with the rise and fall in bacterial population. (Slides 6, 7, and 8.) These slides illustrate the three fractions as degradation takes place and reflect the downward trend of the nitrogen values.

An additional analysis, that for hydroxyproline, also reflects the destruction of connective tissue. As the collagenase enzyme of the marine bacteria tears apart the collagen of the connective tissue, hydroxyproline is released and appears in the three fractions mentioned above (Slides 9 and 10). These slides show a portion of the data and illustrate the increase in hydroxyproline. The destruction of the connective tissue results in soft shrimp that is especially unfit for canning.

pH has been used as an indicator of the quality of shrimp by many firms in the canning industry. Our pH values were carefully checked and rechecked, since they appeared to be higher than those commonly used. The starting pH at Day 1 was 7.0 for the pink and the brown shrimp, but it rose to 7.3 for the well-washed white shrimp. All three species of shrimp reached a pH of 8 before the taste panel noted an "off" flavor. (Slides 11, 12, and 13.) These slides illustrate the rise in pH as the shrimp are aged in ice.

At the beginning of this study, we decided that we would try to show the deterioration of shrimp tissues by means of histological preparations—we wanted to dramatize the effects produced by both intrinsic, or autolytic factors, and extrinsic, or bacterial factors. Although we cannot histologically demonstrate which of these factors caused the greater damage, the results clearly show the overall damage caused by a combination of these factors.

The methods used in this study were intended to show differences in the connective tissue and the relation of this tissue to the other tissues of the shrimp. The reasons for directing our methods primarily toward connective tissue were two-fold: (1) since this tissue serves as a supporting tissue, its loss will result in the loss of overall tissue integrity, which, in turn, results in a soft, or mushy shrimp; and (2) the overall composition of this tissue includes such specific elements as collagen and elastin, which are subject to attack by collagen-and elastin-specific enzymes from both land and marine microorganisms.

The following slides dramatize the degradative or spoilage processes that take place in the tissue of shrimp during prolonged ice storage:

Slide No. 14 — Hindgut of a 1-day shrimp
Slide No. 15 — Same area in a 14-day shrimp
Slide No. 16 — Outside of 1-day shrimp
Slide No. 17 — Outside of 7-day shrimp
Slide No. 18 — Outside of 14-day shrimp
Slide No. 19 — Post-mortem bacterial invasion
Slide No. 20 — Post-mortem bacterial invasion
Slide No. 21 — Post-mortem bacterial invasion

In conclusion, I hope that this brief discussion has brought into focus some of the problems that must be solved in the handling, processing, and storing of fish and shellfish if the consumer is to be offered a Grade A product.

SOME EFFECTS OF ENDRIN ON ESTUARINE FISHES

JACK I. LOWE
U. S. Bureau of Commercial Fisheries
Biological Laboratory
Gulf Breeze, Florida

ABSTRACT

Laboratory experiments were conducted to determine the acute and chronic effects of endrin to estuarine fishes. Short-term bioassays in flowing seawater determined 24-hour LC_{50}'s for spot (*Leiostomus xanthurus*), mullet (*Mugil cephalus*), menhaden (*Brevoortia patronus*), longnose killifish (*Fundulus similis*), and sheepshead minnows (*Cy-*

prinodon variegatus). A population of spot was exposed continuously for eight months to a sublethal concentration (0.05 ppb.). No pathology was found in the spot after seven months of exposure, but, a three-week exposure to a near-lethal concentration (approximately 0.075 ppb.) produced pathology characterized by systemic lesions involving the brain and spinal cord, liver, kidneys and stomach.

Residue analyses (gas chromatography) of spot exposed to 0.05 ppb. endrin for five months revealed an accumulation of 78 ppb. (micrograms/kilogram). No endrin could be detected in these fish after a 13-day holding period in uncontaminated water. Chronic exposure of spot to endrin did not affect their tolerance to sudden changes of salinity. Endrin-exposed fish were also able to endure extended periods of starvation.

Sublethal concentrations of endrin do not appear to affect the general physical condition of spot. The threshold of toxicity is extremely critical and the importance of time must not be underestimated in determination of a sublethal concentration of endrin for fish.

INTRODUCTION

The toxicity of endrin to freshwater fish has been demonstrated in many laboratory experiments and it is generally considered to have the highest acute toxicity of any of the commonly used insecticides (Henderson, Pickering, and Tarzwell, 1959; Katz and Chadwick, 1961; and Mount, 1962). Bridges (1961) reported on observations from a field study which suggested that the acute toxicity of endrin in the field situation is not as great as might be expected on the basis of laboratory studies. Endrin has recently been implicated in massive fish kills which have occurred annually on the lower Mississippi River since 1960 (U. S. Public Health Service, 1964—unpublished report).

