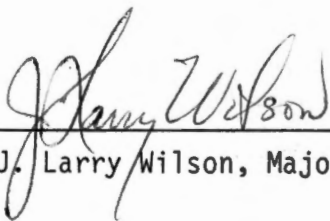
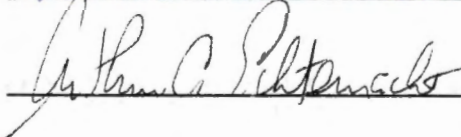


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ACCUMULATION OF DDT BY CHANNEL CATFISH (ICTALURUS PUNCTATUS)
HELD IN CAGES IN THE HUNTSVILLE SPRING BRANCH/
INDIAN CREEK COMPLEX

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Lee Ann Woodward

March 1985

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ABSTRACT

Catfish fingerlings (Ictalurus punctatus) were used to determine if direct contact with residues of DDTR (DDT and its metabolites) in the sediments of Huntsville Spring Branch/Indian Creek in Alabama were responsible for DDTR concentrations in the fillets. Fish were stocked in cages where sediment levels of DDTR averaged 404, 178, and 36 ppm. Analyses indicated no significant differences in DDTR concentrations in fish fillets between contact and no-contact conditions. Significant differences were found in averaged fillet concentrations of DDTR among all sites. Concentrations of dissolved DDT in water (for filtered samples) were approximately 1.0 ppb for all sites. The diet of the fish contained 67% invertebrates (mainly Chironomidae) with high DDTR residues and significant differences were found between Sites A and C, and B and C. Since diet was a possible source of DDTR contamination and dissolved DDTR concentrations in the water did not vary among Sites A, B, and C, it is hypothesized that diet was the more significant factor in the mean concentrations of DDTR found in the fish fillets from these sites. More definitive studies are needed for confirmation.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. LITERATURE REVIEW.	6
III. PROPERTIES OF DDT.	9
IV. SITE DESCRIPTION	11
V. METHODS.	14
VI. RESULTS AND DISCUSSION	20
VII. SUMMARY.	30
LIST OF REFERENCES	32
VITA	38

CHAPTER I

INTRODUCTION

In 1947, the Calabama Chemical Company began production of DDT on the Redstone Arsenal. Calabama operated this plant, leased from the Arsenal, for seven years with capacity at approximately 1.13×10^7 kilograms (kg) of DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane] per year. The plant was located so that wastewater was discharged directly into Huntsville Spring Branch (HSB). The effluent contained calcium chloride, hydrochloric acid, monochlorobenzene, hypochlorites, chloral, sulfuric and sulfonic acid, and DDT. DDT levels ranged up to 0.8 ppm, mainly in particulates.

Olin Matheson Chemical Company bought Calabama Chemical Company in 1954 and continued the manufacture of DDT. Production under Olin's supervision was about 1.13×10^7 kg per year at full capacity. Effluent at this time was 5.68×10^6 L daily with no provisions for treatment. In 1965, settling ponds were constructed in an attempt to meet new discharge rates of 10 ppb DDT. It is estimated that 5,443 kg of DDT accumulated in these ponds in a span of three to four months. Two years later (1967) these ponds were enlarged. A new drainage ditch was constructed when the DDT levels in the sediments of the old ditch were high enough to be considered a source of DDT transfer itself. The old ditch was treated with lime and ferrous sulfate and filled. A year later, the Federal Water Pollution Control Administration

(FWPCA) determined that the HSB immediately below the drainage ditch contained an average of 3.58 ppb DDT in water, thus meeting standards of 10.0 ppb DDT.

In 1969, the FWPCA set new standards for DDT in wastewater at 0.02 ppb. Olin installed a carbon filter at the outlet to the settling pond, but, unable to meet FWPCA standards, stopped production of DDT at the plant on 30 June 1970. Two other pesticides were produced at the plant, trichloroacetone (TCAN) and methoxychlor. Production of these was discontinued after less than a year and in 1972 the plant was demolished. Short-term containment measures were begun shortly thereafter and finished in 1977. These included filling and sealing the old settlement basin, the diversion of drainage around the site, installation of two dams in the drainage ditch to create sediment retention ponds, and installation of a water filtration/carbon absorption unit to treat water leaving the drainage ditch. Late in 1979, surface soils at the old plant site were removed to an approved landfill located on another part of the Arsenal. Further excavation of contaminated sediments from the ditches and ponds followed by filling was done to preclude surface erosion of DDT. It was assumed that further migration of DDT from the site to HSB should be negligible [O'Shea et al. 1980, Water and Air Research (WAR) 1980, Fleming and Cromartie 1981].

Fish surveys of 1979-80 indicated that fish in the Huntsville Spring Branch/Indian Creek (HSB/IC) complex exhibited high concentrations of DDT. Channel catfish (Ictalurus punctatus), in particular,

were affected with all fish sampled containing above 5 ppm and many greater than 50 ppm DDTR (DDT and its metabolites). Smallmouth buffalo (Ictiobus bubalus) were contaminated to a lesser degree. Largemouth bass (Micropterus salmoides) generally had concentrations less than 5 ppm DDTR, although some individual fish had concentrations greater than 10 ppm DDTR (WAR 1980).

Capture-recapture studies of channel catfish, blue catfish (I. furcatus), flathead catfish (Pylodictis olivaris), white crappie (Pomoxis annularis), and white bass (Morone chrysops) in Wheeler Reservoir, done by TVA in 1978, showed a possible range of movement of these fish. Recapture data showed that 85% of the fish had moved from the point of release. Channel catfish, in particular, had a 52% recapture rate upstream from the point of release and 48% downstream. Some catfish were recaptured 32 km from the point of release within 10 days. Thus, fish contaminated by the residues in HSB/IC can move out of the system to the Tennessee River (TR) and its reservoirs.

