Kepone toxicity to rabbits, rats, and dogs. A study by Good et al. (1965) showed cumulative effects of Kepone on mammals. Vivid evidence of sublethal Kepone effects

on man can be seen in employees who worked at Life Science Products and received very large doses of Kepone in its particulate form. These people exhibited serious neurological disorders and sterility, but presently seem to be recovering from the toxic manifestations of Kepone poisoning (Guzelian et al., 1980).

Research on the uptake and toxicity of Kepone from seawater has accelerated in the last few years. Schimmel and Wilson (1977) determined 96 hour LC₅₀ levels for four estuarine organisms, using flow through bioassays. The organisms and their 96 hour LC₅₀s were: grass shrimp (Palaemonetes pugio), 121 ug/l; sheepshead minnows (Cyprinodon variegatus), 69.5 ug/l; spot (Leiostomus xanthurus), 6.6 ug/l; and adult blue crabs (Callinectes sapidus), 210 ug/l. The blue crab appeared to be relatively resistant to Kepone in solution. The pesticide caused pronounced toxic, reproductive, and teratogenic effects in lifecycle tests with estuarine mysids (Mysidopsis bahia) (Nimmo et al., 1977) and sheepshead minnows (Hansen et al., 1977). Growth was inhibited at aqueous concentrations of Kepone as low as 0.072 ug/l for mysids and 0.08 ug/l for sheepshead minnows. Concentrations of 29.5 ug/l Kepone were found to be toxic to lugworms (Arenicola cristata) (Rubenstein, 1977). Toxicity and accumulation have been demonstrated in four species of marine unicellular algae. Seven day growth EC₅₀ values as low as 0.35 mg/l have been reported (Walsh et al., 1977). Since these levels of Kepone are higher than levels found in the James River, actual field toxicity to phytoplankton may not occur, but uptake by algae could result in accumulation at higher trophic levels. Finally, Bourquin et al. (1978) found that Kepone is toxic to certain types of aquatic bacteria at concentrations as low as 0.02 mg/l.

Bahner et al. (1977) found that Kepone is bioconcentrated by oysters, mysids, grass shrimp, sheepshead minnows, and spot from Kepone concentrations in seawater as low as 0.023 ug/l. All species showed nearly equilibrated tissue concentrations within 8 to 17 days and bioconcentration factors ranged from 2,300 to 13,500 in long term (28 day) bioconcentration tests. Depuration varied widely from species to species. Oysters exhibited rapid depuration when put in a Kepone-free system, with no Kepone detected in their tissues after 20 days. Grass shrimp and fish took 24 to 28 days to depurate 30 to 50 percent of the Kepone in their tissues. It is important to note that mysids, sheepshead minnows, and grass shrimp play an important role as food sources in estuarine food chains. They act as essential omnivores linking energy transfer from detritus and benthic flora and fauna to carnivores at higher trophic levels. Oysters and spot are commercially valuable seafoods which, if contaminated with Kepone, could have direct health effects on humans.

Haven and Morales-Alamo (1977) showed that mollusks (Crassostrea virginica, Macoma balthica, and Rangia cuneata) concentrate Kepone from contaminated suspended sediments by a factor of 10^3 . Oysters bioconcentrated Kepone from seawater by a factor of 10^4 (Bahner et al., 1977).

These studies indicate that there is active uptake of Kepone from seawater and suspended sediments at various trophic levels in the estuarine food chain. Early work on DDT indicated the importance of dietary inputs to DDT body burdens in organisms. Jarvinen et al. (1977) indicated that dietary uptake of DDT by fathead minnows could account for 25 to 62 percent of the total DDT body burden of these fish. Woodwell

et al. (1967) reported biomagnification of DDT in an estuarine salt marsh. Concentrations of DDT increased with the trophic level through more than three orders of magnitude, from 0.04 ug/g in plankton to 75 ug/g in ring-billed gulls. The highest concentrations were found in scavenging and carnivorous fish and birds.

A study with Mirex and blue crabs has shown that this pesticide can be transferred through a grass shrimp - juvenile blue crab food chain to yield concentrations high enough to kill the crabs (Lowe et al., 1971). This study also indicated that Mirex contaminated fish (1.0 ug/g) caused the death of juvenile blue crabs upon ingestion. In a study of Mirex uptake in a catfish food chain, Colins et al. (1973) showed that fish denied access to the natural food chain in Mirex treated ponds did not accumulate the pesticide. Free living catfish in the same ponds contained as much as 0.65 ug/g Mirex after 6 months. This last study indicates the importance of food chain pesticide uptake in scavenger/predator organisms in nature.

A recent paper by Macek et al. (1979) questions the importance of dietary uptake and biomagnification of contaminants to the total residue levels in aquatic organisms. Biomagnification is defined here as the process by which the tissue concentrations of bioaccumulated chemical residues increase as these materials pass up the food chain through two or more trophic levels. Bioconcentration is defined as uptake directly from water while dietary accumulation indicates uptake from the food alone. Total uptake from both sources is termed bioaccumulation (Brungs and Mount, 1978). Macek's group suggest that bioconcentration of most chemical pollutants, including Kepone, clearly represents the most

significant input to organism residue levels. The early papers concerning the importance of biomagnification and dietary inputs were based on DDT which the authors found to be one of the few chemicals for which dietary inputs do represent a significant percentage of the total organism residue level. This, they state, led scientists to believe that all other organochlorine contaminants would act in the same way, a belief they found to be unsound. The authors also state that the most useful data for identifying those chemicals for which dietary sources may contribute statistically significant residues to the total body burden are data on the depuration rates of the chemicals from biological systems. A case in point is DDT, which is a much more persistent chemical after it enters the body than the other chemicals studied. The idea is that the slower the depuration rate, the more likely that dietary inputs will play a more important part in total residue levels.

Although Macek et al. (1979) indicate that dietary inputs of Kepone should not play a significant role in organism residue levels, current data seems to suggest otherwise, at least for the blue crab. Kepone in seawater appears to be relatively non-toxic to adult blue crabs (LC₅₀ - 210 ug/l) (Schimmel and Wilson, 1977). Bioconcentration did occur, but measurably only at levels greater than 110 ug/l. Measurable Kepone residues were not found in crabs exposed to concentrations lower than this. Butler (1963) indicated the relative low toxicity of Kepone and Mirex in solution when compared to DDT. Lowe et al. (1971) found that Mirex, when ingested, was more toxic to juvenile blue crabs than when applied in solution and Mahood et al. (1970) indicated that crabs readily ingested Mirex bait when available, with fatal results. Leffler (1975)

showed that Mirex, upon ingestion, was more potent than DDT in eliciting subacute responses such as increased metabolic rate, inhibition of the autotomy reflex, and reduction of carapace thickness. These studies indicate that Kepone and Mirex are much more toxic as stomach poisons than contact poisons.

Schimmel et al. (1979) exposed juvenile blue crabs to Kepone in water (0.03 and 0.3 ug/l), in food (0.25 ug/g in oyster tissue), and in combinations of these. When fed contaminated oyster tissues, crabs accumulated Kepone to an average of approximately 0.1 ug/g in both their muscle and remaining tissue. There was no measurable concentration of Kepone in crabs exposed only to Kepone in the water. Furthermore, there was no difference in Kepone uptake between crabs exposed to Kepone in food alone and in food and water simultaneously. This indicates that very little if any of the Kepone body burden of crabs comes from Kepone in water. After 28 days in a Kepone free environment the crabs had not depurated the pesticide.

In a companion study, juvenile crabs were fed naturally contaminated James River oysters (0.15 ug/g) and oysters contaminated with Kepone in the laboratory (0.15 and 1.9 ug/g). The experiment lasted for 56 days. Mortalities among crabs which were fed James River oysters and oysters containing 1.9 ug/g Kepone were significantly greater than control crabs. Increased mortalities for crabs fed James River oysters over those crabs fed oysters contaminated equally in the laboratory were thought to result from high metal concentrations in James River oysters (aluminum, zinc, and copper). Molting was also reduced significantly at all Kepone doses tested. Crabs depurated 32% of the Kepone in their edible tissues

when fed uncontaminated oysters for 8 weeks. Most of the apparent depuration was attributed to growth dilution. Crabs fed oysters contaminated with 1.9 ug/g Kepone exhibited signs of poisoning similar to crabs fed fish contaminated with 1.0 ug/g Mirex (Lowe et al., 1971). These included initial crab excitability followed by lethargy, convulsions and death.

These studies suggest that uptake of Kepone by blue crabs in the James River is mainly through food sources and that depuration is slow. Bender et al. (1977) found that most natural food sources of the blue crab in the river are contaminated with Kepone levels as high as 2.0 ug/g. Therefore, it is reasonable to assume that Kepone residues will remain high in blue crabs as long as detectable concentrations of the pesticide remain in the environment.

Annual blue crab fishery statistics show a major decline in blue crab landings from the James River from 1972 to December, 1975, when a ban on crabbing in the river was instituted due to Kepone contamination (National Marine Fishery Service, 1963-1975). This decline must be viewed cautiously though, since crabbing in the river has been very sporadic in the past, long before Kepone was ever manufactured. The decline in landings (2,328,600 pounds in 1972 to 32,400 pounds in 1975) may be due to a number of factors, most of which are related to the high degree of industrialization in the James River basin. It is interesting to note however that the decline in crab catches corresponds to the period of peak production of Kepone. This decline in crab catch plus the subsequent moratorium on crab and finfish harvesting due to high Kepone levels has caused massive economic problems to the fishermen in the area.

