MONTANA STATE DEPARTMENT OF FISH AND GAME
FEDERAL AID IN FISH RESTORATION SECTION
HELENA, MONTANA

JOB COMPLETION REPORT
INVESTIGATIONS PROJECTS

State of Montana

Project No. F-21-R-2 Name Evaluation of DDT Spraying

Job No. III Title Bio-assays on the Toxicity of DDT to Fish and Fish-food Organisms

Period June 1, 1957 - April 30, 1958

Abstract:

During the summer of 1957, eighty three experiments under controlled cold water conditions were conducted at the Ovando Hatchery to determine the effects of DDT on cutthroat trout, rainbow trout, and mountain whitefish.

A partial statistical analysis of the data reflects conflicting results.

Chemical analysis to determine the amount of DDT present in fish tissue samples from the Ovando Hatchery were conducted by the Chemistry Department at Montana State College.

Objectives:

Extensive studies have been and are being made on trout streams by a cooperative study of the Montana Fish and Game Department and the U. S. Forestry Service. These extensive studies are being made to determine effects of forest spraying with DDT on fish and fish-food organisms. To better evaluate the extensive studies and the test stream study it is necessary to conduct laboratory investigation on the toxicity of DDT to fish and fish-food organisms under various conditions. It is the objective of this job to: (1) study the toxicity of various concentrations of DDT considering physical and chemical conditions, and time and type of exposure; (2) study the physiological effects on the fish.

Techniques Used:

During the summer of 1957, a standard procedure was adopted for conducting bio-assays at the Ovando Hatchery.

Hatchery reared rainbow trout and cutthroat trout were used in 66 experiments. These fish were obtained from the Anaconda and Arlee Fish Hatcheries. Wild rainbow trout, cutthroat trout and mountain whitefish were used in 18 experiments. These were collected by shocking from streams near Ovando and then transported to the Ovando holding ponds by a hatchery truck. Wild fish were not subjected to testing until a week after collecting to reduce any adverse effect from shocking.

The bio-assays were conducted in hatchery troughs containing 200 liters of water. To this water a known amount of DDT suspended in oil was added. Each experiment lasted 48 hours, during which time the same water was circulated at 17 gallons per minute by paddle pumps. The return pipe was run through a cold water bath to reduce temperature fluctuations in the experiment troughs. Plastic pipe was used in the pumping systems to facilitate cleaning between experiments. The number of fish used in each experiment were as follows: large fish (5 inches and larger) 10 fish per experiment; medium size fish (2 inches to 5 inches) 25 fish per experiment; small fish (2 inches and under) 50 fish per experiment. The troughs were covered with hardware cloth to prevent the fish from jumping out.

The experiments were conducted in blocks of six, one of these being a control. The control trough was alternated between the six troughs used. The usual practice was to use the trough which in the previous block had the highest concentration as the control trough in the following block. This was done to test the efficiency of cleaning between experiments.

The troughs and all equipment contaminated with DDT were thoroughly cleaned between each block of experiments. The toxic water was pumped into a sump pit to prevent contamination of a nearby stream. The troughs and equipment were flushed with cold water and then scrubbed with hot soapy water which was allowed to circulate through the pumping system for ten minutes. This was followed by a hot water flush for a few minutes and a cold water flush for another hour.

The procedure used in the experiments started with a 24 hour pre-experiment hold. Fish were taken from the holding pond and placed in the head troughs. This was to allow the fish to adjust to the new environment and to facilitate observations of the fish to be tested. Fish that were injured during the seining and handling could be detected and the fish removed. After the pre-experiment hold the fish were transfered to the experiment troughs, each containing 200 liters of circulating water. At this time the DDT was added, temperature and starting time recorded for each experiment. During the experiment the temperature in each trough was recorded hourly, and at eight hour intervals a Winkler oxygen analysis was conducted. The number of fish showing signs of distress were recorded hourly for each trough. When a fish died during the experiment the weight, length and time of death within a 15 minute range was recorded. The dead fish were put into labeled plastic bags and frozen.

At the conclusion of each experiment the temperature of the experiment trough was adjusted to the temperature of the head trough over a 30 minute period. The fish surviving the test were then transfered back to the head trough for a 24 hour post-experiment hold. Any mortality during the post-experiment hold was recorded similar to that during the experiment. At the end of this period the fish were tagged with opercle tags and released in the holding pond for further observations.

Findings:

A considerable amount of time was spent during the summer devising techniques, standardizing the procedure, and determining the limitations of the bio-assays.

Eighty three experiments under controlled conditions were conducted during the summer of 1957 to determine the effects of DDT on cutthroat trout,

rainbow trout, and mountain whitefish under cold-water conditions. See Table I.