A summary of the conditions of HSB/IC as given by WAR (1980) or Smithson (Olin Corp., pers. comm.) are as follows:

1. an extensive amount, approximating 400 metric tons, of DDTR as in the sediments of HSB/IC;
2. this DDTR is being slowly moved through the HSB/IC complex to the Tennessee River, mainly by sediment transport;
3. fish, particularly channel catfish, are highly contaminated with some having levels above the 5 ppm limit for fillets set by the Food and Drug Administration's (FDA) interstate food allowances;

4. contamination of fish in the TR results from low levels of DDTR that now exist in water and/or sediment downstream of IC; and

5. contaminated fish in the TR appear to migrate from contaminated areas.

The small village of Triana is located approximately 18 km downstream from the old plant drainage ditch, at the juncture of Indian Creek and the Tennessee River. This community of 600-1,000 has used the river for generations as a source of water, food, and recreation. Fish were the mainstay of the inhabitants' diet and provided the sole source of income for many fishermen (Reynolds 1980). The discovery of high concentrations of DDT in the TR by the Army's Environmental Agency (O'Neill 1979) led to the examination of some Triana residents for DDT residues. A study done by the Center for Disease Control (Atlanta) found that of the 499 individuals tested, 28% had DDT blood serum levels more than 10 times greater than the geometric mean of the average person in the United States (15 ppb). Twenty-three people had DDT blood serum levels greater than 500 ppb and 6 greater than 1,000 ppb. Age and fish consumption were found to be the two best correlates with DDT residue levels (Kumnant 1982).

The Olin Corporation is responsible for the clean-up of the impacted area. Of major concern is the reduction of DDTR levels to below the 5 ppm limit set by the FDA for the fillets of commercial fish in the area. The fish most affected is the channel catfish, which is also the most utilized by the local people; for these reasons the channel catfish is the focus of this study.

Catfish typically inhabit the water column near the bottom where they may at times scavenge in the sediment for food, thus frequently coming in contact with the contaminated sediments. This behavior is unlike other local food fish which live higher in the water column and do not contact the sediment with any frequency. It is the impact of this sediment-contacting behavior on contaminant uptake that was addressed in this study. A field investigation was made to help determine if DDTR is transferred from sediment to channel catfish in the natural environment. More specifically, these experiments were designed to show whether direct contact with the contaminated sediments is necessary to achieve high (greater than 5 ppm) DDTR concentrations in fish fillets.

CHAPTER II

LITERATURE REVIEW

Since the advent of pesticides, much research has been done to determine their environmental fate. Due in part to their economic importance, fish have been much utilized in the study of pesticide fate in aquatic ecosystems. Reviews of this work are to be found annually since 1968 in the Journal of the Water Pollution Control Federation (e.g., Spehar et al. 1981, Spehar et al. 1982, Buikema et al. 1983), and Johnson (1968), Kenaga (1972), and Hamelink and Spacie (1977). These compendia include chronic and acute test data, uptake rates, uptake amounts, mortality rates, bioconcentration factors in relation to partition coefficients, and methods of accumulation. Of interest to this study is the method of contaminant acquisition in the fish; that is, the element of the fish's environment which is the primary source of contamination.

For DDT the debate has centered on whether the diet or the water is the primary source of the toxicant. Woodwell et al. (1967) found that DDT residue concentrations were found in increasing amounts with the higher trophic levels of a food web in an estuary, suggesting that the chemical is accumulated through a contaminated diet. Holden (1962) found that fish, in the absence of food, absorbed DDT through the gills. Thus the Woodwell et al. (1967) findings indicated that, in addition to direct uptake from the water, the diet also contributed to body

residues but they did not elucidate whether it was accumulation exclusively from the diet, water, or both.

Jarvinen et al. (1977) clarified how DDT residues in a fish's diet can be either contributory or non-significant. They used flow-through aquaria utilizing high and low (0.5 and 2.0 ppb) DDT contaminated water. One set of fish was fed a contaminated food (45.6 ppm radio-labeled DDT in dried clams) while in the duplicate set of tanks, fish were fed a DDT-free diet. Third and fourth sets of aquaria were maintained with non-contaminated water, one set contained fish fed the contaminated diet and the other served as the control. Results indicated that contaminated food plus contaminated water were additive yielding a higher accumulation than contaminated food or contaminated water alone. Accumulation from the water was the main contributor at high water concentrations of DDT but at the lower DDT concentrations, accumulation from the food increased from 30 to 60% of the total uptake. This is evidence that contaminated diet plays a differential role dependent upon relative water residue concentrations.

Water and food are not the only sources of contamination. Johnson (1968) and Huang (1971) have suggested that bottom muds should be more closely examined as to their role in accumulation of toxicants in aquatic systems. Connell and Miller (1984) concluded that the sediment plays an important role in the transport and exit of pollutants in the environment by "(1) providing sorption surfaces, (2) acting as a buffer system, and (3) serving as sinks for pollutants." The question of how much the concentration of the pesticide in the sediment directly affects the final body residues of the associating organisms has been

explored little for DDT. Most work has been done with polychlorinated-biphenyls (PCB's) or herbicides that have similar characteristics such as high partition coefficients and high bioconcentration factors that lend themselves well to comparisons.

Young et al. (1977) and Giddings and Eddlemon (1977) showed that sediments do act as "sinks" for pollutants which then serve as sources for contamination. They found that higher levels of residues in the sediments were correlated with the higher concentrations in the examined benthic species. The same direct correlation was found by Nimmo et al. (1971) between concentrations of PCB in the sediment to PCB in the bottom-dwelling shrimp (Penaeus duorarum) and the Addler crab (Uca pugilator) held in flow-through aquaria. In all these studies, low levels of the contaminants were also found in the water so that it cannot be concluded whether all or part of the body residues came from the sediments or from the water.

CHAPTER III

PROPERTIES OF DDT

DDT, dichlorodiphenyl trichloroethane or 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane, was synthesized by Zeidler in 1874. It was not until 1939 that Müller discovered its properties as a potent insecticide. Pure DDT (melting point 108 - 109°C, vapor pressure 1.5×10^{-7} mm Hg) (Metcalf 1971) has low solubility in water, about 1 ppb, but is soluble in many organic solvents (Kenaga 1972).