The study of Kepone effects on blue crabs has usually involved exposure in water. The present study was designed to determine the 65 day LD₅₀ for Kepone when ingested by crabs. The Kepone doses that were selected were based on the experimental results reported by Schimmel et al. (1979) and a consideration of the range of Kepone values found in estuarine vertebrates and invertebrates which constitute the major natural food source for the blue crabs in the James River. Sublethal effects on parameters such as growth, metabolic rate, and carapace thickness were also measured for all crabs surviving the LD₅₀ experiments. Juvenile crabs were studied, since in most cases juvenile organisms represent a more sensitive life stage than adults. Any reduction in the juvenile crab population would cause a subsequent reduction in the adult population.

MATERIALS AND METHODS

Culture and Maintenance

Juvenile blue crabs (Callinectes sapidus Rathbun) of approximately the same molt stage (Stage C - Hard) were used in these experiments. Crabs tested in Experiment I had a mean spine to spine width of 34.7 mm (range 26.8 to 45.7 mm) and a mean wet weight of 3.05 g (range 1.26 to 7.03 g). Those tested in the second experiment were slightly larger with a mean width of 38.1 mm (range 26.1 to 55.0 mm) and a mean wet weight of 4.63 g (range 1.63 to 13.44 g). An initial subsample of crabs showed no detectable Kepone (<0.02 ug/g; whole body) in either crab population.

Crabs were captured by seine and dip net. Those used in Experiment I were collected at Mile 3 of the York River in Virginia during June of 1978, while those used in the second experiment were collected from the Indian River (30⁰/oo salinity) near Jensen Beach in eastern Florida during February of 1979. The Florida crabs were used because juvenile blue crabs are unavailable in the Chesapeake Bay area during winter months.

In the Florida collection about 10 percent of the 300 crabs collected were Callinectes ornatus. Since sampling time was limited, all juvenile Callinectes sp. collected were shipped in modified ice chests by commercial airline. The insides of the chests were lined with chicken

wire to allow for ventilation. Crabs were layered between wet burlap. The burlap was not treated with chemical preservatives. Ventilation holes were drilled in the tops of each cooler. Each chest contained 150 crabs and was carried in the pressurized, cabin temperature baggage compartments. Total travel time, from packing to release was approximately 10 hours. Mortalities during shipment were less than 5 percent.

To avoid salinity shock, crabs were kept for a few days in recirculating water tanks with a salinity of 20‰. After 5 days, crabs were placed in the experimental tanks for final acclimation to ambient salinity (15‰). There was mortality during the acclimation period resulting from salinity shock or delayed effects of transport. Despite these mortalities, there were enough crabs for Experiment II. Approximately 12 percent Callinectes ornatus were included in the test population. These crabs were distributed randomly in the experimental tanks. In reporting results from Experiment II, only data for Callinectes sapidus were used since C. ornatus exhibited greater mortality.

Before the experiments, crabs were held in the test tanks during the 14 day acclimation period. Tanks were 37.5 liter all-glass aquaria divided into 16 chambers by fiberglass sheets. Each chamber, approximately 10 x 7.5 x 30 cm, held one crab. An equal number of males and females was used in each tank.

The exposure apparatus is detailed in Figure 1. Six treatments were used in each experiment, with duplicate tanks per treatment. The tanks were covered with plastic grating to prevent crab escape. The fiberglass sheets forming the chamber walls had 6 mm holes drilled in them to allow for good water circulation. Eight holes were used per

Figure 1. Diagram of system used to maintain blue crabs.

chamber wall. The holes began approximately 2 cm from the bottom to reduce the chance of cannibalism of recently molted crabs by crabs in adjacent compartments. York River water filtered to 10 microns was pumped to a header tank, heated as necessary and distributed to the 12 test tanks by siphons at 500 ml/min (Figure 1). According to Sprague's guidelines (1969) this provided a 90 percent replacement in approximately 2.5 hours. This allowed for adequate flushing and maintenance of dissolved oxygen concentrations.

Crabs were fed uncontaminated fish during the acclimation period. Feeding was discontinued 5 days prior to the beginning of experiments. At that time the crabs were measured (spine to spine width and mid-body dorsal-ventral thickness), weighed, and examined for missing appendages and molt stage. If a crab molted between this time and the start of an experiment, it was remeasured.

The experimental temperature was 28°C for the first experiment and 21°C for the second. The temperature for each experiment was selected based on the highest ambient temperature expected during the 65 day test period. Equipment was not available to cool experimental water during the summer, so a single temperature for both experiments was impossible. Temperatures were thermostatically controlled using four 1,000 watt Vicor heaters.

Contaminated Food Preparation

Experiment I

The food source was naturally contaminated striped bass (Morone saxatilis) from the James River. Six concentrations of contaminated

food were prepared from fish filets which had already been analyzed for Kepone. Striped bass of different concentrations were mixed only when necessary to produce the desired Kepone concentration. Each food batch was blended to a "mushy" consistency and subsamples were analyzed for Kepone. Food at six different Kepone concentrations was prepared in this manner. Measured Kepone in these food preparations were: non-detectable (<0.02 ug/g), 0.42, 0.80, 1.20, 1.60, and 2.50 ug/g.

Following analysis, each food mixture was molded in holes in a plexiglass sheet. These holes had been drilled to the diameter necessary to obtain food pellets weighing 0.2 g (approximately 5 percent of the mean wet weight of the crabs). The plexiglass sheets were slightly frozen, and the food pellets were forced out of the molds and the amount needed for each daily feeding was packaged and frozen for later use.

Experiment II

The food for Experiment II was laboratory contaminated oysters (Crassostrea virginica). All oysters were collected from the Rappahannock River. Kepone free oysters (<0.02 ug/g) were contaminated by exposing different groups to a nominal aqueous Kepone concentration of 1.0 ug/l for varying time periods. The Kepone was administered in a flow-through system. The pesticide was dissolved in acetone and dosed into flowing seawater by a peristaltic pump. Actual uptake figures are presented in Figure 2. The oysters from each exposure period were blended and sampled for Kepone. These various exposure period mixtures were utilized to prepare food pellets containing the desired Kepone concentrations. Oysters of different concentrations were mixed only when necessary to produce the desired concentration.

Figure 2. Kepone uptake by *Crassostrea virginica* exposed to nominal Kepone concentrations in water of 1.0 ug/l for various time intervals.

Prior to final Kepone analysis, a small amount of unflavored animal gelatin was added to each batch of oyster meat to improve its consistency. The mixture ratio was approximately 1 g gelatin/60 g oyster mixture. Kepone analysis of these final mixtures gave the following six Kepone concentrations: nondetectable, 0.36, 0.82, 1.10, 1.64, and 2.26 ug/g. Food pellets were prepared as in Experiment I, except pellet size was increased to 0.4 g. Crabs were fed every other day rather than daily, so they were still being fed at approximately 5 percent mean body weight per day.

Preparation of food in this manner allowed control over both the Kepone concentration in the food and the actual amount fed to each crab. Weight of food pellets in Experiment I was 0.197 g (± 0.001 g; SE), in Experiment II, 0.395 g (± 0.001 g). Daily doses of Kepone (food concentration x weight food) were: Experiment I - 0.0, 0.08, 0.16, 0.24, 0.32, 0.50 ug, and Experiment II - 0.0, 0.07, 0.16, 0.22, 0.33, 0.45 ug.

Exposure Protocol

Following acclimation of the crabs the 65 day LD₅₀ experiments were initiated using the equipment described earlier. Crabs were allowed to feed for 24 hours at which time any remaining food was removed and weighed to determine actual food consumption. Only crabs which consumed all of the food presented them were used in data analysis. These crabs would therefore have a daily ingestion rate of 5 percent their mean wet weight at the beginning of the experiments. The feeding rate was not adjusted for crab weight gain during the experiment. Bioassay procedures were consistent with the guidelines of the American Public Health

Association (1975) and procedures published by the EPA (Stephan, 1975). Dissolved oxygen, temperature, and pH were measured twice daily. Salinity was not controlled but was measured daily. Flow rates were calibrated twice daily. Crab compartments were cleaned weekly by siphon vacuum.

The crabs were observed twice daily during the experiments. The initial observation followed the first feeding. Molts were counted and old carapace parts were removed. As crabs died during the experiments they were removed from the tanks, measured, weighed, and frozen for Kepone analysis.

The same experimental system was utilized for both experiments. The major differences between the two experiments can be summarized as follows:

- 1) Experiment I was performed from August to October, 1978 (28°C). Experiment II was performed from March to May, 1979 (21°C).
- 2) The food source for Experiment I was striped bass while that for Experiment II was oysters. The change was made to better approximate conditions of the earlier experiment of Schimmel et al. (1979) which indicated a 56 day LD50 for juvenile blue crabs of less than 1.9 ug/g Kepone in oyster tissue.
- 3) Crabs were fed every day during Experiment I and every other day during Experiment II. The change was made when it was noticed that during the first experiment, some crabs would often skip a feeding period but eat normally during the following period. In order to avoid this in the second experiment, the food pellet size was doubled, and crabs were fed every other day to keep them "hungry". Over the 65 day experimental periods, the average amount of food consumed was the same for crabs in both experiments.
- 4) During Experiment I there was no effort to control photoperiod or to shield the crabs from other laboratory activity. Experiment II was performed with a 14 hour light, 10 hour dark cycle and the crabs were shielded from other lab activity.

