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BIOACCUMULATION AND EFFECTS OF KEPONE®
ON SPOT, LEIOSTOMUS XANTHURUS

A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary

In Partial Fulfillment

of the Requirements for the Degree of

Master of Arts

---

by

Linda Louise Stehlik

1980

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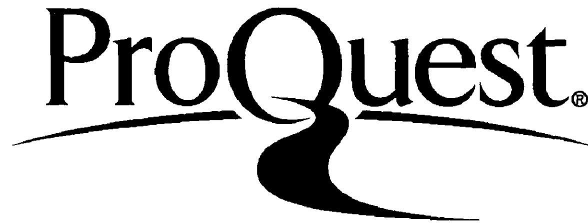

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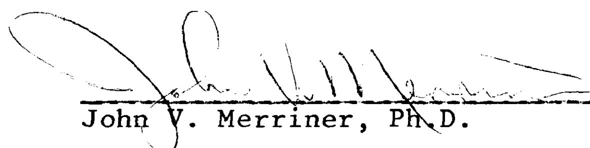
This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

Linda Louise Stehlik

Approved, August 1980



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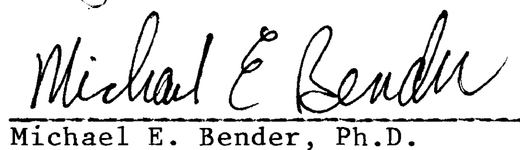

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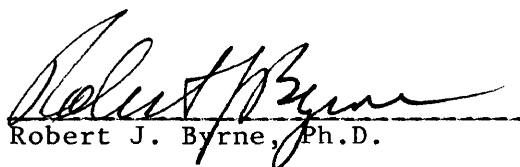

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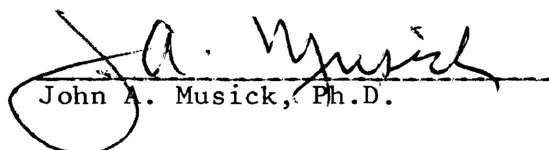

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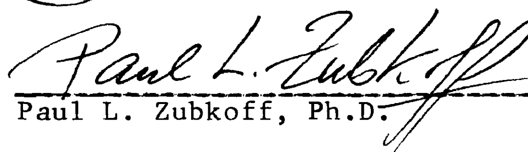

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ABSTRACT

Juvenile spot were administered Kepone® (chlordecone) by ingestion and their rates of accumulation and development of symptoms noted. The Kepone was on ground squid particles fed daily on a percent body weight basis. Pesticide accumulation was constant and additive because little Kepone was depurated by the fish. In two of three experiments in which Kepone at levels greater than three ppm was fed, the spot developed muscular tremors, scoliosis, and broken vertebral centra, and eventually died. Radiographing, clearing and staining, and dissection were employed to observe fractured bones. In fish exposed three weeks or more, all but a few vertebrae became hyperplasic and were surrounded by a thick layer of connective tissue. This new tissue growth was collagenous, causing elevated collagen percentages in the bones of treated fish assayed for their composition. Growing control spot held in the laboratory five weeks had lower bone collagen percentages than did wild fish.

BIOACCUMULATION AND EFFECTS OF KEPONE®

ON SPOT, LEIOSTOMUS XANTHURUS

INTRODUCTION

The water, sediments, and biota of the James River, Virginia, are contaminated with the organochlorine pesticide Kepone® (chlordecone). The pesticide was released into the river in effluent from Allied Chemical Corporation and Life Science Products, Inc. in Hopewell, Va., from 1966 to 1975. After Kepone was detected, the river was closed to finfishing by the Governor of Virginia. The Kepone molecule is seldom metabolized (Blanke et al., 1978), and is broken down otherwise only by ultraviolet light or temperatures exceeding 900°C. Although Kepone is slightly soluble in water, it adheres to solids, especially organic solids. Most of the Kepone in James River water is actually attached to suspended sediment particles (Huggett et al., 1979). The largest Kepone sinks in the river are organic-rich sediments and the biota. Spot (*Leiostomus xanthurus*), inhabitants of the estuarine James for nine months of the year, had concentrations above the EPA action level for human consumption (0.3 ppm) (Bender et al., 1977). As analyzed by Virginia Institute of Marine Science (VIMS) Department of Ecology and Pollution, James River spot in 1975 had a mean Kepone concentration of 1.70 ppm (n = 3), in 1976 a mean of 0.95 ppm (n = 14), and in 1977 a mean of 1.49 ppm (n = 22), range 0.031 - 4.15 ppm. Similar concentrations were found in resident and migratory fish species in the James River below the spill site.

Coincident with Kepone pollution, millions of estuarine fishes were found in the James River in 1973-1976 with deformed or broken and repaired vertebral columns (Bellanca and Bailey, 1977). The "broken

back" symptom has been noted in fish exposed to sublethal concentrations of several pesticides (Mayer et al., 1977). It has also been experimentally induced by vitamin deficiency, temperature, salinity, or oxygen changes, irradiation, electric shock, and heavy metal pollution; as reviewed by Bengtsson (1975).

Several studies on the uptake of Kepone and the closely related compound Mirex® in fishes and invertebrates have been reported. Schimmel and Wilson (1977) determined 96 hour LC_{50}s for invertebrates and fishes, including spot, using Kepone dissolved in seawater. Chronic, sublethal effects on sheepshead minnow (*Cyprinodon variegatus*) adults, eggs, and fry were assessed by Hansen et al. (1977a) and Couch et al. (1977). Bahner et al. (1977) introduced Kepone into spot and sheepshead minnows by bioconcentration (uptake from water) and bioaccumulation (from food) in food chain experiments, then fed clean food to contaminated spot one month for depuration (loss of compound from the body). Buckler (1979) conducted acute and chronic bioassays of Kepone and Mirex on fathead minnows (*Pimephales promelas*). Van Veld (1980) fed and injected channel catfish (*Ictalurus punctatus*) with ^{14}C-Kepone. Croaker (*Micropogon undulatus*), spot, and American eels (*Anguilla rostrata*) from the James River were held for Kepone depuration by Doyle et al. (1978), Hedgepeth et al. (1979), and Hedgepeth and Stehlik (1979). Schimmel et al. (1979) and Fisher (1980) observed bioconcentration and bioaccumulation by blue crabs (*Callinectes* sp.). Van Valin et al. (1968) investigated the uptake of Mirex in bluegill (*Lepomis*

macrochirus) and goldfish (Carassius auratus) by water and food. Simultaneous uptake of Mirex from water and food in pinfish (Lagodon rhomboides) was documented by Lowe et al. (1971). Channel catfish (Ictalurus punctatus) obtained Mirex through food chains of experimentally contaminated ponds (Collins et al., 1973). Hogchokers (Trinectes maculatus) concentrated Mirex from water and sediment in experiments by Kobylinski and Livingston (1975).

The toxicity of Kepone is to the nervous system and it accumulates in the brain, muscles and vital organs. Symptoms in humans included tremors, blurred vision, and coordination difficulty (Sterrett and Boss, 1977). In many of the chronic toxicity studies mentioned above, fishes and crabs exhibited muscular tetany and loss of coordination. Other effects include inhibition of catfish brain ATPases (Desaiah and Koch, 1975); increased activity of several liver enzymes (Mehendale, 1978); excitability and elevated metabolic rate in blue crabs (Leffler, 1975). Concentrations of ^{14}C-Kepone after ingestion by channel catfish were highest in the blood, followed by the brain and vital organs, and lowest in mesenteric fat and the carcass (Van Veld, 1980). Kepone was found to be associated with high density lipoproteins and albumin, components of blood and neural tissue (Skalsky, 1979). In contrast, DDT and other organochlorine pesticides accumulate in triglyceride-rich low density lipoproteins (Morgan et al., 1972) found in mesenteric fat and liver.

The spot, Leiostomus xanthurus, is an abundant estuarine fish of the family Sciaenidae. Spawning of the Chesapeake Bay population is

postulated to occur well offshore from November to March (Dawson, 1958). The young metamorphose and drift inshore, reaching river mouths in March or April, as postlarvae 12 mm (standard length) or greater. They school and feed in shallows, moving upriver as they grow. One and two year old spot forage in Chesapeake Bay estuaries from March through September (Chao and Musick, 1977). In fall, spot migrate seaward, leaving the Bay when the water temperature is approximately 10°C. Most spot spawn at the end of their second year (Dawson, 1958) and may rarely survive three or four years (Welsh and Breder, 1923). Spot winter offshore, moving deeper and southward from their summer foraging areas (Dawson, 1958). Spot are omnivorous, feeding on benthic annelids, molluscs, crustacea, copepods, microzooplankton and detritus (Chao and Musick, 1977). Juveniles consume small zooplankton, especially copepod nauplii. Larger fish feed near the bottom and suck up benthic and epibenthic organisms with their ventral mouths (Wetzel et al., 1979). Spot are adaptable to varying temperature and salinity; they have been found in 0-60 ppt salinity, and acclimate to 10°-35°C (Parker, 1971).

Standard bioassay procedures for measuring uptake and effects of toxicants upon aquatic organisms have been reviewed by Sprague (1969, 1970, 1971), Tarzwell (1975) and others. With aquarium maintenance, environmental conditions, introduction of pollutants, predation, and the age, sex, size, number and nutrition of organisms are placed under control of the experimenter. Realistic concentrations, exposure times, and media (contact, respiration, or ingestion) must be chosen

(Leduc, 1977) so that experiments can approximate toxicant exposure in nature. The lethal level of a substance in water is measured by of the LC_{50}, or concentration at which half the organisms die in 96 hours of exposure. Uptake is usually quantified by administering known amounts of substance (contact or ingestion) and sampling periodically yielding a curve of increasing concentration with time. The curve is a diagonal line when organisms store a substance without breakdown or egestion. It flattens when organisms reach equilibrium with the substance in their bodies (equal uptake and depuration rates). Assimilation or extraction efficiency is the ratio of the amount of substance retained to that exposed to or ingested. The bioconcentration factor (BCF) is the constant of proportionality between the concentration in water (C_W) and that in organisms (C_o) (Veith et al., 1979):

$$C_o = C_W \cdot BCF$$

Bioaccumulation factor (BAF) is used for ingested substances (Bahner et al., 1977).

Although the LC_{50} is an important tool in determining toxicity, lethal concentrations may never occur in the field. Long-term bioassays must then be designed at appropriate sublethal concentrations. As defined by Sprague (1969) an acute stressor is severe enough to cause response in a fish within four days; a chronic stressor causes response over a longer period, at least one-tenth a fish's lifetime. Short-term and long-term are synonymous with acute and chronic but the time implied is less definite.

Symptoms and physiological parameters for measuring stress caused by a toxicant should be chosen with care. Selye (1976) defined stress as a set of physiological responses by which an animal tries to restore a normal metabolism against a physical or chemical force. A stressor will be lethal when it demands greater physiological compensation than a fish can endure (Wedemeyer, 1976). A sublethal stressor can affect hematocrit, blood glucose, respiration rate or even tissue structure without immediate death and these changes are reversible if the stressor is removed. Prolonged application of the stressor to a population may cause harm, which has been typified as reduced survival, growth, or reproduction (Mayer et al., 1977), reduced scope for activity (Leduc, 1977), or lowered disease resistance (Wedemeyer, 1970). Measured symptoms or parameters in chronic bioassays should be those which irreversibly inhibit any of the above five necessary conditions for the survival of the population. Growth is commonly measured, although it is sensitive to many sublethal stressors and it may be impossible to prove that growth alterations were caused by the toxicant in question (Sprague, 1971). It is better to choose parameters specifically sensitive to the toxicant, which are easily measurable, quantifiable, and of known natural variability. Respiration, swimming performance, avoidance behavior, histopathology, fecundity, hatchability, and scope for activity are often used for sublethal bioassays (Sprague, 1971; Leduc, 1977).

