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A FINAL REPORT ON THE TOXICITY THRESHOLD EXPERIMENTS INDICATING THE EFFECTS ON FISH LIFE OF THE PRODUCTS DERIVED FROM THE POLYSULFIDE TREATMENT OF CYANIDE WASTES

by

Carl L. Hubbs and George N. Washburn

Chemists of the E. I. du Pont de Nemours Company of Niagara Falls, New York have developed a process of denaturizing the wastes from cyanide plants. This process consists of treating the various cyanide wastes with calcium polysulfide (CaS_n). The polysulfide reacts with the CN producing thiocyanide. An excess of the polysulfide is said to be required for the completion of the reaction.

Mr. Milton P. Adams, Director of the Stream Control Commission, and Dr. B. H. Vollertsen, research chemist of the du Pont de Nemours Company, requested in a conference that the Institute for Fisheries Research run some toxicity-threshold experiments on the products derived from this method of treatment of cyanide wastes.

On June 8, 1942, the Institute received from the Niagara Falls Plant of the du Pont de Nemours Company a supply of the salts and treated wastes needed for such tests. Accompanying the shipment was a report of the analytical data concerning the same, which is quoted as follows:

> "We are at this date shipping you three one gallon samples of treated cyanide salts and solutions and one pound of sodium sulfocyanide as described below.
>
> While calcium polysulfide can be readily and cheaply made by boiling together slaked lime and flower of sulfur in the proportions of one pound of lime to two pounds of sulfur, for this experimental work commerical lime sulfur was used.
>
> <u>Experimental Data</u>
>
> Preparation of calcium polysulfide solution -- 5 lbs. lime sulfur mixed into 7.8 lbs. water (specific gravity 1.28, 32° Be. at 60° F.). All tests for residual cyanide were made by the addition of a large excess of lead carbonate to remove

sulfides, filtered and titrated with 0.01 N $AgNO_3$ using neutral KI indicator.

In all samples treated, 20% excess lime sulfur was used.

Calculated for every 45.3 gms. of NaCN, 0.146 liters of lime sulfur is required.

Disposal of Residue from Carburizing Salt Bath

Weight of salt residue _ 1090 gms. (inerts, chlorides and carbonates of barium and sodium)

Salt mixed with water and solution made up to 3000 cc.

Total NaCN in solution -------------------40.86 gms.

Lime sulfur solution required ------------ 0.146 liters

Log of Run

3:30 p.m. Lime sulfur added to cyanide residue solution
3:40 p.m. 10 cc. titrated took 0.05 cc. 0.1 N $AgNO_3$
4:10 p.m. 50 cc. titrated took 0.3cc. 0.01 N $AgNO_3$
calculated 6 ppm NaCN

10:00 a.m. following day
50 cc. sample tested 6 ppm NaCN
Supernatant tested for barium - none detected
Insolubles filtered off and clear solution made up to one gallon.

Disposal of Waste Solution from Copper Plating Bath

Sample analyzed

Free NaCN -------------------------0.34 oz./gal.
Cu---------------------------------9.81 oz/ gal.
NaCN to form Na_2CuCN_3 ------------22.73 oz./gal.

Total NaCN of solution------------23.07 oz./gal.

One liter of solution was diluted with 5 liters of water and heated to 100° C.

Calculated lime sulfur required for NaCN---0.618 liters
Calculated lime sulfur required for Cu-----0.208 liters
Total lime sulfur required 0.826 liters

Log of Run

1:45 p.m. Lime sulfur added to solution at 100° C. and stirred.
4:45 p.m. Heat discontinued.
8:30 a.m. Following morning solution tested 8 ppm NaCN.
Solution filtered and made up to one gallon.

Disposal of Residue from NaCN Bath

Weight of sample - 420 gms. (inerts, chlorides and carbonates of sodium)

Salt mixed with water and solution made up to 2000 cc.

Total NaCN in solution - 98.39 gms.

Lime sulfur required - 0.354 liters

Log of Run

9:55 a.m. Lime sulfur added to cyanide solution
10:25 a.m. Analysis showed 2 ppm NaCN, solution was filtered and made up to one gallon.

Sodium Sulfocyanide Analysis

NaCNS -----------98.8% -----------NaCL---------- .3%
Na_2S ----------- .004% -------- Cu ---------- .002%"

On June 21, we received the following additional information pertaining to the shipment of June 5:

"The original solution of carburizing salt contained 9000 ppm as NaCN. After reacting with a 20% excess of calcium polysulfide solution this tested 8 ppm NaCN and 11,000 ppm NaCNS.

The NaCN solution contained 42,000 ppm and after treatment tested 2 ppm NaCN and 50,000 ppm NaCNS.

