1980

Uptake, distribution, metabolism and clearance of kepone by channel catfish (Ictalurus punctatus)

Peter A. VanVeld
College of William and Mary - Virginia Institute of Marine Science

UPTAKE, DISTRIBUTION, METABOLISM AND CLEARANCE OF KEPONE BY CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

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

Peter A. VanVeld

1980

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts in Marine Science

Peter A. VanVeld

Approved,

Michael E. Bender, Ph.D.

Robert J. Byrne, Ph.D.

John V. Merriner, Ph.D.

Morris H. Roberts, Jr., Ph.D.

J. Ernest Warinner, III, M.A.

TABLE OF CONTENTS

	Page
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	vii
INTRODUCTION	2
MATERIALS AND METHODS	14
RESULTS	26
DISCUSSION	44
SUMMARY AND CONCLUSIONS	62
SUGGESTIONS FOR FUTURE RESEARCH	64
LITERATURE CITED	67
VITA	74

ACKNOWLEDGMENTS

I thank the members of my committee for their enthusiasm and support. Mike Bender suggested the topic and helped make my time at VIMS a truly educational experience. I can not thank Mory Roberts enough for guidance that improved the quality of this work tremendously.

Field collections were made possible through the expertise of John Zearnes, Danny Fisher, Joe Lascara and Bob Middleton.

I thank Marc Fariss of MCV for allowing me to use his lab and for providing pesticide standards.

LIST OF TABLES

Table		Page
1	Mean values with their standard errors for the concentration of Kepone in tissues of channel catfish during the dietary exposure study	32
2	Mean values with their standard errors for the percentage of the total body burden Kepone found in the tissues of channel catfish sacrificed on day 70 of the dietary exposure study	34
3	Kepone concentration in tissues and percentage of the total body burden Kepone found in the tissues of channel catfish 48 hours and 120 hours after receiving IM injections of Kepone.	36
4	Mean values plus their standard errors for the concentration of Kepone in tissues and the percentage of the total body burden Kepone found in tissues of white catfish sacrificed on day 30 of the dietary exposure study	37
5	Recovery of Kepone from gill rinses, skin mucus and urine of channel catfish 48 hours after receiving IM injections of Kepone	40
6	Results of analysis of channel catfish bile and blood for chlordecone, chlordecone alcohol and glucuronide conjugates of chlordecone and chlordecone alcohol	41
	Dietary accumulation factors (DAF) of organochlorine pesticides in fish	45
8	Daily dietary dosage levels, uptake rate constants and clearance rate constants for organochlorine pesticides in fish	48

LIST OF FIGURES

Figure		Page
1	Structures of chlordecone (Kepone) and chlordecone monohydrate	4
2	Structures of chlordecone (Kepone), chlordecone alcohol and the glucuronide of chlordecone alcohol.	10
3	Total body burden Kepone concentration in channel catfish receiving a ^{14}C-Kepone contaminated diet (1.36 μ g/gm) at a rate of two percent of their body weight per day for a period of 90 days followed by a Kepone free diet for a period of 30 days. . . .	28
4	Semi-log plots of total body burden Kepone concentrations (dry weight) in channel catfish during the clearance phase of the dietary exposure study and in channel catfish and white catfish which received intra-muscular injections of Kepone	31
5	Sample chromatographs of the Kepone standard, chlordecone alcohol standard, chlordecone emulphor solution and bile from channel catfish receiving IM injections of Kepone . . .	43
6	Kepone concentrations in channel catfish collected from the James River versus length of fish.	54

ABSTRACT

Channel catfish (Ictalurus punctatus) fingerlings were exposed to a ^{14}C-Kepone (19.68 µCi/µmole) contaminated diet (1.36 µg/gm) at a rate of two percent of their body weight per day for a period of 90 days, followed by a Kepone free diet for 30 days. The uptake and clearance rate constants were 0.237 day^{-1} (wet weight basis) and 0.080 day^{-1} respectively. The half-life of Kepone in these fish was 8.7 days. The dietary accumulation factor (DAF) defined as the equilibrium concentration of pesticide in fish/daily dietary dosage level was calculated to be 3.0. The clearance rate of Kepone in channel catfish is independent of fish size or initial total body burden. The clearance rate for white catfish (Ictalurus catus) receiving intramuscular injections was 0.043 day^{-1} ($t_{1/2}$ = 16.1 days).

Highest concentrations of Kepone in channel catfish were found in the blood followed by the brain. Greater than 80 percent of the Kepone present in whole blood is associated with the plasma fraction. Lowest concentrations were consistently found in mesenteric adipose tissue, followed by carcass. White catfish exposed to the ^{14}C-Kepone contaminated diet contained highest concentrations in the brain followed by liver and intestines. Lowest concentrations were measured in the mesenteric adipose and carcass.

In channel catfish, Kepone may be eliminated with bile via the intestinal contents. There is evidence for an extrabiliary source of fecal Kepone in channel catfish. Kepone may be excreted across the gills and sloughed off with epidermal mucus. Urinary excretion is negligible.

Channel catfish are capable of reducing Kepone to chlordecone alcohol as shown by the presence of this metabolite in the bile of fish following intramuscular injections of Kepone. Chlordecone is not conjugated with glucuronic acid in the bile.

UPTAKE, DISTRIBUTION, METABOLISM AND CLEARANCE OF KEPONE BY CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

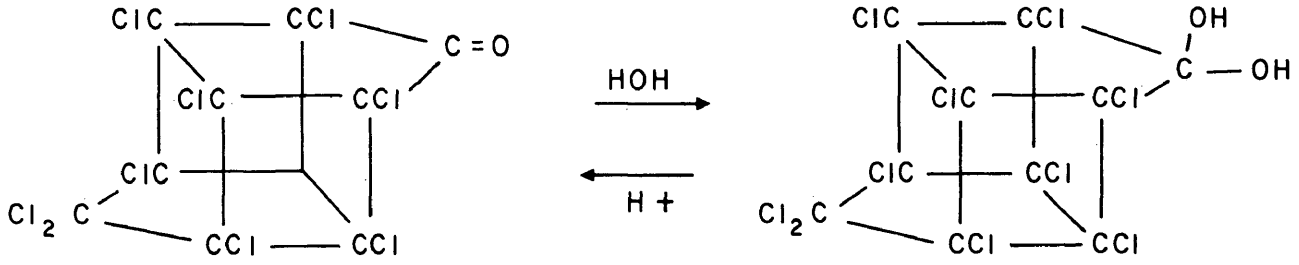
INTRODUCTION

Kepone^{R}, the trade name for chlordecone is one of the class of chlorinated hydrocarbon insecticides including chlordane, heptachlor, dieldrin, endrin and mirex which are manufactured from a hexachlorocyclopentadiene intermediate. Pure Kepone is a colorless, odorless, crystalline solid with a melting point of 349°C and a vapor pressure of 10^{-5} mm Hg (Huff and Gerstner, 1978). The anhydrous compound becomes hydrated when exposed to room temperature and humidity. The trihydrate of Kepone $C_{10}Cl_{10}O \cdot 3H_2O$ is the most stable form (Dawson, 1978). In biological tissues, Kepone exists as a monohydrate (Figure 1) which exhibits some characteristics of a weak acid (Guzelian et al., 1980).

The water solubility of Kepone is 1.5 to 2.0 mg/l in the pH range of 4 to 6, increasing to 5 to 70 mg/l in the 9 to 10 pH range (Dawson, 1978). At 100°C, the water solubility may be as high as 4000 mg/l (Martin and Worthing, 1974). Kepone is readily soluble in acetone and lower aliphatic alcohols but slightly less soluble in benzene, toluene and hexane (Dawson, 1978).

Kepone was first synthesized in 1951 and introduced into the market by Allied Chemical Corporation in 1958 for use as a pesticide. Its use in the United States was restricted to an ingredient in ant and roach baits but has been used in other countries for control of banana and potato pests. In accordance with require-

Figure 1. Structures of chlordecone (Kepone) and chlordecone monohydrate.



CHLORDECONE

CHLORDECONE HYDRATE

ments for registry of Kepone as a pesticide, Allied Chemical in 1958 conducted toxicological studies on the acute, subchronic and chronic toxicity of Kepone in several species (Jaeger, 1976). The LD_{50} (defined as the single dose required to kill 50 per cent of the test organisms) for rabbits, rats and dogs was reported to be 65, 95, and 250 mg/Kg respectively. The results indicated that Kepone is a cumulative toxin which may require long periods of time to exert its effects and that residues of 10 μ g/gm in the diet causes cancer in mice and rats.

Allied Chemical Corporation produced approximately 1.5 million pounds of Kepone between 1966 and 1973. Under contract with Allied Chemical, Life Science Products manufactured approximately 1.7 million pounds during 16 months of operation in 1974 and 1975. Production of Kepone was voluntarily halted in 1975 because Virginia State Health officials became aware of health hazards to workers from Kepone exposure. More than 50 per cent of the 110 employees at Life Science Products showed high blood levels of Kepone. Approximately 20 workers were hospitalized with clinical evidence of damage to the central nervous system, liver and testes.

Other serious health and environmental hazards have resulted from the discharge of Kepone into the upper tidal portion of the James River estuary via effluent from the Hopewell sewage treatment plant. A study conducted by the United States Environmental Protection Agency (1975) revealed that Kepone was present in air, soil and water near Hopewell. Bender et al. (1977) reported residues in James River biota collected during an extensive monitoring program. Average residue concentrations expressed on a wet weight basis, varied from

0.04 µg/gm in channel catfish (Ictalurus punctatus) to 2.4 µg/gm in largemouth bass (Micropterus salmoides). Residues in long-term resident estuarine fish varied from 0.6 µg/gm in American eel (Anguilla rostrata) to 2.7 µg/gm in white perch (Morone americanus). Short-term resident marine fish (e.g. American shad, Alosa sapidissima; Atlantic menhaden, Brevoortia tyrannus) had residues averaging less than 0.1 µg/gm. Blue crab (Callinectes sapidus) residues averaged 0.19 µg/gm for females and 0.81 µg/gm for males. Huggett et al. (1979) reported Kepone contamination in all trophic levels of the James River ecosystem. Residue concentrations in phytoplankton and detritus expressed on a dry weight basis averaged 1.3 µg/gm and 0.7 µg/gm respectively while residues found in zooplankton averaged 4.8 µg/gm on a wet weight basis. As a result of the widespread contamination of James River biota, all fishing in the river was banned in 1975. Later, the ban was eased to permit the harvesting of clams, oysters, catfish, herring, and shad. In addition, some areas of the James River seaward of the James River bridge (Route 17) are open for the harvest of crabs.

Based on the results from monitoring of Kepone residues in sediments, an estimated 30,000 kilograms of Kepone may reside in James River sediments (Huggett, personal communication), Because water residues of dissolved Kepone have averaged in the low ng/l range (Huggett et al., 1979) it appears that the sediments are the major sink for Kepone in the James River. Kepone is associated primarily with organic material in the upper and middle estuary and with fine-grained sediment in the lower estuary. The majority of the Kepone bound to suspended material accumulates in the area of the turbidity maximum in the middle estuary.

Prior to the Hopewell incident, information concerning the aquatic toxicology of Kepone was scarce. The 24 hour and 96 hour LC_{50}'s (defined as the concentration of chemical in water required to kill 50 per cent of the test organisms in the specified period of time) for rainbow trout (Salmo gairdneri) were reported to be 66 µg/l and 20 µg/l (Cope, 1965). The 24 hour and 48 hour LC_{50}'s for white mullet (Mugil curema) were 500 µg/l and 55 µg/l and for longnose killifish (Fundulus similis), 300 µg/l and 84 µg/l (Butler, 1963).

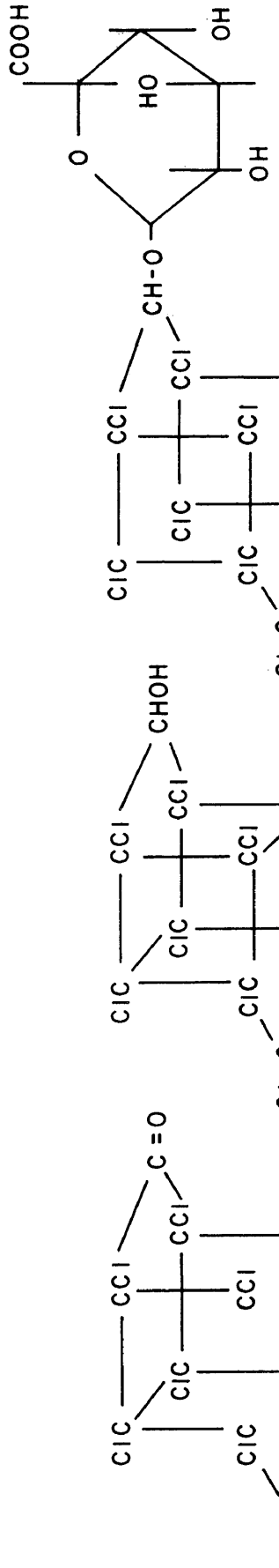
Following the discovery of Kepone contamination of James River biota, aquatic toxicology studies of Kepone have focused on species native to the James River estuary. Schimmel and Wilson (1977) reported that the 96 hour LC_{50}'s for spot (Leiostomus xanthurus), sheepshead minnow (Cyprinodon variegatus), grass shrimp (Palaemonetes pugio), and blue crab were 6.6 µg/l, 69.5 µg/l, 121 µg/l and >210 µg/l, respectively. Aqueous exposure of Kepone produced chronic effects on all life stages of sheepshead minnow (Hansen et al., 1977) and chronic effects in life cycle tests with mysids (Mysidopsis bahia) (Nimmo et al., 1977). Schimmel et al. (1979) reported mortality in blue crabs during a 28 day feeding experiment involving exposure to the pesticide via the diet at levels of 0.15 and 1.9 µg/gm. Couch et al. (1977) found that Kepone induces lateral curvature of the spine (scoliosis) in sheepshead minnow resulting in severe histological effects. Mechanisms of the toxic actions of Kepone on aquatic organisms remain poorly understood although ATPase inhibition has been established (Desaiah and Koch, 1975).