The present study was undertaken to determine the acute and chronic effects of endrin on some estuarine fishes. Initial bioassays were conducted on spot (*Leiostomus xanthurus*), mullet (*Mugil cephalus*), menhaden (*Brevoortia patronus*), longnose killifish (*Fundulus similis*), and sheepshead minnows (*Cyprinodon variegatus*). Twenty-four-hour LC_{50}'s (concentration of endrin in flowing seawater which killed 50 percent of the fish) were determined for these species. The spot, a common sciaenid fish in both Atlantic and Gulf waters, was selected for use in long-term studies to evaluate the sublethal effects of endrin to an estuarine species.

MATERIALS AND METHODS

Endrin is the common name of one of the chlorinated hydrocarbon insecticides (1,2,3,4,10,10 - hexachloro-6,7-epoxy - 1,4,4a,5,6,7,8,8a-octahydro-1, 4-endo-5,8-dimethanaphthalene). The endrin used in these experiments was technical grade, 98% purity, obtained from the Shell Chemical Corporation. Endrin is classified as a general poison, insecticidally active both as a stomach and contact poison.

The experiments were conducted in a continuous-flow system in which test solutions were renewed continually. The laboratory saltwater supply is pumped directly and continuously from Santa Rosa Sound in northwest Florida. The fish were held in plastic acquaria with a capacity of approximately 25 liters. Seawater flowed through the aquaria at a constant rate of 154 liters per hour. Stock solutions of endrin in acetone were mixed in aspirator bottles and metered into the constant flow of seawater to obtain the desired concentration. This constant-flow system eliminated the need for aeration and assured a steady concentration of the toxicant.

Fish used in the experiments were seined from local waters and acclimated in the laboratory before use. Fish held longer than three or four days were fed a mixture of ground fish and oyster meat.

ACUTE TOXICITY OF ENDRIN TO FISH

Endrin has been tested on fish under such a variety of conditions that it is difficult to compare the results of various studies. Katz and Chadwick (1961) determined the toxicity of endrin to chinook and

coho salmon, rainbow trout, bluegill, mosquitofish, guppies, and the marine threespine stickleback. Coho salmon, the most sensitive, had a 96-hour TL_m (median tolerance limit—concentration killing 50 percent) of 0.27 ppb. (micrograms/liter) endrin; sticklebacks, the most tolerant, had a 96-hour TL_m of 1.65 ppb. These tests were conducted in standing water. In continuous-flow tests, Mount (1962) found that the 96-hour TL_m values for bluntnose minnows ranged from 0.27 ppb. for fish 30 mm. standard length to 0.47 ppb. for fish 60 mm. standard length.

By the continuous-flow procedure and bioassay techniques similar to those described by Doudoroff et al. (1951), 24-hour LC_{50}'s were determined for five marine species (Table 1). Median tolerance limits ranged from 0.23 ppb. for the killifish to 2.6 ppb. for mullet. All fish in these tests were juvenile. The temperature and salinity figures are averages for the 24-hour period.

Table 1. Acute toxicity of endrin to five species of marine fish.

Species	24-hour LC_{50} (ppb.)	Temperature (°C.)	Salinity (%)
Striped mullet	2.6	29	21
Spot	0.45	17	23
Menhaden	0.80	27	29
Sheepshead minnow	0.32	28	29
Longnose killifish	0.23	25	19

Short-term bioassays are of limited value in determining the degree of toxicity of organic insecticides to fish. They are useful in evaluating the relative toxicity of different compounds but they cannot be used to determine safe concentrations. Length of exposure is extremely important in determining a sublethal concentration of a toxicant. Data in Table 2 illustrate this point. Separate groups of spot were exposed to several critical concentrations of endrin for periods up to 19 days. After five days of exposure all fish were dead in concentrations of 0.1 ppb. and above. The next lower concentration of 0.075 ppb. killed no fish until the ninth day of exposure and 19 days of continuous exposure were required to kill 57% of the population. The 0.05-ppb. concentration proved to be sublethal.

Table 2. Percentage mortality of spot (*Leiostomus xanthurus*) exposed to a series of concentrations of endrin for periods ranging from 2 - 19 days.