- 5) Crabs were randomly assigned to treatment tanks in both experiments. Randomization of treatment tanks on the sea table was utilized only in the second experiment. This was done to eliminate any possible effects of tank location on the table.

Metabolic Rate Determination

Metabolic rates were determined by measuring oxygen consumption of crabs in a closed chamber at the termination of the LD₅₀ experiments. All determinations were made between 1000 and 1600 hours to avoid diurnal variations. The test apparatus is shown in Figure 3. Each test chamber consisted of a plexiglass cylinder with a volume of approximately 1 liter. This chamber volume was utilized by Leffler (1972) with good results. Each chamber had a hole in one end into which a rubber stopper could be inserted. Each stopper had the severed neck of a 300 ml BOD bottle inserted through it. When the bottle stopper was inserted into the neck and the whole rubber stopper assembly inserted into the hole in the plexiglass cylinder, an airtight chamber was formed.

The chambers were kept in a water bath utilizing the same temperature water as the LD₅₀ experiment. Experiments were conducted using 10 chambers at a time (9 crabs + 1 blank). The chambers were separated by plastic sheets to avoid any visual stimulation of the crabs. The dissolved oxygen concentrations were monitored by a Yellow Springs oxygen probe inserted into the BOD bottle necks.

A live intermolt crab was placed in each chamber. Water for the chambers was supplied from the header tank used in the LD₅₀ studies. An airstone was installed in each chamber during a $\frac{1}{2}$ hour acclimation period. Following acclimation, the airstones were taken out and all

Figure 3. Apparatus for oxygen consumption determinations

air bubbles were removed from the chamber. The rubber stopper assembly was then inserted into the chambers and initial dissolved oxygen readings were taken. An electric stirrer was utilized during the measurements to maintain uniform chamber dissolved oxygen. The bottle stoppers were replaced carefully after each measurement to avoid introduction of air bubbles. At the end of 30 minutes a final dissolved oxygen reading was taken from each chamber.

The weight and displacement volume of each crab was determined after oxygen consumption measurements. Oxygen consumption ($\mu\text{l O}_2/\text{g wet weight}\cdot\text{hour}$) was computed according to the following formula:

$$\text{O}_2 \text{ consumed} = \Delta\text{O}_2/t \cdot V/W \cdot 0.7 \text{ ml O}_2/\text{mg O}_2 \cdot 1,000 \text{ ul/ml},$$

where:

$$\Delta\text{O}_2 \text{ (mg/l)} = (\text{O}_2 \text{ initial}) - (\text{O}_2 \text{ at time } t)$$

$$t \text{ (hrs)} = \text{elapsed time}$$

$$V \text{ (ml)} = \text{chamber volume} - \text{crab volume}$$

$$W \text{ (g)} = \text{crab wet weight.}$$

Q₁₀ Calculations

Q₁₀ values were computed using the mean crab oxygen consumption values between comparative Kepone doses in the two experiments. The formula used was:

$$Q_{10} = (R_2/R_1)^{10/\Delta T},$$

where:

R_2 = mean oxygen consumption during Experiment I

R_1 = mean oxygen consumption during Experiment II

ΔT = temperature difference.

Actual crab metabolic rate changes over the 7°C temperature range (Q7) were computed as simply (R_2/R_1).

Autotomization

After all metabolic determinations were completed, the capacity of the crabs to autotomize limbs when exposed to an extreme generalized stimulus was investigated. Crabs were individually placed into 60°C water and the number of limbs autotomized was recorded. This method was used by Leffler (1975) to study the effect of Mirex and DDT on crab autotomization.

Growth Measurements

All crabs died within seconds following their placement in the 60°C water. Crabs were rinsed with water to insure that Kepone contaminated food had not become absorbed into their shells. Dead crabs were weighed (wet weight), and width, body thickness, and carapace thickness were determined (Figure 4). Carapace thickness was measured by calipers. The mean of 3 measurements was recorded. All crabs were frozen for later Kepone analysis.

Kepone Analysis

Blue crabs were analyzed for Kepone by the Virginia Institute of Marine Science, Department of Ecology-Pollution. Because of small crab

Figure 4. Diagram of crab dimensions measured:
A: spine to spine width; B: mid-body
dorsal ventral thickness; C: enlarged
section of mid-dorsal carapace.

sizes and limited funds, samples were often pooled by size and sex. Whole body samples only were analyzed. Samples were thawed, weighed, blended, and desiccated with a 9:1 mixture of Quso^R G30 (precipitated silica) and sodium sulfate. The desiccated sample was homogenized and the Kepone was extracted in a Soxhlet extractor for 16 to 18 hours with a 1:1 mixture of petroleum ether and ethyl ether. Following this the sample was concentrated and cleaned with a series of solvents through a Florisil column to remove possible contaminants which could interfere with the Kepone analysis. The purified sample was analyzed for Kepone by electron capture gas chromatography (Hodgson et al., 1978; Moseman et al., 1977; Huggett et al., 1979).

Statistical Analysis

Mortality data were analyzed by a χ^2 Test of Independence at the 0.05 α level. A t-test was used on the sublethal data from treatment replicates to insure replicate equality. The data from the replicates were pooled and analyzed by a one-way analysis of variance (ANOVA). When the ANOVA showed a significant variance between treatments for any sublethal parameter ($\alpha = .05$), a Student-Newman-Keuls test was performed to determine which treatments were significantly different.

RESULTS

Exposure Water Conditions

Exposure water conditions for both experiments are presented in Table 1. Temperature was higher in the first experiment, as was salinity. Dissolved oxygen concentrations were lower during this experiment. These differences were due to the fact that Experiment I was conducted during the summer. Average pH values were the same but the range was greater during the first experiment due to the "red tide" appearance in the York River. The pH values fluctuated greatly from day to day depending on the intensity of the "red tide". These dinoflagellate blooms were dominated by Cochlodinium heterolobatum.

Mortality

Statistical analysis of total mortality data at the end of each 65 day experiment showed that, within each experiment, there were no significant differences throughout the Kepone dose ranges tested. Percent mortalities in Experiment I were found to average 34.9 percent while those for Experiment II averaged 24.2 percent (Table 2). For the second experiment, percent mortality was based on Callinectes sapidus only. The Callinectes ornatus data were excluded because there appeared to be greater mortality at the higher Kepone doses tested. Since the number

Table I
Exposure Water Conditions

	Temperature ($^{\circ}\text{C}$)		Dissolved Oxygen (mg/l)		Salinity ($^{\circ}/\text{oo}$)		pH	
	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range
Experiment I	28.0	27.0 - 29.0	6.1	2.3 - 7.9	19.9	19.0 - 21.0	7.9	7.6 - 8.4
Experiment II	21.1	20.0 - 22.5	7.8	6.4 - 9.0	15.1	14.0 - 17.5	7.9	7.8 - 8.2

Table 2

Crab mortality data for 65 day LD₅₀ experimentsEXPERIMENT I

Food Kepone Conc.	Control		0.42 $\mu\text{g/g}$		0.80 $\mu\text{g/g}$		1.20 $\mu\text{g/g}$		1.60 $\mu\text{g/g}$		2.50 $\mu\text{g/g}$	
	I	II	I	II	I	II	I	II	I	II	I	II
Tank Replicates												
No. dead	7	6	13	6	7	5	4	3	6	6	4	6
% Mortality	43.8	37.5	81.3	37.5	43.8	31.3	25.0	18.8	37.5	37.5	31.3	43.8
No. dead per treatment	13		19		12		7		12		10	
% Mortality per treatment	40.6		59.4		37.5		21.9		37.5		31.3	$\bar{X} = 34.9^{\circ}/o$

EXPERIMENT II - Callinectes sapidus only

Food Kepone Conc.	Control		0.36 $\mu\text{g/g}$		0.82 $\mu\text{g/g}$		1.10 $\mu\text{g/g}$		1.64 $\mu\text{g/g}$		2.26 $\mu\text{g/g}$	
	I	II	I	II	I	II	I	II	I	II	I	II
Tank Replicates												
No. dead	2	3	3	3	5	5	1	3	4	3	5	2
% Mortality	16.7	23.1	25.0	20.0	41.7	35.7	7.7	23.1	33.3	21.4	35.7	15.4
No. dead per treatment	5		6		10		4		7		7	
% Mortality per treatment	20.2		22.2		38.5		15.4		26.9		25.9	$\bar{X} = 24.2^{\circ}/o$

of C. ornatus was small (2 to 4 per treatment), this trend could not be statistically demonstrated. Actual crab survival data is presented in Appendix A.

There was increased mortality for crabs fed the lowest Kepone concentration in the first experiment (Table 2). Examination of these data shows that crabs in one of the duplicate tanks at this concentration experienced greater mortality (81 percent) than crabs at any other Kepone concentration tested. Since this high mortality was not evident at any other Kepone concentration tested, it was thought not to be Kepone related.