There is no evidence reported in the literature for metabolic transformation of Kepone by an aquatic organism. Borsetti and Roach (1978) identified 9-chloro and 8-chloro homologs of Kepone in mullet (Mugil sp.) collected from the James River. It is likely, however, that these homologs were produced either by photodegradation or during industrial production of Kepone.

Evidence for a reduced form of Kepone has been reported by Blanke et al. (1978). The metabolite designated as chlordecone alcohol is believed to be produced in the liver (Guzelian et al., 1980). In human bile 98 percent of the chlordecone alcohol is conjugated with glucuronic acid (Boylan et al., 1979). The structures of chlordecone, chlordecone alcohol and the glucuronide of chlordecone alcohol are shown in Figure 2. Conjugation of a lipophilic compound with glucuronic acid often results in enhanced excretion by decreasing the lipid solubility of the compound.

Aquatic organisms are generally believed to acquire the bulk of pesticide residues from aqueous and/or dietary sources. Bahner et al. (1977) studied the accumulation of Kepone from water and food by estuarine organisms in laboratory bioassays designed to represent simple estuarine food chains. In flow-through bioassays, the bio-concentration factors (defined as the ratio of the concentration of Kepone in the test organisms to that found in the water) for mysids, grass shrimp, sheepshead minnows and spot averaged as high as 13,000, 11,000, 7,000 and 3,000, respectively. In studies involving exposure to Kepone via dietary sources, the bioaccumulation factor (defined as the ratio of the concentration of Kepone in the predator to that found in the prey) for mysids fed Kepone contaminated brine shrimp was 0.53 and for spot which consumed these mysids 0.85. The

Figure 2. Structures of chlordecone (Kepone), chlordecone alcohol and the glucuronide of chlordecone alcohol.



GLUCURONIDE OF CHLORDECONE ALCOHOL

CHLORDECONE ALCOHOL

CHLORDECONE (KEPONE)

initial bioconcentration of Kepone from water by food organisms was the dominant source of Kepone in these food chains. From this data, Macek et al. (1979) concluded that the contribution of dietary sources to the total body burden Kepone in aquatic organisms is negligible when compared to the potential for bioconcentration from water alone. However, in the James River where residues measured in water are in the low ng/l range, the importance of aqueous exposure may be considerably less than these laboratory studies indicate.

Depuration of Kepone has been observed in a number of estuarine organisms. Following aqueous exposure to Kepone, oysters held in Kepone free water lost 35 per cent of their total body burden in 24 hours and following dietary exposure, oysters fed a Kepone free diet lost more than 90 per cent of their total body burden within 10 days (Bahner et al., 1977). Huggett et al. (1979) estimated the half-life of Kepone in oysters to be approximately one week in the summer and 40 days during the winter suggesting that there is a temperature effect upon depuration. Bahner et al. (1977) found that depuration of Kepone by grass shrimp and spot was slower than that reported for oysters. Kepone residues were reduced 30 to 50 per cent in 24 to 28 days in these species. Hedgepeth et al. (1979) found that depuration by spot from the James River held in Kepone-free water was much slower (72 per cent loss in 200 days).

Information regarding the partitioning of Kepone in the tissues of aquatic organisms is scarce and incomplete. Bahner et al. (1977) reported that Kepone residues in laboratory exposed spot were highest in brain followed by liver, gills and muscle after 30 days of depuration. Values for other tissues were not given. Hedgepeth and

Stehlik (1979) analyzed composite samples of selected tissues of American eels from the James River. Highest residues were found in a composite sample of gall bladders followed by liver, muscle, gonads and fat. Roberts (1980) found that blue crabs collected from the James River contained higher residue concentrations in the reproductive tissues (ovary and testes) than the hepatopancreas.

Of particular relevance to patterns of Kepone accumulation in aquatic organisms is the range of residue levels exhibited by resident freshwater fish. Bender et al. (1977) observed that the total body burden of Kepone was related to the residence time of fish in the river and the distance from the source of contamination. However, the range of average residue concentrations in different species of resident freshwater fish spans almost three orders of magnitude between 0.04 µg/gm in channel catfish and 2.4 µg/gm in largemouth bass. Residue concentrations in white catfish (Ictalurus catus) averaged 0.25 µg/gm, almost one order of magnitude higher than concentrations observed in channel catfish. Differences in residue concentrations attained by fish may largely be influenced by different feeding habits and spatial distribution. However, a range in concentrations covering three orders of magnitude suggests that there may be species differences in uptake, tissue distribution, metabolism and elimination of Kepone.

The present study was undertaken to provide information on the importance of these processes in determining the degree to which Kepone will accumulate in channel catfish.

The major objectives of this study were to determine:

1) rates of uptake and clearance of dietary Kepone by channel catfish

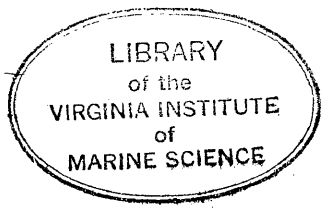
2) the dietary accumulation factor DAF (defined as the residue concentration of pesticide in fish after equilibrium has been reached with dietary pesticide divided by the daily dietary dosage level) for Kepone in channel catfish.

3) the pathways of elimination of Kepone in channel catfish and the relative importance of each .

4) the tissue distribution of Kepone in channel catfish.

5) the tissue distribution of Kepone in white catfish.

6) whether biotransformation byproducts of Kepone are present in channel catfish.



MATERIALS AND METHODS

Choice of Primary Methodology

Radiotracer methodology was chosen for this study because the sensitivity of detection offered exceeds that of other available methods. The ^{14}C-Kepone (Pathfinder Laboratories, St. Louis, Mo.) was a gift from Allied Chemical Corporation. The specific activity of the labeled compound (19.68 μCi/μmole) allowed for detection of Kepone residues in tissues at levels as low as 0.01 ng/mg for samples as small as twenty milligrams or levels as low as 0.2 ng/mg for samples as small as one milligram. Other methods of analysis involving electron capture gas chromatography (Moseman et al., 1977) recommend relatively large sample weights (10-20 gms) for a minimum practical detection level of 0.01-0.02 ng/mg. In the present study, it was assumed that the radio-labeled Kepone behaved in a chemical and physical manner identical to the unlabeled compound.

Dietary Exposure Studies

For studies involving the exposure of fish to Kepone from a dietary source, the experimental diet was prepared by first dissolving the labeled pesticide in a 1:1 solution of cod liver oil and corn oil. Next, twenty milliliters of the oil was sprayed onto four kilograms of commercial grade trout chow. The mixture was blended in a stainless steel bucket using a stainless steel spoon. Five one gram samples of the contaminated chow were analyzed for radioactivity. The

diet contained 124.2 disintegrations per minute (DPM) per milligram of chow (S.E. = ±4.7) which is equivalent to a Kepone residue level of 1.36 µg/gm.

Based on residue levels measured in James River biota (Bender et al., 1977) the concentration of Kepone incorporated into the diet was believed to approximate that which wild James River channel catfish would be exposed to in a natural diet. The level of radioactivity in the diet was sufficiently high that significant dilution of the labeled pesticide could occur within the body tissues and still allow accurate detection of radioactivity. Preliminary work indicated that the oil solution remained bound to the food for extended periods of time following immersion in water. Tests involving force feeding of gelatin capsules full of Kepone contaminated oil to channel catfish indicated that fish could readily assimilate a high percentage (65%) of the oil-bound Kepone into their tissues. Therefore, it was assumed that the uptake of Kepone from the experimental diet, in which relatively small amounts of oil were used, was not restricted by the oil digestion capabilities of the fish.

Channel catfish (average weight = 6.5 gms; S.E. = ±0.25) were collected from the Mattaponi River. Kepone residues were non-detectable in these fish according to the results of analysis by electron capture gas chromatography. Fifty fish were placed in a 500 liter fiberglass tank having a well water flow of two 1/min. The water in the tank had the following characteristics: dissolved oxygen 8.0 mg/1; pH 6.9; hardness as $CaCO_3$ 146 mg/1. The entire experiment was conducted at 19 ± 1°C. Fish were acclimated to these conditions for two weeks and fed uncontaminated trout chow at a

rate of two per cent body weight per day. Following the two week acclimation period fish were exposed to the ^{14}C-Kepone contaminated diet at a rate of two per cent body weight per day. The feed was readily consumed by the fish each day throughout the entire experiment. The daily dietary dosage level averaged 0.027 micrograms Kepone per gram of wet weight fish. Five fish were sacrificed on days 5, 15, 30, 50, 70 and 90. The amount of food provided was adjusted at each sacrifice period so that two percent body weight per day was provided throughout the study. At the end of 90 days, the pesticide contaminated diet was replaced with uncontaminated trout chow so that clearance could be observed. Five fish were sacrificed on days 5, 15 and 30 of the clearance phase. There were no mortalities observed in channel catfish during the entire experiment.

Fish were anesthetized with tricaine methanesulfonate (TMS) prior to dissection. Whole blood was collected by drainage from a severed caudal peduncle into stainless steel pans. Fish were dissected into stomach, intestines, intestinal contents, liver, gall bladder (including bile), kidneys, gills (filaments plus arches), brain and mesenteric adipose. The remainder of the fish, predominately muscle, skin and bones was homogenized and will be referred to as carcass Gonads were not developed in these fish.

White catfish fingerlings (average weight 6.5 gms; S.E. = $\pm$0.212) were collected from the Mattaponi River at the same time and location as channel catfish. Under laboratory conditions identical to those described above for channel catifsh, white catfish were exposed to the ^{14}C-Kepone contaminated diet. These fish did not remain healthy and after fifteen days of exposure to the experimental

diet, food consumption began to drop rapidly. Therefore, these fish were removed from the tanks after day 30. On days 5, 15 and 30, five fish were sacrificed and dissected according to the same procedure used for channel catfish.

IM Injections

Intramuscular (IM) injections of Kepone were administered to groups of fish in order to supplement and support data collected from the dietary exposure study. ^{14}C-Kepone was dissolved in one part ethanol followed by dropwise addition of one part emulphor (GAF Corporation) followed by dropwise addition of eight parts water. IM injections of this medium offered an effective and convenient method of administering a known amount of Kepone to fish.

Intramuscular injections of Kepone were performed on one group of fish in order to provide samples of gill rinses, skin mucus and urine for radiometric analysis. Analysis of these components was necessary for the determination of their importance in Kepone elimination. For this study, five channel catfish (average weight 80 gms) were administered a dose equivalent to 0.20 µg/gm (wet weight) and placed in a flowing well water tank held at 19 ± 1°C. In addition, two fish which had not received injections were placed in the tank to account for adsorption of excreted pesticide onto mucus. The fish were held for 48 hours after which they were removed from the tank and anesthetized with TMS. Urine was collected by drying the skin with a paper towel and then gently squeezing the area surrounding the urogenital papilla (Lee et al., 1972). As urine was expelled it was collected into a 50 µl pipet. Mucus samples were gently scraped off the skin surface with a stainless steel spatula. An attempt was

made to collect mucus from the entire surface of the fish. Excised gills were gently swirled in scintillation vials containing well water. The percent of the total gill mucus removed from the gills under these conditions is unknown.

A second group of fish received IM injections of Kepone to determine if the half-life of Kepone in channel catfish is related to the size of the fish or the total body burden Kepone in the fish. White catfish were also used in this study so that an estimate of the half-life of Kepone in this species could be made. In addition, the channel catfish in this study were dissected as in the dietary exposure study to determine if the tissue distribution of Kepone was affected by the method of dosing and/or the relative size of the dose. Three fish of each species (average weight 90 gms) were administered a dose equivalent to 0.20 µg/gm and placed in flow-through aquaria held at 19 ± 1°C. One fish of each species was sacrificed on days 2, 5 and 15 following injection.

A third group of fish were injected (IM) with Kepone in order to provide samples of bile and blood for analysis of chlordecone alcohol and glucuronic acid conjugates of chlordecone alcohol. Blood samples also yielded data on the relative amount of Kepone present in the plasma versus the blood cells. Four channel catfish (average weight 150 gms) received a dose of Kepone equivalent to 1.0 µg/gm and were placed in a flowing well water tank held at 19 ± 1°C. After one week, the fish received an additional dose of Kepone equivalent to 0.50 µg/gm. Fourteen days following the initial injection, the fish were sacrificed and bile was drained from the gall bladders into stainless steel pans. Blood samples were collected

in heparinized tubes and the plasma and cells separated by centrifugation at 2000 rpm for five minutes.

Liquid Scintillation Counting

All samples, exlcuding urine, were lyophilized to a constant dry weight and homogenized when necessary with a mortar and pestle. Subsamples (10-20 mg if possible) were weighed and placed in scintillation vials. The samples were wetted and then digested with 1 ml NCS^R tissue solubilizer at 50°C until all tissue was dissolved. Crushed bone was never completely digested with NCS. Fifteen milliliters of scintillation cocktail (5 gms PPO per liter toluene) were then added to each sample. Samples were dark adapted for 48 hours to eliminate background interference from chemoluminescence. The samples were counted in a Beckman LS-150 liquid scintillation counter at a 2.0 percent counting error. The external standard channels ratio method was used for quench calibration so that the counting efficiencies for each sample could be determined. Count rates were converted to Kepone residue levels (ng Kepone/mg dry weight tissue) by use of the following calculation:

$$\frac{\text{CPM(sample)} - \text{CPM(background)}}{\text{CE} \times \text{sample weight}} = \text{DPM/mg}$$

DPM/mg x 0.011 ng Kepone/DPM = ng Kepone/mg tissue

or DPM/mg x 11 pg Kepone/DPM = pg Kepone/mg tissue

where CPM = counts per minute

CE = counting efficiency of sample

DPM = disintegrations per minute

Urine samples (50 µl) were placed in scintillation vials followed by addition of 10 milliliters of Aquafluor^R cocktail.