For mammals, DDT is relatively non-toxic with an oral LD₅₀ of 200 mg/g and a dermal LD₅₀ of 3,000 mg/g for the rat (Metcalf 1971). In channel catfish, the 96-hour LC₅₀ is 0.02 ppm (Wellborn et al. 1984). DDT is a broad spectrum insecticide affecting the peripheral nervous system; however, the biochemical mode of action is unknown. DDT is dehydrochlorinated, in the presence of strong alkali, to the non-insecticidal 2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene (DDE) (Metcalf 1971). This can be oxidized to p,p'-dichlorobenzophenone. These reactions account for much of the abiotic formation of DDT residues. DDT is commonly metabolized to DDD [2,2-bis-(p-chlorophenyl)-1,1-dichloroethane] by the liver and intestinal microflora of animals. DDT in insects is metabolized by dehydrochlorination to form DDE or by α -hydroxylation to form 2,2-bis-(p-chlorophenyl)-2-hydroxy-1,1,1-trichloroethane (kelthane). In mammals, DDT is enzymatically dehydrochlorinated to DDE; this is converted to DDA, bis-(p-chlorophenyl)-

acetic acid, which is water soluble and can be excreted in the urine (Metcalf 1971). This conversion usually occurs in the endoplasmic reticulum of the liver in vertebrates and Malpighian tubules of insects. This transformation does not proceed at a constant rate and will vary among species depending on their metabolic capabilities. Nonetheless, dehydrochlorination is a powerful defense mechanism developed by animals against many potentially harmful substances (Walker 1975).

A factor of great interest when studying the fate of a chemical in an aquatic environment is its partition coefficient (P). The partition coefficient is the concentration ratio at equilibrium of an organic compound between a non-polar solvent (e.g., n-octanol) and

water, $\left(P = \frac{\text{concentration in non-polar solvent}}{\text{concentration in water}} \right)$. The partition

coefficient has been negatively correlated with aqueous solubility, and positively correlated with physical adsorption on solids, biomagnification/accumulation, and lipophilic storage. The higher the P value, the lower the solubility in water and, conversely, the more hydrophobic, the greater adsorption on solids. This relationship has been shown to correspond to a higher bioaccumulation in organisms (Metcalf et al. 1971, Neely et al. 1974, Chiou et al. 1977, Southworth et al. 1980). This is commonly measured as a bioconcentration factor (BCF) (Neely et al. 1974) which is a ratio of the chemical concentration in the organism, or a specific tissue of the organism to that of its abiotic environment. DDT's partition coefficient is between 1×10^5 and 1×10^6 (Hamelink et al. 1971).

CHAPTER IV

SITE DESCRIPTION

The DDT plant was located on Redstone Arsenal near Huntsville in northern Alabama (Figure 1). Adjoining the Arsenal is Wheeler National Wildlife Refuge. Due to a long-standing agreement, the Arsenal has incorporated under joint management 2,385 hectares of the Refuge into its boundaries. The Refuge composes 13,972 hectares stretched along 32 km of the Tennessee River and Wheeler Reservoir. Included are some 2,430 hectares of seasonally-flooded waterfowl reserves, wintering from 40,000 to 60,000 ducks and 30,000 geese. Lowland areas contain wildlife food crops and higher areas support habitats of hardwood and pine forests.

Through this area flows the Huntsville Spring Branch which converges with Indian Creek, a tributary of the Tennessee River at TR kilometer 513.6 (TRK 513.6). The HSB/IC are slow, meandering, shallow rivers which periodically overflow, spreading far out onto mudflats, swamps, and hardwood bottoms during times of high water. The rivers flow through predominantly Huntington silt loam, Lindsides silty clay loam, and Melvin silty clay loam (Swenson et al. 1958).

The HSB/IC complex has been delineated into three areas according to percent of the total mass distribution of DDTR found in the sediments (WAR 1980). Reach A extends from HSBK 8.6 (HSB kilometer) to HSBK 3.8 and contains approximately 98% of the total DDTR in the

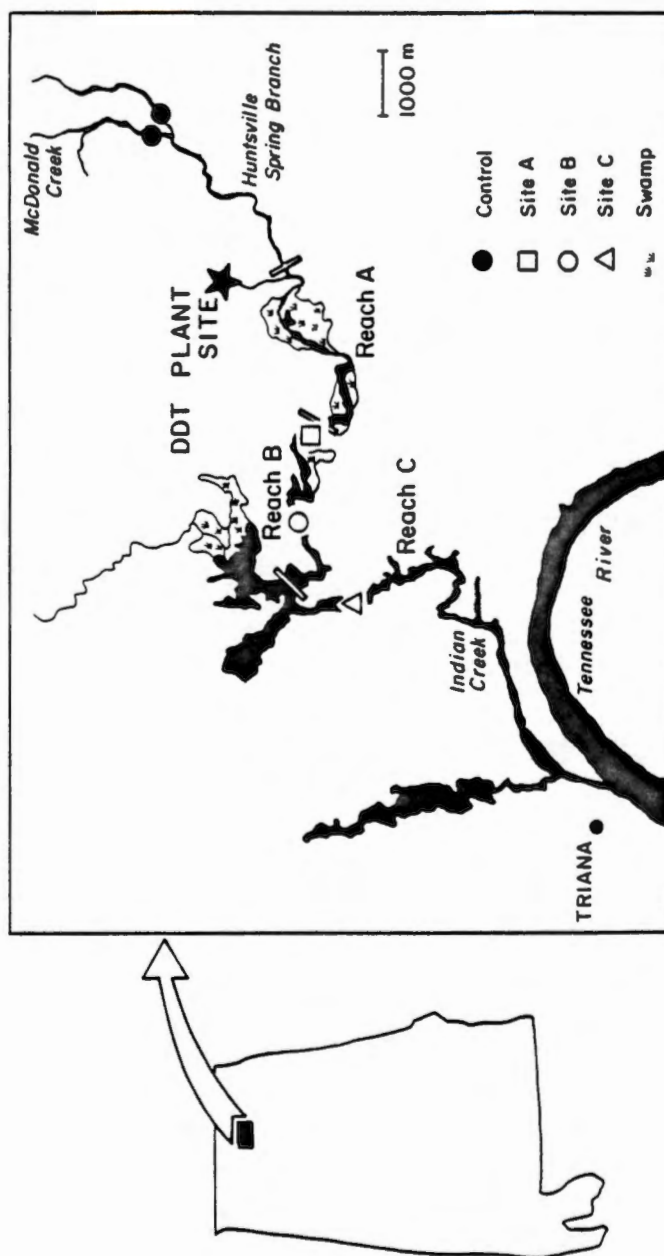


Figure 1. Location and identification of the reaches and sites in the Huntsville Spring Branch/Indian Creek complex.