Dose/Uptake

Data concerning crab Kepone body burdens is presented in Figure 5. These data represent whole body concentrations. Examination of this graph indicates that after 65 days of ingestion, Kepone concentrations in the crabs were equal to the Kepone concentrations of the food at most of the treatment concentrations tested in both experiments. The only exception was the highest Kepone concentration tested in Experiment I (2.5 ug/g). At this treatment level the crabs showed a higher concentration of Kepone in their tissues than was in their food.

Dose/uptake curves of Kepone over time by the crabs are shown in Figures 6 and 7. These data indicate that equilibrium was not reached at any Kepone concentration tested in either experiment during the test periods. The data presented do not indicate "true" uptake by juvenile crabs since they represent crabs which died during the LD50 experiments and therefore could not be considered healthy specimens. In an actual

Figure 5. Whole body crab Kepone concentrations after 65 days of ingesting Kepone contaminated food.

Figure 6. Dose/uptake over time for crabs fed various Kepone doses during Experiment I.

Figure 7. Dose/uptake over time for crabs fed various Kepone doses during Experiment II. (Ordinate scale doubled from Figure 6)

dose/uptake experiment healthy crabs would be sacrificed for Kepone analysis at various time periods. This may not be as important in these experiments for a number of reasons. First, Kepone does not appear to be depurated by juvenile blue crabs. Second, the crabs appeared to eat normally up to the time of death.

Dietary accumulation of Kepone by the crabs after 65 days is presented in Table 3. It should be remembered that these values do not represent equilibrium values. These data indicate an increase in the dietary accumulation by crabs at the highest Kepone concentrations of Experiment I. There is also an apparent increase in Kepone assimilation by crabs during Experiment I when compared to that by crabs in the second experiment.

Male vs. Female Uptake

Male versus female uptake was compared by a t-test for each Kepone concentration tested. There was no significant difference between male and female uptake during Experiment I. The sample sizes were small due to extensive pooling of the crabs for analysis. Sample sizes for Experiment II were greater since there was less pooling. Statistical analysis of these data indicates a greater Kepone uptake by females than males at two of the five Kepone concentrations tested (0.82 and 1.64 ug/g) ($\alpha = .05$). At the other three Kepone concentrations there was no significant difference in Kepone uptake between female and male crabs after 65 days.

Table 3

Dietary accumulation factors of crabs after 65 days
(Crabs fed 0.2g food/day)

	(A) Food Kepone Conc. ($\mu\text{g/g}$)	(B) Total Kepone Fed/Crab (μg)	(C) Mean Crab Kepone Conc. ($\mu\text{g/g}$)	(D) Total Kepone Per Crab (μg)	(E) Kepone Assimilation (%)*	(F) Dietary Accumulation Factor**
Experiment I	0.42	5.46	0.38	3.23	59	13
	0.80	10.40	0.76	6.91	66	18
	1.20	15.60	1.42	12.85	82	24
	1.60	20.80	1.73	16.05	77	22
	2.50	32.50	4.61	32.70	100	37
Experiment II	0.36	4.68	0.39	2.36	50	22
	0.82	10.66	0.57	3.02	28	14
	1.10	14.30	1.28	7.09	50	23
	1.64	21.32	1.48	9.44	44	18
	2.26	29.38	2.54	14.15	48	23

* Kepone Assimilation (%) = (D)/(B) X 100

** DAF = $C_a / C_f(W_f/W_a)$; Denominator = daily dietary dosage level per gram crab

Sublethal Effects (data presented in Appendix B)

Growth

Percent increase in width, mid-body thickness, and wet weight per molt was calculated for each live crab at the end of the LD₅₀ experiments. None of these parameters showed any significant change over the range of Kepone concentrations tested during either experiment. The average percent increase/molt (standard deviation) during Experiment I was, width - 16.2 (4.7); mid-body thickness - 18.5 (4.7); and wet weight - 81.2 (24.8). That during Experiment II was, width - 10.2 (2.7); mid-body thickness - 9.6 (4.2); and wet weight - 33.3 (12.3).

Carapace Thickness/Width Ratio

Carapace thickness/width ratios were compared for all live crabs which molted during the experiments. For crabs in Experiment I this ratio was inversely related to Kepone concentration ($\alpha = .01$). The ratio ranged from a mean of 5.15×10^{-3} for control crabs to a mean of 3.40×10^{-3} for crabs fed food contaminated with 2.5 ug/g Kepone (Figure 8a). Crabs from Experiment II did not show this relationship (Figure 8b). There was no significant difference between carapace thickness/width ratios over the range of Kepone concentrations studied.

Molting

The average number of molts per crab did not differ significantly at any Kepone concentration tested in either experiment. Crabs in the first experiment molted an average of 2.14 times while those in Experiment II averaged only 0.8 molts per crab (Table 4).

Autotomization

There were no significant differences in the number of limbs

Figure 8. The relationship between Carapace Thickness/Width Ratio and Kepone dose for Experiment I(a) and II(b).

Table 4

Molt data for crabs fed various Kepone doses for 65 days

<u>Experiment</u>	<u>Food Kepone Conc. (μ g/g)</u>	<u>Total # Molts</u>	<u>Crabs surviving</u>	<u>Average Molts/Crab</u>
I	0.00	42	18	2.33
	0.42	31	15	2.07
	0.80	35	17	2.06
	1.20	53	22	2.41
	1.60	38	19	2.00
	2.50	41	21	1.95
II	0.00	15	21	0.71
	0.36	19	20	0.95
	0.82	12	16	0.75
	1.10	19	23	0.83
	1.64	12	20	0.60
	2.26	20	21	0.95

autotomized by crabs exposed to the various Kepone treatments. The average percent limbs autotomized during Experiment I was 61.2 (SD = 26.0), while the average for Experiment II was 56.3 (SD = 25.3).

Oxygen Consumption

The highest Kepone concentration caused a significant increase ($\alpha = .01$) in oxygen consumption in both experiments (Figures 9a and 9b). A Student-Newman-Keuls Test performed on the data from Experiment I indicated that the mean oxygen consumption (318.0 ul O_2 /g·hr) of crabs exposed to the highest Kepone treatment was significantly greater than the levels observed for crabs at all other treatments. Crab oxygen consumption was not significantly different at the five lowest treatments. The mean oxygen consumption of control crabs was 210.9 ul O_2 /g·hr.

Data from Experiment II indicates a similar trend, but not as pronounced. As in the first experiment, oxygen consumption by crabs fed the highest Kepone treatment (157 ul O_2 /g·hr) was significantly greater than that at the three lowest treatment levels (control, 0.36, and 0.82 ug/g). The oxygen consumption of the crabs at the highest concentration was not significantly different from that for crabs fed 1.10 and 1.64 ug/g Kepone.

Q7 and Q10 Information

Q7 and Q10 values for crabs at various Kepone treatments in the two experiments are presented in Table 5. These data indicate that there is an average doubling in metabolic rate of juvenile blue crabs when the temperature is increased from 21°C to 28°C.

Figure 9. The relationship between Oxygen Consumption and Kepone dose for Experiment I(a) and II(b).

Table 5 - Q_{10} and Q_7 values computed from average oxygen consumption for equal Kepone treatments from the two experiments

Food Kepone treatment ($\mu\text{g/g}$)	EI EII	Control Control	0.42 0.36	0.80 0.82	1.20 1.10	1.60 1.64	2.50 2.26
Mean crab Kepone conc. ($\mu\text{g/g}$)	EI EII	<0.02 <0.02	0.38 0.39	0.76 0.57	1.42 1.28	1.73 1.48	4.61 2.54
Average Temp. ($^{\circ}\text{C}$)	EI EII	28 21	28 21	28 21	28 21	28 21	28 21
Mean O_2 consumption ($\mu\text{l } \text{O}_2 / \text{g}\cdot\text{hr.}$)	EI EII	210.9 88.3	198.7 78.5	220.5 68.8	243.0 117.8	225.4 132.2	318.0 157.7
Q_{10}^*		3.46	3.77	5.28	2.81	2.14	2.72
Q_7^{**}		2.39	2.53	3.20	2.06	1.70	2.02

$$*Q_{10} = (\text{O}_2 \text{ consumption E.I} / \text{O}_2 \text{ consumption E.II})^{(10/\text{temp. E.I} - \text{temp. E.II})}$$

$$**Q_7 = (\text{O}_2 \text{ consumption E.I} / \text{O}_2 \text{ consumption E.II})$$

Behavioral Observations

The behavior of the crabs was observed throughout the experiments, especially during feeding periods. The only significant behavioral difference between crabs in different treatments was related to what Leffler (1975) and Schimmel et al. (1979) called "excitable feeding". Very early in each of the present experiments, crabs at the highest treatment levels tended to exhibit highly excited feeding behavior. During Experiment I, 78 percent of the crabs at the highest Kepone treatment exhibited this behavior, while during the second experiment the figure was 67 percent at the highest treatment level. The number of crabs exhibiting consistently excitable behavior was significantly greater (g-test, $\alpha = .05$) at the highest Kepone concentration than at all other treatments in both experiments.

The behavior appeared to be related to a loss of nervous coordination, as will be discussed later. It should be noted that the type of excitable feeding behavior differed between experiments. During Experiment I the excitability was evident in the form of rapid, random movement throughout the compartment and, quite often, the inability of the crab to find its food. During the second experiment, the crabs were able to locate the food more easily, but would hold it for a time without eating while moving around their compartments. When feeding began, the crabs appeared to have a much more difficult time in controlling their mouthparts, and with actual food manipulation. Mouthparts were moved rapidly with much breaking apart of the food. Control crabs tended to hold the food calmly with little food breakage.