Concentration of endrin (ppb.)	Days of exposure										
	2	3	4	5	6	7	8	9	10	11	19
0.75	100	—	—	—	—	—	—	—	—	—	—
0.56	100	—	—	—	—	—	—	—	—	—	—
0.32	100	—	—	—	—	—	—	—	—	—	—
0.18	0	100	—	—	—	—	—	—	—	—	—
0.10	0	30	90	100	—	—	—	—	—	—	—
0.075	0	0	0	0	0	0	0	7	7	14	57
0.050*	0	0	0	0	0	0	0	0	0	0	0
0.0 (control)	0	0	0	0	0	0	0	0	0	0	0

* This concentration proved to be sublethal in a continuous exposure of eight months.

CHRONIC EXPOSURE OF SPOT TO ENDRIN

Since the level of pesticide pollution in estuaries is likely to be of a low chronic nature, the effects on marine fish of long-term exposures to pesticides are more indicative of what might occur in the estuarine environment.

Two groups of 50 juvenile spot each were held in the laboratory from January until December 1964. One group (the other was a control) was exposed continuously for eight months (April to December) in

flowing seawater to a sublethal concentration of 0.05 ppb. endrin. This concentration was selected on the basis of results obtained from preliminary toxicity tests.

The experimental fish exhibited no symptoms of poisoning during the eight-month exposure. Mortality was approximately 15 percent in both control and experimental groups. Fish of both groups grew from a mean total length of 22 mm. in January to a mean standard length of 81 mm. in December. The mean weight of 25 fish from each group at the end of the experiment was 12 grams.

Since spot spawn offshore no observations could be made on reproduction, but gonads of both control and endrin-exposed fish were approaching sexual maturity; reproduction might have taken place had the fish been in their natural habitat. In studies on bluntnose minnows, Mount (1962) found that endrin in concentrations of 0.5 ppb. or less did not affect gonadal development in individuals which could tolerate such concentrations. He also reported that endrin concentrations as low as 0.5 ppb. appeared to prevent reproduction in guppies.

No attempt was made to control temperature and salinity of the seawater flowing through the aquaria. Average monthly water temperatures during the eight-month exposure varied from 20° to 29°C; maximum and minimum temperatures were 32° and 11°C. Average monthly salinities varied from 23 to 30o/oo; salinity extremes were 11 and 33o/oo.

Tests were made at the end of the experiment to determine whether or not chronic exposure to endrin had affected the resistance of the fish to the insecticide. Separate groups of fish from both experimental and control aquaria were exposed to previously determined lethal concentrations of endrin; the results of these tests are summarized in Table 3. Fish from the chronic exposure were less tolerant than the control fish to lethal concentrations of endrin during the first 24 hours of exposure. At the end of 72 hours of exposure, however, the two groups showed similar survival. Boyd and Ferguson (1964) reported on resistance and cross-resistance in populations of Mississippi Delta mosquitofish *(Gambusia affinis)* with a history of exposure to insecticides. They presented evidence favoring a genetic basis for this resistance through the selective action of insecticides. Ferguson et al. (1964) reported a 200-fold difference in 36-hour TL_m values with endrin tested on blue gills from resistant and non-resistant populations.

Table 3. Acute toxicity of endrin to treated and untreated spot (*Leiostomus xanthurus*).

Source of fish	Number of fish tested	Concentration of endrin (ppb.)	Number surviving 24 hours	48 hours	72 hours
Control fish from chronic-exposure experiment (not previously exposed)	5	0.75	5	0	0
	5	0.56	5	0	0
	5	0.32	5	4	2
	5	0.18	5	5	4
	5	0.0 (control)	5	5	5
Experimental fish from chronic-exposure experiment (previously exposed)	5	0.75	0	0	0
	5	0.56	2	0	0
	5	0.32	5	3	0
	5	0.18	5	5	3
	5	0.0 (control)	5	5	5

RESIDUE ANALYSIS

Fish that survived the eight-month exposure to 0.05 ppb. endrin were analyzed by thin-layer chromatography to determine the accumulation of endrin in the tissues. Whole-body analyses revealed a residue of 67.0 ppb. (micrograms/kilogram). A similar sample of the control fish contained no detectable endrin.

Fish samples from a second chronic-exposure test (5-month ex-

posure to 0.05 ppb.), conducted between February and July 1965, were analyzed by gas chromatography for endrin residues. A residue of 78.0 ppb. was detected by this method. No endrin could be detected in fish from this chronic exposure after 13 days in uncontaminated water. This rapid disappearance of endrin from the tissues is surprising, since we have found that fish retain DDT residues several weeks after removal from DDT-contaminated seawater.

