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MICHIGAN DEPARTMENT OF CONSERVATION
COOPERATING WITH THE
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LABORATORY TESTS ON THE TOLERATION OF FISHES TO DINITRO-O-CYCLOHEXYLPHENOL AND PHENOTHIAZINE, ORGANIC COMPOUNDS PROPOSED TO BE USED IN MIDGE FLY LARVAE CONTROL AT SEWAGE TREATMENT PLANTS.

by

George Washburn and John Funk[1]

During the warm summer months midge fly larvae have been a considerable nuisance in the sewage treatment plant at the Caro State Hospital. In order to eliminate this condition, the control of the larvae by chemicals has been suggested. Extensive research work on the control of the midge larvae has been done with the use of pyrethrum, but due to war restrictions this chemical is not available at the present time.

In exploration of possible substitutes, Mr. W. F. Shephard, Chief, Division of Sewage and Sewage Treatment, Bureau of Engineering, Michigan Department of Health, reports that two compounds, Dinitro-o-cyclohexylphenol and phenothiazine, furnished by the Dow Chemical Company have been tried out and appear to offer control possibilities. Of course, Mr. Shephard was interested in the toxic effect of these substances to fishes, as the treated sewage effluent would be released in public waters, (the Cass River). At the request of W. F. Shephard, the Institute For Fisheries Research has agreed to cooperate with the Michigan Department of Health and run a series of fish toleration tests with these chemicals.

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[1] Dr. Carl L. Hubbs, Curator of Fishes, Museum of Zoology, University of Michigan directed these tests.

Fish used in Toleration Tests.

Eight species of fish were used in these tests. With the exception of the goldfish, all of the forms are considered as warm water fishes, characteristic of an ordinary stream fauna found in southern Michigan. Listed below in systematic order are the species used.

Goldfish (Carassius auratus)
Northern creek chub (Semotilus atromaculatus atromaculatus)
Golden shiner (Notemigonus crysoleucas auratus)
Common shiner (Notropis cornutus frontalis)
Northern Yellow bullhead (Ameiurus natalis natalis)
Northern black bullhead (Ameiurus melas melas)
Pumpkinseed (Lepomis gibbosus)
Bluegill (Lepomis macrochirus macrochirus)
Black crappie (Pomoxis nigro-maculatus)

The goldfish, creek chub, golden shiner, pumpkinseed, bullheads and the black crappies were taken by seine from the Saline River, one mile south of the Village of Saline in Washtenaw County. The common shiners were collected from the Huron River, four miles west of Ann Arbor in Washtenaw County. The bluegills were furnished by the Wolf Lake State Fish Hatchery in Kalamazoo county. The size range of these eight species varied from two to four inches.

The fish used in these tests were placed in holding tanks in the Experimental Aquarium at the University Museum Building. The fish were held two or three days prior to the tests, to allow ample time for adjustment to the new surroundings. Daily mortality records were kept on the stock supply in order to assure good test material. If the loss became greater than five percent, the stock was abandoned and a new supply obtained. *

Methods Employed in Toleration Tests.

All of the toleration tests were performed in the Experimental Aquarium located in the University Museum of Zoology Building. The water used in holding the stock supply of test fish and for dilutions in preparation of

* Note. The common and golden shiners did not hold well in the stock tanks and new supplies had to be secured.

the various concentrations of Dinitro-o-cyclohexylphenol (which will be referred to hereafter as Dow D-N) was the softened, filtered circulating aquarium water which in many past tests has proven to be satisfactory for the maintenance of fish life. The chemical analysis of this water at the beginning of the test indicated a pH of 7.4, Dissolved Oxygen of 8.1 p.p.m. and a hardness (methyl orange alkalinity) of 46 p.p.m.

In contrast, different types of water were used during the toleration tests with phenothiazine. The stock supply of fish was held in water secured from the stream in which the fish were obtained, thus eliminating a possible shock before actual testing. In addition, two types of water were used in preparation of the various test concentrations; one the soft aquarium water and the other a relative hard water (pH 8.1 and methyl orange alkalinity of 270.) secured from Flemming Creek (a typical stream containing a moderate fish population representing 15 to 20 species) in Washtenaw County, four miles east of Ann Arbor.

The variation from the standard procedure which was used during the toleration runs with the Dow D-N was brought about by a recent article published in the April, 1944 issue of Ecology by L. F. Miller.[2] His studies on the toxicity of various types of soft waters to fishes, indicated that most filtered waters were toxic, the degree depending upon the type of softener employed. Now, as we have been using soft water in all of the past tests, even though the control fish survived the test period, it seemed desirable to check this point by using a typical stream water. In this way, at least theoretically, comparable data of contrasting waters will be available.