The content of collagen in fish bone is a useful parameter for

sublethal bioassays. Alterations of collagen and hydroxyproline in fish bone have been studied by Halver et al. (1969), Halver (1972), Wilson and Poe (1973), Lovell and Lim (1978), Sato et al. (1978), Yoshinaka et al. (1978); specifically with pesticides by Mayer et al. (1977, 1978) and Mauck et al. (1978). Bone is composed of osteocytes, a matrix of collagen fibers, and crystalline calcium, phosphorus, and other minerals. The amino acid hydroxyproline makes up about nine percent of the collagen molecule and must be hydroxylated from proline after procollagen is formed. Ascorbic acid is necessary for the hydroxylation and is also used for detoxification of drugs and chemicals in the liver (Wagstaff and Street, 1971). Mayer et al. (1977) hypothesized that pesticide detoxification may compete with collagen synthesis for available ascorbic acid, thus causing reduced collagen or hydroxyproline in fish bone. Reduced protein in bone means relatively greater mineral content which makes bones brittle and easily breakable. Fish that were chronically exposed to toxaphene, Aroclor® 1254, Mirex, and other chemicals developed broken vertebral columns (Mayer et al., 1977; pers. comm.). Measurement of collagen is time consuming but not difficult and the results are quantifiable. Collagen is affected only by long-term stressors, and is known to be affected by pesticides.

My objectives were to investigate rates of Kepone accumulation by spot and effects of that uptake, especially bone damage. Hansen et al. (1977b) suggested that Kepone's chronic toxicity and bioaccumulation potential are more probable hazards in the environment

than acute toxicity. Estuarine fishes certainly obtain Kepone from the contaminated food chain of the James River, yet only Bahner et al. (1977) and Van Veld (1980) investigated Kepone uptake in fish by ingestion. Reactions of fish to Kepone may depend on its mode and rate of entry as well as resultant body concentration. Vertebral damage may occur from neuromuscular rigidity in an acute exposure, or in a chronic exposure from reduced bone collagen content.

MATERIALS AND METHODS

The first experiment was a short-term feeding of a high concentration of Kepone to determine the level of ingestion that caused physical symptoms. Experiments three and four continued these aims, using Kepone-soaked squid instead of fish flesh. Experiment two was a three-month feeding of two low Kepone concentrations that would not cause death or impairment for observation of biochemical changes in fish bones. It was shortened to two months after heavy mortalities of unknown cause.

Culture Methods

Young-of-the-year spot were collected by beach seine at several locations in the lower York River from May through July 1978 and 1979. For all experiments, fish were held in aerated tanks of continuously flowing York River water filtered through 5μ filter bags. Average flow rate into 23 gallon capacity (87 liter) glass aquaria was 1-2 liters per minute; average flow rate into 100 gallon capacity (379 liter) fiberglass tanks was 9 liters per minute. Salinity and temperature fluctuated with incoming water. Tank water exited through standpipes and drains to the river. For the third and fourth experiments, outflow from the treated tank passed through a holding tank of oysters, *Crassostrea virginica*, collected from the York River.

Protocol for bioassays (Tarzwell, 1975) dictates that organisms should be of uniform size, but available spot were of various sizes, so they were culled by size range. In experiment three, control and

treated spot in size ranges 26-30 mm, 31-35 mm and 36-40 mm were grouped in separate tanks. In experiment four, fish were separated into 40-49 mm and 50-69 mm groups.

Feeding Methods

In the first experiment spot were fed finely chopped flesh of striped bass (*Morone saxatilis*). Control fish were fed bass caught at the Chesapeake Bay mouth with no detectable Kepone. Treated fish were fed striped bass caught in the James River containing 3.9 ppm of Kepone.

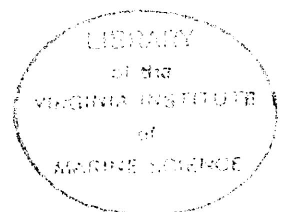
For the rest of the experiments, spot were fed ground squid which contained no detectable Kepone when bought. Pens were removed and squid were chopped in a meat grinder, then sieved and rinsed to remove fine particles. The meat grinder was scrubbed with Alconox® and rinsed with acetone beforehand to prevent Kepone contamination of control food. Ground squid was weighed and frozen in foil packages.

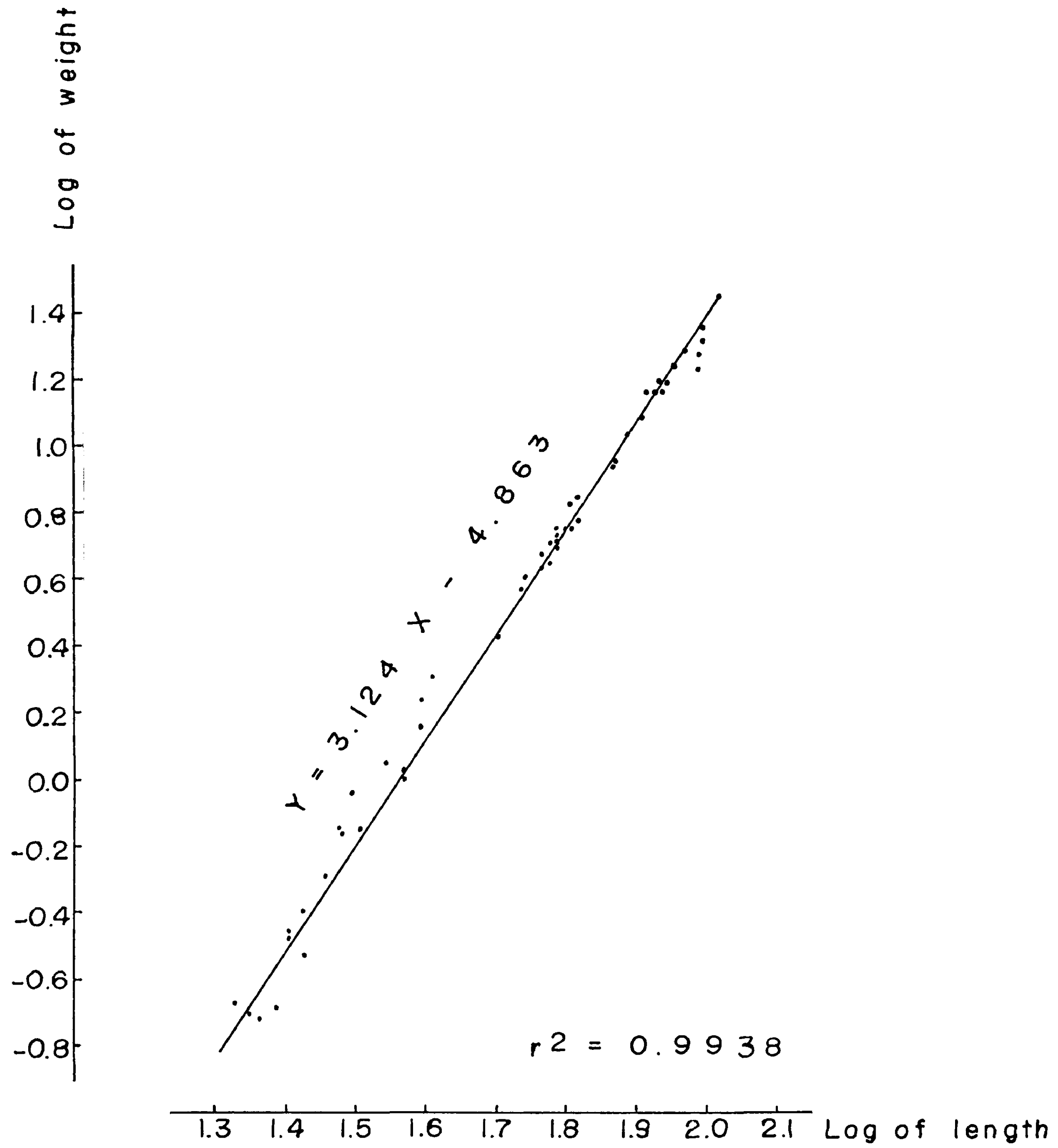
Pieces of squid were soaked in Kepone solution based on the method of Leffler (1975). By his method, liver and shrimp were soaked in acetone containing the pesticide, then airdried. However, acetone residues in dried squid were lethal to spot, so a water solution was used. For experiment two, 2200 g of drained, ground squid was soaked in 2200 ml of distilled water to which Kepone in 1 ml acetone had been added to yield 1.8 ppm Kepone. After 24 hours soaking at 5°C, squid was drained until its weight was 2200 g, then packaged in foil and frozen. Thirty gram samples contained 1.4, 1.4, 0.8, 1.2, and 1.2 ppm

Kepone by analysis, averaging 1.2 ppm. In one treated group, fish were fed the contaminated food on alternate days with uncontaminated food, for an average concentration of 0.59 ppm. In the other treated group, the contaminated food was fed every four days, for an average of 0.30 ppm Kepone in the diet. For experiment three, 400 g of squid was soaked in 400 ml of water and 1 ml of acetone to yield 6.1 ppm. Samples contained 2.9, 3.1, 3.1, 4.2, and 3.3 ppm, averaging 3.2 ppm. Thawing, refreezing, and thawing a 10 g package of squid caused a loss of 1-2 g of water. The last two food samples above were thawed, drained, refrozen, then analyzed, and do not show a loss of Kepone with the water loss. For experiment four, 1200 g of squid was soaked in 1200 ml of water at 10 ppm. Food samples were 3.2, 3.3, 3.2, 3.5, and 3.4 ppm, averaging 3.31 ppm Kepone.

Fish were fed by percentage of body weight in control and treated tanks. This uniformly restricted feeding design (Tarzwell, 1975) prevents inequalities of ration between tanks or individual fish. Percent body weight feeding was used in bioassays by Van Valin et al. (1968) feeding adult bluegills 5% per day; juvenile salmonids 1.5% (Macek and Korn, 1970); 1.5-5.5% (Phillips and Buhler, 1979); and 4-12% per day (Phillips and Buhler, 1978). As juvenile spot were acclimating it was observed that they would eat 5% or higher at one feeding. The percent of food to body weight was fixed at the start of each experiment, based on what the fish were consuming during acclimation in one daily feeding. The fish were weighed, or measured and weight calculated by regression (Figure 1) at the start of each

Figure 1. Regression of log length on log weight for juvenile spot, measured after death, unfrozen.





Log of weight
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
Y = 3.124 X - 4.863
r2 = 0.9938
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0
2.1
Log of length

experiment. A subsample from each tank was measured at biweekly intervals to recalculate weight. Fish in experiment four were fed their daily ration over two feedings. Severely poisoned fish that did not eat were omitted from the count. After fish were fed, tanks were cleaned of uneaten particles. Those particles too large to swallow were chopped and refed; inedible portions subtracted from the weight and discarded.