Internal standardization was used to determine the counting efficiency for urine samples. Count rates were converted to Kepone concentrations (pg Kepone/µl urine) by use of the following calculations:

$$\frac{\text{CPM(sample)} - \text{CPM(background)}}{\text{C.E.} \times \text{sample volume (50 µl)}} = \text{DPM/50 µl}$$

$$\text{DPM/50 µl} \times 11 \text{ pg/DPM} \times 1/50 = \text{pg/µl}$$

The total body burden of Kepone in fish was calculated by summing the products of the residue level in each tissue times the total weight of each tissue. The percentage of the total body burden Kepone found in each tissue was determined for all tissues with the exception of mesenteric adipose. Mesenteric adipose was excluded from these calculations because of the difficulty involved in removing all of this tissue from the mesentery. Similarly, the percent of the total body burden Kepone present in the blood was not determined because the total weight of blood in the fishes was unknown. In percent of total calculations, the contributions from that adipose tissue and blood removed from fish were added to carcass values.

Calculation of Uptake Rate, Clearance Rates, Half-life, Equilibrium Body Burden and Dietary Accumulation Index (DAI)

Assuming that (1) the sole source of Kepone available to fish in the dietary exposure study was that which was available in the food and (2) the dosage rate was held constant during the entire uptake phase of the study, then the change in the residue level of Kepone in channel catfish over time can be described by a first order kinetic model written as

$$dC_a/dt = K_1 \quad Q - K_2 C_a \qquad (1)$$

where

K_1 = uptake rate constant (day^{-1})

K_2 = clearance rate constant (day^{-1})

C_a = residue level in fish at time t (µg/gm)

Q = daily dietary dosage level (µg/gm)

(i.e. amount of Kepone available to fish each day)

This model for dietary accumulation is equivalent to the model for bioconcentration of pesticides from food presented by Metcalf (1977) and is similar to the widely accepted (Hamelink, 1977; Branson et al., 1975; Blanchard et al., 1977) model for bioconcentration of a contaminant from water.

Integration of equation (1) yields

$$C_a = \frac{K_1}{K_2} Q \ (1-e^{-K_2 t}) \qquad (2)$$

The dietary exposure study consists of two phases, an uptake phase where Q is held constant at 0.027 µg/gm and a clearance phase where Q = 0. During the clearance phase equation (1) reduces to

$$dC_a/dt = -K_2 \ C_a \qquad (3)$$

Integration of equation (3) yields

$$\ln C_a = -K_2 t + a \qquad (4)$$

where

a = ln C_a at time 0 of the clearance phase

The clearance rate constant of Kepone was determined by fitting data from the clearance phase to equation (4) by the method of least-squares regression. Clearance rate constants will be the same whether calculated from wet weight body burden values or dry weight values.

The half-life ($t_{1/2}$) of Kepone was determined by using the calculated value of K_2 and equation (4) in which

$$\ln 0.5\ C_{a_o} = -K_2 t_{1/2} + \ln C_{a_o}$$

$$K_2\ t_{1/2} = \ln C_{a_o} - \ln 0.5\ C_{a_o}$$

$$t_{1/2} = \frac{\ln 2}{K_2} \qquad (5)$$

where

C_{a_o} = residue level in fish at beginning of clearance phase

During some early portion of the uptake phase, the clearance term (K_2C_a) is negligible because C_a is small. Therefore, equation (1) reduces to

$$\frac{dC_a}{dt} = K_1 Q \qquad (6)$$

Integration of equation (6) yields

$$C_a = K_1 Q\ t + c \qquad (7)$$

where

$c = C_a$ at time 0

The determination of the uptake rate constant (K_1) of Kepone by channel catfish was determined as follows:

1) A plot was made of the total body burden Kepone versus time during the dietary exposure study.

2) Using the data for the initial, essentially straight line portion of the uptake curve (i.e. an interval over which K_2C_a seems to have a negligible effect) a trial value of the uptake rate K_1 was calculated using least-squares regression for equation (7) rearranged as

$$\frac{C_a}{Q} = K_1 t + c' \qquad (8)$$

where $c' = \frac{c}{Q}$

3) With the K_2 calculated by fitting data to equation (4) and the K_1 derived from equation (8), predicted values for each measured C_a were calculated over the entire uptake phase of the study using equation (2). The sum of squared deviations of predicted values from measured values of C_a was calculated.

4) Step 3 was repeated using several alternative values of K_1 subjectively selected to reduce the sum of squared deviations between predicted and observed C_a values. The value of K_1 that yielded the least residual sum of squared deviations was accepted as the best estimate of uptake rate (K_1) of dietary Kepone by channel catfish.

The K_1 calculated using wet weight C_a values will differ from the K_1 calculated using dry weight C_a values by a factor of 0.25 (0.25 = dry weight channel catfish/wet weight channel catfish).

At some point in time, the terms for uptake (K_1Q) and clearance ($-K_2C_a$) in equation (1) may become equal and equilibrium is reached (i.e. $dC_a/dt = 0$). If uptake rate, clearance rate and

dosage level are known, then the equilibrium body burden (C_e) can be calculated even if equilibrium has not been reached.

$$C_e = \frac{K_1}{K_2} Q \tag{9}$$

If equilibrium has been reached (as observed by a plateau in the uptake curve) then equation (9) can be rearranged to yield an estimate of K_1 as

$$K_1 = \frac{C_e K_2}{Q} \tag{10}$$

For determination of the dietary accumulation index

$$DAF = \frac{C_e}{Q} = \frac{K_1}{K_2} \tag{11}$$

Significance of difference between clearance rate constants (regression coefficients) were tested by analysis of covariance (Snedecor and Cochran, 1967).

Measurement of Chlordecone Alcohol

Bile and blood collected from fish receiving IM injections of Kepone were analyzed for chlordecone alcohol and glucuronide conjugates of chlordecone alcohol according to the methods outlined by Boylan et al. (1979). The bile and blood from each of four experimental fish were pooled with that of another experimental fish yielding two pooled samples of bile and two of blood. These samples were then split resulting in a total of four bile and four blood samples. The bile and blood of the two control fish were pooled and split yielding two samples of bile and two of blood. Each sample was weighed and placed in culture tubes followed by addition of

approximately four milliliters of water. In addition, two milliliters of sodium acetate buffer (pH = 4.5) were added to each tube, and, 5000 units of partially purified β-glucuronidase were added to half of the split samples. All samples were incubated overnight at 37°C, the optimum temperature for enzymatic digestion. Following the addition of two milliliters of 60 percent sulfuric acid, the samples were autoclaved for one hour. Samples were extracted twice with hexane/acetone (85/15) and cleaned with concentrated sulfuric acid. The solvent was evaporated and the extracted material redissolved in five percent methanol in benzene. Kepone and chlordecone alcohol concentrations in the solvent mixture were measured with a model 5730A Hewlett Packard gas chromatograph equipped with a ^{63}Ni electron capture detector. The carrier gas (95% argon and 5% methane) was maintained at 50 ml/min. Operating temperatures were 220°C for the column oven, 250°C for the injection port and 360°C for the detector. Column packing was 110/120 mesh Chromosorb W HP coated with 1.5% OV-17 and 1.95% QF-1.

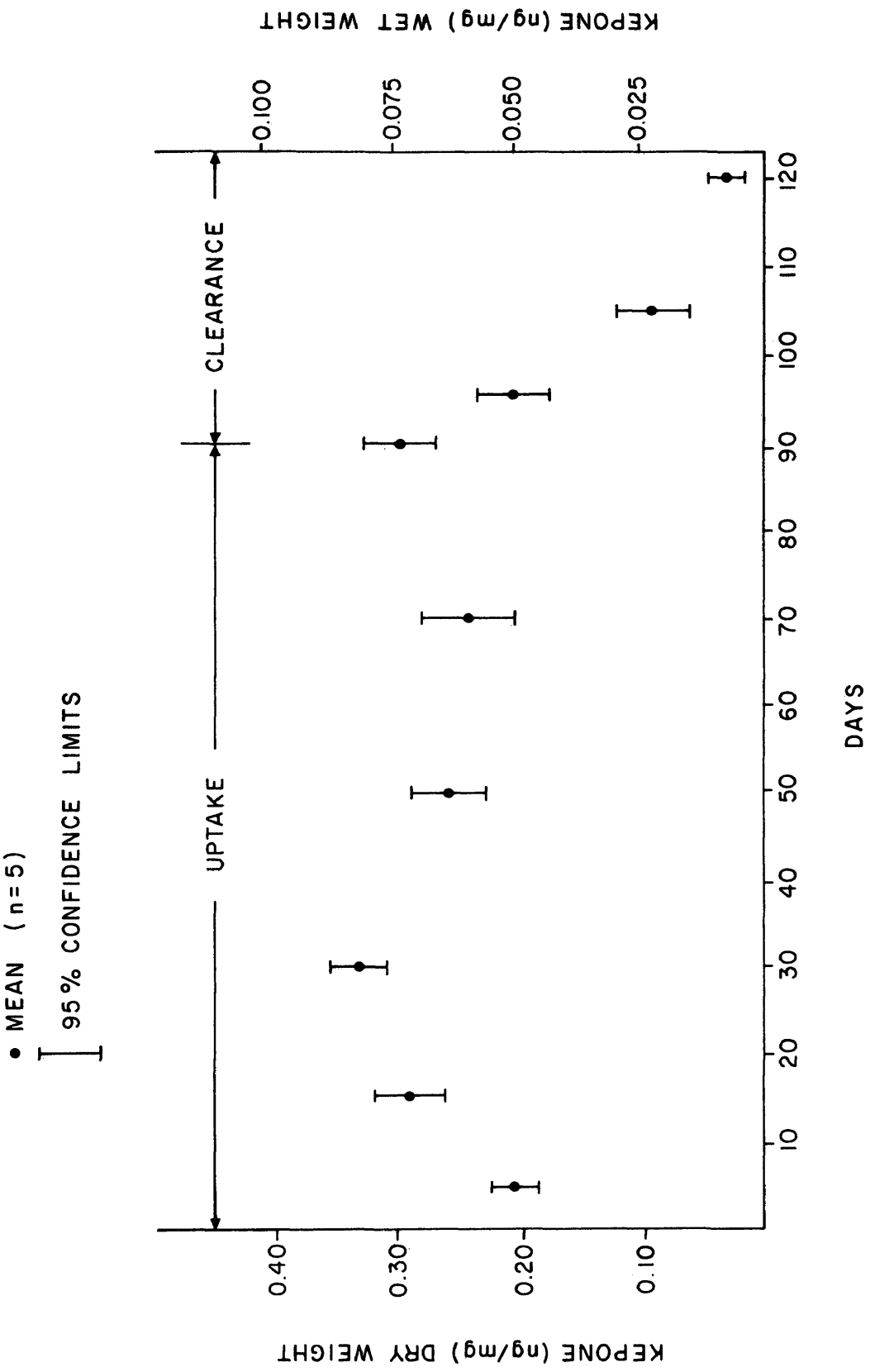
RESULTS

The feeding rate of two percent body weight per day resulted in an average weight gain of 63 percent by the end of the 120 day experiment.

Uptake Rate, Clearance Rates, Half-life, Equilibrium Body Burden and Dietary Accumulation Index (DAI)

Observation of Kepone residue levels in channel catfish during the dietary exposure study (Figure 3) suggests that these fish achieved a state of equilibrium with dietary Kepone. Although a flat equilibrium plateau is not obvious, the equilibrium residue level is estimated to lie between 0.24 and 0.33 ng/mg on a dry weight basis or between 0.06 and 0.08 ng/mg on a wet weight basis. The uptake rate constant K_1 for dietary Kepone in channel catfish was calculated to be 0.237 day^{-1} (wet weight) or 0.948 day^{-1} (dry weight). The clearance rate constant for dietary Kepone in channel catfish was 0.080 day^{-1}. Application of these values to equation (9) yields a predicted equilibrium residue level (C_e) of 0.32 ng/mg (dry weight basis) or 0.08 ng/mg (wet weight basis). Thus the predicted value for the equilibrium residue level lies within the range estimated by examination of the uptake curve. On day 15, the C_a (observed) was approximately 90 percent of C_e (predicted) suggesting that the fish were very close to equilibrium at that time.

Figure 3. Total body burden Kepone concentration in channel catfish receiving a ^{14}C-Kepone contaminated diet (1.36 µg/gm) at a rate of two percent of their body weight per day for a period of 90 days followed by a Kepone free diet for a period of 30 days.


KEPONE (ng/mg) WET WEIGHT
0.100
0.075
0.050
0.025
CLEARANCE
UPTAKE
• MEAN (n=5)
95% CONFIDENCE LIMITS
KEPONE (ng/mg) DRY WEIGHT
0.40
0.30
0.20
0.10
10
20
30
40
50
60
70
80
90
100
110
120
DAYS

Based on the predicted value for C_e, the dietary accumulation index for Kepone in channel catfish is 11.9 (dry weight basis) or 3.0 (wet weight basis).

Semi-log plots of Kepone residue levels in fish during the clearance phase are linear (Figure 4) indicating that clearance is adequately described by first order kinetics. The clearance rate for channel catfish receiving IM injections of Kepone at a dose equivalent to 0.20 µg/gm (wet weight) was 0.087 day^{-1}. The difference between this value and that obtained from the dietary exposure study (K_2 = 0.080 day^{-1}) is not significant at the 5% level (F = 2.79, df = 1,3). The half-life of Kepone in channel catfish based on the values of K_2 was 8.7 days for fish in the dietary exposure study and 8.0 days for fish receiving IM injections. These results indicate that the residence time of Kepone in channel catfish is not greatly affected by the size of the fish or the residue level in the fish at the beginning of the clearance phase.