All of the toleration tests were run at room temperatures (72° to 76° F.). The test equipment consisted of wide-mouth two-quart glass jars, each equipped

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[2] Miller, L. F. 1944. An Experimental Study Of The Effect Of Softened Waters On Fish. Ecology, Vol. 25:2, pp. 249-253.

with an air line disperser and a lid. The fish were tested in a 1500 ml. medium, well aerated before introduction and continually aerated during the test run or until the fish died. Two fish were used in each test jar which represented one specific concentration of the substance to be tested. The time was recorded when the fish were first introduced and again recorded at death or the termination of the 96-hour test run. This time limit has been used in many past toleration experiments and in our experience has seemed satisfactory for general routine laboratory toxicity tests. Periodic examinations of the test runs were conducted during the day, recording general observations on the fishes' behavior. No checks were made at night, (6:00 P.M. to 8:00 A.M.) and if the fish died during this period, the average survival time was computed as a mean between the two extremes.

If the fish survived the 96-hour test period, the run was terminated and that particular concentration was considered innocuous under laboratory conditions. In order to arrive at the consistent tolerance limit (the highest concentrations of the test substance at which all of the fish can be expected to survive the 96-hour period) various concentrations were prepared and tested, supplementing new concentrations between the two extremes when it seemed necessary. If at any time death of the fish in any of the test jars was not consistent, new trials were run and all of the data recorded are presented in this report.

Results of Toleration Tests on Dow D-N

The first substance to be tested was the Dow D-N, a yellow crystallin chemical readily soluable in the dilution water. A ten liter stock solution, containing 100 p.p.m. of Dow D-N, was prepared and used as a basis for all of the tests. The toxicity of this substance was tested on six species of fish namely: goldfish, creek chub, golden shiner, common shiner, pumpkinseed and the black crappie.

Dow D-N proved to be highly toxic, producing death to all species tested at very low concentrations (see Table I) in a relatively short time. The most tolerant species was the goldfish which survived a 96-hour test in 0.4 p.p.m. Lethal effects were produced within a few minutes of exposure at higher concentrations. Due to the high toxicity encountered, no consistent toleration limit for any species was attained. If more precise tests were conducted, one might expect that the toleration limit would fall between 0.1 p.p.m. and 0.5 p.p.m. for all six species. Observations, conducted on exposed fish in the test jars, revealed a severe constriction of the body muscles after death. No undue agitation in reaction to the chemical was exhibited by the fish when first placed in the test jars.

Table I

Survival Time of Six Species of Fish In Different Concentrations of Dow D-N

Concentrations in p.p.m. of Dow D-N	Survival time for two of each species											
	Goldfish		Creek chub		Golden shiner		Common shiner		Pumpkinseed		Black crappie	
	Hrs.	Min.	Hrs.	Min.	Hrs.	Min.	Hrs.	Min.	Hrs.	Min.	Hrs.	Min.
10								4½ 4½				
9								5 5				
8								5 5				
7								5 5				
6								5 5				
5								5 6				
4								6 6				
3								7½ 10				
2		24 30						11 14				
1.8												
1.6		31 36										
1.4												
1.2		29 46								37 39	1 1	20 05
1.0						26 33		35 41				
0.9				31 52				35 52	1	46 20	1	57 00
0.8	1 1	23 53				20 34		35 55				
0.7							1	35 06				
0.6			1	34 02		43 55	1 1	01 06		50 58		131 155
0.5							1 1	08 08				
0.4	96+ 96+				1 2	40 10	2 2	05 25				
0.3			1	53 18			2 73	05 20	2 2	04 52	2 3	45 10
0.2					2 4	35 20	21 24	50				
0.1							96+ 96+					
Control	96+ 96+		96+ 96+		96+ 96+		96+ 96+				96+ 96+	

Results of Toleration Tests on Phenothiazine

Toleration tests with both the Dow D-N and phenothiazine were planned to be run simultaneously, but due to complications in preparing the latter substance for testing, the Dow D-N was completed before tests were made with the phenothiazine. Phenothiazine is a whitish crystalline chemical insoluable in water, hence the difficulty encountered in preparation of the various test concentrations. Correspondence with Mr. W. W. Allen, Assistant Manager of the Technical Service and Development Division of the Dow Chemical Company, indicated that phenothiazine was soluble in various organic compounds. It appeared from his data sheet that acetone as a solvent warranted a trial, as the solubility in this liquid was 27% by weight. This, however, proved to be of little value, as initial preparations were unsuccessful. As soon as the dissolved phenothiazine and acetone were mixed with water, the former substance again assumed its crystalline form. In order to prevent this reaction, a third substance would have to be introduced to act as an emulsifying agent which would further complicate the test procedure. The idea of testing phenothiazine in solution was then abandoned and tests were undertaken using this substance as a suspensoid. (This is the method to be employed in the control of midge larvae.) Ten liters of stock solution were prepared by mixing one gram in 10,000 grams of water. In order to assure as even a distribution of size particles in the solution, the phenothiazine was wetted and ground in a mortar before mixing with the full 10 liters of dilution water. When various concentrations were prepared, the total volume of the stock solution was highly agitated to bring about an even distribution of the phenothiazine crystals as possible.

