For Administrative Use Only

June 6, 1962

Progress Report on Bioassay

Montana Fish and Game Department & Montana State Board of Health

During the past four months toxicity tests have been conducted in the bioassay laboratory at Bozeman, Montana. The laboratory has been financed jointly by the Montana Fish and Game Department and Montana State College. The primary purpose of the work thus far has been to evaluate the toxicity of impounded wastes from the Waldorf-Hoerner pulp and paper mill at Missoula to rainbow trout. This information is necessary in order to recommend concentrations for discharge which will exert a minimum effect on the fish life of the Clark Fork River.

The test organisms used have been primarily fingerling rainbow trout. Guppies were also used in tests with a herbicide. The U. S. Fish and Wildlife Hatchery at Bozeman furnished the trout used in the tests through May 16, 1962. The cooperation by this hatchery in supplying the test organisms has saved the state considerable expense, since there are no state hatcheries reasonably close to Bozeman. Fish were obtained from the State Fisheries Station at Arlee for the last series of tests on May 24, 1962. By this time, fish at the Bozeman Hatchery had become too large for use in static assays.

The bioassay methods used follow those described by Doudoroff et.al. (1951) and Henderson and Tarzwell (1957). The tests run to date have been static, that is, there is no water exchange. The temperature of the test solutions was controlled at 56°F ($\pm$ 1°) by means of a constant temperature bath. Dechlorinated Bozeman City water and Clark Fork River water (taken from the river immediately above pulp andpaper waste discharges) were used for the tests. Dissolved oxygen analyses were run on the strongest solution in each series tested at 24 hour intervals throughout the experiments. COD (chemical oxygen demand), BOD (biochemical oxygen demand) and total dissolved solids (conductimetric) analyses were conducted on waste samples following collection. Methods outlined by the American Public Health Association et. al. (1960) were used in the chemical analyses.

An electrically controlled metering pump system is being built by the Instrumentation Laboratory at Montana State College to serve a continuous flow bioassay system. This pumping system will meter toxicant into a chamber where it will be mixed with dilution water coming in by gravity flow from a constant-head tank. From the mixing chamber, the toxic solution will run into the container with the test organisms by gravity flow. This will allow tests of at least 30 days to be conducted. Exposure of the test organisms for at least this length of time will be necessary in order to determine a reasonably safe concentration based upon chronic toxicity.

It is apparent from results obtained on pulp mill waste (Table 1) that considerable variation exists within a waste storage pond probably due to physical and chemical stratification. Samples from pond 7 were collected from the surface and from 2 feet below the surface. Considerably different toxicity results were obtained by this manner of sampling. In order to get a more accurate picture of the toxicity of each pond it seems best to sample the effluent when the pond is being emptied.

Table 1. The interpolated 24, 48, 72 and 96 hour median tolerance limits of rainbow trout fingerlings to pulp mill waste in percent concentration and some analyses of the characteristics of the waste samples.

Sample	Date	Dilution Water	ppm COD	ppm BOD	ppm TDS (Cond.)	TLm 24	48	72	96
Composite[1]	3-23	Lab.	253	210	-	-	9.7	7.2	6.5
Pond 7 (Sample #1)[2]	4-18	C.F.	525	175	513	13.0	8.0	7.0	7.0
" (Sample #2)[2]	4-19	"	"	"	"	-	-	10.0	10.0
Pond 9(effluent)	4-18	"	650	220	684	7.8	4.2	4.2	4.2
" (duplicate)	4-19	"	"	"	"	7.2	5.6	4.7	4.2
Pond 3 (effluent seepage)	5-16	Lab.[3]	522		735	6.3	4.7[4]	3.2[4]	3.2[4]
" "	5-16	C.F.[3]	"		"	5.6	3.25[4]	-	-
Pond 6 (effluent)	5-16	Lab.	735		359	3.2[4]	1.8[4]	1.8[4]	1.8[4]
" "	5-16	C.F.	"		"	3.2[4]	<1.0[4]	<1.0[4]	<1.0[4]
Pond 4 (surface)	5-24	Lab.	454	147	855	10	7.5	7.5	7.5
" "	5-24	C.F.	"	"	"	>10[4]	>10[4]	>10[4]	>10[4]
Pond 10 (surface)	5-24	Lab.	369	142	462	2.6	2.6	2.6	2.6
" "	5-24	C.F.	"	"	"	4.25	4.25	4.1	4.1

[1] Composite of all ponds

[2] Samples taken from surface to two feet deep.

[3] 62 percent mortality in control

[4] DO too low for test to be reliable (usually below 5.0 ppm)

C.F. - Clark Fork River

The reaction of the test organisms to pulp mill waste was usually slow. They would first become listless and would not respond to an external stimulus, i.e. rapping on the test containers. As long as 24 hours later they would lose their equilibrium and death would follow. This type of reaction was common in all tests except with the pond 6 and 10 wastes. In these tests (stronger of the series) the fish were in distress immediately after introduction into the test solutions (Table 2).