The clearance rate for Kepone in white catfish receiving IM injections was calculated to be 0.043 day^{-1}. The difference between this value and that obtained from channel catfish injected with Kepone is significantly different at the 5% level (F = 36.5, df = 1,4). The half-life of Kepone in white catfish was calculated to be 16.1 days.

Tissue Distribution

During the first five days of the dietary exposure study, there was a relatively rapid increase of Kepone residues in all tissues of channel catfish (Table 1). Between days five and fifteen the average residue levels in all tissues increased but at a slower

Figure 4. Semi-log plots of total body burden Kepone concentrations (dry weight) in channel catfish during the clearance phase of the dietary exposure study and in channel catfish and white catfish which received intramuscular injections of Kepone.

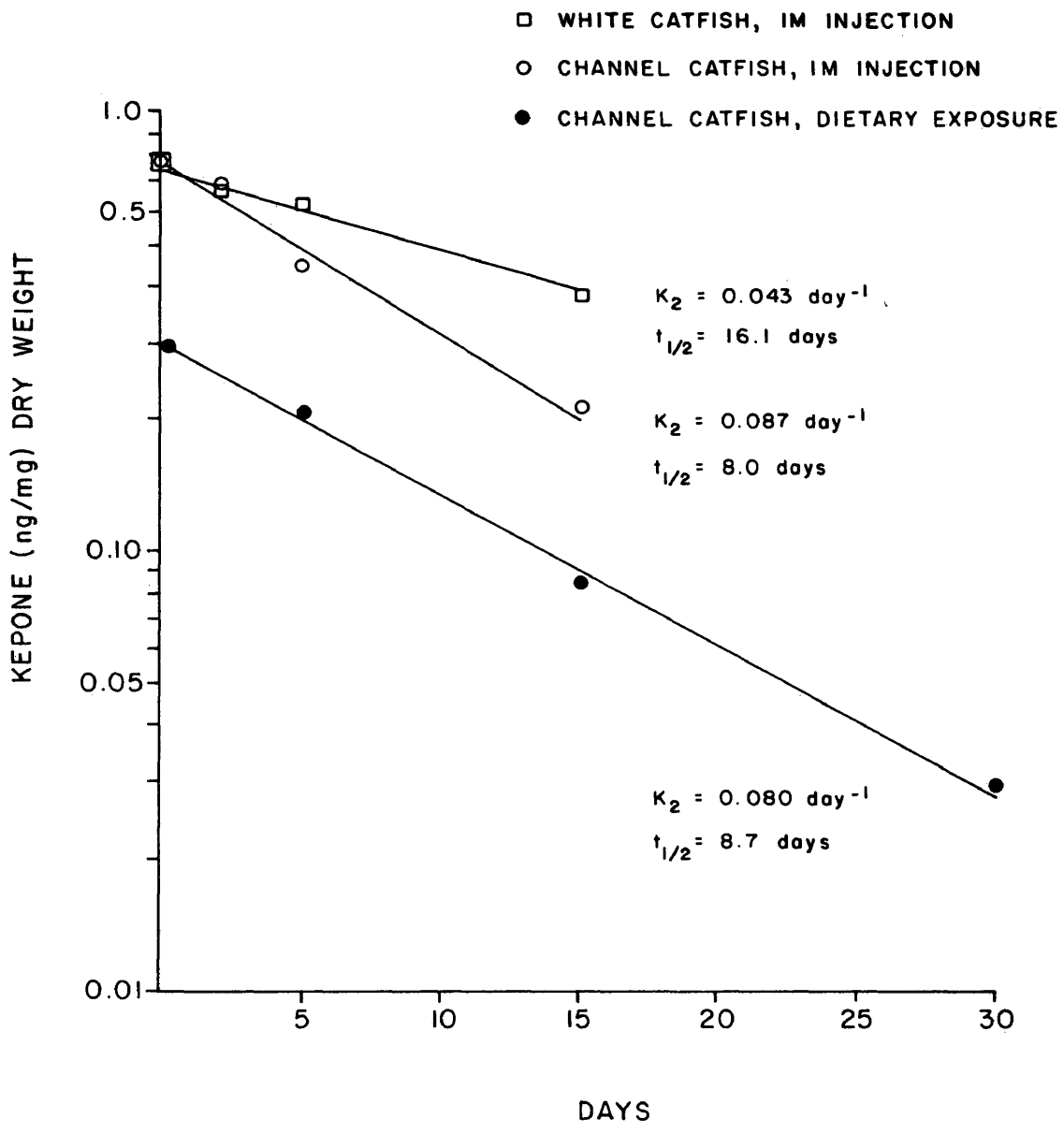

WHITE CATFISH, IM INJECTION
CHANNEL CATFISH, IM INJECTION
CHANNEL CATFISH, DIETARY EXPOSURE
KEPONE (ng/mg) DRY WEIGHT
1.0
0.5
0.10
0.05
0.01
5
10
15
20
25
30
DAYS
K_2 = 0.043 day^{-1}
$t_{1/2}$ = 16.1 days
K_2 = 0.087 day^{-1}
$t_{1/2}$ = 8.0 days
K_2 = 0.080 day^{-1}
$t_{1/2}$ = 8.7 days

TABLE 1

MEAN VALUES WITH THEIR STANDARD ERRORS FOR THE CONCENTRATION OF KEPONE IN TISSUES OF CHANNEL CATFISH (pg/mg-DRY WEIGHT) DURING THE DIETARY EXPOSURE STUDY. NUMBER OF SAMPLES EQUALS FIVE UNLESS OTHERWISE INDICATED.

	UPTAKE						CLEARANCE		
	5 DAYS	15 DAYS	30 DAYS	50 DAYS	70 DAYS	90 DAYS	5 DAYS	15 DAYS	30 DAYS
carcass	179 ± 8	224 ± 19	274 ± 11	222 ± 11	208 ± 15	253 ± 12	176 ± 17	75 ± 10	25 ± 3
blood	609 ± 53	1209 ± 83	958 ± 88	859 ± 63	853 ± 43	1000 ± 43	783 ± 130	411 ± 84	221 ± 27
intestines	300 ± 25	316 ± 125	735 ± 10	649 ± 80	672 ± 75	833 ± 75	470 ± 65	254 ± 31	64 ± 14
stomach	362 ± 21	648 ± 73	568 ± 31	646 ± 78	642 ± 46	622 ± 50	338 ± 32	177 ± 21	54 ± 6
liver	568 ± 13	579 ± 60	605 ± 21	453 ± 29	451 ± 31	576 ± 41	461 ± 40	187 ± 20	57 ± 12
gall bladder	461 ± 55	734 ± 32	662 ± 57	697 ± 37	606 ± 55	694 ± 46	465 ± 17	173 ± 16	-
kidneys	353 ± 19	750 ± 84	587 ± 60	598 ± 45	545 ± 40	615 ± 42	451 ± 20	209 ± 36	67 ± 16
gills	339 ± 32	702 ± 148	447 ± 11	437 ± 66	322 ± 50	428 ± 36	274 ± 24	140 ± 15	42 ± 7
brain	541 ± 45	821 ± 51	809 ± 15	716 ± 45	683 ± 55	820 ± 51	541 ± 51	248 ± 29	62 ± 13
mesenteric adipose			166 ± 0 (n=1)	139 ± 14 (n=3)	154 ± 35 (n=2)	-	111 ± 9	57 ± 6	26 ± 3
intestinal contents	353 ± 0 (n=1)	695 ± 48 (n=3)	494 ± 42	537 ± 58	358 ± 99 (n=3)	485 ± 57	209 ± 34	66 ± 9	67 ± 9

rate. After day fifteen there was little change in the relative distribution of Kepone in various tissues indicating that either the tissues were approaching equilibrium at the same rate or that the tissues were at equilibrium with dietary Kepone at this time. The exception was found in the intestines where the average residue level doubled between days fifteen and thirty. After day thirty, residues in the intestines remained at essentially the same level.

No tissues had residues approaching the levels found in the diet (1.36 µg/gm). Highest residues were found in blood followed by brain. Analysis of plasma and blood cell fractions of whole blood from channel catfish receiving IM injections of Kepone indicate that more than 80 percent of the Kepone present in blood is associated with the plasma. Residue levels in adipose tissue were consistently lower than in any other tissue. Carcass values were slightly higher than those found in the adipose but lower than those found in any of the organs. Although carcass residue levels were relatively low, due to its bulk, the carcass represents the major reservoir for Kepone storage (Table 2). The percentage of the total body burden of Kepone found in each tissue was calculated from residues measured in fish sacrificed on day 70 but are representative of all channel catfish between day thirty and day ninety of the uptake phase.

Between day zero and day fifteen of the clearance phase, the relative distribution of Kepone was essentially the same as that observed during the uptake phase indicating that the clearance rates for all tissues were approximately the same. However, on day thirty of the clearance phase, the relative amount of Kepone in blood (blood/

TABLE 2

MEAN VALUES WITH THEIR STANDARD ERRORS FOR THE PERCENTAGE OF THE TOTAL BODY BURDEN KEPONE FOUND IN THE TISSUES OF CHANNEL CATFISH SACRIFICED ON DAY 70 OF THE DIETARY EXPOSURE STUDY. NUMBER OF SAMPLES IS GIVEN IN PARENTHESES.

	% of Total ± S.E.	
carcass	81.7	±1.25(5)
intestines	3.6	±0.45(5)
stomach	2.7	±0.22(5)
liver	5.3	±0.31(5)
gall bladder	0.3	±0.04(5)
kidneys	2.1	±0.18(5)
gills	3.1	±0.40(5)
brain	2.0	±0.18(5)
	100.5%	

carcass = 8.8) is higher than the average relative amount observed at other times (blood/carcass = 4.3).

The tissue distribution of Kepone in channel catfish 48 hours after IM injection of Kepone (0.20 µg/gm) is similar to that in fish from the dietary exposure study (Table 3). However, exposure to a large dose resulted in a relatively high concentration in the bile as shown by the gallbladder/blood ratio in the 48 hour fish (= 7.1) compared to the average ratio in channel catfish from the dietary exposure study (gall bladder/blood = 0.7). Residues of Kepone in the brain of fish after 48 hrs were higher than residues found in the blood. The relative distribution of Kepone in other tissues is similar to that found in channel catfish in the dietary exposure study with lowest residue concentrations in adipose tissue followed by carcass.

The residue pattern of Kepone in tissues of channel catfish 120 hours after injection is essentially identical to that found in fish from the dietary exposure study with highest residue levels found in blood followed by brain, lowest residue levels found in adipose tissue followed by carcass and a gall bladder/blood ratio equal to 0.7.

The tissue distribution and percentage of the total body burden Kepone found in tissues of white catfish sacrificed on day thirty of the dietary exposure study are shown in Table 4. Food consumption by this time had become erratic and fish were eating less than two per cent of their body weight per day. Highest residue levels were found in the brain followed by liver and intestines. The levels of Kepone in these tissues exceeded those found in the

TABLE 3

KEPONE CONCENTRATION IN TISSUES AND PERCENTAGE OF THE TOTAL BODY BURDEN KEPONE FOUND IN THE TISSUES OF CHANNEL CATFISH 48 HOURS AND 120 HOURS AFTER RECEIVING IM INJECTIONS OF KEPONE AT A DOSE EQUIVALENT TO 0.20 µg/gm (WET WEIGHT).

	48 HOURS		120 HOURS	
	ng/mg	% of TOTAL	ng/mg	% of TOTAL
carcass	0.61	84.6	0.39	82.2
blood	1.80	-	1.63	-
intestines	1.70	2.8	0.97	4.8
stomach	1.10	1.4	0.93	1.7
liver	1.38	4.2	1.26	5.8
gall bladder	12.75	2.7	1.17	0.5
kidneys	1.80	1.7	1.48	2.9
gills	1.32	1.0	1.11	1.5
brain	2.09	0.6	1.58	0.7
mesenteric adipose	0.30	-	0.20	-
		100.0%		100.0%

TABLE 4

MEAN VALUES PLUS THEIR STANDARD ERRORS FOR THE CONCENTRATION OF KEPONE IN TISSUES AND THE PERCENTAGE OF THE TOTAL BODY BURDEN KEPONE FOUND IN TISSUES OF WHITE CATFISH SACRIFICED ON DAY 30 OF THE DIETARY EXPOSURE STUDY. NUMBER OF FISH GIVEN IN PARTENTHESES.

	ng/mg ± S.E.(n)	% of Total ± S.E.
carcass	0.49 ± 0.037(5)	73.9 ± 1.26
blood	1.13 ± 0.087(5)	-
intestines	1.52 ± 0.139(5)	4.5 ± 0.45
stomach	1.02 ± 0.121(5)	1.7 ± 0.62
liver	1.54 ± 0.118(5)	9.5 ± 0.45
gall bladder	0.95 ± 0.078(4)	0.4 ± 0.07
kidneys	1.20 ± 0.032(5)	4.6 ± 0.36
gills	0.84 ± 0.033(5)	3.6 ± 0.47
brain	1.64 ± 0.098(5)	2.7 ± 0.38
mesenteric adipose	0.45 ± 0.020(2)	
intestinal contents	0.47 ± 0.051(5)	-
		100.9%

experimental diet. As in channel catfish, lowest residue levels were found in the adipose tissue. However, the adipose/carcass ratio of Kepone in white catfish (= 0.9) is higher than that found in channel catfish (= 0.6) in the feeding study. The relative amount of Kepone found in the blood of white catfish (blood/carcass = 2.3) is less than that found in the blood of channel catfish (blood/carcass = 3.5). The percentage of the total body burden Kepone found in white catfish carcass (75%) was less than that found in channel catfish carcass (82%).