Sampling and Analysis

During the experiments fish were observed daily for pesticide poisoning symptoms, and samples were removed periodically (weekly, or at five day intervals) for Kepone and bone analysis. Healthy fish without symptoms were removed approximately 24 hours after their last meal, and frozen in foil until analysis. Fish for collagen measurement were similarly frozen. Fish destined for clearing and staining were preserved in 10% buffered formalin. Those for Kepone and collagen analysis were radiographed at the VIMS Ichthyology Department. Length measurements on live fish were made at two week intervals. Temperature was measured daily and salinity and dissolved oxygen sampled weekly, then determined with a Beckman conductivity meter and Winkler titration, respectively.

Kepone analysis of water, fish, oysters, and fish food was performed by the VIMS Ecology and Pollution department. Fish were measured, weighed, gutted and chopped with a knife, then placed in tared mason jars and reweighed. A sample as small as one gram could

be used; smaller fish were pooled. The samples were frozen overnight to rupture cells, or if they had already been frozen this step was omitted. A mixture of 9:1 anhydrous sodium sulfate and Quso® G-30 precipitated silica (Philadelphia Quartz Co.) was added for dessication, twice the sample weight. The samples were then mixed and refrozen. The procedures to follow were carried out by Mr. Harold Slone and assistants. The dessicated samples were ground to powder in a blender, then Soxhlet extracted in paper thimbles with 1:1 ethyl ether:petroleum ether for 16 hours. Extracts were concentrated by evaporation and purified by activated florisil column chromatography. The elutriate containing Kepone was analyzed by electron capture gas chromatography. Oysters, fish food, and tank sediment were analyzed similarly; water samples were extracted with benzene. Kepone concentrations were reported on a wet weight basis.

Fish were cleared and stained with alizarin and alcian blue by a modification of the method of Dingerkus and Uhler (1977). Cleared fish were examined under a binocular microscope and abnormalities of the vertebral skeleton and appendages counted. The first gill arch, right or left, in some fish was cut out and examined.

Collagen was determined in fish bones by the method of Flanagena dn Nichols (1962), modified by Mayer et al. (1977). Vertebral columns were dissected out then brushed under cool running water. The central two-thirds of each column (16 vertebrae) which is homogeneous in collagen content (P. M. Mehrle, pers. comm.) was used. Under a dissecting microscope, vertebrae were separated, neural and hemal

spines cut off, and all remaining flesh and connective tissue cut off. Vertebrae were left whole, not pulverized. They were air dried, then rinsed in acetone and defatted overnight in 2 ml of 2:1 chloroform and methanol per sample (Sato et al., 1978). The solvent was removed, vertebrae were dried in weighing dishes one hour at 110°C, cooled 45 minutes in a dessicator, and weighed. Samples were then extracted 24 hours with 2 ml of 0.1 N sodium hydroxide to remove alkaline-soluble proteins. They were rinsed with and placed in 2 ml of 10% sodium EDTA (pH adjusted to neutral) on a shaker table in a refrigerator. The EDTA solution was changed twice in 48 hours. After demineralization, samples were placed in a vacuum filter and washed with distilled water, then acetone, then returned to test tubes and extracted one hour in 2 ml 1:1 ethanol and ether. The solvent was removed and the insoluble collagen dried one hour at 110°C, cooled, and weighed.

Rappahannock, James, and York River young-of-the-year spot were collected on VIMS summer survey trawl cruises in 1977, 1978, and 1979 and frozen until analysis.

RESULTS

The first experiment, high dosage feeding with 3.9 ppm Kepone in striped bass, lasted 17 days until nearly all treated spot exhibited poisoning symptoms and most were dead. Initially the spot measured 20-42 mm standard length, 50 control and 150 in treated tanks. They were acclimated to the laboratory nine days. Temperature, salinity, and dissolved oxygen are listed in Table 1. Symptoms appeared by the fifth day of Kepone feeding, including slow movement, reduced appetite, and loss of equilibrium. Small fish died earliest, and the survivors on the 17th day were all larger fish (31-51 mm). Those still fed and appeared robust, but had poisoning symptoms indicating imminent death.

Spot eating 0.59 and 0.30 ppm Kepone on squid for two months (experiment two) exhibited no pesticide symptoms. There were 300 fish (35-100 mm), acclimated one month in three 100 gallon tanks. Unexplained deaths occurred in the second month in controls and in the higher dosage treated group, but remaining fish appeared healthy and well-fed. Dying fish were examined by Dave Zwerner, a VIMS parasitologist, but no cause of death was ascertained. Survivors after two months were kept an additional month eating Kepone-free food and showed no poisoning symptoms or pesticide depuration. A few of the largest individuals grew but there was little change in average fish length in any tank from days 0-84.

Table 1. Water conditions during experiments.

Days	Mean Temperature °C	Day	Tank	Salinity ppt	Dissolved Oxygen mg/l
I. HIGH DOSAGE UPTAKE FROM STRIPED BASS. JUNE 1978.					
0-6	24.3	6	C[a]	18.3	7.1
			T	18.3	4.6
7-13	25.7	13	C	17.8	6.0
			T	17.4	7.1
14-16	24.1				
II. LOW DOSAGE UPTAKE FROM SQUID. OCT.-DEC. 1978.					
0-6	22.9	7	A (all)	20.3	6.2
7-13	20.4	14		20.3	5.8
14-20	20.1	21		20.5	6.6
21-27	18.4	28		20.8	6.8
28-34	16.8	35		20.9	7.2
35-41	16.6	43		21.8	7.1
42-48	16.4	49		21.4	7.2
49-55	15.9				
56-69	14.1	63		21.8	7.8
70-84	11.5	70		21.1	7.9
		84		19.7	9.2
III. HIGH DOSAGE UPTAKE FROM SQUID. JUNE 1979.					
0-6	20.1	6	C (all)	18.3	6.0
7-13	21.2	13		17.9	5.9
IV. HIGH DOSAGE UPTAKE FROM SQUID. JULY 1979.					
0-6	23.9	7	C[a]	18.0	5.1
			T	18.0	5.7
7-13	26.1	13	C	17.7	4.5
			T	17.7	5.2
14-20	26.7	19	C	18.0	4.3
	26.5		T	18.0	4.5
21-27	27.7	28	C	18.0	4.5
	27.8		T	17.9	5.5

[a] C = control tank; T = treated tank.

In the third experiment, spot were fed 3.2 ppm Kepone on squid for 15 days but developed no symptoms of poisoning. Control and treated spot were separated by size groups, totalling 100 control and 200 treated fish. Because they were acclimated only four days, many were still learning to swallow squid particles during the experiment and died of starvation or capture stress. Their delicate bodies were also damaged while measuring for growth. Mortality in all tanks was over 50%. Average growth of surviving controls was 2 mm, zero in treated fish.

Spot in the fourth experiment, also high dosage, (3.3 ppm) exhibited poisoning symptoms and mortality. Fish were larger, 40-70 mm, and less vulnerable to damage by handling. There were 60 control and 90 treated fish; fish were acclimated and accustomed to eating squid in one week. Lack of coordination and loss of equilibrium developed on the seventh day of the experiment. Affected individuals worsened and died in several days. After four weeks all survivors were severely affected. Control fish were healthy and had grown an average of 8 mm. Surviving treated fish averaged 7 mm growth, although there may have been some bias from deaths of small individuals.

Food Consumption

Food consumption varied daily but stayed close to the chosen percentage of body weight. Inedible particles of squid were removed and caused small daily fluctuations in weight of food eaten. Weekly

averages of feeding rate (Table 2) changed because more food was given if appetites increased. Kepone consumption rates were calculated from food consumption rates and concentrations.

Kepone Concentrations

In the first experiment, spot were fed a high concentration of Kepone in striped bass flesh, and uptake was detected in sampled fish (Table 3; Figure 2a). Composites of several fish were analyzed because the fish were small. Poisoning symptoms first appeared on day five, when the sampled fish contained 1.2 ppm. Near the end of the experiment one liter of tank water contained no detectable Kepone (Table 4).

Fish in the second experiment accumulated Kepone steadily from days 0 to 56 (Table 4; Figure 2b,c). Uptake curves for tanks A and B, days 7 to 56, appeared linear and the regression lines are shown. An analysis on variance on tanks A and B upheld the hypothesis of linearity (for A: $F = 0.2885$, $P > 0.25$; for B: $F = 1.234$, $P > 0.25$) (Zar, 1974). There was a wide range of concentrations in individual fish on each date, but there was no consistent pattern relating concentration to fish weight (Table 5). From days 56 to 84, only clean food was fed, and there was little or no depuration. Kepone detected in controls (composites of five) on days 7, 42 and 84 may have originated from contamination in the experiment or analysis. Bottom sediment from tank A, day 84, had no detectable Kepone.

Table 2. Percent body weight fed and Kepone consumption rates.

Days	% BW[a]	KCR[b]	% BW	KCR	% BW
EXPERIMENT ONE					
	3.91 PPM KEPONE				
0-6	13.1	0.512			
7-13	9.0	0.352			
14-16	6.1	0.238			
$\bar{x}$	10.2	0.398			
EXPERIMENT TWO					
	0.59 PPM		0.295 PPM		CONTROL
0-6	5.7	0.0336	4.8	0.0142	4.1
7-13	6.0	0.0354	5.2	0.0153	4.4
14-20	6.4	0.0378	5.6	0.0165	4.7
21-27	6.8	0.0401	6.1	0.0178	5.1
28-34	6.6	0.0389	7.8	0.0230	8.6
35-41	5.6	0.0330	6.3	0.0186	7.3
42-48	4.7	0.0277	6.0	0.0177	7.2
49-55	4.6	0.0271	6.2	0.0183	5.4
$\bar{x}$	5.8	0.0342	6.0	0.0177	5.9

Table 2. (Continued).

Days	% BW	KCR	% BW	KCR	% BW	KCR	% BW	KCR	% BW	% BW
EXPERIMENT THREE										
	TREATED TANKS FED 3.18 PPM KEPONE						TREATED		CONTROL	
	26-30mm		31-35mm		36-40mm		Pooled		26-30mm	31-35mm
0-6	10.0	0.318	7.2	0.229	7.9	0.245	8.4	0.266	11.3	7.7
7-14	12.3	0.391	11.0	0.350	10.3	0.328	11.2	0.356	12.5	11.3
$\bar{x}$	11.3	0.359	9.4	0.299	9.3	0.296	10.0	0.318	12.0	9.8
EXPERIMENT FOUR										
	3.31 PPM KEPONE								CONTROL	
									40-49mm	50-69mm
0-6	14.1	0.467							14.4	14.7
7-13	15.5	0.513							15.6	15.1
14-20	14.2	0.470							16.7	17.2
21-27	13.8	0.457							12.7	12.0
$\bar{x}$	14.4	0.477							14.9	14.8

[a] $\frac{\text{g food given - food left over (each day)}}{\text{g fish in tank (each day)}} \cdot 100$ = percent body weight eaten per day (% BW)

[b] $\frac{\mu\text{g Kepone}}{\text{g food}} \cdot \frac{\text{g food eaten}}{\text{g fish}} = \frac{\mu\text{g Kepone eaten}}{\text{g fish}}$ Daily Kepone consumption rate (KCR)

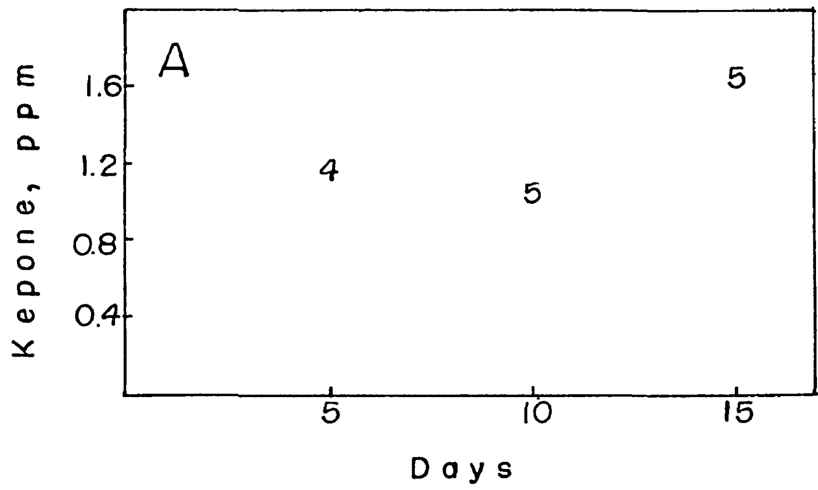
Table 3. Kepone concentrations in parts per million from experiment one, high dosage uptake from striped bass, 3.9 ppm.