Table 2. The rates of mortality observed in the indicated concentrations of pond 6 and 10 pulp and paper mill wastes.

Dilution water	pond	% concentration	% mortality	Time elapsed
Laboratory	6	10	100	5 min.
Clark Fork	"	"	90	30 min.
Laboratory	"	5.6	90	30 min.
Clark Fork	"	"	25	30 min.
Laboratory	10	10	100	10 min.
Clark Fork	"	"	"	15 min.
Laboratory	"	5.6	100	10 min.
Clark Fork	"	"	"	30 min.
Laboratory	"	3.2	100	1 hour
Clark Fork	"	"	10	96 hours

Mortality in the tests with pond 6 and 10 wastes was possibly caused by a volatile material, since observed effects on the test organisms were slight after the first hour. Dissolved oxygen was 7.0 -8.0 ppm in the test solutions during the initial mortality. Since initial mortality was the most severe and the DO was adequate during the period, the 24 hour TLm value of 3.2 percent for pond 6 is considered valid (Table 1).

Some difference was found between the toxicity of the wastes in laboratory dilution water and Clark Fork River dilution water. The test organisms exhibited a lower tolerance in the laboratory dilution water. Comparison tests were made using the pond 9 effluent six days following the tests enumerated in Table 1. In this instance a TLm^{96} (96 hour median tolerance limit, i.e. 50% survival of the test animals) of 7.0 percent was obtained with laboratory dilution water while the TLm was above 10 percent (90 percent survival in the 10 percent solution) in the Clark Fork dilution water. The higher TLm values than those shown in Table 1, with respect to pond 9 effluent, are undoubtedly due to a loss in strength of the waste during the six day delay in starting the second series of tests. The waste samples were held at 46°F in the laboratory during this period.

The tests with pond 4 waste (Table 1) and pond 6 and 10 wastes (Table 2) definitely show a lower tolerance of the test organisms in laboratory dilution water compared to Clark Fork River dilution water. Dissolved oxygen, in the pond 4 tests with Clark Fork water, was at levels too low for the results to be reliable. In spite of this, a higher tolerance of the test organisms was shown in the Clark Fork dilution water.

Although alkalinity and hardness (indication of buffering capacity) was greater in the laboratory dilution water, tolerance of the test organisms was consistently lower in solutions with that water. Some of the buffering capacity of this water is probably impaired since it is passed through a carbon filter in dechlorination.

On February 8, 1962 the laboratory water had a bicarbonate alkalinity of 122 ppm and a hardness of 200 ppm. The Clark Fork immediately above the pulp mill had an alkalinity of 90 ppm and a hardness of 156 ppm in April of 1962, prior to high water. Alkalinity has ranged from 88 to 90 ppm and from 62 to 119 ppm in test solutions with the laboratory and Clark Fork dilution waters respectively. The range in hardness has been from 90 to 124 in laboratory and from 66 to 106 in Clark Fork dilution waters. None of the variations in toxicity could be attributed to differences in alkalinity or hardness.

Difficulties with low dissolved oxygen in tests with pond 3 and 6 wastes (Table 1) were probably a result of the test organisms being too large. The size fish used exceeded the 2 grams/liter limit recommended by Doudoroff, et.al. (1951).

Waste analyses such as COD, BOD and total dissolved solids (conductimetric) have been run on the samples taken for bioassay with the purpose of investigating a relative index of toxicity. To date there does not seem to be any close relationship. More tests should be conducted before an attempt is made to estimate toxicity from COD, BOD or conductimetric TDS.

Tests were also run to determine the tolerance of rainbow trout and guppies to xylene in laboratory dilution water. The 48-hour TLm with trout was 14 ppm and the 96 hour TLm with guppies was 32 ppm. This material is used rather extensively in irrigation canals to control emergent

vegetation and is used at levels considerably above what these tests indicate are tolerable to fish life. The material was found to be quite volatile and toxicity decreased with time, even in static solutions. However, to prevent fish kills a dilution factor by the receiving stream of at least 10Ø should be available. That is, the concentration reaching the stream should not exceed .1.4 ppm (TLm^{48} of 14 ppm ± 10Ø). Precautionary measures must be stressed to people using this material.

Eugene B. Welch, Biologist
Pollution Control

References Cited

American Public Health Association
1961. Standard Methods for the Examination of Water and Waste Water. 11th Ed.. Am. Pub. Health Assn., New York.

Doudoroff, P. et. al.
1951. Bio-assay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage and Industrial Wastes. 23:1380-1397.

Henderson, C. and C. M. Tarzwell.
1957. Bio-assays for control of industrial effluents. Sewage and Industrial Wastes. 29:1002-1017.