sediments. Reach B extends from HSBK 3.8 to the confluence with IC, and contains approximately 1.4% of the total DDTR. The remaining 0.6% of the DDTR is downstream from this point in IC to the TR.

The source of contamination originated from a wastewater ditch which flowed from the plant entering at HSBK 8.6. Testing done in 1980 showed DDTR in sediments highest from HSBK 8.6 to HSBK 3.8 with a range of 50 to 3,000 ppm. This extreme in range was caused by the DDTR not being contained in the main river channel but spreading over the entire flood plain. In 1983, Olin and Recra (an environmental consulting firm) sampled and tested sediments and found ranges to be as follows: HSBK 8.6 = 1,000 - 80,000 ppm; HSBK 3.8 = 30 - 1,000 ppm; and ICK 7.5 = 0 - 30 ppm.

A section of the river complex from about HSBK 5.0 to ICK 0.5 runs through Redstone Arsenal's military "Test Area 1." The test area was in use during the hours of 0800 to 1700 Monday through Friday and occasionally during these hours on the weekend. Due to the possible danger to researchers on the river during tests, entry to this range was restricted to before, after, or between tests.

CHAPTER V

METHODS

Cages, which are a standard method of fish culture for catfish (Schmittou 1969), were used to contain the fish in the river. Caged fish have been used in several instances as pollution indicators (Edwards et al. 1964, Hasselrot 1965). The study cages were constructed of a 1.0 m x 1.0 m welded square angle-iron (treated with rust-resistant paint) to form the top, and at each corner was bolted a 2.7 m long angle-iron to form the frame. Onto this frame was placed a polyvinylchloride-coated (PVC) 1.3 cm x 2.5 cm rectangular mesh hardware cloth inset. Two types of cages were supported by this frame. The open-bottom cage, which allowed the fish direct contact with the sediments, was an enclosure of the PVC-coated hardware cloth, and was 2.0 m in height. The closed-bottom cage was a 1.0 m cube of the PVC-coated hardware cloth.

Selection of the four sites coincided with various levels of DDTR in the sediments. A control was located above Reach A in an area where no DDTR was found in the samples of sediments, and far enough above the contaminated area to prevent any backwash of the pollutant. The other three locations were selected for areas that would enable a comparison of accumulation in the fish of the high, middle, and low DDTR ranges in Reaches B and C of the HSB/IC complex. Reach A was not included in this study. Sites selected were:

(1) HSBK 12.5, control, later relocated (see Results) to McDonald Creek, designated in this study as "Control";

(2) HSBK 3.8, Reach B, "Site A";

(3) HSBK 1.6, Reach B, "Site B";

(4) ICK 7.5, Reach C, "Site C."

On 6 July, 1983, the cages were located at the four sites on HSB/IC. To insure that the tops of the cages were above the water for sampling and feeding, the cages were placed to one side of the channel where the river depths approached 1 m. At Site B the river proved shallow enough for placement at mid-stream. At each location the river bottom was a thick mud, averaging approximately 20 cm, which held the cages securely and covered the bottom edges of the open-bottom cages. For added strength, the four upright posts were pounded into the hard clay substrate below the mud.

At the time of placement, each open-bottom cage (C) was checked for a complete seal around the bottom. This was done by pole, foot, and sometimes hand. Each closed-bottom cage (NC) was checked for distance between the top of the mud and the bottom of the cage. Each was further adjusted by pounding of the uprights until a standard 0.5 m from the bottom was obtained.

The cages were set out in groups of four, thus providing replicates at each site. Cages were numbered according to relative position on the river as Control 1-4 at HSBK 12.5 and proceeding downstream with Site A 1-4 at HSBK 3.8, Site B 1-4 at HSBK 1.6, and Site C 1-4 at ICK 7.5.

Channel catfish fingerlings of 15 to 20 cm were obtained from a local fish farmer on 12 July 1983. These were held in two floating 1 m x 1 m x 2 m cages in a back eddy of the TR below the field laboratory until they could be moved to study cages in the HSB/IC river complex.

Fish were stocked in study cages on 16 and 17 July. Day 0 fish samples were taken before each stocking to establish background levels of DDTR for the fish. Cages were stocked at 60 per NC, and 90 per C cage (approximately 70 catfish/m³). Fish were transported in lots of 38 per 208 L plastic drum, with a maximum of four drums per boat; thus, only two cages could be stocked per trip. Water temperature, dissolved oxygen (DO), pH, plankton sample, sediment sample, and water sample were taken when each cage was stocked (day 0). All trips were coordinated with the activities of the test range. Time spent in each stocking averaged 2-4 hours. Stocking was done on two days instead of continuing into the night on 16 July due to the fatigue of the workers and their unfamiliarity with the rivers. The stocking order was determined by access to the range, i.e., different parts of the range would be open and/or closed at different times.

Fish were fed on alternate days when not prohibited by test range activities. A commercial catfish ration (50% floating pellet, 50% sinking pellet) was used at a minimum of 6% of total body weight. At each feeding, DO, pH, and water temperature were recorded.