Pathways of Elimination

During the uptake phase, the residue level of Kepone in the intestinal contents averaged 36 percent of that incorporated in the diet. The fecal Kepone would be expected to include that which was never absorbed by the gut. However, the presence of fecal Kepone in fish during the clearance phase of the feeding experiment at residue levels approximating those found in the carcass indicates that unabsorbed Kepone is not the sole source of fecal Kepone.

The presence of Kepone in the gall bladder provides evidence that Kepone may be excreted in bile with the feces. The high concentration of Kepone in the gall bladder of channel catfish 48 hours after receiving an IM injection of Kepone suggests that biliary excretion may be enhanced by exposure of fish to relatively high doses of Kepone.

Residue levels in the intestinal contents of fish during the clearance phase were approximately 42 percent of the levels found in bile.

Channel catfish receiving IM injections of Kepone (0.20 μg/gm) and held for 48 hours in a flow-through tank yielded an average of 0.33 and 0.10 percent of the total administered dose in gill rinses and skin mucus respectively (Table 5). These values are small relative to the total administered dose. However, it must be borne in mind that fish are continuously sloughing off mucus from skin and gill surfaces. Net elimination of Kepone via these pathways may therefore be substantial. Kepone residues detected in the gill rinses and skin mucus of control fish are believed to be the result of adsorption of excreted pesticide.

The low concentration of Kepone in the urine of channel catfish indicates that this pathway is not of major importance in this species (Table 5).

Metabolism

Analysis of bile and blood for Kepone and chlordecone alcohol in fish receiving IM injections of Kepone indicate that channel catfish are capable of reducing Kepone to chlordecone alcohol (Table 6). Residues of chlordecone alcohol in the bile accounted for an average of 1.7 per cent of the total biliary chlordecone. Bile samples subjected to conditions of enzymatic digestion with β-glucuronidase had essentially the same measurable residue levels of Kepone and chlordecone alcohol as those in which the enzyme was absent. Sample chromatograms of the Kepone standard, chlordecone alcohol standard, chlordecone emulphor solution and channel catfish bile used in this study are shown in Figure 5.

Chlordecone alcohol was not detected in the blood of channel catfish.

TABLE 5

RECOVERY OF KEPONE FROM GILL RINSES, SKIN MUCUS AND URINE OF CHANNEL CATFISH 48 HOURS AFTER RECEIVING IM INJECTIONS OF KEPONE AT A DOSE EQUIVALENT TO 0.20 µg/gm (WET WEIGHT). (N.D. = NON-DETECTABLE)

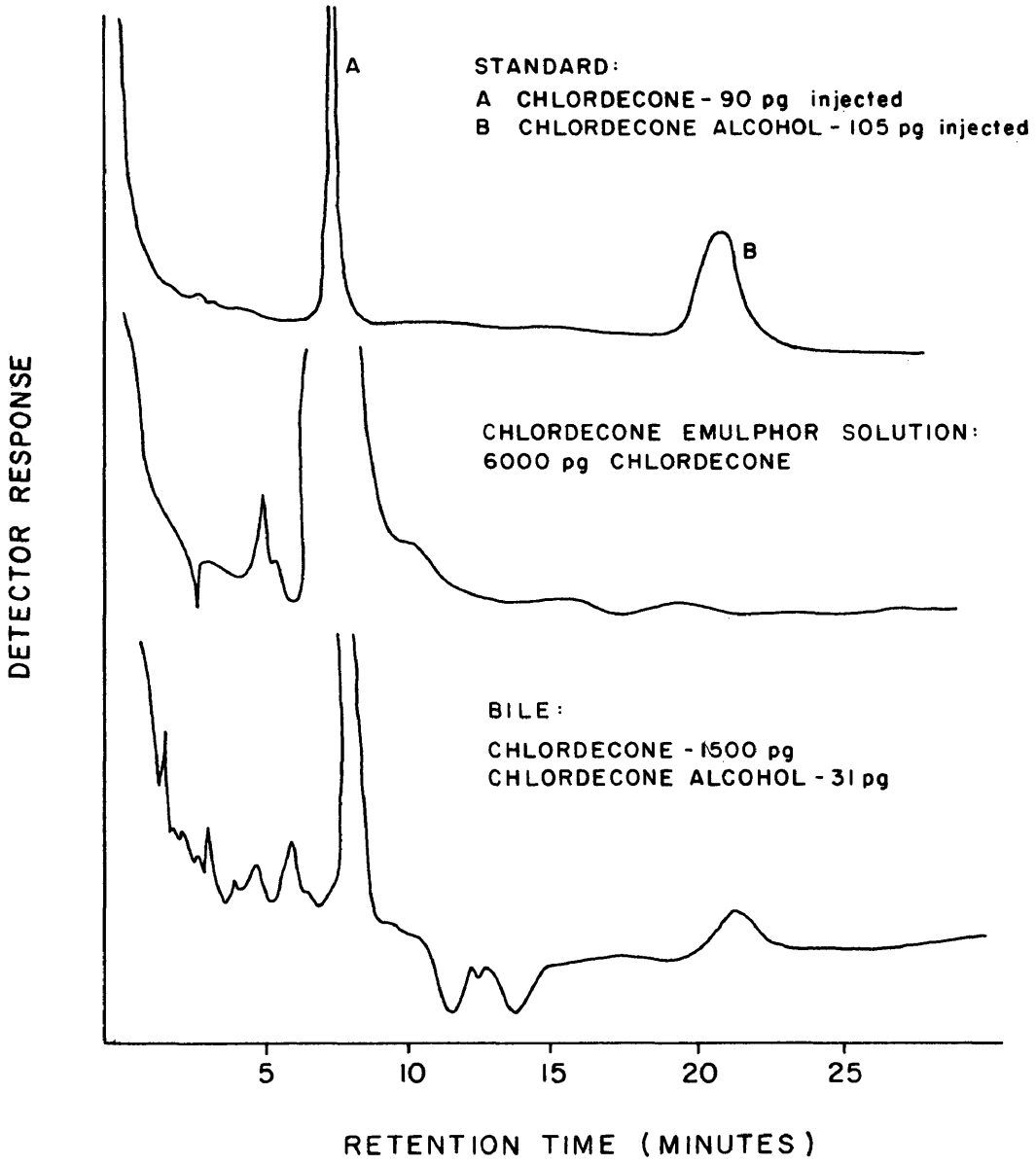
		EXPERIMENTAL FISH					CONTROLS	
		A	B	C	D	E	F	G
GILL RINSE	Nanograms Recovered	58.7	45.2	49.3	52.3	48.0	9.3	8.5
	% of Total Recovered	0.35	0.28	0.30	0.39	0.31		
SKIN MUCUS	Nanograms Recovered	15.7	17.2	14.3	19.4	17.3	1.3	1.1
	% of Total Recovered	0.09	0.10	0.09	0.11	0.10		
URINE (50 µl)	Nanograms Recovered	<0.1	<0.1	<0.1	<0.1	<0.1	N.D.	N.D.
	% of Total Recovered	<0.001	<0.001	<0.001	<0.001	<0.001		

TABLE 6

RESULTS OF ANALYSIS OF CHANNEL CATFISH BILE AND BLOOD FOR CHLORDECONE, CHLORDECONE ALCOHOL AND GLUCURONIDE CONJUGATES OF CHLORDECONE AND CHLORDECONE ALCOHOL. FISH RECEIVED AN IM INJECTION OF KEPONE EQUIVALENT TO 1.0 µg/gm (WET WEIGHT) FOLLOWED BY AN ADDITIONAL DOSE (0.5 µg/gm) AFTER ONE WEEK. FISH WERE SACRIFICED TWO WEEKS AFTER INITIAL DOSE. (N.D. = NON-DETECTABLE; N.S. = NO SAMPLE)

	CHLORDECONE (ng/mg)		CHLORDECONE ALCOHOL (ng/mg)	
	β-Glucuronidase absent	β-Glucuronidase present	β-Glucuronidase absent	β-Glucuronidase present
BILE (Fishes 1 and 2)	9.7	11.3	0.19	0.22
BILE (Fishes 3 and 4)	9.0	11.1	0.13	0.14
BLOOD (Fishes 1 and 2)	12.2	13.9	N.D.	N.D.
BLOOD (Fishes 3 and 4)	10.1	12.6	N.D.	N.D.
BILE (CONTROL)	N.S.	N.D.	N.S.	N.D.
BLOOD (CONTROL)	N.S.	N.D.	N.S.	N.D.

Figure 5. Sample chromatographs of the Kepone standard, chlordecone alcohol standard, chlordecone emulphor solution and bile from channel catfish receiving IM injections of Kepone.


A
STANDARD:
A CHLORDECONE - 90 pg injected
B CHLORDECONE ALCOHOL - 105 pg injected
B
CHLORDECONE EMULPHOR SOLUTION:
6000 pg CHLORDECONE
BILE:
CHLORDECONE - 1500 pg
CHLORDECONE ALCOHOL - 31 pg
DETECTOR RESPONSE
5
10
15
20
25
RETENTION TIME (MINUTES)

DISCUSSION

The dietary accumulation factor (DAF) was created for use in this study to allow comparison of the potential for accumulation of various organochlorine pesticides from food by different species of fish. Equilibrium residue concentrations attained by fish exposed solely to pesticide contaminated diets are largely dependent upon the amount of pesticide available in the diet (Macek et al., 1970; Warlen et al., 1977; Argyle et al., 1975). The amount of pesticide available in the diet is dependent upon the pesticide concentration of the diet and the feeding rate. The bioaccumulation factor (BAF) used by Bahner et al. (1977) (defined as the concentration of Kepone in the predator/concentration in prey) would not be useful in comparing the potential for accumulation of dietary pesticides because it does not include the feeding rate as the DAF does.

Presumably, a relatively low DAF implies a relatively low potential for accumulation of a dietary pesticide. The DAF for Kepone and channel catfish (DAF = 3.0) is slightly lower than the value calculated from the data presented by Argyle et al. (1975) on the accumulation of dietary dieldrin by channel catfish (DAF = 3.8; average of two groups of fish) (Table 7). The data of Macek et al. (1970) yielded a DAF for dieldrin and rainbow trout of 16.0 (average of two groups of fish) and for DDT and rainbow trout 36.8 (average of two groups of fish). Warlen et al. (1977) observed that Atlantic menhaden (Brevoortia tyrannus) did not reach equilibrium with dietary DDT at

TABLE 7

DIETARY ACCUMULATION FACTORS (DAF) OF ORGANOCHLORINE PESTICIDES IN FISH. VALUES ARE EXPRESSED ON A WET WEIGHT BASIS.

SPECIES	PESTICIDE	DAF	SOURCE OF DATA
spot	Kepone	11.4	Bahner et al., 1977
channel catfish	Kepone	3.0	present study
white catfish	Kepone	6.1	present study
rainbow trout	Dieldrin	15.5	Macek et al., 1970
channel catfish	Dieldrin	3.8	Argyle et al., 1975
rainbow trout	DDT	31.0	Macek et al., 1970
menhaden	DDT	57.5	Warlen et al., 1977

the end of a 48 day exposure period. Values for K_1 were not determined. However, a dietary accumulation factor of 57.5 (average of three groups of fish) can be calculated using the total body burden of DDT at the end of the exposure period. Because fish were not at equilibrium with dietary DDT, the calculated DAF is an underestimation. Similarly, from Bahner et al. (1977), on the accumulation of dietary Kepone by spot, a dietary accumulation factor of 11.4 calculated from the total body burden of Kepone in spot at the end of a 30 day exposure period is an underestimation because equilibrium had not been reached. Based on a daily dietary dosage level of 0.027 µg Kepone/gm fish and the average residue level in fish at the end of the thirty day exposure period, the DAF for white catfish is 6.1. This value is expected to be an underestimation because fish were not regularly consuming the daily ration.

Comparison of DAF's suggests that dietary Kepone and dieldrin have a relatively low potential for accumulation in channel catfish. However, in contrast to Kepone, for which residues in James River fish are lowest in channel catfish, dieldrin levels in channel catfish collected from Iowa streams were higher than in any other species analyzed (Morris and Johnson, 1971). These authors attributed the high residues of dieldrin in channel catfish to this species spending more time close to dieldrin-laden bottom sediment than other species.

Although the dietary accumulation index is defined as the equilibrium residue level of dietary pesticide in fish divided by the amount of pesticide available in the diet, equations (10) and (11) show that the DAF is equivalent to K_1/K_2. Comparison of uptake and

clearance rate constants for various organochlorine pesticides in different species reveals that the K_1 observed for Kepone in channel catfish is slightly lower than values of K_1 for dieldrin in channel catfish and rainbow trout but higher than K_1's observed for DDT in rainbow trout (Table 8). Clearance rate constants for Kepone and dieldrin in channel catfish exceed those observed for pesticides in all other species. Therefore, the low DAF's observed for channel catfish may largely be a result of rapid clearance.

Although the potential for accumulation of a pesticide in fish is largely controlled by the nature of the pesticide itself, examination of James River field data, dietary accumulation factors and clearance rates indicate that there are species differences in the ability to accumulate Kepone residue.

Species differences in the tissue distribution of Kepone are not outstanding. For example, spot contained highest residues of Kepone in the brain followed by liver, gills and muscle (Bahner et al., 1977). These authors did not report the relative distribution of Kepone in other tissues. However, the relative amount of Kepone found in these tissues of channel catfish and white catfish follows the same pattern. Analysis of tissues in James River eels (Hedgepeth and Stehlik, 1979) indicated that, as in channel catfish and white catfish, eels maintain higher concentrations of Kepone in muscle tissue than adipose tissue. Differences do occur in the fat/muscle ratio: the fat/muscle ratio of eels (= 0.9) is identical to that of white catfish (= 0.9) but not that of channel catfish (= 0.6).