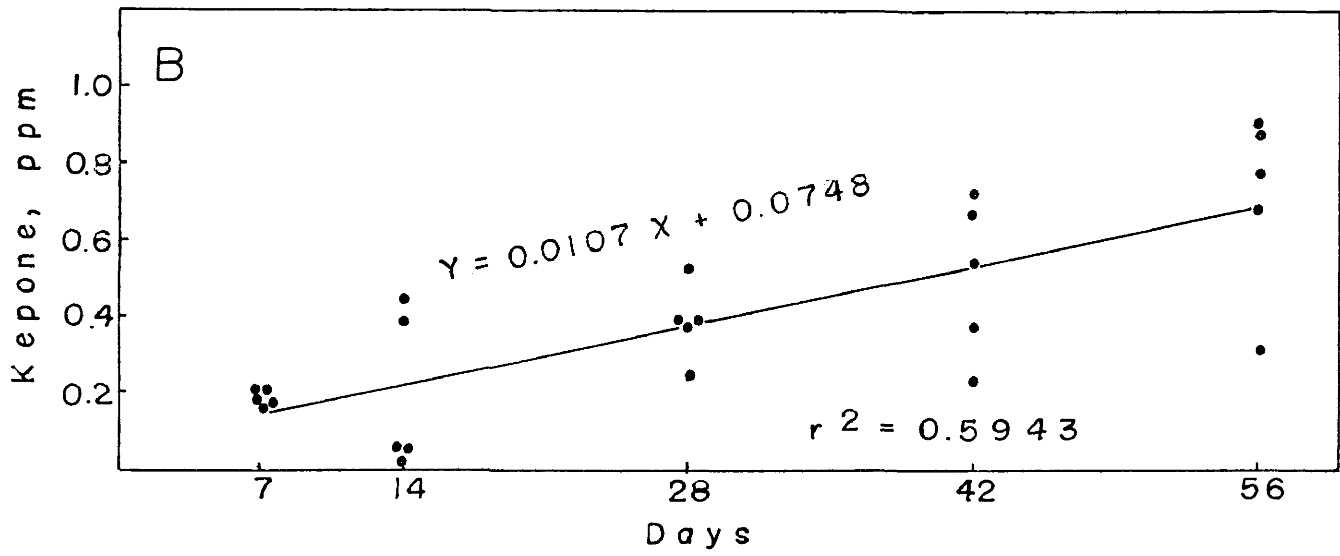
Days:	0	5	10	15
number of fish	39	4	5	6
fish	≤0.01	1.17	1.05	1.65
water				≤0.005

Table 4. Kepone concentrations in parts per million from experiment two, low dosage uptake from squid.

Days:	0	7	14	28	42	56	Depuration 84
TANK A 0.59 PPM IN SQUID							
Individual fish		0.22	0.44	0.38	0.53	0.77	0.57
		0.17	0.38	0.39	0.37	0.31	0.40
		0.19	0.02	0.24	0.66	0.67	0.79
		0.22	0.04	0.39	0.23	0.87	1.1
		0.18	0.04	0.52	0.71	0.89	0.81
Mean		0.20	0.18	0.39	0.50	0.70	0.74
Composite (n = 5)	≤0.01						
Sediment							≤0.01
TANK B 0.30 PPM IN SQUID							
Individual fish		0.11	0.02	0.15	0.26	0.34	0.20
		0.08	≤0.01	0.18	0.39	0.29	0.14
		0.04	0.02	0.23	0.22	0.32	0.36
		0.09	0.03	0.17	0.37	0.32	0.26
		0.02	0.02	0.20	0.32	0.36	0.33
Mean		0.07	0.02	0.19	0.31	0.33	0.26
Composite (n = 5)	≤0.01						
TANK C CONTROL							
Composite (n = 5)	≤0.01	0.01	≤0.01	≤0.01	0.01	≤0.01	0.03

Figure 2. Kepone concentrations in spot by days of feeding.
A. Experiment one. Points are composite samples, and show the number of fish per sample.
B. Experiment two, Sublethal dosage of 0.59 ppm on squid. Each point represents one fish.
C. Experiment two, Sublethal dosage of 0.30 ppm on squid. Each point represents one fish.


A
Kepone, ppm
1.6
1.2
0.8
0.4
4
5
5
5
10
15
Days


B
Kepone, ppm
1.0
0.8
0.6
0.4
0.2
Y = 0.0107 X + 0.0748
r 2 = 0.5943
7
14
28
42
56
Days

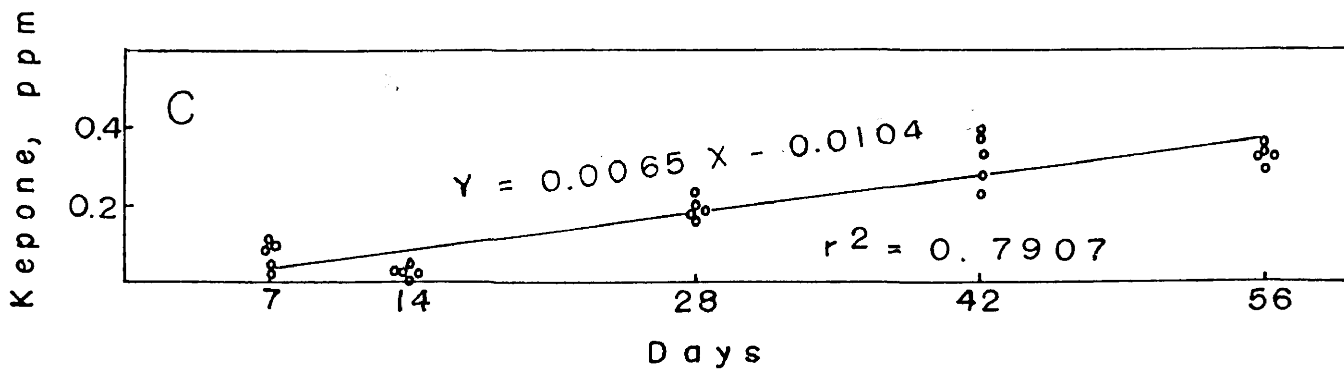

C
Kepone, ppm
0.4
0.2
Y = 0.0065 X - 0.0104
r 2 = 0. 7907
7
14
28
42
56
Days

Table 5. Kepone uptake and weight of spot in second experiment.

	Fed 0.59 ppm		Fed 0.30 ppm	
Day	Weight, g	Kepone ppm	Weight, g	Kepone ppm
42	21.9	0.53	13.9	0.26
	17.2	0.37	10.3	0.39
	11.0	0.66	8.1	0.22
	6.3	0.23	8.0	0.37
	1.9	0.71	6.9	0.32
56	23.6	0.31	22.1	0.34
	14.2	0.67	14.7	0.29
	12.4	0.77	11.8	0.32
	2.8	0.87	10.2	0.36
	2.8	0.88	4.2	0.32
84	18.2	0.57	21.2	0.20
	8.2	0.79	16.7	0.30
	3.5	0.81	5.8	0.14
	2.9	1.1	4.3	0.26
	1.8	0.40	4.1	0.33

Kepone accumulated in the bodies of spot in the third experiment with increasing variability over time (Table 6; Figure 3a). Composite samples were analyzed because fish were small. Dead fish were analyzed, as well as the planned samples. There was no apparent difference in concentrations between the size groups, or between live and dead fish so all samples were pooled. Oysters living in treated tank effluent concentrated Kepone.

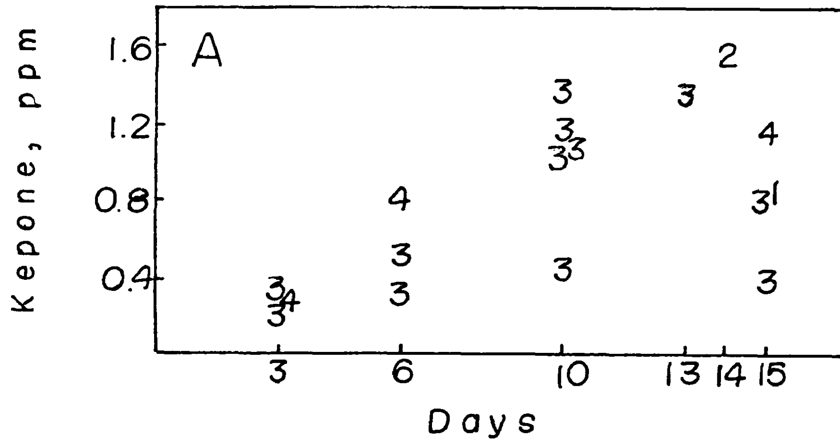
Uptake in the fourth experiment (Table 7; Figure 3b) was linear, as verified by analysis of variance for linearity ($F = 1.160$, $P > 0.25$) between days 1 to 28. Variability among individual fish was high. On day seven, the fish at 1.1 ppm, the highest of the five, had externally obvious poisoning symptoms such as muscular tremors and loss of equilibrium. All five fish on day 14 contained over one ppm, but only one of those had shown symptoms. All treated fish analyzed from the day 28 sample were alive but severely affected. Controls had no detectable Kepone. Oysters accumulated appreciable amounts of Kepone from treated tank effluent. On day seven, 0.1 ug/l was detected in tank effluent.

Table 6. Kepone concentrations in parts per million from experiment three, high dosage uptake from squid, 3.2 ppm.

Days:	0	3	6	10	13	14	15
TREATED TANKS							
26-30 mm							
n		4	4	3			4
		0.28	0.80	0.45			1.1
31-35 mm							
n		3	3			2	3
		0.20	0.33			1.5[a]	0.84
36-40 mm							
n		3	3	3 each:	3		3, 1
		0.36	0.49	1.4	1.4[a]		0.37
				1.1[a]			0.82[a]
				1.2[a]			
				1.1[a]			
Composite (n = 5)	≤0.01						
Oysters	≤0.01		0.03				0.02
CONTROL TANK							
Composite (n = 5)	≤0.01						≤0.01

[a] fish were dead when sampled

Figure 3. Kepone concentrations in spot by days of feeding. A.
A. Experiment three, lethal dosage of 3.2 ppm on squid. Points are composite samples, and show the number of fish per sample. Size classes pooled.
B. Experiment four, lethal dosage of 3.3 ppm on squid. Each point represents one fish.