DISCUSSION

Mortality

The LD₅₀ value for juvenile crabs fed Kepone is above a daily dose of 0.5 ug (2.5 ug/g x 0.2 g food per day). In neither experiment was there a significant difference in mortality between control crabs and crabs at the highest Kepone treatment.

The low percent mortality reported here is at odds with previously reported data of Schimmel et al. (1979). They found 80 percent mortality after 56 days for juvenile blue crabs fed 1.9 ug/g Kepone contaminated oysters (temperature of 25.5°C). The crabs were fed 0.5 g of food twice per week, or 0.95 ug Kepone per feeding, assuming the crabs ingested all they were fed. This is not a normal feeding pattern for crabs, which in nature are almost continuously feeding. This high feeding dose, separated by 3.5 days of no feeding, could have been abnormally stressful to the crabs. Although the crabs in the present study were fed a greater amount of Kepone per given time period than in Schimmel's study (1.75 ug vs. 0.95 ug Kepone per 3.5 days at the highest treatment levels), they were not dosed heavily and then starved for 3.5 days. This continuous daily feeding schedule better represents normal crab feeding behavior.

Another possible explanation for the differential mortality between the two studies may be the contribution of some "outside" factor. In

the present study one of the low Kepone treatment levels caused 81 percent mortality for crabs in one of the duplicate tanks. The duplicate tank for this treatment and all bracketing treatments did not show this high crab mortality. Therefore, the deaths were attributed to some unidentified second toxicant or infection. Schimmel may have had a similar problem but failed to recognize it due to the lack of replication and bracketing Kepone treatments.

A decrease in total percent mortality (including controls) was observed from Experiment I to II. This reduction may have been due to the reduced experimental temperature of the second experiment. Leffler (1972) found that mortality in juvenile blue crabs is directly proportional to temperature between 13° and 34°C and is especially high during molting at elevated temperatures. This has been related to the crabs increased metabolic rate at the higher temperature and during molting, and the reduced oxygen carrying capacity of the water. Crabs would die from asphyxiation at the higher temperature, especially during molting when oxygen consumption doubles. In this study it was found that crab molting frequency tripled at the high temperature. Chances of molting deaths would therefore increase.

It is possible that physical changes in the bioassay system prior to Experiment II may have helped decrease mortalities. These changes were outlined in the Materials and Methods section. The absence of the "red tide" during the second experiment may also have reduced mortalities. Although there were no deaths directly attributable to "red tide" in the first experiment, an increase in stress caused by the rapid fluctuation of pH values caused by the "red tide" may have reduced the crab's survival ability.

Dose/Uptake

Crabs did not reach an equilibrium body burden at any Kepone dose tested in either experiment (Figures 6 and 7). Kepone analysis of crabs at the end of the 65 day experiments does indicate that at the highest Kepone dose in Experiment I, there was biomagnification of Kepone above concentration levels in the food.

Biomagnification has been defined as the process by which the tissue concentrations of bioaccumulated chemical residues increase as these materials pass up the food chain through two or more trophic levels (Macek et al., 1979). In order to have biomagnification, a pesticide must be readily absorbed from food, and once assimilated, it must be relatively resistant to metabolism or excretion. Schimmel et al. (1979) found that Kepone is not depurated once assimilated by juvenile blue crabs. Bryan (1979), in his review of bioaccumulation of pollutants, indicates that in organisms which store a contaminant without excretion, there appears to be a tendency to concentrate that contaminant with age unless growth of new tissue is sufficiently rapid. Concentrations of cadmium in the bivalve Scrobicularia plana do not increase with size or age at low levels of exposures, but increase appreciably at higher levels. A similar type of observation was made by Boyden (1977) on the limpet, Patella vulgata, which also stores cadmium. The concentration of cadmium undergoes growth dilution at low levels but at higher levels, growth of new tissue is not fast enough to offset the increased cadmium level. It is possible that growth dilution of Kepone at low doses in juvenile crabs keeps the tissue concentrations low, but at high doses the growth of new tissue is overwhelmed and high tissue concentrations

occur. Biomagnification would occur when crab tissue concentration levels became higher than the food concentration levels.

Another possible explanation for the increased Kepone uptake at the high doses comes from earlier work on organochlorine compounds and their relationship to the lipid content of an organism. Buhler et al. (1969) found increasing lipid content in coho salmon with increasing DDT content in the food. Macek et al. (1970) showed the same effect of DDT and Dieldrin on lipid content of rainbow trout. These authors noted these changes in their lipid studies but had no explanation for them. The same relationship could hold for Kepone and lipid content in crabs. Since Kepone is lipid soluble, an increase in lipid content would allow crabs at high Kepone doses to assimilate more than those at low doses.

The Dietary Accumulation Factors (DAF) presented in Table 3 indicate the ability of blue crabs to take up Kepone from their food. The formula is derived from first order kinetic equations for uptake/deposition (Hamelink, 1977; Metcalf, 1977). These equations take into account the daily dietary dosage level per gram of crab, not per gram of food. The DAFs presented indicate that at the highest Kepone dose in Experiment I crabs accumulated almost twice the amount of Kepone than at any other treatment level. DAFs at the other treatment levels were similar. Kinetic uptake/deposition theory predicts that the DAFs should be the same at all treatments, once equilibrium is reached. Since the DAFs presented are not equilibrium values, they represent underestimates of the final DAF values. Reasons for the high DAF at the highest treatment level in Experiment I are not clear. The increased metabolic rates of the crabs at this treatment level may have had some effect on the uptake rate constants. Further study is needed on the effects of changing

metabolic rates and temperatures on uptake/depuration rate constants of the blue crab.

It is suggested from this uptake data, and other work on Kepone uptake from water, that dietary accumulation of Kepone from food is the more important mechanism for explaining environmental Kepone residues in the blue crab. Kepone in seawater appears to be relatively nontoxic to adult blue crabs (Schimmel and Wilson, 1977). Bioconcentration did occur, but was measurable only at Kepone levels in the water greater than 110 ug/l. Measurable Kepone residues were not found in crabs exposed to concentrations lower than this. Schimmel et al. (1979) indicated that crabs fed for 28 days on oysters contaminated with 0.25 ug/g Kepone accumulated a mean whole body Kepone concentration of 0.1 ug/g. This was true regardless of whether they were exposed during feeding to clean water or water contaminated with 0.3 ug/l Kepone. Crabs exposed only to water contaminated with 0.3 ug/l Kepone for 28 days showed no detectable levels in their bodies.

Crabs, therefore, would not be expected to bioconcentrate Kepone from the estuarine waters of the James River to any appreciable degree since water concentrations of Kepone in the river ranged from non-detectable levels to 1.20 ug/l, with most of the values skewed towards the non-detectable level (Lunsford et al., 1980). On the other hand, natural vertebrate and invertebrate food sources of blue crabs in the James are contaminated with 0.09 to 2.0 ug/g Kepone on the average (Bender et al., 1977). Holland et al. (1971) found that juvenile crabs can consume up to 55 percent of their body weight per day under natural conditions. The chance of a juvenile crab consuming large quantities

of Kepone contaminated food in nature is therefore much greater than its chance of coming in contact with highly contaminated water masses.

Another possible source of Kepone for crabs is bottom sediment or suspended sediment. Crabs spend much of their time buried in and moving on the sediment layers where Kepone has been found to be highly concentrated. This possible source of contamination needs to be investigated to determine its input, if any, to crab Kepone residues.

Macek et al. (1979) recently concluded that dietary accumulation and biomagnification of chemical residues within aquatic food chains is quantitatively insignificant when compared with the process of bioconcentration of chemical residues directly from water. They state that DDT appears to be the only chemical for which they found a potential for significant dietary accumulation through an aquatic food chain. These conclusions are based on a relatively small number of experiments on simple food chains. Work on catfish and Mirex (Colins et al., 1973) and blue crabs and Kepone (Schimmel et al., 1979; Schimmel and Wilson, 1977) indicates that other simple food chains give completely different results. In these food chains dietary accumulation appears to be the major source of chemical residues. It should be noted that Macek et al. (1979) indicated that Kepone should show little dietary accumulation in a food chain, based on work done by Bahner et al. (1977).

The Macek et al. (1979) publication also concluded that the persistence of a chemical in the environment, as measured by its depuration rate, would be an indicator of the relative importance of dietary accumulation to residues levels in organisms. DDT is given as an example. Its very slow depuration rate indicates that dietary accumulation would

play an important role in DDT residue levels in organisms. The general first order kinetic equations for uptake and clearance of a pollutant (Hamelink, 1977; Metcalf, 1977) show that clearance rates are independent of the type of uptake occurring (i.e., bioconcentration or dietary accumulation). If these kinetic equations are accepted along with the hypothesis of additivity, there does not appear to be much basis for the hypothesized relationship between depuration rates and accumulation. Unless Macek et al. (1979) have other data to show a specific link between slow depuration and high dietary accumulation, then their depuration conclusion represents nothing more than a simple observation on a very small sample.