Unlike tissue distribution of pesticides reported by other investigators, the data from the present study are expressed in terms

TABLE 8

DAILY DIETARY DOSAGE LEVELS, UPTAKE RATE CONSTANTS AND CLEARANCE RATE CONSTANTS FOR ORGANOCHLORINE PESTICIDES IN FISH.

SPECIES	PESTICIDE	Q(μg/gm)	$K_1(day^{-1})$	$K_2(day^{-1})$	half-life(days)	SOURCE OF DATA
channel catfish	Kepone	0.027	0.237	0.080	8.7	present study
white catfish	Kepone	0.027	not available	0.043	16.1	present study
spot	Kepone	0.088	not available	0.023	30	Bahner et al., (1977)
channel catfish	dieldrin	0.017	0.308	0.077	9.0	Argyle et al., (1975)
channel catfish	dieldrin	0.034	0.262	0.072	9.6	Argyle et al., (1975)
rainbow trout	dieldrin	0.029	0.285	0.017	40	Macek et al., (1970)
rainbow trout	dieldrin	0.145	0.257	0.017	40	Macek et al., (1970)
rainbow trout	DDT	0.029	0.134	0.004	160	Macek et al., (1970)
rainbow trout	DDT	0.145	0.161	0.004	160	Macek et al., (1970)
mosquitofish (Gambusia affinis)	Mirex	not available	not available	0.005	130	Ivie et al., (1974)

of dry weight and are unaffected by water contents of different tissues. Residues in tissues having a high water content (e.g. blood) will appear relatively high when expressed in terms of dry weight as opposed to wet weight.

While the Kepone distribution pattern shows similarities in different species of fish, this trend differs from the distribution pattern of other pesticides in fish. The most obvious difference is the low accumulation of Kepone in adipose tissue of white catfish, eel and particularly channel catfish. The literature is replete with evidence that adipose tissue is a major storage depot for many lipophilic compounds including: chlordane in goldfish (Carassius auratus) in which a fat/muscle ratio of approximately 22 was found (Moore et al., 1977); DDT and dieldrin in rainbow trout in which fat/muscle ratios of approximately 80 and 43 were found (Macek et al., 1970) and DDT and dieldrin in goldfish in which fat/muscle ratios of 23 and 30 were found (Grzenda et al., 1970; Grzenda et al., 1971). A similar trend is evident in birds and mammals (Findlay and de Freitas, 1971; Ivie et al., 1974; Mehendale, et al., 1972).

In addition to adipose tissue, liver has been found to be a major storage site of pesticides in some species including DDT in dogfish (Squalus acanthias) (Dvorchik and Maren, 1972) and DDT and mirex in winter flounder (Pseudopleuronectes americanus) (Pritchard et al., 1973).

The predominant lipids found in the adipose tissue and liver of fish are triacylglycerides, often referred to as "neutral lipids" because of their non-polar nature. Adipose tissue is composed of approximately 90 percent triacylglycerides in most animals. The tendancy for lipophilic compounds to accumulate in these tissues

suggests that triacylglycerides are the major compounds involved in pesticide storage. This line of thought is supported by the observation that PCB residue levels in pigeon tissues (de Freitas and Norstom, 1974) and DDT residues in cod tissues (Mitchell et al., 1977) show a smaller range of values when expressed on a triacylglyceride basis rather than on a total lipid basis. However, low residue levels of Kepone in the adipose tissue of white catfish, American eel and particularly in channel catfish indicate that triacylglycerides are not major storage compounds for accumulation of Kepone in these species.

A lack of association between Kepone and triacylglycerides is also indicated by results of plasma binding studies of Kepone in human blood (Skalsky et al., 1979). Gel filtration chromatography revealed an association of Kepone with high density lipoproteins and to a lesser, though substantial, degree with albumin in human serum. The major lipid fraction of high density lipoproteins in mammals are cholesterol esters and phospholipids. In contrast, DDT and DDE have been shown to associate primarily with triacylglyceride-rich low density and very low density lipoproteins (Morgan et al., 1972).

Plasma lipoproteins serve as transport vessels for various water insoluble compounds. Differences in the distribution of pesticides may therefore be influenced by differences in blood transport mechanisms. For example, the very low density lipoproteins and low density lipoproteins transport triacylglycerides and presumably associated pesticides (e.g. DDT) to adipose deposits. Pesticides not associated with triacylglyceride rich lipoproteins (e.g. Kepone) would not be expected to accumulate to a large degree in adipose tissue.

Although major differences in lipid metabolism and plasma lipoprotein composition exist between mammals and fish (Cowey and Sargent, 1972; Mills and Taylaur, 1971; Kayama and Iijima, 1976), the trend towards low accumulation of Kepone in the adipose tissue of mammals (Egle et al., 1978) and fish suggests that factors influencing the partitioning of Kepone may be similar in both animal classes.

Following the logic of Skalsky et al. (1979) the predominance of Kepone in the plasma fraction of whole blood and negligible concentrations of Kepone in the urine is evidence that, as in humans, Kepone may be bound to plasma lipoproteins in the blood of channel catfish. Relatively high residue levels of Kepone in the phospholipid and cholesterol ester rich brain tissues indicates that Kepone may also be transported by the high density lipoproteins in fish. The cholesterol level in the plasma of teleost fish is much higher than that of mammals (Larsson and Fange, 1977). If there is an association of Kepone with cholesterol in the blood of fish, then there would be a relatively large number of vessels available for transport of Kepone to cholesterol rich tissues (e.g. brain) resulting in relatively large amounts of Kepone in these tissues.

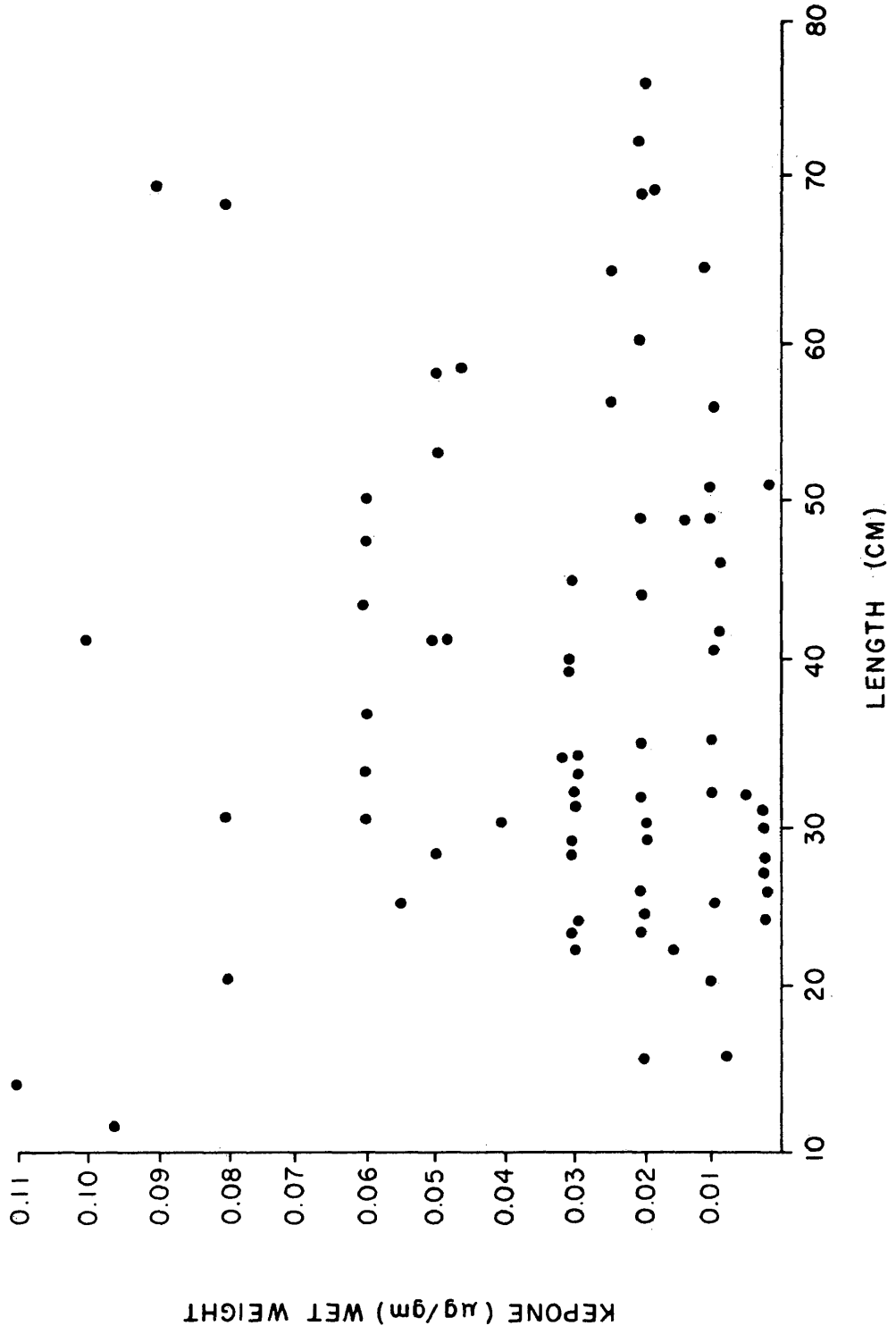
Undoubtedly, the structure of Kepone itself plays a major role in its partitioning patterns. There is substantial evidence that the accumulation of chlorinated hydrocarbon pesticides is directly related to the relative solubility of the compounds in water and fats (Hamelink et al., 1971; Chiou et al., 1977). Any factor which increases the water solubility or decreases the fat solubility of a compound will reduce the degree to which it accumulates in tissues. Structurally, Kepone resembles mirex, differing only in the substitution of two

chlorine atoms in mirex for a carbonyl group in Kepone. The possession of this carbonyl group results in a polar molecule which is 2,000 times more soluble in water than mirex. The water solubilities of Kepone and mirex are 2.0 and 0.001 mg/1 respectively (National Academy of Science, 1978) as compared to 0.05-0.25 mg/1 for dieldrin and 0.0012 mg/1 for DDT (Metcalf, 1976). Based on its high water solubility alone, Kepone would not be expected to have as great a potential for accumulation in fats as the less water soluble compounds.

Residue concentrations of some lipophilic compounds have been found to be related to the size of a given species. For example, Bulkley et al. (1976) found that large channel catfish accumulated higher residue concentrations of dieldrin than smaller fish fed at the same rate. Residues of DDT in lake trout (Salvelinus namaycush) and coho salmon (Oncorhynchus kisutch) increased as the size of the fish increased (Reinert and Bergman, 1974). Generally, fat (i.e. triacylglyceride) content increases with size and age in most species of fish (Shul'man, 1960). Therefore, residues of pesticides which have a high affinity for body fat will tend to increase as a function of size. Low accumulation of Kepone in the triacylglycerides of fish may help to explain why Bender et al. (1977) found no relationship between Kepone residue concentrations and fish size in any of the species of James River fish tested including channel catfish (Figure 6).

The low potential for accumulation of Kepone in channel catfish may be partially attributed to the low affinity of Kepone for the adipose tissue of this species. However, as the results of this and other studies have shown, the relative distribution of Kepone in different species of fish is similar. The major differences lie in

Figure 6. Kepone concentrations in channel catfish collected from the James River versus length of fish. (data from Bender et al., 1977)


KEPONE (µg/gm) WET WEIGHT
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
10
20
30
40
50
60
70
80
LENGTH (CM)

the absolute concentrations of Kepone residues that the tissues of different species will accumulate. The rapid clearance rate of Kepone by channel catfish indicates that the low potential for accumulation of Kepone in this species is the result of a relatively efficient excretion mechanism(s).

Biliary excretion of a number of chlorinated hydrocarbon pesticides has been documented. In mammals, biliary excretion via the fecal material is the ultimate pathway for elimination of these compounds. However, extensive excretion via this pathway often occurs only after metabolic transformation of these compounds. For example, in rats receiving intravenous doses of DDT, most of the biliary pesticide was in the form of the more water soluble metabolite DDA (Smith, 1976). Similarly, aldrin and dieldrin are excreted in the bile of rats almost entirely in the form of hydrophilic metabolites (Smith, 1976). The principal organ involved in metabolism of foreign compounds is the liver, but metabolism of these compounds has also been shown to occur in other tissues such as the gastrointestinal tract, kidneys, blood and intestinal flora (Smith, 1976).

Biliary excretion of chlordecone (Kepone) has been observed in former employees of Life Science Products, Inc. (Boylan et al., 1979). The amount of chlordecone and chlordecone alcohol excreted into the intestines with the bile was four times greater than that which was eliminated in the stool. These results indicate that biliary chlordecone may be reabsorbed by the gut along with the bile salts during enterohepatic recirculation.

The significance of the biotransformation of chlordecone to chlordecone alcohol and subsequent glucuronide conjugation with

respect to excretion efficiency in unclear. Generally, glucuronide formation results in the more rapid excretion of a foreign compound because of its decreased lipid solubility. In human bile, 10-15 per cent of the chlordecone and 98 per cent of the chlordecone alcohol were present as glucuronide conjugates. However, these conjugates are not present in the stool. Stool contained chlordecone (40 µg excreted in 24 hours) and chlordecone alcohol (161 µg excreted in 24 hours) in the unconjugated form. These results indicate that unconjugated chlordecone alcohol is the predominate form in which the pesticide is excreted by humans.