A
Kepone, ppm
1.6
1.2
0.8
0.4
3
6
10
13
14
15
Days

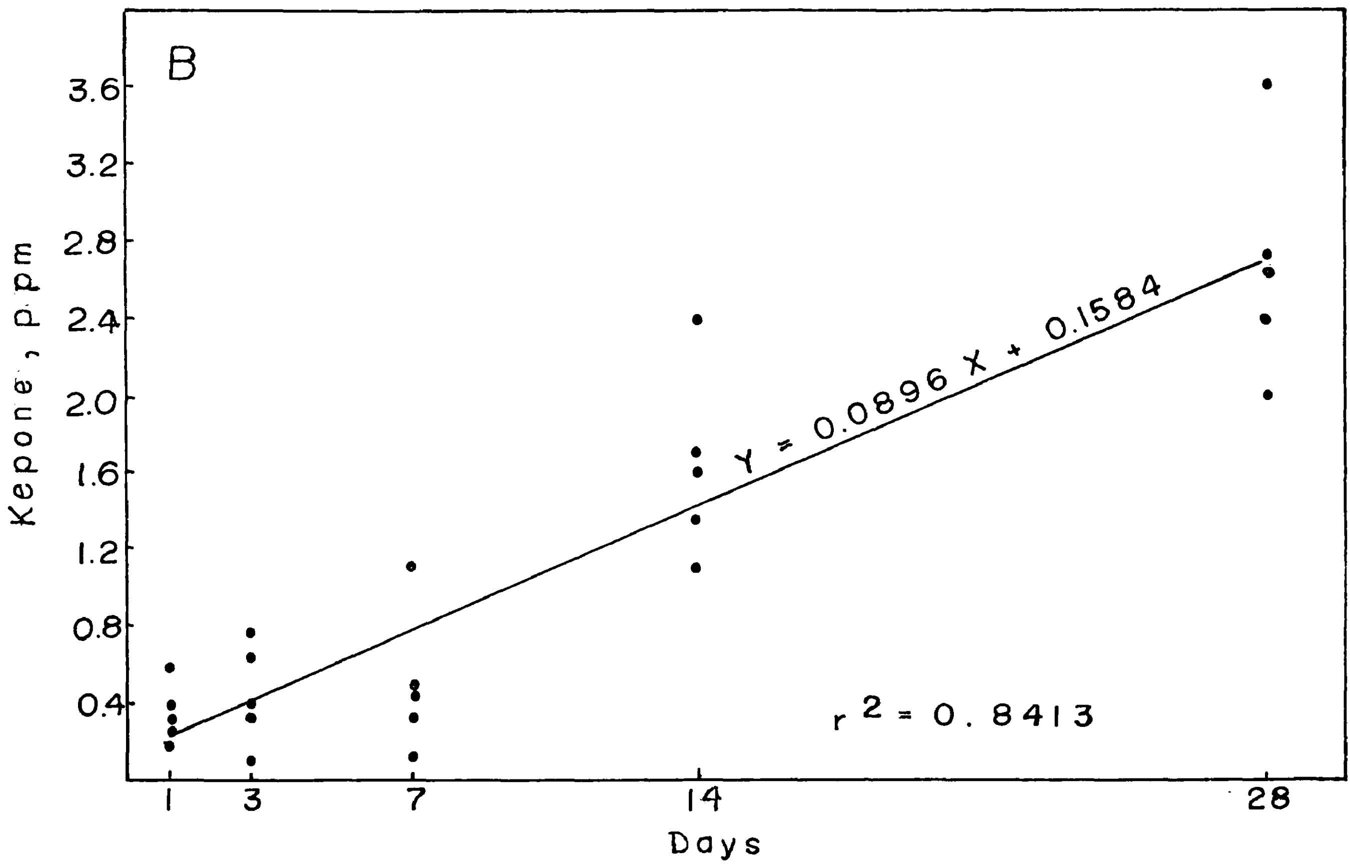

B
Kepone, ppm
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
Y = 0.0896 X + 0.1584
r 2 = 0.8413
1
3
7
14
28
Days

Table 7. Kepone concentrations in parts per million from experiment four, high dosage uptake from squid, 3.3 ppm.

Days:	0	1	3	7	14	28
TREATED TANK						
Individual fish		0.28	0.64	1.1[a]	1.1[a]	2.6[a]
		0.39	0.77	0.11	1.3	2.4[a]
		0.35	0.38	0.32	1.7	2.7[a]
		0.18	0.09	0.49	2.2	2.0[a]
		0.58	0.35	0.47	1.6	3.6[a]
Mean		0.36	0.45	0.50	1.6	2.7
Composite (n = 5)	≤0.01					
Water				0.001		
Oysters	≤0.01			0.07	0.13	0.18
CONTROL TANK (n = 5)						≤0.01

[a] fish had pesticide poisoning symptoms

External Symptoms of Kepone Poisoning

Externally visible pesticide poisoning symptoms appeared in spot in two of the high dose experiments. Hemorrhages beneath the skin of the head and above the operculum were common. Diminished activity, cessation of feeding and loss of equilibrium preceded death. In the first experiment, smaller spot became emaciated and darkly pigmented. In the fourth experiment, the earliest symptoms of poisoning were slow swimming and slight scoliosis. At this stage, however, they still fed eagerly. Severely affected fish drifted head up or head down, and their bodies were bent at a right angle with hemorrhages at the flexure. Death followed in one or two days.

There was some mortality in all tanks (Table 8). In the two experiments in which Kepone symptoms appeared, mortality in treated tanks greatly exceeded that in control tanks. In the second experiment, unexplained deaths in control and high dose tanks occurred in the second month. Mortality in all tanks of the third experiment exceeded 50%.

Internal Symptoms

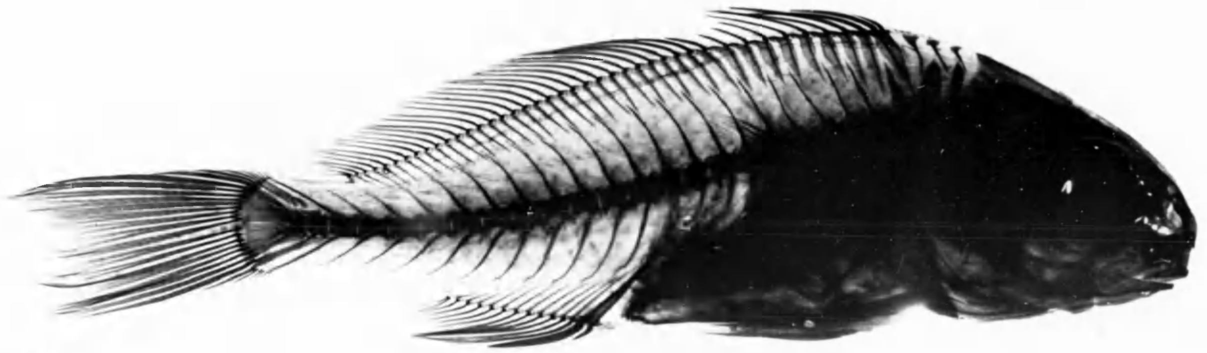
Radiographs and cleared specimens revealed bone abnormalities in spot from all Kepone tretments, with the majority of cases in the lethal experiments. Control spot (Figure 4a) had vertebral centra which were longer than wide and translucent in cleared specimens. Some treated spot had one or more cracked vertebral centra surrounded by abnormally enlarged bone and connective tissue (Figure 4b). Centra

Table 8. Mortality of test fish in Kepone ingestion experiments.

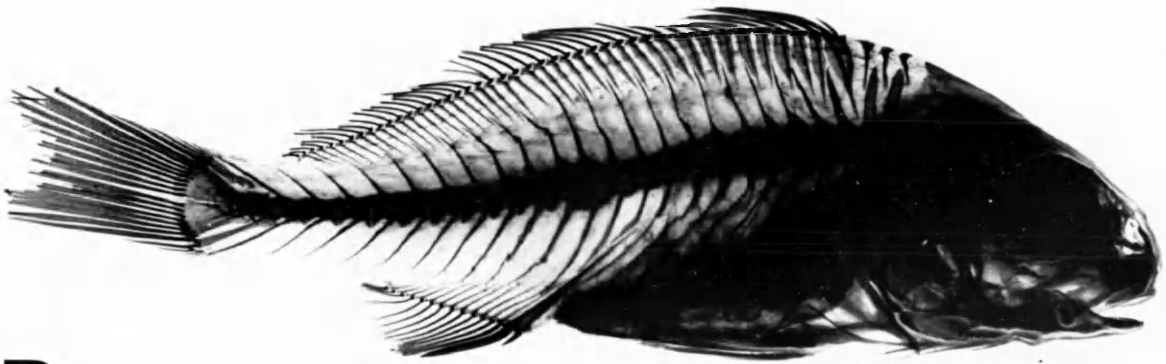
Experiment	Days	Group	N	% Mortality
I[a]	16	Control	50	10.0
		Treated	150	84.7
II	56	Control	92	10.9
		0.59 ppm	99	22.2
		0.30 ppm	93	4.3
III	15	Control 26-30 mm	59	71.1
		Control 31-35 mm	40	50.0
		Treated 26-30 mm	55	58.1
		Treated 31-35 mm	101	40.6
		Treated 36-40 mm	55	65.5
IV[a]	28	Control 40-49 mm	28	25.0
		Control 50-69 mm	28	10.7
		Treated 40-69 mm	90	43.3

[a] Kepone symptoms in treated tanks.

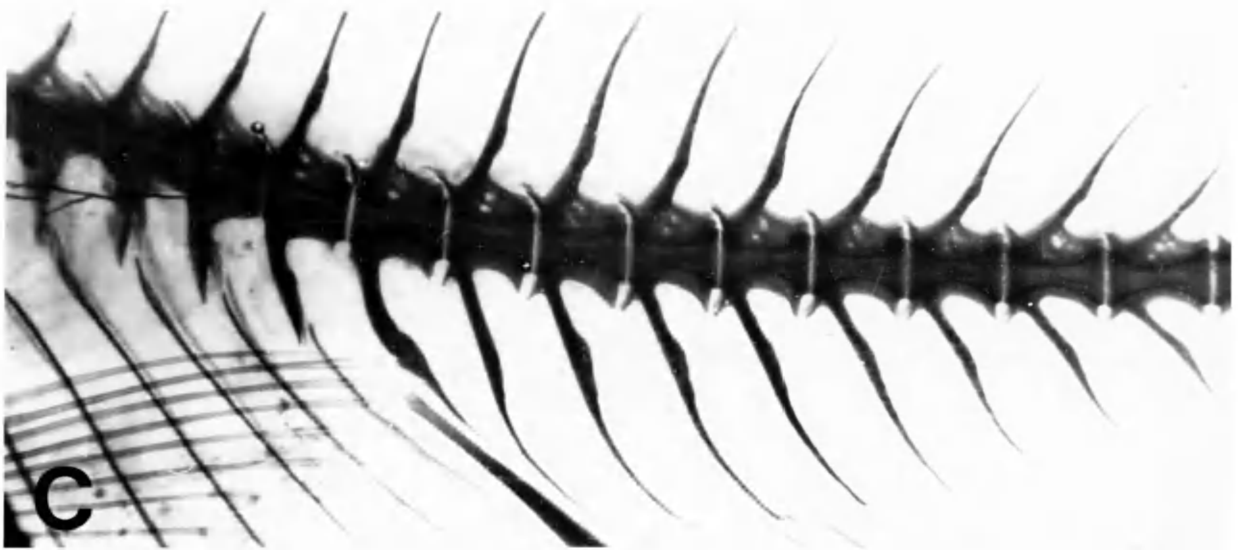
Figure 4. Cleared and stained spot illustrating bone deformities.
A. Normal spot (55 mm), from control tank, fourth experiment.
B. Spot (55 mm) from treated tank, fourth experiment, with vertebral fracture and hyperostosis.
C. Treated spot from fourth experiment, with bent ends of neural and hemal spines.