Sampling of the fish was done on days 2, 5, 15, 25, 35, 45, and 55 of the study. This was to accommodate the initial fast uptake of DDT as shown by Meeks (1968) and Eberhardt et al. (1971). Time of

sampling was determined by access to the test area, usually resulting in sampling occurring before 0800 hours or after 1700 hours, and/or during 0700-2000 hours on weekends. On several occasions sampling was interrupted to be continued several hours later. Each sampling included the following:

(1) Three fish were removed from each cage at each site with a net, and fillet samples were prepared for analysis. Ten percent of all offals were also prepared for analysis, along with split samples for quality control. Replicate samples were taken after day 25.

(2) A 1.0 L water sample was taken at a depth of approximately 0.5 m from each cage for DDTR analysis.

(3) Vertical plankton tows were collected from each cage for DDTR analysis (days 15 - 55).

(4) A random-grab sediment sample from each open-bottom cage and immediately surrounding the closed-bottom cages was taken for DDTR analysis.

(5) DO, pH, and temperature were determined in the field for each cage pair, using portable standardized meters.

(6) Gut contents were frozen and later inspected for the presence of food items. DDTR concentrations were determined (days 25 - 55).

Water, sediment, and plankton samples were put on ice when collected in the field and were maintained cold until analyzed by Olin. Fish were put on ice and then frozen upon arrival at the field laboratory until preparation. Sample preparation for the fish consisted of removing skin and visible fat, filleting, and then grinding (with a

mortar and pestle) and thoroughly mixing each fillet. A mixed fillet sample (approximately 5 gm) and a replicate were then refrozen.

Guts were removed, cut open, and rinsed of all contents with deionized water. Visual inspection with a dissecting microscope was used to determine types and numbers of organisms. These were recorded as was the wet weight of the contents, then refrozen. Contents were then sent to Olin for DDTR analysis (Olin Corporation 1983). All samples of fillets, gut contents, and offals analyzed were of individual fish.

All water, plankton, and sediment samples were taken in bottles and jars which had been washed with detergent and then thoroughly rinsed with tap water, acetone, tap water again, and then deionized water. Only glass or metal sample containers, collectors, or preparation tools were used and caps were lined with either teflon or aluminum foil due to DDTR's adsorptive properties. Each was labeled for date, site, and cage of collection.

Analysis for DDTR was done by Olin's laboratory in Charleston, Tennessee, and analyses were subjected to a thorough quality control (QC) for both precision and accuracy. Exact QC procedures for each type of analysis can be found in Olin's laboratory procedures guide (1983). In general, each QC procedure consisted of: (a) 10% of all samples were analyzed in duplicate, (b) accuracy limits were established for gas chromatographic recoveries, (c) standard reference materials and blanks were analyzed for approximately 10% of the field samples, (d) p,p'-DDT standards were analyzed daily for instrumental

checks, and (e) surrogate recoveries were utilized as measures of extraction efficiency.

The procedure for DDTR analysis was new to the laboratory, and some analyses were rejected because of QC criteria. When available, replicate samples were reanalyzed, but due to storage limitations these were few and restricted to some fish fillets.

Data were analyzed with the aid of The University of Tennessee Computing Center's Decsystem-10 computer and the Statistical Analysis (SAS) 1982 software package. Student's *t* was used for comparisons of contact versus no-contact conditions, the Bonferroni *t* procedure was used for among site comparisons of water, catfish fillets, and gut contents.

CHAPTER VI

RESULTS AND DISCUSSION

Three separate stockings were made for the control site, once at HSBK 12.5 and twice on McDonald Creek, a tributary to HSB. Each attempt failed apparently due to a sudden decrease in DO (less than 1.0 ppm) caused by a flush of sewage-laden water from a treatment plant upstream of HSBK 12.5. Each trial lasted 1, 16, and 9 days, respectively. No DDTR was detected in fish fillets, water, or sediment taken from any control site. All subsequent results shall deal only with Sites A, B, and C.

Quality control criteria caused rejection of some analyses; replicate samples were only available for a few fish fillets. DDTR values for water rejected encompassed half of the samples obtained for day 5 and all samples for day 45. Plankton values rejected were 3 of 8 samples for day 15, 2 of 12 for day 25, and all samples for day 45. All values obtained for fish offals were rejected based on quality control criteria. Due to the total loss of DDTR analyses of offals and a large percentage of plankton DDTR analyses, plankton and fish offals were not included in any of the statistical tests.

For 55 days of exposure, no significant difference (Student's t , $p \leq 0.05$) in DDTR concentrations was found in fish fillets in contact and no-contact conditions. Therefore, DDTR values in fillet,

gut contents, and sediments were pooled and averaged by site at each sampling for further statistical comparisons (Bonferroni t procedure).

Water height at the cages fluctuated less than 1 m. Highest water was in July at the beginning of the study and gradually fell approximately 0.5 m over the course of the study. Water quality parameters were considered suitable for channel catfish growth during the study period.

Parameter	Mean $\pm$ Std. Dev.	Range
Dissolved Oxygen (ppm)	7.3 $\pm$ 2.8	1.7 - 15.0
Temperature (C)	21.9 $\pm$ 10.3	25.8 - 33.0
pH	7.95 $\pm$ 0.77	6.15 - 9.35

Concentrations of DDTR in the unfiltered water samples ranged from 1.9 to 26.1 ppb with the highest amounts found at Site A and the lowest at Site C. Values were significantly different among all sites at $p \leq 0.0001$. Filtered water samples (particulates and humic acids removed) had DDTR concentrations of approximately 1.0 ppb at all sites (Table 1). This indicated that the amount of soluble, unbound DDTR in the water did not vary from site to site and therefore would not be a factor influencing the mean concentrations of the fish fillets at different sites.

Table 1. DDTR concentrations and bioconcentration factors (BCF) for fillets, filtered water (fw), sediment (s), and gut contents (gc) by sites sampled. Values for filtered water and sediment are representative for the entire study; fillet and gut content values are for days 25 - 55.