In fish, the mechanisms involved in the metabolism of foreign compounds are generally believed to be less well developed than those operating in mammals (Forster and Goldstein, 1969). Nonetheless, studies on the enzyme systems of fish have revealed metabolic and conjugation mechanisms which are involved in the excretion of pesticides. Trout, salmon and cod contain uridine diphosphate glucuronic acid, and glucuronide formation has been demonstrated using trout liver homogenates in vitro (Forster and Goldstein, 1969). In studies involving the metabolism of Bayer 73 (a sea lamprey larvicide) Statham and Lech (1975) recovered 40 per cent of the compound in the bile of rainbow trout following aqueous exposure. The material in the bile was a glucuronide conjugate of Bayer 73. Goldfish exposed to a DDT contaminated diet contained 24 per cent DDT and 76 percent DDE in their bile (Grzenda et al., 1970). No metabolites of dieldrin were found in goldfish fed a dieldrin contaminated diet (Grzenda et al., 1971). Pritchard et al. (1973) reported that biliary excretion of DDT and mirex by winter flounder equaled 6.5 percent and 2.2 percent

of the total body burden of these pesticides over a one week period. The authors did not report the methods used in determining these values. No metabolites of either pesticide were detected during the one week period. High residues of unaltered chlordane in the bile of goldfish suggest a biliary route of excretion for this pesticide (Ducat and Khan, 1979).

The results of enzymatic digestion of bile and blood samples with β-glucuronidase indicate that either there were no glucuronide conjugates of Kepone and its metabolites or that the conditions for enzymatic digestion were not adequate to break the conjugates.

The relatively low percentage of chlordecone alcohol in the bile of channel catfish relative to that found in human bile suggests that the mechanisms involved in the reduction of chlordecone are not as well developed in this species as in humans. Though chlordecone alcohol was shown to be the predominate form of chlordecone excreted in the stool of humans, the low levels of chlordecone alcohol in the bile of channel catfish would not be expected to significantly influence net fecal excretion of chlordecone. However, differences observed may be the result of humans being exposed to the pesticide for years as compared to days of exposure in the fish receiving IM injections of Kepone. Metabolic mechanisms of transformation may require long periods of time before operating a full potential.

Evidence for a non biliary mechanism for the excretion of Kepone with the feces of man and rats has been presented by Boylan et al. (1979). As mentioned previously, diversion of the bile from the human intestines terminated fecal excretion of chlordecone alcohol. However, the amount of fecal chlordecone increased following bile

diversion. These authors concluded that chlordecone enters the intestinal contents from a nonbiliary source and that bile inhibits excretion via this nonbiliary route. The mechanism of the nonbiliary pathway is unknown but may involve desquamation of chlordecone-laden cells lining the intestine or transport directly across the intestinal mucosa (Boylan et al., 1978).

To the author's knowledge, there is no literature pertaining to an extrabiliary source of fecal pesticide in fish. To verify such a pathway, either the bile of the fish in question would have to be diverted or knowledge of the total contribution of biliary pesticide to the excrement would be needed. However, channel catfish in the depuration phase of the feeding study contained relatively high levels of Kepone residues in the intestinal contents as well as in the intestines themselves. Residue concentrations in the intestinal contents of fish during the depuration phase were approximately 42 percent as high as the concentrations found in the bile. If the total fecal Kepone was derived from biliary Kepone alone, then the contribution of bile to the total weight of the intestinal contents would be 42 percent. Such a large contribution of bile to the total weight of the intestinal contents is unlikely particularly since fish are consuming two per cent of their body weight per day in feed. The results suggest that an extrabiliary source of fecal Kepone is present in channel catfish.

Excretion of pesticide via the gills of fish is poorly understood. Residues of pesticides detected in the gills of fish have been cited as evidence of gill excretion (Moore et al., 1977). However, mere presence of pesticides in gill tissues is not substantial evidence that this process occurs. Determinations of the total amount

of pesticide excreted across the gills are complicated by reabsorption of excreted pesticides to epidermal surfaces of fish (Gakstatter and Weiss, 1967).

Fromm and Hunter (1969) demonstrated that isolated perfused gills of rainbow trout could absorb dieldrin from water into the vascular system as long as plasma proteins were present in the perfusion fluid. Since the dieldrin concentration in the perfusion fluid was always less than that found in the water, the proposed mechanism for entry into the vascular system was one of simple diffusion.

Following this logic, for fish in which the concentration of pesticide in the blood in higher than that of the surrounding water, the pesticide may be excreted across the gills by the process of diffusion. However, Dvorchik and Maren (1972) found that gill excretion of DDT by the dogfish was negligible. The authors attributed this observation primarily to the extremely low water solubility of DDT and to the binding of DDT to plasma proteins. These factors, in addition to the rapid uptake of DDT by tissues would severely limit the possibility of diffusion across the gill. Similarly, Pritchard et al. (1973) attributed the negligible gill excretion of mirex and DDT by winter flounder to plasma binding of these pesticides.

In channel catfish, significant excretion of Kepone across the gills may be explained in part by the high water solubility of Kepone compared to mirex and DDT. Also, in contrast to DDT uptake by dogfish tissues, Kepone had a relatively low affinity for the tissues of channel catfish. This resulted in relatively high levels of Kepone in the blood stream of channel catfish in contrast to dogfish in which essentially all of the administered DDT was present in the

liver 72 hours after exposure. Thus, in channel catfish there is a relatively large amount of Kepone available in the bloodstream for excretion across the gills by whatever mechanisms are involved. Tissue distribution studies indicate that the relative amount of Kepone present in the blood of channel catfish (blood/carcass ratio = 3.5) is greater than that found in the blood of white catfish (blood/carcass ratio - 2.3) suggesting that the amount of Kepone available for excretion across the gills is greater in channel catfish than white catfish.

Very little information is available on the elimination of hydrocarbons across the skin surface of fish. Guiney et al. (1977) reported that rainbow trout accumulated tetrachlorobiphenyl in the skin and eliminated this compound across the skin very slowly. Varanasi et al. (1978) demonstrated that rainbow trout are capable of eliminating significant amounts of naphthalene and its metabolites via the epidermal mucus. To the author's knowledge, there is no evidence in the literature for the elimination of chlorinated hydrocarbon pesticides in this way; nor is there evidence that this pathway has been investigated for these compounds. Epidermal mucus is constantly being sloughed off and renewed by fish. Therefore, the presence of Kepone in the mucus of channel catfish is a strong indication that this is an important pathway for the excretion of this compound. Albumen is a major constituent of the epidermal mucus (Van Oosten, 1957) as well as the blood plasma of fish (Feeney and Brown, 1974). Albumen has also been found to exhibit a high degree of association with Kepone in human blood (Skalsky et al., 1979). Although these observations may have relevance to the topic of Kepone excretion in epidermal mucus,

any mechanism proposed at this time for excretion of pesticide by this route would be based on conjecture.

Generally urinary excretion of chlorinated hydrocarbon pesticides has been found to be slight in most species. Urinary excretion of chlordecone by man (Skalsky et al., 1979) and rats (Egle et al., 1978) has been found to be negligible. There is no evidence for significant excretion of these compounds with urine in fish. There is evidence that an important factor governing the urinary excretion of a compound is its molecular weight. Compounds having a molecular weight of 300 or greater are generally not efficiently excreted with urine (Smith, 1976). Negligible quantities of Kepone (molecular weight, 491) would therefore be expected in the urine of channel catfish and other species.

SUMMARY AND CONCLUSIONS

Calculation and comparison of dietary accumulation factors provides evidence that dietary Kepone has a relatively low potential for accumulation in channel catfish. The low value for the DAF may be partially attributed to the lack of association between Kepone and the triacylglyceride-rich tissues often associated with storage of chlorinated hydrocarbon pesticides. Kepone appears to have a greater affinity for tissues rich in phospholipids and cholesterol esters. However, this trend is evident in species which exhibited higher dietary accumulation factors.

The uptake rate constant for dietary Kepone in channel catfish (K_1 = 0.237 day^{-1}) lies within a range of K_1's calculated for the uptake of dieldrin and DDT by various fish species. However, the clearance rate constant for dietary Kepone in channel catfish (K_2 = 0.080 day^{-1}; $t_{\frac{1}{2}}$ = 8.7 days) is greater than that determined for all other species. Therefore, the low accumulation of dietary Kepone in channel catfish may be the result of relatively efficient excretion mechanisms.

This study provides evidence that Kepone may be excreted with the intestinal contents via biliary and extrabiliary pathways. The latter pathway may involve passage of Kepone directly across the intestinal wall into the intestinal contents. In addition, Kepone may be excreted across the gills and epidermal surfaces of channel

catfish. The relatively high levels of Kepone in the blood of channel catfish compared to that found in the blood of white catfish suggests that more Kepone is available in the former for excretion across the gills. The relative importance of these pathways has not been determined. Urinary excretion of Kepone by channel catfish is negligible possibly due to the molecular size of the pesticide.

The presence of chlordecone alcohol in the bile of channel catfish receiving IM injections of Kepone indicates that this species is capable of metabolizing Kepone into a form which is more readily excreted via the intestines. However, the significance of the metabolism of such a small percentage of the total body burden Kepone with respect to elimination rates is unknown.

SUGGESTIONS FOR FUTURE RESEARCH

The present study addressed the uptake and clearance of Kepone by one species of fish. Similar studies need to be performed on other species to allow determination of their uptake rates, clearance rates, and dietary accumulation factors. Dietary exposure rates should be varied to determine the relationship between exposure rate and equilibrium concentrations.

Dietary Kepone represents only one route of exposure. Species differences in the potential for bioconcentration of Kepone directly from water should be investigated. The relative importance of the two modes of exposure should be determined using concentrations in water and diet which are representative of those encountered in the James River ecosystem. As with dietary exposure, it is important to determine the relationship between bioconcentration of Kepone from water and exposure concentration. In addition to dietary and aqueous exposure, it would be desirable (though complicated) to determine the relative importance of exposure to Kepone-laden bottom and suspended sediments to the residue levels attained by fish. These sources represent the major reservoirs for Kepone storage in the James River ecosystem. Studies such as those mentioned above would aid in predicting the possible effects of Kepone abatement upon the residue levels exhibited by James River biota.

The present study has identified several possible excretory pathways for Kepone in channel catfish. Future work should be directed

towards an understanding of the relative importance of these pathways in this and other species. Studies involving the metabolism of Kepone deserve further attention. Chlordecone alcohol should be sought in bile of other species. Long term exposure studies should be performed in case the induction of metabolic activity takes place only after extended periods of exposure.

The distribution of Kepone within the tissues of fish may largely be influenced by blood transport mechanisms. It is generally assumed that chlorinated hydrocarbon pesticides are associated with plasma lipoproteins. To gain insight into the specific classes of compounds responsible for the transport and storage of Kepone in fish the following procedure could be followed:

1) Mechanically separate the high density lipoproteins, low density lipoproteins and very low density lipoproteins of fish plasma.
2) Determine the amount of Kepone in the high density lipoprotein, low density lipoprotein and very low density lipoprotein fractions of plasma.
3) Identify and quantify the major lipid groups associated with each fraction (i.e. phospholipid, triacylglyceride, cholesterol, cholesterol esters, free fatty acids).
4) Express Kepone residues in terms of the lipid content of each fraction (e.g. µg Kepone/gm phospholipid, µg Kepone/gm free fatty acid, etc.). In other words, for each of the three major density fractions, residue levels will be expressed in terms of each of the five major lipid groups.

5) Compare the range of residue levels found in the three major density fractions when expressed on the basis of the different lipid groups. If Kepone is primarily associated with one particular lipid, then the range of residue levels when expressed in terms of this lipid will be smaller than the range of residue levels expressed in terms of the other lipids.

The method described above may seem to be an involved method of determining which lipid groups are involved in the transport and storage of Kepone. However, direct measurement of Kepone residues in different lipid groups would be difficult because methods used for the separation of the major lipid groups (e.g. TLC) require the use of solvents which result in the extraction of Kepone during separation.

LITERATURE CITED

Argyle, R. L., G. C. Williams, and C. B. Daniel. 1975. Dieldrin in the diet of channel catfish (Ictalurus punctatus): uptake and effect on growth. J. Fish. Res. Bd. Canada 32: 2197-2204.

Bahner, L. H., A. J. Wilson, Jr., J. A. Sheppard, J. M. Patrick, Jr., L. R. Goodman, and G. E. Walsh. 1977. Kepone bioconcentration, bioaccumulation, loss, and transfer through estuarine food chains. Chesapeake Sci. 18: 299-308.

Bender, M. E., R. J. Huggett and W. J. Hargis, Jr. 1977. Kepone residues in Chesapeake Bay biota. pp. 14-37. In: Proceedings of the Kepone Seminar II, U.S. EPA, Region III, Philadelphia, Pa.

Blanchard, F. A., I. T. Takahashi, H. C. Alexander, and E. A. Bartlett. 1977. Uptake, clearance, and bioconcentration of ^{14}C-sec-butyl-4-chlorodiphenyl oxide in rainbow trout. pp. 162-177. In: F. L. Mayer and J. L. Hamelink (eds.) Aquatic Toxicology and Hazard Evaluation, ASTM Spec. Tech. Publ. 634.

Blanke, R. V., M. W. Fariss, P. S. Guzelian, A. R. Patterson, and D. E. Smith. 1978. Identification of a reduced form of chlordecone (Kepone) in human stool. Bull. Environ. Contam. Toxicol. 20: 782-785.

Borsetti, A. P., and J. A. G. Roach. 1978. Identification of Kepone alteration products in soil and mullet. Bull. Environ. Contam. Toxicol. 20: 241-247.

Boylan, J. J., J. L. Egle, and P. S. Guzelian. 1978. Cholestyramine: Use as a new therapeutic approach for chlordecone (Kepone) poisoning. Science 199: 893-895.

Boylan, J. J., W. J. Cohn, J. L. Egle, Jr., R. V. Blanke, and P. S. Guzelian. 1979. Excretion of chlordecone by the gastrointestinal tract: Evidence for a nonbiliary mechanism. Clin. Pharma. Therap. 25: 579-585.