A



B

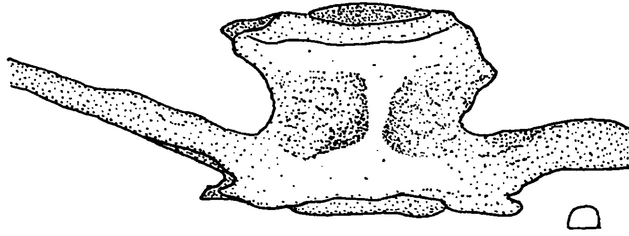


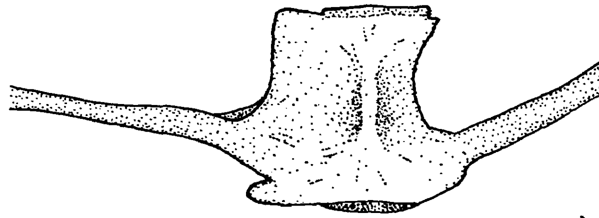
6-13 were the sites of rupture. Vertebrae near the fracture were vertically and laterally thickened, and appeared denser in radiographed and cleared fish. Many spot had bent, broken, or lumpy neural and hemal spines and pleural ribs.

Details of thickening could be seen in vertebral centra dissected and cleaned for collagen analysis. The centra of normal spot (Figures 5a, c) from the Rappahannock River were thin and smooth, surrounded by a small amount of connective tissue that was easily scraped from the surface. Abnormal centra from a fish of experiment four (Figures 5b, d) had enlarged ridges of coarse, spongy bone around the anterior and posterior faces of articulation, basapophyses (lateral bars), zygapophyses, and neural and hemal arch bases. Surrounding the most severely enlarged centra was a thick mass of connective tissue which had become hardened in some places and was difficult to remove. One specimen had blood clots between two centra. In fish surviving 28 days of the fourth experiment, vertebrae 5 to 16 or 20 were thickened.

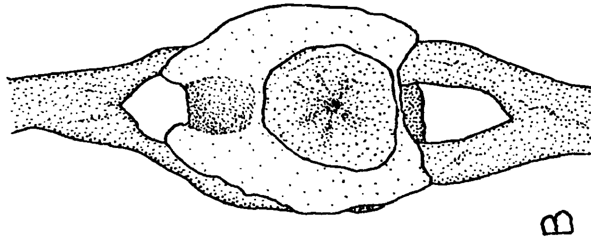
The occurrence of bone defects by experiment is summarized in Table 9. Spot in the first and fourth experiments developed broken centra, first seen on days 10-13 and 7 respectively. There was one case each in the second and third experiments. Bent neural and hemal spines commonly occurred in fish with broken centra, and also in 29% of the 45 sampled fish from experiment two. Thickened vertebrae appeared in treated fish late in the fourth experiment, rarely in the other experiments. Radiographed or dissected fish from experiment two

Figure 5. Dissected and cleared vertebrae of spot.
A,C. Vertebrae of normal spot (60 mm) from Rappahannock River.
B,D. Enlarged vertebrae of treated spot (61 mm) from fourth experiment, after four weeks of Kepone ingestion.


D


C

1 mm


B

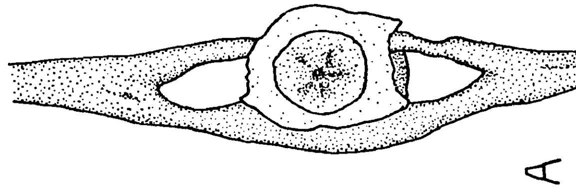

A

Table 9. Summary of bone damage.

Experiment	Radiographed	Cleared	No Damage[b]	Bent Spines	Broken Vertebrae	Thickened Vertebrae	Bent Spine Ends	Gills Examined	Bent Gill Raker Ends	Deformed Gill Filaments
I.										
Control days 6-13[a]		12	12	0	0	0	12	3	2	0
Treated days 10-13[a]		46	39	1	5	2	43	5	2	0
Treated days 15-17[a]		28	19	2	8	0	28	5	3	2
Treated day 17		8	3	5	2	0	8	4	4	1
II.										
Control day 56	9		7	2	0	0				
Treated day 56	20		13	7	1	0				
Control day 84	2		2	0	0	0				
Treated day 84	14		10	4	0	0				
III.										
Seined from York River		205	205	0	0	0	3	10	10	9
Control days 8-9		30	30	0	0	0	9			
Treated days 8-9		28	28	0	0	0	10			
Treated days 8-9[a]		19	19	0	0	0	3			
Control day 15		12	12	0	0	0	11			
Treated day 15		36	30	3	1	3	36			
IV.										
Control day 14	5		5	0	0	0				
Treated day 14	5		2	2	3	0				
Treated days 13-14[a]	2		1	0	1	0				
Treated days 15-21[a]	20	2	7	6	10	3	1	2	2	2
Treated days 22-28[a]	11	5	1	6	8	7	5	5	4	4
Control day 28	10	5	10	0	0	0	3	5	1	2
Treated day 28	14	3	1	7	11	13	3	3	1	1

[a] Sampled fish had died in the tanks.
[b] No broken or thickened vertebrae or spines (next three columns).

had vertebral thickening. In wild-caught, control, and treated spot, the ends of gill rakers were often twisted or bent, and occasionally there were enlarged lumps of tissue or twists on gill filaments.

Bends or twists in the fine ends of neural and hemal spines were often visible in cleared fish (Figure 5c). The deformities arose in thin, hyaline bone projecting from the thicker, more opaque portion toward the vertebrae. Some fish had only a few gently undulating spine ends, in others nearly every vertebral spine was bent. Angles of almost 90° were often discernable in the first four hemal spines. Incidence of bent spine ends increased in control and treated fish with time in captivity. Of 205 cleared spot captured simultaneously with those destined for the third experiment, three fish had a few slightly bent spines. After 12-13 days captivity, 29% of the cleared fish (n = 77) had them, most only slightly. After 19 days, 92% were afflicted, often conspicuously (n = 48). The ends of pleural ribs were often bent. No other bones in cleared specimens were noticeably deformed.

Bone Collagen

Bone collagen percentages in spot (Table 10) were found to be similar to literature values for other species (Mayer et al., 1977) after modification of analytical technique and with practice. Since the procedure was to weigh before and after demineralization, error could result from inaccurate weight or loss of pieces of bone. Bones of fish below about 60 mm standard length or with an initial weight of

Table 10. Collagen analysis of wild-caught, control, and treated spot. Percentage collagen in dry bone of 16 vertebrae.

Sample	mm SL	% Collagen	Initial Dry Weight mg
Rappahannock River, 8-78	93	19.2	45.8
	90	32.2	34.8
	85	28.9	33.9
	82	27.5	28.0
	75	25.2	23.6
	90	27.3	46.1
	78	25.8	28.2
	68	26.6	18.0
		$\bar{x}$ = 26.6 ± 1.3 (SE)	
James River, 8-77	110[a]	37.9	157.9
	87	28.2	46.4
	86	25.7	40.1
	84	26.5	36.3
	83	26.7	37.1
	80	26.1	40.6
	77	27.2	32.2
	75	24.8	24.0
		$\bar{x}$ = 26.5 ± 0.4 (not including 110 mm)	
York River, 8-79	91	27.6	53.3
	83	24.7	35.6
	79	23.4	27.2
Experiment Four, Day 28, Treated	63[a]	32.7	21.1
	62[a]	27.7	20.8
	60[a]	30.8	21.8
	59[a]	27.0	19.3
	59[a]	34.6	21.3
		$\bar{x}$ = 30.6 ± 1.4	
Experiment Four, Day 28, Control	75	25.1	14.8
	74	22.8	17.3
	71	21.7	13.6
	69	21.7	13.8
	69	23.5	13.6
	69	22.5	13.8
		$\bar{x}$ = 22.9 ± 0.5	

Table 10 (concluded)

Sample	mm SL	% Collagen	Initial Dry Weight mg
Experiment Two,	93	26.4	42.0
Day 0	89	26.5	39.9
	86	28.0	30.0
	83	29.4	24.0
	79	24.5	24.6
	63	26.7	12.7
	62	28.5	9.8
	55	25.8	8.0
		$\bar{x} = 27.0 \pm 0.6$	
Experiment Two,	97	30.1	43.5
Day 57	94	29.7	34.6
A - High Dose	83	31.1	23.1
	75	25.8	14.9
	74	24.4	17.7
	74	29.7	13.8
	71	28.3	15.5
		$\bar{x} = 28.4 \pm 0.9$	
Experiment Two,	109	35.2	51.3
Day 57	99	28.9	33.5
B - Low Dose	89	30.6	24.5
	85	29.2	19.5
	82	29.4	16.3
	80	29.6	23.0
	78	29.6	14.5
	75	28.6	13.6
		$\bar{x} = 30.1 \pm 0.8$	
Experiment Two,	94	29.8	28.5
Day 57	92	29.7	28.6
C - Control	89	29.3	24.2
	84	28.8	22.2
	84	26.3	24.3
	78	25.0	15.6
	61	35.0	6.0
		$\bar{x} = 28.2 \pm 0.8$ (not including 61 mm)	

[a] Vertebrae thickened.

less than 10 mg were too light to weigh accurately when demineralized. The 61 mm fish in experiment two (day 57 C) was excluded from the mean. Averages of two or three weighings were used. Thickened vertebrae were discovered in one fish from the James River and all treated fish from experiment four. Thickened bones were heavier than those of normal fish of the same lengths. Collagen percentages did not correlate with fish length or initial sample weight.

Wild-caught fish were compared with those kept in the laboratory. Because of the difference in variances of the James and Rappahannock River samples, they were compared by a non-parametric Mann-Whitney test, and found to be the same at the $\alpha = 0.01$ level (Zar, 1974). Similarity between wild (Rappahannock) fish and six control spot of experiment four was rejected at the $\alpha = 0.05$ level in a Mann-Whitney test, and similarity between wild fish and five treated fish from experiment four was accepted at $\alpha = 0.05$. An analysis of variance was performed between wild fish (both rivers pooled), day 0, day 57A, B, and C. Equality of means was rejected at the $\alpha = 0.05$ level. When a Newman-Keuls multiple range test was done, the dissimilar groups were tank B and wild fish at $\alpha = 0.01$ and tank B and day 0 fish at $\alpha = 0.05$.