Male vs. Female Uptake

There appeared to be some differential Kepone uptake between the sexes at two of the five treatment levels in Experiment II. At these treatment levels whole body Kepone concentrations of females were significantly greater than concentrations in males. Bracketing Kepone treatments however did not indicate this differential uptake between the sexes.

Differential internal partitioning of Kepone by adult crabs has been demonstrated by Roberts (1980). His work indicates that the hepatopancreas of female crabs concentrates Kepone while that of the male does not. This is thought to be one of the reasons for the higher Kepone concentrations in the backfin muscle of the male crab. Another possible reason would be the extrusion of Kepone in the egg masses of female crabs. This differential Kepone partitioning has not been related

to adult whole body Kepone concentrations and has not been studied to any extent in juvenile crabs. It does indicate that there are differences between the way Kepone behaves in female and male crabs. Partitioning studies, utilizing labeled Kepone in food, would be necessary to determine if differential partitioning exists in juvenile crabs, and if so, whether it could affect whole body Kepone concentrations.

Sublethal Effects

Growth

Increased Kepone treatment levels did not have any significant effect on crab width increase, mid-body thickness increase, or wet weight increase per molt. In order to extensively study growth in juvenile crabs, a much longer experimental period may be necessary. For example, Leffler (1972) used a test period of 540 days to show that increased temperatures resulted in a reduction of growth per molt in crabs.

Carapace Thickness/Width Ratio

Carapace thickness/width ratios were inversely related to Kepone dose in Experiment I. This is indicative of a decrease in carapace thickness since there were no significant differences in shell width increases per molt between crabs. Leffler (1975) found the same carapace thinning effect in his Mirex studies on juvenile blue crabs. Reduced carapace thickness could have a detrimental effect on juvenile crab survival. Thin carapaces which occur following Kepone ingestion would reduce shell strength. The probability of successful predator attacks on such crabs would increase. This could cause an overall reduction in the number of juveniles reaching the reproductive stage.

Persistent organochlorine pesticides have been implicated in the past in thinning or reduction in bulk of other calcified tissues. Immature Roman snails (Helix pomatia L.) fed small amounts of DDE grow thinner shells than controls (Cooke and Pollard, 1973). Peakal (1970a and b) found that several organochlorine hydrocarbons, when ingested by birds, caused a significant reduction in egg shell thickness. Recent work by Eroschenko and Place (1977, 1978) showed that ingestion of Kepone by Japanese quail (Coturnix coturnix japonica) resulted in significant shell thinning and weakening.

Brown (1978) concluded that egg shell thinning induced in birds by DDE is due to a calcium deficiency. The probable cause of this deficiency was linked to ATP-ase inhibition by DDE. Other possible causes included inhibition of the carbonic anhydrase enzyme, malfunctions of the thyroid gland, and an upset in the balance of the reproductive hormone estrodial. A combination of these factors is most likely involved. Fingerman (1973) indicated that crustacean molting and carapace formation is a complex process involving numerous enzymatic reactions mediated by neuro-secretions and hormones. A complex inhibition process by Kepone and Mirex is most likely involved in the reduced carapace deposition in crabs.

The carapace thinning effect was not seen in the second experiment, a fact which is likely a function of the reduced molting frequency. With only an average of 0.8 molts per crab in this experiment, the thinning of the carapace may not have had a sufficient chance to manifest itself. This supports the earlier contention that Kepone induced growth effects are highly dependent on the length of the experiments.

Molting

The average number of molts per crab was not significantly different at any of the Kepone doses tested in either experiment. The average number of molts was reduced significantly in the second experiment. Schimmel et al. (1979) indicated that molting was reduced with increased Kepone dose. In fact, they found decreased molting frequencies at Kepone concentrations in the food as low as 0.15 ug/g (0.08 ug Kepone per feeding).

It is difficult to explain the discrepancies in the results for molting rates between the two experiments. The number of molts by control crabs in both studies was similar when one considers the temperature effects noted by Leffler (1972). An unidentified second toxicant or infection may have weakened the crabs in the study of Schimmel. Another factor may have been the feeding schedule utilized by Schimmel, as discussed earlier. In nature a crab is almost continuously searching for food and eating. The abnormal feeding regime utilized by Schimmel may have put increased stress on the crabs. As mentioned earlier, crab molting is a complex process involving neurosecretions (X-organ-sinus gland-complex) and hormones (Y-organ). Because of the close relationship between the crabs nervous system and endocrine system, any undue external stress could result in inhibition of the molt cycle.

Autotomization

The ability of crabs to autotomize limbs did not appear to be significantly reduced by increased Kepone doses. Leffler (1975) found that Mirex, when fed to juvenile blue crabs over a 5 week period, caused a significant reduction in the autotomization ability when the crabs

were exposed to an extreme generalized shock (60°C water). All three Mirex doses tested (1.5, 3.0, and 3.5 ug/g) had the same effect on autotomization.

It appears that Kepone does not act like Mirex in this regard. For some reason, it works more like DDT which does not cause autotomy inhibition at subacute levels (Leffler, 1975). The reason for this is not clear. It would seem that Kepone would act more like Mirex in its effect on crabs, being such a close chemical relative. In fact, its effects on crab carapace thinning and metabolic rate elevation are very similar to Mirex, as would be expected.

Oxygen Consumption

Kepone concentrations in food of 2.5 ug/g in the first experiment and 2.26 ug/g in the second caused significant increases in juvenile blue crab metabolic rates, as measured by oxygen consumption. This finding agrees with earlier studies concerning the effects of organochlorine pesticides on crab metabolic rates.

DDT and other organochlorine pesticides have an unstabilizing effect on normal nerve and muscle function (Yeager and Munson, 1945; Roeder and Weigont, 1945, 1948; Bodenstein, 1946; Welsh and Gordon, 1947). These unstabilizing effects are caused by alteration of axon membrane permeability to Na^+ and K^+ ions which derange or inhibit action-potential transmission (Brown, 1978). The unstabilizing effects include lowered response thresholds and repetitive after discharges of nerves (Narahashi and Yamasaki, 1960; Narahashi and Haas, 1968). Leffler (1975) found a similar response for crabs exposed to dietary Mirex. The increased muscular and nervous activity must substantially increase the

metabolic rates of these tissues, thereby increasing the overall crab metabolic rate.

The highest metabolic rates were exhibited by crabs fed the highest Kepone dose at the highest experimental temperature (28°C). Temperature levels of this magnitude occur during mid to late summer in the James River, a time when there is rapid growth of juvenile blue crabs. Corresponding low dissolved oxygen levels accompany these high temperatures. Crabs suffering these Kepone related metabolic rate increases during these periods could have difficulty in obtaining adequate oxygen, especially during molting when metabolic rates normally double in healthy crabs. Leffler (1972) found that increased mortality during molting does in fact occur when metabolic rates are elevated by high temperatures. In areas which normally suffer reduced dissolved oxygen concentrations during the summer months, the increased metabolic rates of Kepone contaminated crabs could clearly lead to increased mortality.

Q7 Values and Temperature Related Effects

Crab metabolic rates doubled with the increase in temperature from 21°C to 28°C. This doubling was evident in control crabs as well as experimental crabs. One effect of this temperature induced metabolic rate difference was reduced mortality during Experiment II, as discussed earlier.

The increase in molting frequency at the higher temperature was also quite pronounced. Leffler (1972) found that molting frequency in juvenile blue crabs increases with temperature. Schimmel et al. (1979) found the average number of molts per control crab to be 1.4 for 56 days

at 25.5°C. Using the average molts per control crab in the present study and the study of Schimmel et al. (1979), one sees an almost linear increase in molting rate with increasing temperature from 21° to 28°C, as predicted by Leffler.

Behavioral Observations

High levels of Kepone fed to juvenile crabs caused a significant increase in excitability, especially during feeding. This excitability is most likely caused by the unstabilizing effect of the pesticide on nerves and muscles, as discussed earlier. This could result in a loss of nervous and muscular coordination which could manifest itself as "excitable behavior".

This excitability could make food capture more difficult for the crabs. Crabs at the highest Kepone treatment doses were sometimes so excited that they were unable to find their food, even in the small compartments. The problems of food location would be tremendously increased in nature. This excitable behavior could also tend to make contaminated crabs easier prey. Crabs in nature tend to remain very still to avoid detection, only moving when vigorously threatened. The crabs then move quickly for a short distance and hide again. After-discharges of nerves and muscles could act to give away the crab's location to its predators. Combined with reduced carapace thicknesses, this could act to decrease the crab's ability to avoid and survive predation.

CONCLUSIONS

Blue crab populations in the James River have continuously declined over the past decade. The results of this study suggest the possibility that Kepone contamination of the river may be a factor in this decline. Studies on actual field populations of crabs in the James need to be undertaken to determine if the effects shown in these laboratory experiments can be detected in naturally contaminated crabs. If so, a stronger relationship between Kepone and crab population decline could be shown.

Acute mortality does not appear to be the problem. The experimental data indicates a 65 day LD₅₀ value greater than 0.5 ug Kepone per day (2.5 ug/g x 0.2 g food per day). Bender et al. (1977) found Kepone contamination of blue crab food sources to range from 0.09 to 2.0 ug/g. Thus, crabs in nature would not likely come in contact with large amounts of food with a Kepone concentration greater than 2.5 ug/g.