Branson, D. R., G. E. Blau, H. C. Alexander, and W. B. Neely. 1975. Bioconcentration of 2, 2', 4,4'-Tetrachlorobiphenyl in rainbow trout as measured by an accelerated test. Trans. Am. Fish. Soc. 104: 785-792.

Bulkley, R. V., R. L. Kellogg, and L. R. Shannon. 1976. Size-related factors associated with dieldrin concentrations in muscle tissue of channel catfish (Ictalurus punctatus). Trans. Am. Fish. Soc., 105: 301-307.

Butler, P. A. 1963. Pesticide wildlife studies - A review of fish and wildlife investigations during 1961 and 1962. U.S. Fish and Wildlife Circ. 167: 11-25.

Chiou, C. T., V. H. Freed, D. V. Schmedding, and R. L. Kohnert. 1977. Partition coefficient and bioaccumulation of selected organic chemicals. Environ. Sci. Tech. 11: 475-478.

Cope, O. B. 1965. The effects of pesticides on fish and wildlife. U.S. Fish and Wildlife Circ. 226: 51-63.

Couch, J. A., J. T. Winstead, and L. R. Goodman. 1977. Kepone-induced scoliosis and its histological consequences in fish. Science. 197: 585-587.

Cowey, C. B., and J. R. Sargent. 1972. Lipid nutrition in fish. Comp. Biochem. Physiol. 57B: 269-273.

Dawson, G. W. 1978. The feasibility of mitigating Kepone contamination in the James River Basin. Appendix to the Kepone Mitigation Project Report. Prepared by Battelle Memorial Institute, Pacific Northwest Laboratory. varied pagination.

Desaiah, D. and R. B. Koch. 1975. Inhibition of ATPases activity in channel catfish brain by Kepone and its reduction product. Bull. Environ. Contam. Toxicol. 13: 153-158.

deFreitas, A. S. W., and R. J. Norstom. 1974. Turnover and metabolism of polychlorinated biphenyls in relation to their chemical structure and the movement of lipids in the pigeon. Can. J. Physiol. Pharmacol. 52: 1080-1094.

Ducat, D. A. and M. A. Q. Khan. 1979. Absorption and elimination of ^{14}C-cis-chlordane and ^{14}C-photo-cis-chlordane by goldfish (Carassius auratus). Arch. Environm. Contam. Toxicol. 8: 409-417.

Dvorchik, B. H. and T. H. Maren. 1972. The fate of p,p'-DDT (2,2-bis(p-chlorophenyl-1,1,1-trichloroethane) in the dogfish (Squalus acanthias). Comp. Biochem. Physiol. 42A: 205-211.

Egle, J. L., S. B. Fernandez, P. S. Guzelian and J. F. Borzelleca. 1978. Distribution and excretion of chlordecone (Kepone) in the rat. Drug. Metab. Dispos. 6: 91-95.

Feeney, R. E. and W. D. Brown. 1974. Plasma proteins in fishes. pp. 307-327. In: M. Florkin and B. T. Scheer (eds.) Chemical Zoology, Volume VIII, Deuterostomians, Cyclostomes and Fishes. Academic Press. New York.

Findlay, G. M. and A. S. W. de Freitas. 1971. DDT movement from adipocyte to muscle cell during lipid utilization. Nature. 229: 63-65.

Forster, R. P. and L. Goldstein. 1969. Formation of excretory products. pp. 313-350. In: W. S. Hoar and D. J. Randall (eds.) Fish Physiology, Volume I, Excretion, Ionic Regulation and Metabolism. Academic Press. New York.

Fromm, P. O. and R. C. Hunter. 1969. Uptake of dieldrin by isolated perfused gills of rainbow trout. J. Fish. Res. Bd. Canada 26: 1939-1942.

Gakstatter, J. H. and C. M. Weiss. 1967. The elimination of DDT-C^{14}, dieldrin-C^{14} and Lindane-C^{14} from fish following a single sublethal exposure in aquaria. Trans. Am. Fish. Soc. 96: 301-307.

Grzenda, A. R., D. F. Paris and W. J. Taylor. 1970. The uptake, metabolism and elimination of chlorinated residues by goldfish fed a ^{14}C-DDT contaminated diet. Trans. Am. Fish. Soc. 99: 385-395.

Grzenda, A. R., W. J. Taylor and D. F. Paris. 1971. The uptake and distribution of chlorinated residues by goldfish (Carassius auratus) fed a ^{14}C-dieldrin contaminated diet. Trans. Am. Fish. Soc. 100: 215-221.

Guiney, P. D., R. E. Peterson, M. J. Melancon, Jr., and J. J. Lech. 1977. The distribution and elimination of 2,5,2',5'-(^{14}C) tetrachlorobiphenyl in rainbow trout (Salmo gairdneri). Toxicol. Appl. Pharmacol. 39: 329-338.

Guzelian, P. S., G. Vranian, J. J. Boylan, W. J. Cohn, and R. V. Blanke. 1980. Liver structure and function in patients poisoned with chlordecone (Kepone). Gastroenterology. 78: 206-213.

Hamelink, J. L. 1977. Current bioconcentration test methods and theory. pp. 149-161. In: F. L. Mayer and J. L. Hamelink (eds.) Aquatic Toxicology and Hazard Evaluation, ASTM Spec. Tech. Publ. 634.

Hamelink, J. L., R. C. Waybrant and R. C. Ball. 1971. A proposal: exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. Trans. Am. Fish. Soc. 100: 207-214.

Hansen, D. J., L. R. Goodman, and A. J. Wilson, Jr. 1977. Kepone^{R}: chronic effects on embryo, fry, juvenile, and adult sheepshead minnows (Cyprinodon variegatus). Chesapeake Sci. 18: 227-232.

Hedgepeth, M. T., R. T. Doyle, and L. L. Stehlik. 1979. A laboratory analysis of Kepone depuration by spot (Leiostomus xanthurus). Virginia Institute of Marine Science, SRAMSOE No. 229. Gloucester Pt., Va. 21 pp.

Hedgepeth, M. Y. and L. L. Stehlik. 1979. The distribution and depuration of Kepone in American eels (Anguilla rostrata) from the James River, Virginia. Part I. Virginia Institute of Marine Science SRAMSOE No. 230. Gloucester Point, Va. 26 pp.

Huff, J. E., and H. B. Gerstner. 1978. Kepone: a literature summary. J. Environ. Path. Toxicol. 1: 377-395.

Huggett, R. J., M. M. Nichols and M. E. Bender. April 1979. Kepone contamination of the James River estuary. Proceedings of Symposium on Contaminants. Honolulu, Hawaii, American Chemical Society, in press.

Ivie, G. W., J. R. Gibson, H. E. Bryant, J. J. Begin, J. R. Barnett, and H. Wyman Dorough. 1974. Accumulation, distribution, and excretion of Mirex-^{14}C in animals exposed for long periods to the insecticide in the diet. J. Agr. Food Chem. 22: 646-653.

Jaeger, R. J. 1976. Kepone Chronology. Science 193: 95-96.

Kayama, M. and N. Iijima. 1976. Studies on lipid transport mechanism in fish. Bull. Jap. Soc. Sci. Fish. 42: 987-996.

Larsson, A. and R. Fange. 1977. Cholesterol and free fatty acids (FFA) in the blood of marine fish. Comp. Biochem. Physiol. 57B: 191-196.

Lee, R. F., R. Sauerheber and G. H. Dobbs. 1972. Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish. Mar. Biol. 17: 201-208.

Macek, K. J., C. R. Rodgers, D. L. Stalling and S. Korn. 1970. The uptake, distribution and elimination of dietary ^{14}C-DDT and ^{14}C-dieldrin in rainbow trout. Trans. Am. Fish. Soc. 99(4): 689-695.

Macek, K. J., S. R. Petrocelli, and B. H. Sleight, III. 1979. Considerations in assessing the potential for, and significance of biomagnification of chemical residues in aquatic food chains. pp. 251-268. In: L. L. Marking and R. A. Kimerle (eds.) Aquatic Toxicology, ASTM STP 667, American Society for Testing and Materials.

Martin, H. and C. R. Worthing (Editors): Pesticide Manual, Fourth Edition. Worchester, England. British Crop Protection Council. 1974. p. 96.

Mehendale, H. M., L. Fishbein, M. Fields, and H. B. Matthews. 1972. Fate of Mirex-^{14}C in the rat and plants. Bull. Environ. Contam. Toxicol. 8: 200-207.

Metcalf, R. L. 1976. Organochlorine insecticides, survey, and prospects. pp. 223-285. In: R. L. Metcalf and J. J. McKelvey, Jr. (eds.) Advances in Environmental Science and Technology, Volume VI, The Future for Insecticides: Needs and Prospects. John Wiley and Sons. New York.

Metcalf, R. L. 1977. Biological fate and transformation of pollutants in water. In: I. H. Suffet (eds.). Fate of Pollutants in the Air and Water Environments, Part 2. Chemical and Biological Fate of Pollutants in the Environment. John Wiley and Sons. New York.

Mills, G. L. and C. E. Taylaur. 1971. The distribution and composition of serum lipoproteins in eighteen animals. Comp. Biochem. Physiol. 40B: 489-501.

Mitchell, A. I., P. A. Plack, and I. M. Thomson. 1977. Relative concentrations of ^{14}C-DDT and of two polychlorinated biphenyls in the lipids of cod tissues after a single oral dose. Arch. Environ. Contam. Toxicol. 6: 525-532.

Moore, R., E. Toro, M. Stanton, and M. A. Q. Khan. 1977. Absorption and elimination of ^{14}C-alpha- and gamma- chlordane by a fresh-water alga, daphnid and goldfish. Arch. Environ. Contam. Toxicol. 6: 411-420.

Morgan, D. P., C. C. Roan, and E. H. Paschal. 1972. Transport of DDT, DDE, and dieldrin in human blood. Bull. Environ. Contam. Toxicol. 8: 321-326.

Morris, R. L. and L. G. Johnson. 1971. Dieldrin levels in fish from Iowa streams. Pesticides Monitoring Journal 5: 12-16.

Moseman, R. F., H. L. Christ, T. R. Edgerton and M. K. Ward. 1977. Electron capture determination of Kepone residues in environmental samples. Arch. Environ. Contam. Toxicol. 6: 221-231.

National Academy of Sciences. 1978. Kepone/Mirex/Hexachlorocyclopentadiene: An environmental assessment. Washington, D. C. 73 pp.

Nimmo, D. R., L. H. Bahner, R. H. Rigby, J. M. Sheppard and A. J. Wilson, Jr. 1977. Mysidopsis bahia: An estuarine species suitable for life-cycle bioassays to determine sublethal effects of a pollutant. pp. 109-116. In: F. L. Mayer and J. L. Hamelink (eds.) Aquatic Toxicology and Hazard Evaluation. ASTM Spec. Tech. Publ. 634.

Pritchard, J. B., A. M. Guarino and W. B. Kinter. 1973. Distribution, metabolism, and excretion of DDT and Mirex by a marine teleost, winter flounder. Environmental Health Perspectives. Experimental issue: 45-54.

Reinert, R. E. and H. L. Bergman. 1974. Residues of DDT in lake trout (Salvelinus namaycush) and coho salmon (Oncorhynchus kisutch) from the Great Lakes. J. Fish. Res. Bd. Canada 31: 191-199.

Roberts, M. H. 1980. Kepone distribution in selected tissues of blue crabs Callinectes sapidus collected from the James River and lower Chesapeake Bay. VIMS manuscript.

Schimmel, S. C. and A. J. Wilson, Jr. 1977. Acute toxicity of Kepone to four estuarine animals. Chesapeake Sci. 18: 224-227.

Schimmel, S. C., J. M. Patrick, Jr., L. F. Faas, J. L. Oglesby and A. J. Wilson, Jr. 1979. Kepone^{R}: Toxicity and bioaccumulation in blue crabs. Estuaries 2: 8-15.

Shul'man, G. E. 1960. Dynamics of the fat content of the body of fish. Russ. Rev. Biol. 49: 209-222.

Skalsky, H. L., M. W. Fariss, R. V. Blanke and P. S. Guzelian. 1979. The role of plasma proteins in the transport and distribution of chlordecone (Kepone) and other polyhalogenated hydrocarbons. Ann. N. Y. Acad. Sci. 320: 231-237.

Smith, R. L. 1976. The Excretory Function of Bile: The Elimination of Drugs and Toxic Substances in Bile. Chapman and Hall, London. 281 pp.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods. The Iowa State University Press, Ames 593 pp.

Statham, C. N. and J. L. Lech. 1975. Metabolism of 2', 5-dichlor-4'-nitrosalicylanilide (Bayer 73) in rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Canada. 32: 515-522.

U. S. Environmental Protection Agency. 1975. Fact sheet on Kepone levels found in environmental samples from the Hopewell, Va. area. Health Effects Research Laboratory EPA, Research Triangle Park, N. C. Unpublished report 15 p.

Van Oosten, J. V. 1957. The skin and scales. pp. 207-244. In: M. E. Brown (ed.) The Physiology of Fishes. Academic Press, New York.

Varanasi, U., M. Uhler, and S. I. Stranahan. 1978. Uptake and release of napthalene and its metabolites in skin and epidermal mucus of salmonids. Toxicol. App. Pharmacol. 44: 277-289.

Warlen, S. M., D. A. Wolfe, C. W. Lewis and D. R. Colby. 1977. Accumulation and retention of dietary ^{14}C-DDT by Atlantic Menhaden. Trans. Am. Fish. Soc. 106: 95-104.

VITA

Peter Alyea VanVeld was born in West Lafayette, Indiana on February 11, 1952. He graduated from Rose High School in Greenville, North Carolina in 1970 and received a B.S. in biology from the University of North Carolina - Chapel Hill in 1975. He is presently employed by the Environmental Protection Agency in Gulf Breeze, Florida.