	N	DDTR mean (sd)	BCF (fw)	BCF (s)	BCF (gc)
FILLET (ppm)					
Site A	37	32.6 (18.6)	32600	0.08	0.35
Site B	35	14.6 (6.5)	14600	0.08	0.22
Site C	41	5.6 (2.4)	4700	0.15	0.44
GUT CONTENTS (ppm)					
Site A	30	92.2 (59.8)	92200	0.23	
Site B	26	66.2 (47.2)	66200	0.37	
Site C	40	12.8 (7.2)	10700	0.35	
FILTERED WATER (ppb)					
Site A	39	1.0 (0.4)			
Site B	39	1.0 (0.3)			
Site C	39	1.2 (0.4)			
SEDIMENT (ppm)					
Site A	24	404.2 (181.8)			
Site B	27	177.8 (106.4)			
Site C	27	36.4 (14.4)			

Sediment samples were taken on each sample day from each cage. The mean DDTR dry weight concentrations are found in Table 1. All sites were significantly different from each other at $p \leq 0.001$.

Examination of 106 guts from the sampled fish showed that only 24% of the diet (based on the wet weight) consisted of catfish ration, 67% was insects, and 9% was miscellaneous. Substrate and vegetative matter were absent from 99% of all samples. Chironomidae (midge larvae and pupae) were the most abundant insects with the highest numbers at Site C and the lowest at Site A.

Chironomids are very common and abundant in aquatic sites such as these. The larvae are found in the water column and commonly in burrows in the substrate where they are filter feeders, collectors, scrapers, and scavengers. Those inhabiting the sediment will remain there until the last larval ecdysis (shedding of the larval integument) and then swim to the surface to emerge (Coffman 1978). Chironomids eaten by the caged fish probably were some combination of the free-ranging larvae and emerging pupae which were washed downstream into the cages. If these insects were coming from areas of much higher DDTR concentrations they could have DDTR body burdens in excess of the sediment and water concentrations at the cage sites. Also, as these were only insect parts (mainly exoskeletons), as a result of partial digestion, DDTR concentrations of the whole insects would probably be higher.

DDTR concentrations of the gut contents ranged from 2.3 to 285.7 ppm with the lowest at Site C and the highest at Site A (Table 1, Figure 2). These were higher than concentrations obtained for these

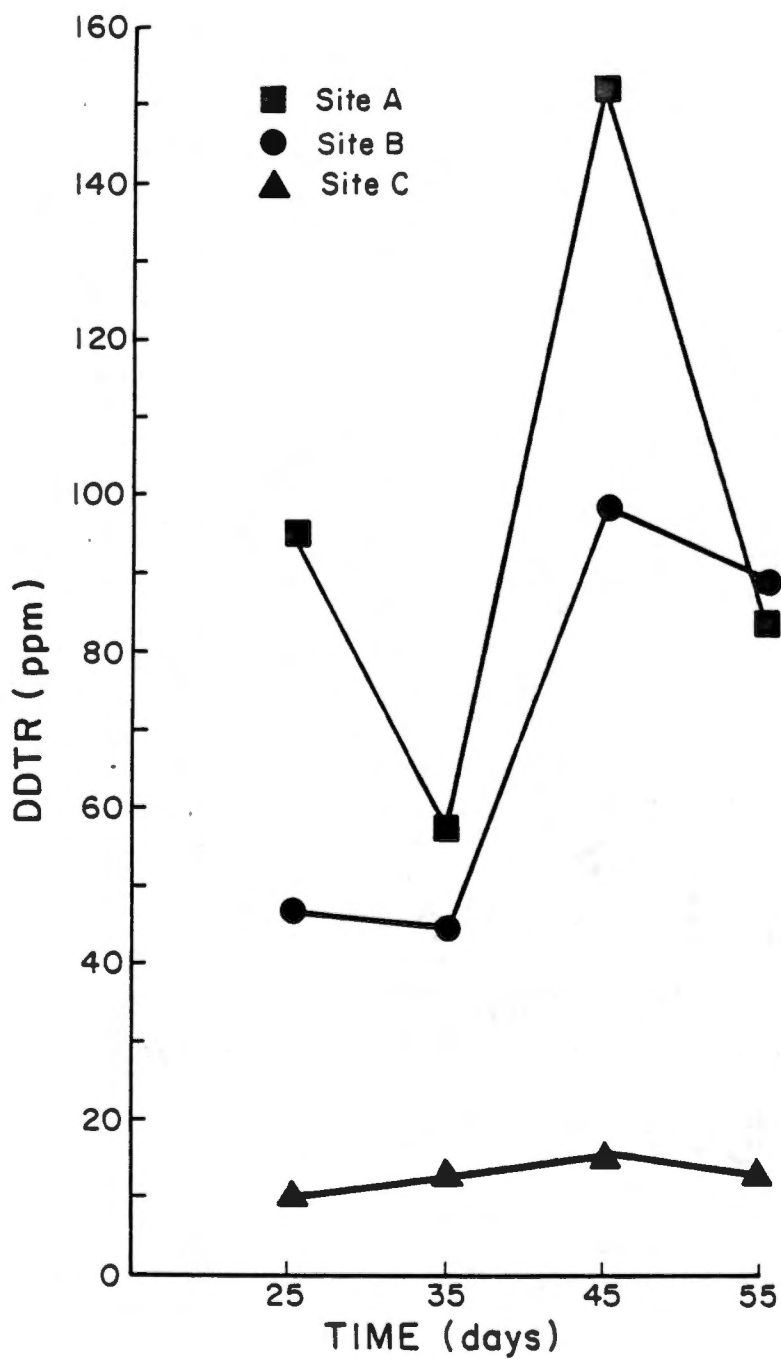


Figure 2. DDTR concentrations (ppm) for the gut contents of channel catfish (wet weight) maintained in cages in the Huntsville Spring Branch/Indian Creek complex. Time is graduated in days of the study (from 16 July to 9 September).

sites for either water or fish fillets. Comparisons of mean DDTR concentrations of gut contents at each site showed significant differences between Sites A and C, and Sites B and C, but no significant difference was found between Sites A and B ($p \leq 0.05$).