PATHOLOGY

A complete histopathological examination of fish from the chronic experiment was made by a pathologist. Three groups of five fish each from the control, 0.05 ppb. endrin (seven-month exposure) and a "positive control" were sent to the E. M. Wood Diagnostic Laboratories, Tacoma, Washington. The "positive control" fish were the survivors (35% of the test population) of a three-week exposure to a critical concentration (approximately 0.075 ppb.) of endrin. These fish were preserved when they had reached a moribund condition. Dr. Wood requested these "positive controls" to identify target tissues and for use in evaluating sublethal concentrations.

No pathological symptoms were found in either the control or sublethal (0.05 ppb.) exposure groups; the two groups could not be separated by microscopic examination. The "positive control" fish were characterized by systemic lesions involving the brain and spinal cord, liver, kidneys, and stomach. Dr. Wood reported, "Degenerative and necrotic changes were present in scattered axon cell bodies of the spinal cord and in Purkinje cells of the cerebellum. The kidneys exhibited diffuse degeneration of the renal tubules without apparent change to the glomerular apparatus or to the adjacent hematopoietic tissue. The liver showed a uniform loss of all glycogen and lipid with focal lesions of necrosis and inflammation. The stomach showed an inhibition of mucous secretion by gastric mucosal lining without other degenerative or inflammatory changes."

In Dr. Wood's opinion the lesions of the central nervous system, kidneys, and stomach are a primary effect of endrin as are probably the necrotic liver lesions. The loss of hepatic fat and glycogen is probably secondary to the systemic toxicity. The complete absence of these hepatic changes in the experimental group in seven months is an indication that 0.05 ppb. endrin would never have a morphological effect on the liver of spot. He further stated that the described lesions may be somewhat nonspecific and that other pesticides may produce the same pathology.

PHYSIOLOGICAL STRESS

Fish that survived the five-month chronic exposure to endrin were subjected to periods of starvation and a rapid drop in salinity to determine any change in their ability to adapt to these physiological stresses. Eight fish from both the experimental and control aquaria were starved for 13 days with no apparent adverse effects. As previously stated, the endrin-exposed fish contained no endrin residues at the end of this starvation period.

Four fish from the experimental group and four from the control were taken from the high-salinity water (26o/oo) of the test aquaria and placed directly into tanks of standing water with a salinity of 13o/oo. When the fish showed no signs of distress, the salinity was gradually lowered to 8o/oo within 48 hours. The control and endrin-exposed fish showed no apparent difference in reaction.

CONCLUSIONS

The tests with endrin show this insecticide to be as toxic to marine and estuarine fish as it is to freshwater species. At least one species can tolerate prolonged exposure to sublethal concentrations of endrin in its environment. The threshold of toxicity is extremely critical; a very slight increase in the amount of endrin in seawater produces pathology and eventual death to spot (Table 2). The duration of

exposure is most important when determining a sublethal concentration of endrin to fish.

Endrin residues in the tissues of living fish do not appear to be detrimental during periods of starvation when fat is being mobilized. Further study is needed to evaluate more fully the effects of physiological stress on insecticide-exposed fish.

ACKNOWLEDGMENTS

The staff of the Gulf Breeze Biological Laboratory gave assistance and advice in the conduct of these studies.

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STUDIES OF COMMERCIAL SHRIMP POSTLARVAE IN MISSISSIPPI SOUND AND ADJACENT WATERS

INTRODUCTION

As a result of the discovery of new fishing grounds in the Gulf of Mexico, expansion of the shrimp fishery has been rapid since 1950. In 1952 the United States shrimp catch of 227.2 million pounds was valued at 55.1 million dollars, exceeding the dollar value of any other fishery (Anderson and Power, 1952). In the period 1950 through 1963 the catch from the Gulf of Mexico averaged 83 per cent of the total catch.

In the Gulf, brown shrimp *(Penaeus aztecus)*, white shrimp (*Penaeus fluviatilis*) and pink shrimp (*Penaeus duorarum*) contribute nearly all of the commercial catch. Before 1950 the catch was dominated by white shrimp. Since 1956 the Gulf shrimp catch has been reported by depth and area of capture, species, size, number of trips and days fished (Gulf Coast Shrimp Data) by the U. S. Bureau of Commercial Fisheries. These data show that about half of the Gulf catch since 1956 has been brown shrimp with the remainder about equally divided, on the average, between pink and white. The catch of white shrimp remains below the volume taken in the mid-thirties. Total catch was greatly reduced in 1957 and again in 1961.

Biological research on Gulf of Mexico shrimp stocks began just before the outbreak of World War I. Kutkuhn (1962) pointed out that the efforts of pioneer biologists, working with limited funds, established a firm life history base for the current expanded effort to bring