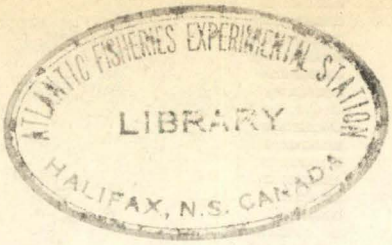

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Author

R. H. M'Gonigle.

REPORT ON TESTS FOR TOXICITY OF VARIOUS MATERIALS SUBMITTED TO THE BIOLOGICAL BOARD OF CANADA, JULY 1936.

In accordance with plans arranged, the various solutions for testing for toxicity were conveyed to the Saint John Hatchery, where with the cooperation of the Superintendent and his staff, experiments were carried out using Speckled or Brook Trout fry (S. fontinalis) of the year's hatch, as the test animals.

These fish are quite susceptible to poisons, and were hence exceptionally valuable for this purpose, as solutions non-toxic to this species may be considered as non-toxic to other species, and for older ages of this same species.

The experiments were carried out in wooden tubs to prevent any action between the solution, and a metallic tub, which might have given rise to toxic solutions had such tubs been used.

The acidity value (pH) was determined in all cases possible. It was not convenient to determine oxygen values, but attempts to provide saturation values were made by vigorous, repeated shakings of the materials. In cases where dilutions were made, the diluting water used was that in the hatchery troughs. The temperature range of the experiments was 16°C. (60.8°F.) at the commencement, and 18.5°C (65°F.) at the conclusion.

The method adopted in testing these solutions was to make a test dilution namely one part of the test solution to seven parts of water (1:8). If this was without effect in an hour, another part of the solution was added, and so on, preparing successively dilutions of increasing strength as follows: beginning with 1:8, then 2:9 (1:4.5), then 3:10 (1:3.3), and finally, if none of these was effective, employing the undiluted, original solution. In

addition, another method of testing was used, in which the test specimens were placed in the undiluted solution first, and if this were found toxic, then dilutions were prepared by adding successively 8 parts of water to the original one part of toxic solution. This latter method was only used for one solution, as will be evident below. In all cases controls were carried out in similar containers, with pure hatchery water, for identical periods.

It is to be pointed out that an hour's exposure is no real test of toxicity. 24 to 48 hours' exposure is really required if the effect of dilute pollutions is to be determined. This tests the effect of the poisons on the gills, skin etc. If the poisons do not act on these organs, but act after swallowing several days (up to 10) are needed to test for such action.

The results secured in testing the various solutions in the above manner were that only one of these was dangerously toxic; another one gave evidence of toxicity in the hour; and another was found to be toxic in full strength due to its great alkalinity, while all others were apparently non-toxic, so far as our method went.

The solutions taken to the Saint John hatchery for testing were from two sources, namely, the Montague Gold Mines, Dartmouth, N. S., and from the Bathurst Pulp and Paper Mill, Bathurst, N. B.

The solutions tested were as follows, and in referring to them in the text below, the number placed beside the description will be used, for brevity.

1. Stream water, from near the Montague Gold Mines.
2. Tailings (effluent, much solid material), Montague Gold Mines.

3. Sulphite effluent from the Bathurst Pulp Mills.
4. Wood Pulp (ground wood sewer), Bathurst Pulp Mills.
5. Suspension of "Green Sludge" (1:64), Bathurst Pulp Mills.
6. Effluent of Main sewer, Bathurst Pulp Mills.

Of these, the first three were without any effect on brook trout fingerlings after an hour in the undiluted solutions.

Number 4 proved to have an effect, as in about thirty-five minutes the test specimen turned over, and appeared to be dead in fifty minutes. It was transferred to pure water, where it recovered, though it remained abnormal for the rest of the afternoon. The control specimen remained apparently normal. It is possible that this effect might have been produced by lack of oxygen, since the finely-ground wood pulp has a rather high oxygen demand. The solution had been thoroughly agitated and shaken up, to produce as efficient an aeration as possible before the fish was put in, but the time involved would permit a sufficient reduction of the oxygen, due to the combined oxygen demand of both fish and residue. A further factor may also enter, a mechanical one, in that the fish kept actively swimming, stirring up the fine pulp, so that the gills of the fish may have become thoroughly clogged with this material, so that respiration was impaired. There was not sufficient material to check upon which of these (or other) factors might have been responsible. This material was harmless in dilutions of 1:3.3.