Sublethal effects of Kepone on juvenile crabs could reduce the probability of juvenile survival to maturity. Increased excitability, reduced carapace thicknesses, and increased metabolic rates resulting from high Kepone body burdens could lead to increased juvenile mortality. Crabs could be less successful in finding food and avoiding predation. Low dissolved oxygen levels in the river water could result in high crab mortality as a result of Kepone induced high metabolic rates.

The above mentioned sublethal effects developed quite rapidly. An average of only two molts was necessary to show significant changes in carapace thicknesses and metabolic rates. Nervous, excitable behavior was evident after only a few weeks. Even though crabs develop from egg to egg in approximately two years, I believe these changes are quick enough to affect populations, especially when one considers the persistence of Kepone in the environment.

APPENDIX A
Crab Survival Data
(Survival Time in Days)

Experiment I
Number Of Days Each Crab Survived

Kepone Treatment (ug/g) Reps	Control		0.42		0.80		1.20		1.60		2.50	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab												
A	65		27		65		51		65		25	
B	65	20	43	65	42	65	65	65	65	65	65	65
C	20	65	22	65	48	65	34	65	55	65	65	55
D	49	65	35	41	65	65	65	65	65	65	65	65
E		53		47		65		65		65		65
F	37		65		24		65		35		29	
G	65	65	58	65	65	15	65	65	34	65	65	65
H	65	65	42	29	65	65	65	65	65	65	19	65
I	34	65	15	65	65	8	65	65	51	65	65	44
J		65		65		23		65		15		44
K	65		33		49		65		65		65	
L	24	29	59	65	65	65	65	65	41	47	65	17
M	38	65	65	65	65	65	65	65	65	65	65	65
N	65	65	24	65	37	65	23	54	65	37	35	65
O		65		17		13		65		48		54
P	65		35		40		65		65		65	
Q	25	52	36	53	65	65	21	48	65	65	65	65
R	65	35	34	65	28	65	65	65	65	56	32	42
S	65	48	65	46	65	53	65	48	34	65	65	65
T		65		65		65		65		65		65

Experiment II

Number Of Days Each Crab Survived
(#° - indicates C. ornatus species)

Kepone
Treatment
(ug/g)
Reps

Crab	A	Control		0.36		0.82		1.103		1.64		2.26	
		I	II	I	II	I	II	I	II	I	II	I	II
		65°		65°		57°		65		65		50°	
	B	65	56	59°	65	54°	65	26	65	56°	65	65	44°
	C	20	65	65°	65°	58	65	65	65	65	56°	30	65
	D	65	37	65	65	40	65	65	65°	54°	49	65	45
	E		65		65		65		65°		45°		62°
	F	65°		65		65		61°		56°		33°	
	G	65	65	65	65	65	9	48°	65	65	65	65	49
	H	65°	65°	65	65	65	65°	65	65	65	46	65	65
	I	65	65	65	65	65	65	65	65	65	65	65	65
	J		65		65		65		65		65		65
	K	12		45		38°		65		56°		20	
	L	65	63	65	61°	65	12	65°	52°	10	65	60	65
	M	65	65	35	65	65	65	65	54	65	65	24	65
	N	65	65°	65	29	65	49	65	65	65	65	65	65
	O		65		65		53		60		65		65
	P	64°		12		65		65		59		65	
	Q	65	65	65°	65	45	65	65	65	25	47	65	65
	R	65	65°	65	65	35	12	65	65	65	65	56	65
	S	65	65	65	65	54	65	65	65	32	65	65	65
	T		65		32		62		55		65		39

APPENDIX B

Sublethal Data

Note: Data on all living crabs at the end of the LD₅₀ experiments were not used for the sublethal effect analysis. Only crabs which ingested all the food given them were used. Crabs also had to molt at least once during the study to be included in the growth and carapace thickness analysis. Crabs which had lost more than two appendages were not used in the sublethal work.

Experiment I

1 - % Width Increase/Molt

Kepone

Treatment

(ug./g)

Reps

	Control		0.42		0.80		1.20		1.60		2.50	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab												
A	23.1				12.0	19.7	20.7	22.0	15.6	13.2	23.8	12.2
B	10.5					13.1		15.4	9.0	13.7	10.9	
C		9.5				12.4	19.2	18.5		9.6	12.9	10.6
D					17.6			15.6		15.6		12.7
E							18.6					
F			12.7									
G	30.4	30.2	7.5	14.5	18.2			11.9		13.7	15.2	17.5
H	15.4	14.9			15.6	14.9	20.7	13.0	18.8	15.3		11.7
I		7.1		24.7			10.0	11.7		15.8	14.5	
J		16.2		16.0				8.7				
K	14.7								14.9		14.5	
L			3.7	19.6	19.8	18.5	21.8	18.2			15.2	
M		21.7	12.9	20.1	17.0	17.4		17.9	10.9	11.5	19.5	20.3
N		15.8		27.9		23.5			18.5			12.2
O		11.4						14.3				
P	20.9						11.8		17.3		18.8	
Q					13.6	15.6		15.9		13.1	29.5	28.8
R	21.7			11.8		16.4	13.0		18.5			
S	13.7		13.7		19.2		13.1	16.1		11.7	18.4	11.9
T		17.6		10.1						14.5		10.8

Experiment I

2 - % Mid Body Thickness Increase/Molt

Crab	Treatment (ug/g) Reps	Control		0.42		0.80		1.20		1.60		2.50	
		I	II	I	II	I	II	I	II	I	II	I	II
A	19.1					11.9		20.1	17.6	17.1	18.0	29.9	15.9
B	10.8				18.8		20.5		17.6	10.3	18.0	29.9	15.9
C			15.5		15.1		12.3		18.2		18.4	17.3	
D						20.3	16.7	17.7	26.8		16.1	20.9	15.9
E									25.6		21.4		13.7
F				25.3				19.7					
G	32.5		26.6	6.3	17.2	20.2			15.8		14.6	14.3	13.5
H	19.8		19.2			18.0	16.5	20.4	16.1	20.7	21.1		13.5
I			17.4		34.3			15.9	16.1		19.6	17.8	
J			15.4		18.4				13.6				
K	21.7									18.6		20.0	
L				9.1	16.7	22.2	18.4	23.1	21.64			19.0	
M			26.0	19.1	12.5	19.4	18.8		22.8	15.2	15.0	20.2	22.2
N			16.1		31.5		20.4			18.2			15.2
O			16.7						18.0				
P	21.5							16.7		21.0		19.0	
Q							16.9				18.7	27.5	31.7
R	19.1				14.2		17.3	17.8	18.0	20.6			
S	14.7			15.2		17.1		17.1			18.2	20.0	13.5
T			13.3		13.3				21.4		19.5		11.7

Experiment I

3 - Wet Weight Increase/Molt

Kepone Treatment ($\mu\text{g/g}$) Reps	Control		0.42		0.80		1.20		1.60		2.50	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab A	101.5								81.5			
B	49.1			60.9	48.2	95.4	91.1	103.0		78.3	90.2	57.5
C		53.2		62.2		42.5		83.1		74.0	48.8	
D					104.5	60.7	91.5	89.2		36.2	75.6	62.2
E								120.3		78.1		41.1
F			73.1				105.5					
G	154.5	146.1	55.5	98.3	107.6			58.3		52.9	65.7	81.4
H	89.5	75.5			74.7	83.9	112.4	80.1	101.1	91.8		64.8
I		45.8		142.8			69.1	61.3		80.4	76.5	
J		82.1		68.4				31.0				
K	84.3								78.1		67.8	
L			67.1	71.1	115.6	97.7	119.8	100.5			84.3	
M		142.2	78.4	67.1	99.3	55.1		107.6	56.1	48.8	98.4	72.9
N		76.6		136.2		104.5			102.5			78.0
O		43.6						68.9				
P	111.7						67.3		78.4		96.2	
Q					45.7	75.2				71.5	110.4	138.9
R	121.5			57.2		86.9	65.5	84.3	97.1			
S	70.7		93.6		80.7		82.6			72.2	88.7	65.7
T		85.6		63.8				70.3		89.3		70.3

Experiment I

4 - Carapace Thickness/Width Ratio
(X10⁻³)

Kepone Treatment ($\mu\text{g/g}$) Reps	Control		0.42		0.80		1.20		1.60		2.50	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab												
A	6.17				4.29	4.36	4.06	4.86	3.85	4.15		3.00
B	4.49					4.98				4.34	3.98	
C		4.14										
D					4.42	3.57	4.23	3.97	3.57	3.04	3.29	3.49
E								3.78		3.67		3.21
F			3.89									
G	4.17		5.01	4.84	4.15			3.39		3.93	3.52	
H	4.84	6.15			4.09	2.87	3.08		3.70	4.42		4.14
I		4.49					4.90	3.60		3.93	3.95	
J		4.57						3.84				
K	4.81								3.62		2.97	
L			5.51	3.85	4.18	4.97	3.86	4.48			2.86	
M		7.01	4.27	4.68	4.81	4.83		3.96		2.93	3.11	2.27
N		5.28		5.25		4.66			3.02			3.53
O		5.98										
P							3.89		3.40		3.16	
Q					4.41	3.55				4.59	3.62	3.48
R				4.68		3.69	4.36	4.22	3.53			
S	4.87		4.87		3.06		3.92			4.07	3.47	4.04
T				4.16				3.44		3.15		3.57