DISCUSSION

Effects of Kepone Poisoning

Kepone causes chronic harm when high levels are consumed by spot, as evidenced by its effects on survival. In the lethal experiments, the stress from Kepone eventually exceeded physiological compensation ability for most of the fish and they died. The concentrations in the food, 3.9 and 3.3 ppm, were higher than available food items for spot in the James River, based on limited data for zooplankton (Jordan et al., 1979) and a few crustacea (Bender et al., 1977). Invertebrates commonly contained one to two ppm, however. Experimental food concentrations about 1 ppm would more closely simulate river conditions and might possibly induce sublethal effects in test fish over a few months. In my original design, some scoliotic fish were to be fed clean food to see if they would live and recover mobility. Chronically affected moribund fish in the wild would be liable to predation or starvation before death from the pesticide itself. Enlarged, fused vertebrae are not lethal in themselves but may reduce flexibility. Holcombe et al. (1976) noted that scoliotic *Salvelinus fontinalis* were physically unable to spawn. Growth may be arrested while fish recover from vertebral fractures, but I found no growth differences between control and treated fish in these short-term experiments. Appetites of treated fish were not depressed for two or three weeks until neurological disablement was severe. Dissolved Kepone reduced growth in full life cycle studies with *Pimephales*

promelas (Buckler, 1979) and Cyprinodon variegatus (Hansen et al., 1977).

Symptoms from ingestion of Kepone developed similarly to those of contact exposures to Kepone. Fish swam and fed slower than normal, and later developed muscular tremors and slight scoliosis. As spot continued to ingest Kepone, scoliosis worsened, with loss of appetite, loss of equilibrium, hemorrhages, and fractured vertebrae. Smaller fish (< 40 mm) had broken centra but did not exhibit right-angle body bends as did the larger ones. Hansen et al. (1977a) and Schimmel and Wilson (1977) noted all of the above external symptoms in spot and sheepshead minnows. Schimmel and Wilson observed darkening of all or part of the skin in both species, which I saw only in 20-30 mm spot in the first experiment. Couch et al. (1977) and Buckler (1979) reported broken vertebrae as well as the external symptoms.

In acute reactions, bending or breaking of the axial skeleton can be caused by muscular tetany. Salvelinus fontinalis with recently developed scoliosis from lead exposure straightened when placed in MS 222 anesthetic (Holcombe et al., 1976). Those which were scoliotic for several weeks did not straighten out in MS 222; apparently having calcified in the bent position. In histological sections from Cyprinodon variegatus exposed to Kepone, displaced and disoriented myotomes surrounded fractured centra (Couch et al., 1977). These sections illustrate how the fracture of one centrum allows adjacent ones to be pushed into it, causing fusion and shortening of the column (Couch et al., 1977). Bone fragments were pushed into the dorsal

aorta, caudal artery, or nerve cord, causing hemorrhages or paralysis. Bengtsson (1974) observed similar fractures in scoliotic *Phoxinus phoxinus* exposed to zinc.

Healing of a broken bone involves the formation of a callus, thick deposits of temporary bone or cartilage around the site of fracture. Woven or non-lamellar bone is temporarily laid down if bone ends are immobilized; cartilage is laid down if the body is poorly immobilized; then either type is replaced permanently by lamellar bone. If the body is not immobilized at all, scar tissue of connective tissue fibers is laid down and bone is not reconstructed (Walter and Israel, 1970). Histological sections by Couch et al. (1977), Bengtsson (1974) and McCann and Jasper (1972) confirmed the repair of fish centra with both cartilage and bone. Thickenings or lumps on neural and hemal spines also result from callus formation. A few small (30-35 mm) cleared and stained spot from my experiments had fractured vertebrae surrounded by blue-stained cartilage, but the majority of fish of all sizes had red-stained bone around the fractures.

During their third week of Kepone consumption, fish in experiment four began to show a unique symptom, the overall thickening of most vertebrae, not just at breakage sites. The structure of bone is either compact, with collagen fibers parallel, or spongy (cancellous), with a network of fibers or trabeculae for rigidity. Most fish vertebrae are amphicoelous, biconid shapes of compact bone, with lateral bars of spongy bone for stress resistance (Laerm, 1976).

Thickened vertebrae of spot had enlarged lateral bars and a buildup of spongy bone around anterior and posterior faces of articulation, which sometimes spread over the entire centrum wall. They were covered with tough connective tissue many times thicker than normal, sometimes partially calcified to the centrum. The most misshapen centra were shorter longitudinally and up to two times wider dorsoventrally than those of control fish of the same size. Usually the most hyperplasic vertebra was fractured. The buildup of bone can be explained as callus formation and possibly a body response to strengthen the spinal column against buckling pressures (Laerm, 1976) especially at the faces of articulation. The profusion of connective tissue can be explained as scar tissue formation.

Couch et al. (1979) noted extreme vertebral hyperostosis in _Cyprinodon variegatus_ exposed to the herbicide trifluralin for 51 days from zygotes. Their vertebrae were 3-20 times normal width, and most of the column was affected. In histological sections, reticulin (fine, newly formed collagen fibrils), osteoblasts, and fibroblasts proliferated around the enlarged vertebrae. The herbicide-treated fish also had elevated serum calcium. Couch et al. (1979) hypothesized that trifluralin stimulated abnormal activity of fibroblasts and osteoblasts, or else affected hormonal control of calcium. John Couch (pers. comm.) observed hyperostosis to a lesser extent in _C. variegatus_ treated with Trithion and Kepone.

Hyperostosis of most of the body's vertebrae has been reported in laboratory studies only by Couch et al. (1979); but there are two instances in field collections. Spot and croaker caught in the James River in 1975 had thickened vertebral columns (personal observation). Valentine (1975) reported Paralabrax nebulifer caught in southern California with heavily calcified vertebral columns.

It is possible that the vertebral fractures in spot could have resulted from hyperostosis. Although the spot were growing, abnormal vertebral growth would outpace body lengthening, and when there was not enough space for the centra, one would push out laterally and fracture. Neural and hemal spines might also break as their growth outpaced the surrounding myotomes. It is likely that hyperostosis was the cause of many of the fractures in treated spot in the fourth experiment. At the 28th day, 14 were alive, still swimming and feeding, yet radiographs revealed 12 with fractured centra. The average growth was about the same for control and treated fish, however the centra of the treated fish were abnormally large.

Some physical changes noted in this study did not originate from the toxicant. For instance, gills were anticipated to be the earliest site of bone deformities, based on the observations of Halver et al. (1969), Van Valin et al. (1968), and Valentine (1975). Control as well as treated spot developed twisted rakers and abnormal lumps of cartilage on gill filaments. Wild-caught juveniles also had these gill anomalies, suggesting that young spot are naturally susceptible to them.

The increasing prevalence of bent neural and hemal spine ends in control and treated fish (experiment three) with time in aquaria was an effect of captivity. Wild-caught juveniles from beach seining and in 1977 plankton collections had almost no bent spine ends, but bends became obvious and common after holding the fish two weeks in the laboratory. Allyn Powell (National Marine Fisheries Service, Beaufort, N.C.) allowed the examination of cleared and stained spot hatched at his laboratory and some wild spot and croakers raised there for at least one week. Nearly all (8-30 mm TL) had bent spine ends; some fish with a few, some with the end of every spine bent. The tips were completely calcified. In captivity, nutritional deficiencies or muscular tension from general stress may alter growing bones before calcification. Growth in the laboratory beyond one month has not been investigated.

No Kepone-induced change in backbone collagen content was evident in these experiments. There was no difference in control and treated fish in the long-term experiment. I regard the significant difference between Tank B and wild fish as an artifact due to insufficient number of samples. Any changes in vertebral collagen in treated fish from experiment four were masked by the increased levels resulting from the proliferation of thick connective tissue. Collagen percentages in the thickened bones fluctuated from 27.0-34.6%, either due to uneven rates of spongy bone addition or difficulties removing the connective tissue, which was hardened to the bone in some places. One fish from the James River had similar hyperostosis. Control fish at day 57 had

no different vertebral collagen content than river fish. Controls of experiment four, in captivity five weeks, showed a collagen reduction. The latter fish grew at least 8 mm, while the former did not grow. Abnormal bone composition in these growing controls suggests inadequate conditions or diet. Because the treated were able to lay down a great amount of connective tissue, which is largely collagen, it appears that their ability to synthesize collagen was not impaired.

Many factors impinge upon fish bone collagen content and would influence its use for measuring sublethal toxicant effects. The cartilaginous skeleton of young fishes ossifies gradually, and after bone is formed it continues to mineralize. Paul Mehrle (pers. comm.) found the vertebral composition of *Ictalurus punctatus* stabilized at an age of 90 days. Fish bone collagen in percent dry weight ranges from 19-45% (Mayer et al., 1977) depending on species, fish age, or perhaps procedural variations; the authors gave no hypothesis. Within an experiment, the values obtained by Mayer et al. (1977) were fairly consistent. Collagen synthesis is dependent on available ascorbic acid, as demonstrated by Mayer et al. (1977, 1978) and Halver et al. (1969) among others. *Oncorhynchus kisutch* grew on a diet containing 50 mg/kg ascorbic acid, but skin wound repair was poor. For optimum health the fish needed 1000 mg/kg in the diet (Halver et al., 1969). Mayer et al. (1978) found that *Ictalurus punctatus* were healthy eating 670 mg/kg, but had vertebral fractures and low bone collagen at 63 mg/kg. *Loligo vulgaris* muscle contains about 46 mg/kg ascorbic acid (Pundit and Magar, 1972). Spot in my experiments ate squid, with

supplementary amounts of algae, river plankton, and the organs of the squid, but their diet was probably deficient in the vitamin. For long-term bone collagen experiments, ascorbic acid in food, vertebrae and liver should be known. Calcium available in the diet or water also influences bone growth. Since percent collagen is determined by weight difference, a decrease in bone mineral would appear to be a collagen increase. Collagen measurement alone is inadequate if the toxicant affects calcium metabolism. Kepone is known to affect mitochondrial calcium (Carmines et al., 1979) and calcium deposition in quail eggs (Eroschenko and Place, 1978). Altered calcium deposition could have been a masking variable in my experiments.

A decrease in vertebral collagen is a long-term reaction, and experiments must be sufficiently long for new fish bone growth in which to detect a change. Fishes can resorb bone minerals (Weiss and Watabe, 1978) but insoluble collagen is seldom if ever mobilized and redeposited in body structures (Love, 1970). Experimental fish that have not grown are therefore poor candidates for bone collagen assays.

Feeding

Feeding by percent body weight successfully controlled the supply of food to groups of fish but could not eliminate differences in the feeding rate of individuals. Generally all individuals appeared to eat well. Feeding rates of groups within an experiment were similar in all size classes and in both control and treated groups.

The appetite of juvenile spot increased with temperature. At 12-16°C they showed no interest in food after eating 5-6% body weight. Above 20°, 12% body weight was close to satiation. Appetite was measured by Elliot (1975) as the voluntary food intake of an animal. He found that in *Salmo trutta*, the weight of food eaten in one meal plotted against temperature rose steadily, reached a plateau, then plummeted in extreme heat. Appetite should be dependent on the caloric demand of metabolism. In fishes, active metabolism increases with temperature to a temperature of optimum performance (Brett and Groves, 1979). I found no published information on optimum performance temperature or active metabolism in spot.