A solid sludge (number 5) had been supplied. A suspension of this substance was prepared by shaking up one ounce of the material in sixty-four ounces of water, then adding test specimens to the suspension. The fish survived an hour in this suspension

without apparent ill-effect, but upon testing the acidity (pH) the next day, when the supernatant liquid was clear enough to permit a colourimetric determination, a pH value of over 11 was discovered. Previous experience in this laboratory with fish exposed to such high alkalinities have shown that exposure to them is tolerated for two to three days, but after that time all fish die. The fish were held in the suspension, for one and a quarter hours. Dilutions were then prepared of this suspension, using ordinary tap water, to find what dilutions might yield a safe acidity concentration. It was found that the acidity value (pH) of a dilution of 1:10 was pH 8.5 -- which is a safe value. This dilution applies to the cleared supernatant liquid, but if much suspended solid were present, still greater dilutions would be needed, to render such water safe.

The last solution, number 6, was the most toxic. Upon placing fish in the undiluted solution, they were found to die at once. Dilutions were then prepared, with the following results:

1:9,	in	which	the	fish	died	in	10	minutes
1:17	"	"	"	"	"	"	20	"
1:25	"	"	"	"	"	"	38	"
1:33	"	"	"	"	"	"	100	"

As it was 5:30 p.m. when this last dilution had been tested, it was decided to leave the material, and ask Mr. Nichol, the Superintendent to make a dilution of 1:41 the next morning, and add fish to it, noting survival time, and then reporting to us. This Mr. Nichol did, and reported survival time of over 18½ hours, without any sign of ill-effect (in fact, taking food), when the test was discontinued.

In this last test, the observation that dilution by eight parts about doubled the survival time, particularly in the

stronger solutions, suggested the existance of a mathematical relationship between the toxicity and survival times, a fact observed by Powers and Carpenter previously. Working out this relationship gave the mathematical equation

$$Y = 25.5 \log x - 16.5$$

In this equation, Y is the dilution, in parts of water, added to one of the effluent, and x is the survival time in minutes. Since a twenty-four survival may be accepted as proof of non-toxicity, this equation can be used to indicate the necessary dilution to ensure survival for twenty-four hours. Solving the equation gives a dilution of 1:64.

The result secured by Mr. Nichol, of probably unlimited survival in 1:41 can be explained in part, by the fact that the diluted material (1:33) had stood overnight and hence the toxins may have been partly oxidized, so the solution was actually weaker than anticipated. Furthermore, it is not to be expected that a close mathematical relationship would persist, especially in great dilutions, since fish normally live in watery solutions - that is, not in distilled (pure) water, which is in fact in itself toxic.

However, in actual practice, there is not a great difference in the concentration of 1:41 and the theoretical value of 1:64. In fact, it is probable that the fresh, raw effluent should be diluted by the theoretical amount, that is by the value derived from the equation.

It will be evident from the above, that if the effluent of the Bathurst Pulp and Paper Mills be so diluted that the effluent from the Main Sewer is rendered non-toxic, then all of the other

effluents will also be safe, of those submitted for testing, and reported upon herein.

The materials from the Montague Gold Mines proved to be non-toxic under the conditions of testing herein reported.

The following table will give in a clear, concise form, the results obtained in the above tests:

Solution	Character	Acidity pH	Strength used	Remarks
No. 1	Clear	6.8	Full strength	No effect in one hour.
2	Much solid material	6.5	Full strength	No effect in one hour.
3	Clear	7.3	Full strength	No effect in one hour.
4	Much suspended pulp	6.9	1:8	No effect in one hour.
			1:4.5	ditto
			1:3.3	ditto
			Full strength	Turned over in 35 min., removed in 50 min.,with survival.
5	Suspension of "Green Sludge"	11.0+	Full strength	No effect in one hour.
6	Main Sewer effluent	?	Full strength	Immediate death.
			1:9	10 minutes survival.
			1:17	20 ditto
			1:25	38 ditto
			1:33	100 ditto
			1:41	18½ hrs. without signs of distress.

R. H. M'Gonigle.
July 27, 1936.