Experiment I
5 - Molts/Crab

Kepone Treatment (ug/g) Reps	Control		0.42		0.80		1.20		1.60		2.50	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab A	2				2				1			
B	2			2		2	2	2	3	2	1	2
C		2		3		2	3	3		2	1	
D					2	2	3	2		1	2	2
E								3		2		2
F			2				3					
G	2	2	2	3	3			2		2	3	3
H	2	2			1	3	3	3	2	2		2
I		1		2			2	2		2	2	
J		4		2				2				
K	2			2					2		2	
L			1	3	3	2	3	2			2	
M		2	2	2	2	1	3	3	2	2	3	2
N	3	3		2		2			2			2
O		2						2				
P	3						2		2		2	
Q					1	3				2	1	1
R	3			2		2	3	3				
S	2		1		2		2	2		2	2	2
T		3		2				2		2		2

Experiment I
6 - % Appendages Autotomized
(Crabs with 9-10 appendages at the start
of the autotomization experiment)

Kepone Treatment (ug/g) Reps	Control		0.42		0.80		1.20		1.60		2.50	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab A	66.6				80.0							
B				60.0		77.8	90.0	88.9	80.0		77.8	
C								70.0			55.6	
D					90.0	0	60.0	0	80.0			80.0
E								11.1		60.0		90.0
F							70.0					
G		90.0		44.4	77.8							
H	77.8	100.0				66.7	30.0		90.0	80.0		
I				50.0						70.0	88.9	
J		70.0		11.1								
K									60.0			
L					60.0	60.0	50.0	60.0			90.0	
M		10.0	50.0								90.0	
N					60.0	60.0		20.0	50.0			60.0
O												
P	77.8					66.7						
Q						66.7					88.9	20.0
R	60.0					40.0			20.0			
S			0		80.0		20.0			70.0	90.0	70.0
T		70.0		44.4						80.0		60.0

Experiment I

7 - Oxygen Consumption ($\mu\text{l O}_2/\text{g}\cdot\text{hr}$)

Kepone

Treatment

($\mu\text{g/g}$)

Reps

	Control		0.42		0.80		1.20		1.60		2.50	
	I	II	I	II	I	II	I	II	I	II	I	II
Crabs	A	221.17			345.93		212.96	212.01			371.48	293.65
B				278.54		190.44					326.13	
C		142.93				166.21			262.14			
D					251.85	204.31	326.26	206.58	228.81		287.05	298.91
E									233.38			430.77
F			279.05				357.00					
G	193.85	170.16	233.31	199.59	167.81							
H	201.55				118.45	214.47	242.85		242.84	214.90		
I				191.05						212.86	342.91	
J		178.20										
K	249.09								280.52		280.32	
L			147.56			392.99	138.91					300.32
M			159.20	115.28				256.64				
N		284.03						233.79				
O									198.80			
P												
Q												
R	263.11			163.03					182.23			
S	162.93		219.95		152.03					197.26	263.04	303.17
T		252.48										

Experiment II

8 - % Width Increase/Molt

Kepone Treatment (ug/g)	Control		0.36		0.82		1.103		1.64		2.26	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab												
A							14.7	12.4	14.2	7.0		
B	7.8			11.1			7.6					11.1
C		9.9					7.9				13.9	
D			9.7	9.6								
E				11.8								
F			11.6		6.5							
G		10.3		8.5					10.0		12.1	
H			6.6	7.8	9.7		9.1		16.0		8.7	12.5
I					13.0		10.3	9.4			10.2	9.4
J		11.3		12.2				6.9				3.9
K							10.8					
L	4.9		11.1		14.3					11.5	7.9	
M	11.7	14.9		10.8	6.1	11.7	9.2		8.4	6.5		11.1
N								8.4			11.4	10.9
O				11.7								6.51
P					2.7				11.6			
Q	11.9	15.7		6.4		10.5	11.1	9.9			11.7	11.3
R			10.8	10.9			10.1	13.6	7.5	5.9		12.0
S	11.1	10.3	7.9	8.2			12.3	10.7			8.8	
T		14.1								9.0		

Experiment II
9 - % Mid Body Thickness Increase/Molt

Kepone Treatment ($\mu\text{g/g}$) Reps	Control		0.36		0.82		1.103		1.64		2.26	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab												
A							14.6	13.5	14.5	9.6		
B	10.7				14.0		10.2					10.3
C		9.5									14.3	
D			6.8	5.1	13.9		5.6					
E				13.2								
F			12.3									
G					2.8							
H		11.1	6.2	5.3			9.5		5.9		7.2	
I				8.6	4.8		10.8	9.1	9.2		7.0	10.7
J					9.2						3.9	6.7
K		30.1		8.9			9.4	9.4				5.5
L	9.77		7.8		15.1					7.7	7.8	
M	12.9	7.5		12.3	3.4	8.4	5.8		8.2	7.6		8.3
N								12.6			11.6	11.3
O				11.1								5.1
P					5.9				11.7			
Q	8.9	9.6		8.0		12.5	9.8	8.9			10.5	9.4
R			10.7	10.9			10.7	14.6	1.4	2.7		8.5
S	4.6	9.6	12.5	10.5			9.4	6.7			9.6	
T		25.7								10.1		

Experiment II
10 - % Wet Weight Increase/Molt

Kepone Treatment ($\mu\text{g/g}$) Reps	Control		0.36		0.82		1.103		1.64		2.26	
	I	II	I	II	I	II	I	II	I	II	I	II
Crabs												
A							47.0	52.1	49.4	10.7		
B	33.9					47.8	21.9					44.4
C		30.7					11.1				54.2	
D			25.9	34.9		57.6						
E				40.9								
F			26.8		23.0							
G									37.1		33.7	
H		42.6	24.2	24.8	26.9		22.9		22.9		28.5	54.4
I				41.2	55.1		41.9	30.7			33.4	33.7
J						23.2		26.6				10.0
K							38.3					
L	17.9		19.1		49.8					34.1	31.6	
M	22.9	36.2		29.7	25.2	34.2	35.0		33.5	31.7		30.0
N								46.3			45.4	36.0
O				38.2								15.8
P					11.7				52.7			
Q	24.5			19.2		34.8	43.6	24.2			35.5	47.7
R	50.7		41.9	10.2			31.8	51.1	0	13.8		36.0
S	20.7	33.7	23.1	38.2			53.9	34.5			38.7	
T		33.3								31.5		

Experiment II

11 - Carapace Thickness/Width Ratio

Kepone

Treatment

($\mu\text{g/g}$)

Reps

	Control		0.36		0.82		1.103		1.64		2.26	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab												
A							3.30	3.82	3.04	3.45		
B	3.70				2.31							
C		2.65					2.86					
D			2.81		4.01		3.18				4.52	
E												
F			3.51									
G		3.13							2.80		3.09	
H			3.49		3.92				2.78			3.27
I					3.90		3.04				3.81	3.58
J		4.63		3.90				3.57				
K												
L	3.00		3.81		3.95					3.78		
M	3.85	3.69		2.70	3.99				3.13			
N												
O												
P					2.58			2.63			2.48	3.51
Q	3.04	3.67			2.56							
R			2.82	3.39				2.64			2.88	
S	2.70	3.15					3.75	2.83	2.70	2.77		2.64
T		3.38						3.71			4.30	

Experiment II
13 - % Appendages Autotomized
(Crabs with 9-10 appendages at the start
of the autotomization experiment)

Kepone Treatment (ug/g) Reps	Control		0.36		0.82		1.103		1.64		2.26	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab												
A							80.0		80.0		80.0	
B	60.0											70.0
C									0		55.6	
D	50.0		60.0	70.0		80.0						
E				0								
F					50.0							
G		80.0	60.0						70.0	30.0		
H							33.3				20.0	80.0
I	50.0		77.8		80.0		60.0	33.3				
J						90.0		88.9				
K							40.0					
L	60.0				80.0							80.0
M					20.0		0				30.0	
N	70.0											
O				88.9						66.7		
P					40.0							
Q		11.1		60.0		70.0	30.0					80.0
R			60.0				20.0	20.0				
S				60.0			88.9				77.8	
T		55.6								44.4		

Experiment II

14 - Oxygen Consumption ($\mu\text{l O}_2/\text{g}\cdot\text{hr}$)

Kepone

Treatment

($\mu\text{g/g}$)

Reps

	Control		0.36		0.82		1.103		1.64		2.26	
	I	II	I	II	I	II	I	II	I	II	I	II
Crab												
A			163.61				32.79		231.86		192.10	
B	85.12					39.16		111.48				
C		118.21				88.61	187.89		134.97			
D	150.12		43.73	147.36		45.49						
E												
F					64.97							
G								73.33	66.02	136.44	271.50	
H					82.21		132.41	213.26			180.77	138.69
I					51.57		42.86		119.09			121.62
J		79.45						221.47		97.64		
K				49.02			77.02					
L	56.92	63.66	18.40		80.58					70.25		
M		37.62		105.50	61.08	86.98			134.13			
N	55.90		177.87					117.40			163.50	85.74
O										143.08		
P					77.33						132.87	
Q	76.75		85.27	47.97		69.41					150.53	194.89
R	175.86						83.19	120.78	154.71	122.65		110.47
S		118.88	28.35			78.55						149.66
T		41.48		39.17						175.81		

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