The experiment had been designed to limit the food source to only uncontaminated catfish ration since contaminated food has been shown to be a contributing factor in DDTR uptake in fish (Macek and Korn 1970, Jarvinen et al. 1977, Macek et al. 1979). However, it was not the only food source, since 67% of the gut contents was contaminated insects and must be considered as a possible source. Jarvinen et al. (1977) found dietary concentrations of DDTR additive at a ratio of water:food DDTR concentrations of $1:9.12 \times 10^4$, but they were not additive at a ratio of $1:2.28 \times 10^4$. In this study, water:food DDTR concentrations were at least $1:9.22 \times 10^3$ for Site A, $1:9.46 \times 10^3$ for Site B, and $1:3.6 \times 10^3$ for Site C. It appears from these data that the diet would not influence DDTR in fillets, but it should be emphasized that these ratios are taken from partially digested gut contents and do not represent the total dietary intake of DDTR by these fish.

However, if the only available DDTR in the water was in the dissolved state, then the water:food ratios would then be $1:9.22 \times 10^4$ for Site A, $1:6.65 \times 10^4$ for Site B, and $1:1.28 \times 10^4$ for Site C. Thus these ratios indicate that diet could be causing the differences in fillet DDTR concentrations. However, this is not substantiated by gut content analysis; there was no significant difference between DDTR

concentrations found in fish at Sites A and B. Further investigations on the total DDTR body burden of the insects are needed.

DDTR concentrations found in the fish fillets were not significantly different between contact and no-contact conditions. This indicated that direct contact with the contaminated sediments did not influence DDTR accumulation in channel catfish. Values for fish fillets at all sites ranged from 56.0 to 3.4 ppm after 55 days (Table 1). At 25 days all sites had mean concentrations greater than 5 ppm, the maximum allowed by the FDA. Sites B and C appeared to have reached equilibrium by day 25 (Figure 3), while Site A did not appear to reach equilibrium for the 55 days of the study. Statistical comparisons of the mean equilibrium concentrations for Sites B and C and the grand mean of sample days 25 - 55 for Site A showed significant differences in the DDTR concentrations in fish fillets among all sites ($p \leq 0.05$). Water did not appear to be the determining factor due to the lack of difference between dissolved DDTR concentrations of the water at the different sites. Therefore, it was probably the amount of DDTR in the diet which influenced the differences in total DDTR accumulated in the fillets among sites. This is substantiated by the BCF's calculated for DDTR mean concentrations in fillets:filtered water and fillets:gut contents (Table 1). The BCF's for fillets:filtered water reflect the differences among sites that would not occur if dissolved DDTR were the governing source of DDTR concentrations in the fish fillets. The BCF's for fillets:gut contents illustrate that even though Site B fish apparently consumed an equally contaminated diet as Site A fish, it was not concentrated in the fillets equally. This cannot be explained