A partial statistical analysis of the data reflects many conflicting results. In ten experiments using rainbow trout of equal length and subjected to the same concentration of DDT, the mortality in 48 hours ranged from 0 percent to 90 percent. It is evident that physiological and environmental factors play an important role in the experimentation.

Although there was considerable amount of conflicting data certain trends were noted during the experimentation:

(1) Hatchery reared rainbow trout under 1.5 inches in length showed a very high mortality at all concentrations. Out of eight experiments a 100 percent mortality was recorded in seven, while the remaining one had a 92.7 percent mortality at a concentration of 0.02 ppm DDT. The surviving fish were transfered to water that did not contain DDT but within six hours all remaining fish of this experiment were dead.

(2) Wild rainbow trout had a lower mortality rate than hatchery reared rainbow trout. Ten experiments were conducted using wild rainbow trout as test fish. No mortality was recorded for this group during the 48 hour testing period.

(3) Hatchery reared cutthroat trout showed a lower mortality rate than hatchery reared rainbow trout.

(4) There was no significant difference between wild cutthroat trout and wild rainbow trout.

(5) Whitefish had a higher mortality rate than either wild rainbow or cutthroat trout.

(6) In all species and sizes of both wild and hatchery reared fish tested there was a delayed mortality.

(7) When test fish which were exposed to DDT were subjected to a physiological stress, such as a 3° F. temperature change there was an immediate increase in the morality rate. This did not occur in the control fish subjected to the same stress.

The fish that were killed in the experiments were collected and frozen. Several of these fish samples were sent to the Montana State College Chemistry Department for DDT tissue analysis. The results from these analysis ranged from 4 ppm to 19 ppm. One sample of fish that survived the testing period (collected immediately after the experiment) had 23.7 ppm DDT in the tissue. Fish surviving two months after the experiment showed a concentration ranging from 5.5 ppm to 6.2 ppm DDT in the tissue. See Table II.

Recommendations:

That the bio-assay studies be continued under more rigorous controls to reduce the variation between experiments and in an attempt to clarify the conflicting results of the 1957 work.

As time allows, experiments should be conducted considering:

1. Size and age of test fish.

2. Various physical and chemical conditions of the water, (temperature, turbidity and alkalinity).

3. Feeding test fish with insects killed by DDT followed by a starvation period.

4. Physiological condition factor in relation to mortality.

5. Relation of aquatic vegetation to toxicity.

6. Delayed mortality.

7. Bio-assays on other species of particular importance to Montana.

Prepared by Norman Schoenthal Approved by George D. Holton
George D. Holton

Date April 19, 1958

TABLE I

EXPERIMENTS CONDUCTED AT THE OVANDO HATCHERY (1957)

Fish Tested	Concentration of DDT Suspended in Oil p.p.m.												
	T*	.125	.25	.5	1	2	3	4	5	6	7	8	9
Hatchery Rb Fry	1	1	1	1	2	1	1						
Hatchery Rb Fingerlings				4			2						
Hatchery Rb Large				2	2	2	15	2	5	3	1	1	1
Wild Rb Fingerlings				1									
Wild Rb Large			2	2	1	1	2						
Hatchery Ct. Large				1									
Hatchery Ct & Rb Large				1	2	2	5	2	1	3			
Whitefish				2	1	1	1	1	1	1			

* 0.02 p.p.m. DDT in water

TABLE II

ANALYSIS OF DDT IN FISH TISSUE
FROM BIO-ASSAYS CONDUCTED AT OVANDO

Sample #	Concentration DDT in Water* p.p.m.	Chemical Analysis DDT in Water p.p.m.	Species (#)	Status when Collected	p.p.m. Tissue
IA	5	0.33	Rainbow (7)	Dead, collected during the experiment	19.0
IB	5	0.33	Rainbow (3)	Live, collected immediately after the experiment.	23.7
40A	6	0.26	Rainbow (4)	Dead, collected during the experiment	13.7
40B	6	0.26	Cutthroat (2)	Dead, collected during the experiment	15.3
67	4	0.24	Whitefish (4)	Dead, collected during the experiment	4.0
67C	4	0.24	Whitefish (1)	Live, collected 69 days after experiment	6.2
71,72A	2&3	0.56 & 0.56	Cutthroat (5)	Dead, collected during the experiment	8.0
71,72B	2&3	0.56 & 0.56	Cutthroat (2)	Live, collected 66 days after experiment	5.9
83A	0.5		Rainbow (5)	Dead, collected during the experiment	10.3
84A	0.5		Rainbow (1)	Live, collected 63 days after experiment	5.5

* This was the calculated p.p.m. of DDT suspended in oil added to the water.