Four species of fish (bluegills, golden shiners, bullheads, and goldfish) were used in the tests on phenothiazine. Parallel tests were run with the bluegills and golden shiners using the soft aquarium water and the hard water from Flemming's Creek. Table II shows the results of the tests.

Table II

Phenothiazine

Concentrations in p.p.m.	Survival time in hours for two of each species					
	Bluegills		Golden shiners		Bullheads	Goldfish
	Aquarium	Creek	Aquarium	Creek	Creek	Creek
10	...	8	12	...	...	...
	...	8	12	...	...	...
8	10	8	12	24	74	96+
	10	8	12	30	96+	96+
6	10	30	12	30	93-	96+
	12	30	12	30	96+	96+
5	10	8	...	30	96+	96+
	12	8	...	30	96+	96+
4	10	96+	12	21	90-	78-
	30	96+	12	30	96+	96+
3	30	96+	...	18	90-	96+
	30	96+	...	24	96+	96+
2	42	96+	30	21	52-	96+
	54	96+	30	30	52	96+
1	42	96+	96+	30	74	96+
	66	96+	96+	30	90	96+
Control	96+	96+	96+	18	66	96+
	96+	96+	96+	24	66	...

No very definite conclusions can be drawn from such results. The goldfish, known to be a quite tolerant fish, survived even the high concentrations. The bullheads, also quite tolerant, survived in some of the higher concentrations but died in some of the lower. The fact that the control fish died early in the experiment suggests that the mortality was due to some cause other than the test substance.

The golden shiners were tested first in aquarium water. There showed quite consistent results, the controls and those in 1 p.p.m. surviving the entire 96 hours, those in 2 p.p.m. surviving about 30 hours, and all those in higher concentrations dying in 12 hours or less. Such results were not unexpected, for golden shiners are known to be quite delicate and easily injured by rough handling or an unfavorable environment. Later when fish from the same stock were tested in creek water, high but quite inconsistent mortality resulted. Since the control fish were among the first to die it seems probable that the fish had been weakened

due to being held in the aquarium tanks and that this was the chief cause of mortality.

Bluegills were selected as test fish because they are not especially resistant like the goldfish or bullheads nor yet particularly delicate like the golden shiners. The first tests with them were made in creek water and it seemed they could withstand concentrations of 4 p.p.m. but were susceptible to larger amounts. The fish affected by the test substance became sluggish, the respiration rate was slow and irregular, and in the later stages they showed loss of equilibrium, first swimming in a tilted position and finally lying on their sides on the bottom of the test jar. Some lived in this final condition for 24 hours or more before finally dying. A few which were visibly affected did not go beyond the first stages and later recovered. When fish from the same stock were later tested in soft aquarium water, all but the control fish died, although the survival time was longer in the lower concentrations. It is possible that the fish had been weakened by having been held under unnatural conditions and that this was at least a partial cause of mortality.

The results of these tests are too variable for any definite toleration limits to be set up. A number of reasons may be given to explain the variable results. In the first place, since phenothiazine is insoluble in water and was used as a suspensoid, there can be no certainty that the actual concentrations obtained were those desired although great care was taken by thorough agitation to make this error as small as possible. Second, the effect of such substance may vary with the chemical content of the water in which they are used although the attempt made to test this by using waters from two different sources showed no consistent results. To avoid this possibility of error it would be necessary to conduct a series

of tests using the water in which the test substance is to be employed. Finally, it is difficult to maintain a stock of test fish over a period of time without the possibility of their becoming weakened and therefore more readily susceptible to possibly toxic substances. This is especially true of some of our native species which do not take to life under unnatural conditions.

It is suggested, therefore, that a maximum of 4 p.p.m. of phenothiazine is the concentration expected to be used in the midge fly control work, and since at least some fish under some conditions survived in 4 p.p.m. or more of the test substance, and since the effluent containing the 4 p.p.m. of phenothiazine will be diluted immediately upon entering the river into which the sewage plant drains, a trial run using not to exceed 4 p.p.m. be made. The run should be followed closely by Conservation Department personnel, and any effect on the fauna and flora of the river below the sewage plant outlet noted and reported.

INSTITUTE FOR FISHERIES RESEARCH

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