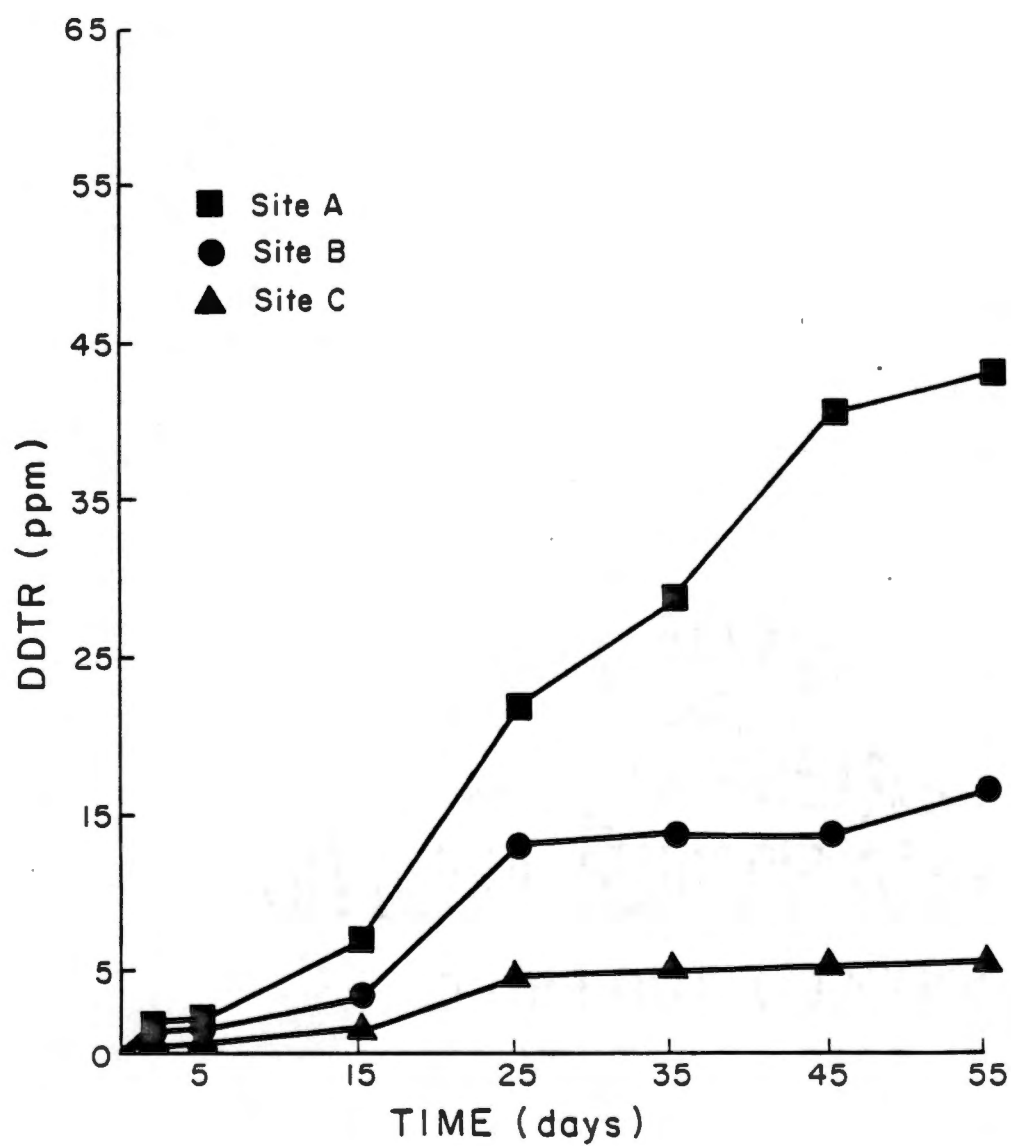


Figure 3. DDTR concentrations (ppm) for channel catfish fillets (*Ictalurus punctatus*), wet weight, maintained in cages in the Huntsville Spring Branch/Indian Creek complex. Time is graduated in days of the study (from 16 July to 9 September).

by this experiment. A study of concentrations of DDTR in whole, undigested insects of the diet is needed for conclusive determination of the major contributing factor of DDTR in the fish fillets of channel catfish in HSB/IC.

If total DDTR concentrations in the water are assessed, these may include amounts bound to humic acids, debris, and other particulates including aquatic organisms. Although mean levels at all three sites were relatively low (< 10.5 ppb), there was a general increase in fillet DDTR concentrations with increasing water column concentrations (Figure 4). Additional studies are needed to determine the availability of the DDTR bound to humic acids and other particulates.

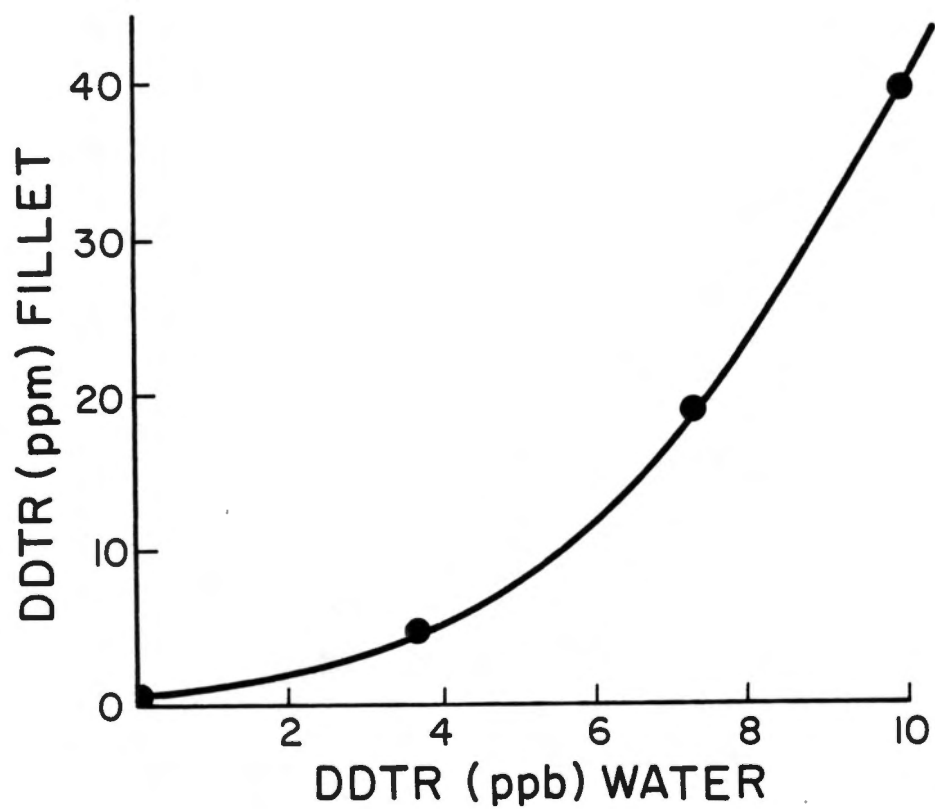


Figure 4. Relationship between mean DDTR concentrations (ppm) for the fish fillets of channel catfish (wet weight) held in cages in the Huntsville Spring Branch/Indian Creek complex and mean DDTR concentrations (ppb) for unfiltered water column samples.

CHAPTER VII

SUMMARY

1. There was no significant difference found in fillet DDTR concentrations of fish in contact with the sediment and those not in contact with the sediment. Thus, direct contact with the contaminated sediment was not necessary for DDTR uptake in the fish and was therefore not a direct factor in the high residue concentrations found in the fish in the HSB/IC system.
2. Differences in averaged fillet concentrations of DDTR were found among all sites. DDTR concentrations of fillets decreased from upstream locations at high sediment concentrations to a low at the downstream locations with relatively low sediment concentrations. These differences in fillet concentrations among sites indicated that the DDTR concentration in the sediment can be indirectly affecting total uptake by the fish.
3. Fish attained the FDA limit of 5.0 ppm DDTR in the fillet within 25 days exposure at all sites.
4. Analysis of the gut contents of sampled fish from days 25 - 55 showed high levels of DDT residues in the fish's diet. In spite of being supplied with a commercial catfish ration, 67% of the diet consisted of highly contaminated invertebrates (mainly Chironomidae). DDTR concentrations in the gut contents far exceeded residue levels found in the corresponding fish fillets, and must

be considered as a possible source of the residues found in the fillets.

5. Water column concentrations of total DDTR were relatively low in comparison with DDTR levels in the sediment, fillets, and gut contents. Highest levels were found at Site A, and generally decreased at downstream locations. There was a general increase in fillet DDTR concentrations with increasing water column concentrations.
6. While total DDTR in water may have been a major factor in uptake, the dissolved portion was not responsible for differences in mean concentrations of DDTR in fish fillets among the sites. Dissolved DDTR levels for filtered water from HSB/IC were approximately 1.0 ppb at all three study sites (M. Smithson, pers. comm.).

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