After filling the gut to capacity at 12% body weight in the morning, the spot would also take an afternoon meal, making possible 16% body weight total feeding in the fourth experiment. Peters and Kjelson (1975) found that in North Carolina juvenile spot foraged continually day and night. They estimated the daily ration of 50-75 mm (TL) juveniles to be 10.1% dry body weight. In this daily ration, animal matter was 59 ± 8% by volume, the rest of the diet being unidentified detritus, phytoplankton, and vascular plant detritus. The spot in my experiments were fed only squid; which is not a natural diet for this species. The fish did grow, however, at a maximum of 9 mm per month at 16% body weight. Over their first year spot grow an average of 11.1 mm per month (Parker 1971). Since they do not grow much in winter, their warm weather growth rate must exceed 11.1 mm monthly. I gave a control group of juveniles at 29° two daily

feedings to satiation. They averaged 23% body weight per day and grew 6 mm in one week. Further Kepone uptake experiments through ingestion should use one or several constant temperatures and a feeding rate adequate for growth of the species.

Kepone Accumulation

Kepone consumption rates (Table 2) reflect both the amount eaten and the Kepone concentration. For example, food in experiment one had a higher concentration than that of experiment four, yet after 15 days both groups of fish had about the same body concentrations because of the large daily ration of the latter. Kepone consumption rates were calculated assuming all particles had the same concentration, but actually they varied, as shown by the food samples analyzed. Striped bass flesh, the preliminary food choice, was probably more evenly contaminated and had the pesticide chemically bound throughout rather than adsorbed on the particle surface only. However, it fouled the tanks, was in short supply, and could have contained heavy metals or other toxicants.

Kepone accumulation from food by spot in my experiments was linear; there was no decrease in the slopes of curves with time. The accumulation curve reflects net accumulation of parts per million as a result of three processes: uptake or extraction, depuration, and dilution by growth. Some organochlorine compounds fed to fish at sublethal levels eventually reached equilibrium, such as DDT + DDE (Jarvinen et al., 1977) dieldrin, and p, p-DDT (Macek et al., 1970).

The accumulation curves of other organochlorines were sloped throught the feeding, with only a slight reduction in slope from growth dilution, as in PCB (Zitko, 1977; Hansen et al., 1976) and Mirex (Van Valin et al., 1968; Ivie et al., 1974). Kepone did not behave the same in all uptake experiments. Spot fed Kepone in my experiments and in those of Bahner et al. (1977) showed steady accumulation, while the concentrations in channel catfish fed Kepone rose for 15 days then decreased somewhat during the rest of the 90 day feeding period (Van Veld, 1980). In aqueous Kepone administration, equilibrium was not reached in spot in 30 days (Bahner et al., 1977) or in _Pimephales promelas_ in 60 days (Buckler, 1979). Blue crabs fed Kepone accumulated it linearly without equilibrium in experiments by Schimmel et al. (1979) and Fisher (1980).

Pesticide accumulation in the body is partly determined by extraction efficiency, or how much of a compound consumed is retained. Extraction efficiency (Table 11) was calculated by dividing the final Kepone concentration accumulated by daily Kepone consumption rate times days of feeding Kepone. Extraction efficiency should be calculated from the linear portion of uptake curves and only if depuration is none or minimal. Extraction efficiency in my high dose experiments was 19.8-27.6%, and in my low dose experiments was 32.9-41.9%. Unretained Kepone was egested or depurated into the water, as evidenced by its accumulation in the oysters. Extraction efficiency of Kepone from mysids by spot for 30 days was 38-39% (Bahner et al., 1977). Using the maximum accumulation of Kepone by

Table 11. Kepone extraction efficiency[a] for spot.

Experiment		Days	ppms accumulated	percent Efficiency	n
I		15	1.65	27.6	6
II	High dose	28	0.39	37.4	5
		56	0.70	36.6	5
	Low dose	28	0.19	41.9	5
		56	0.33	32.9	5
III	All sizes	15	1.01	21.2	16
IV		28	2.65	19.8	5

[a] $\frac{\mu g/g \text{ Kepone in body}}{\text{daily Kepone consumption rate (per g fish)} \cdot \text{days}} \cdot 100$

channel catfish at 15 days, their extraction efficiency was 19.8% (Van Veld, 1980).

Pesticide accumulation in the body is also affected by depuration. Efflux of a compound is possible when there are a limited number of storage sites in the body and/or when the compound can be mobilized from the storage sites. There are species differences in Kepone depuration. Van Veld (1980) noted a half-life of 8.0 - 8.7 days for Kepone in channel catfish. American eels (_Anguilla rostrata_) lost about 90% of their Kepone in seven weeks (Hedgepeth and Stehlik, 1979). Depuration of Kepone from spot is much slower. Adult spot lost 53% of their Kepone in 31 days (Hedgepeth et al., 1979). The juvenile spot in the long-term experiment lost no Kepone from days 56-84 while eating uncontaminated food. Kepone is stored mainly in the brain associated with high density lipoproteins. Less polar organochlorine compounds, such as DDT and Mirex, are stored in liver and fat tissues. The latter would commonly be called upon for energy reserves and the pesticide mobilized, but neural tissue is rarely broken down. A slow depuration rate, as in spot, could be explained as a difficulty in mobilizing the pesticide from wherever it is stored. From the limited data on the sites of Kepone storage in fishes (Van Veld, 1980; Hedgepeth and Stehlik, 1979; Bahner et al., 1977) no species differences are yet known.

Long-term accumulation studies utilize growing fish, therefore dilution of the pesticide concentration with growth will affect accumulation curves. A long-term feeding of PCBs to growing catfish

provides an example: the concentration equilibrated and declined over 252 days, yet the actual weight of PCBs in each fish, expressed in ug/fish, accumulated additively (Hansen et al., 1976). The uptake curves for my long-term experiment might have flattened if the fish had grown. Uptake by the spot in my fourth experiment was linear despite a 66% weight increase in 28 days. In contrast, while the channel catfish grew 63% in 90 days, their Kepone concentrations declined (Van Veld, 1980). The greater depuration ability of channel catfish probably explains the difference in uptake curves with respect to spot. It is also possible that no Kepone feeding experiments with spot have been run long enough to reach equilibrium if it occurs.

Accumulation rate as defined by Phillips and Buhler (1978) is the concentration accumulated divided by days of the experiment. It can be used in cases of little or no depuration. Average Kepone accumulation rates for my experiments and other spot studies are given in Table 12. Whether Kepone was administered in food or water, it was toxic if the accumulation rate was 0.095 or above. At 0.095 and 0.110, death came in seven or more days, but at 0.425 and 0.800, death was much sooner. Accumulation rates show that the buildup of Kepone, regardless of its mode of entry, controlled toxicity.

The duration and rate of Kepone uptake determines onset of symptoms. During the fourth experiment, I noted that one fish in the seven day sample exceeded 1 ppm and was dying, but some in the 14 day sample that exceeded 1 ppm were symptomless. A high accumulation rate in that individual apparently was lethal. Similarly, spot in LC_{50}

Table 12. Kepone accumulation rates[a] for spot.

Experiment	Days	ppms accumulated	Rate	Poisoning Symptoms
1	15	1.65	0.11	deaths
2 High dose	56	0.70	0.0125	none
Low dose	56	0.33	0.0058	none
3 26-30 mm	13-15	1.13	0.075	none
31-35 mm	13-15	1.12	0.067	none
36-40 mm	13-15	0.86	0.057	none
$\bar{x}$		1.01	0.067	
4	28	2.65	0.095	deaths
Schimmel and Wilson, 1977	4 (water)	1.7	0.425	deaths
	4 (water)	3.2	0.800	deaths
Bahner et al., 1977	30 (water)	0.093	0.0031	none
	30 (water)	0.94	0.0313	none
	30 (food)	0.019	0.0006	none
	30 (food)	1.05	0.0350	none

[a] $\frac{\mu g \text{ Kepone accumulated}/\ \mu g \text{ fish}}{\text{days of experiment}}$ = accumulation rate

Table 13. Bioaccumulation factor[a] for spot.

Experiment	ppm fish	ppm food	days	BAF
1	1.65	3.91	15	0.422
2 High dose	0.70	0.59	56	1.190
Low dose	0.33	0.295	56	1.105
3	1.01	3.18	15	0.318
4	2.65	3.31	28	0.801
Bahner et al.,	0.0195	0.023	30	0.85
1977	1.05	1.23	30	0.85

[a] $\frac{\text{ppm fish}}{\text{ppm food}} = \text{BAF}$

assays (Schimmel and Wilson, 1977) that reached 2 ppm died in four days, whereas some spot collected from the James River and held in the laboratory for months contained over 2 ppm and were not ill. Spot that exceeded 2 ppm in the fourth week of experiment four were moribund. Apparently the spot in the river survived by accumulating their Kepone gradually over the season.

Bioaccumulation factor reflects accumulation of a compound from one link in a food chain to another. A bioaccumulation factor (BAF) greater than one means biomagnification will occur. Bioaccumulation factors calculated from my experiments (Table 13) and from Bahner et al. (1977) increase with number of days feeding on Kepone. Beyond 30 days BAFs exceed one, meaning that fish eating in Kepone contaminated rivers for a long enough period will biomagnify the compound slightly (assuming slow depuration). Because bioaccumulation factors exceed one, and Kepone concentrations in epibenthic invertebrates in the James River ranged from 0.1-2.0 ppm in the past few years (Bender et al., 1977) spot in the river in the mid 1970's could have accumulated their observed concentrations in a few months.

Depending on Kepone accumualation rates, some spot in the James River could have developed fractured vertebrae, or possibly died. The ability of Kepone to cause vertebral fractures in spot in the laboratory has now been proved using an intensive dosage, as a result of muscular tension or hypertrophy of vertebral centra. The highest accumulation rates eventually caused mortality. A few cases of bone fractures were found in spot eating sublethal dosages at lower

accumulation rates. In the James River, depending on rates of accumulation, individual spot could acquire enough Kepone to cause bone damage. Fractured and hypertrophied vertebrae of spot from the James River appear very similar to those of spot fed Kepone in the laboratory.

CONCLUSION

1. In juvenile spot feeding on Kepone-contaminated food, Kepone concentrations rose linearly with time. Equilibrium was not reached in the experimental period, 56 days.

2. Juvenile spot did not depurate in 28 days eating Kepone-free food.

3. Accumulation rates 0.95 or above produced toxic effects and deaths. The timing of accumulation to a given concentration controlled occurrence of mortality.

4. There was little difference between food and water uptake of Kepone on external (behavioral) or internal (structural) development of symptoms.

5. Properly executed bioaccumulation experiments must be carried out at constant temperatures and feeding rates. The best growth rate for juvenile spot was at 24-28°C with a ration of 16% body weight.

6. Kepone poisoning induced acute neuromuscular tremors and fractured vertebral centra.

7. After at least three weeks of exposure, Kepone induced abnormal hypertrophy of vertebrae and proliferation of surrounding connective tissue.

8. Those spot with hyperostosis had unusually high percentages of collagen in dry vertebrae. Control spot growing in the laboratory

had unusually low percentages of collagen in bone.

9. Stress and nutrition in captivity caused certain bone deformities, such as bent ends of neural and hemal spines, and may affect bone formation regardless of toxicant application.

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