The copper plating solution contained 3,000 ppm NaCN and after treatment tested 6 ppm [NaCN] and 5,000 ppm NaCNS."

The tests were performed in the Experimental Aquarium of the University of Michigan Museum of Zoology. The water used in this experiment was taken from the filtered circulating aquarium-room water which has proven to be satisfactory for fish life. The water temperature varied from 25.5° C. to 27.7° C. The pH was 7.9 and the D. O. was 7.43 p.p.m. The experimental equipment consisted of wide-mouth two-quart glass jars, each equipped with an air line. Each concentration of the material tested was made up to one liter and two small fish were used in each trial run. The jars were arranged in such a manner as to assure uniformity throughout. A control jar was operated during each run.

Two general procedures were followed. One ("normal aeration") was to aerate the solution for 20 minutes by means of a small flow of fine air bubbles before the fish were introduced and to continue such aeration as long as the fish remained alive or until the end of the run; the other was to preaerate the solution by a heavy flow of fine bubbles for 24 hours before introducing the fish. In a later paragraph, this latter method is explained more fully. In all experimental runs, the time was recorded when the fish were placed in the solutions and again when the fish died. An arbitrary time limit of 96 hours was set as the duration for a trial run; if at the end of this time, the fish were alive an in apparent good health the run was concluded.

In so far as was consistent with the equipment available and the exigencies of the work to be done, the tentatively standard methods for toxicity measurements proposed by Doudoroff, et al (MS) were followed.

Before any experimental work was started with the treated waste products, all three of the solutions were tested for the presence of free NaCN. Mr. Arno Heyn, Teaching Fellow in Analytical Chemistry at the University of Michigan who conducted our chemical tests, reported that no free NaCN could be detected.

The purpose of this experimental work was to determine how much the toxicity can be reduced by this method of treatment of cyanide waste products. In order to reach this conclusion, two main factors had to be taken into consideration and accounted for, namely, the toxicity of the sodium thiocyanide salts and whether or not the presence of the excess polysulfide was toxic. In addition any toxicity due to still other chemicals was to be looked for.

The NaCNS salt (commercial) was used as a basis for testing the toxicity threshold. This threshold once established was used as a basis for testing the toxicity of the other waste products. The difference in the threshold of each waste product and that established by the NaCNS salt was then attributed to the excess polysulfide or other toxic substances present in these wastes. The excess polysulfide was easily removed by a period of preaeration. This was accomplished by passing a heavy stream of fine air bubbles through the stock solution for 24 hours. The chemical reaction was probably as follows:

$$2\ CaS_n + O_2 \longrightarrow 2\ CaO + S_n$$

$$CaO + H_2O \longrightarrow Ca(OH)_2$$

or

$$3\ CaS_n + 4\ H_2O + O_2 \longrightarrow 3\ Ca(OH)_2 + H_2S + (3n-1)S$$

The free sulfur precipitates out as monoclinic crystals, The clear supernatant was poured off and then tested for its toxicity under conditions of normal aeration. The precipitate was likewise tested.

The computations of the concentrations used in these tests were based on the analytical data furnished by B. H. Vollertsen.

Sodium thiocyanide was the first substance to be tested. It was important that the toxicity threshold be known for this salt, which was thought to be the chief active ingredient in the treated cyanide solutions. The threshold established for NaCNS was used as the theoretical basis for computing the dilutions used in the other solutions. The NaCNS preparation used was dried in a drying oven at 103° F. before being used in making up the stock solution. The excess water present was computed as equivalent to 1.5% by weight.

EXPERIMENTAL RUN WITH THE BLUNTNOSE MINNOW, HYBORYNCHUS NOTATUS

Approximately 125 adult bluntnose minnows were seined from the York Pond near Saline, Michigan, on June 24, 1942. These fish were brought to the Experimental Aquarium, placed in stock tanks and allowed an adequate time to become acclimated to the new conditions before being used in tests. The mortality rate was quite high in the holding tanks as 8 died the night of the 24th, 7 on the 25th, 8 on the 26th, and 6 on the 27th. The death was attributed to bacterial fin rot. The tests were started June 28th. After completing the run on the thiocyanide salts, this species was discarded as being unsuitable for a test fish because of the high mortality rate both in the stock tank and in the control jars. The data obtained in the tests with sodium thiocyanide are given in Table I.

TABLE I

SURVIVAL TIMES OF BLUNTNOSE MINNOWS IN DIFFERENT CONCENTRATIONS OF NaCNS

Experiments started June 28, 1942

P.p.m. of NaCNS	No. of fish	Survival time Hrs.	Min.	Sec.	Remarks
50,000	1	0	1	42	
	1	0	1	45	
	1	0	1	51	
	1	0	1	57	
25,000	2	0	6	30	
10,000	1	1	2	..	
	1	1	23	..	
1,000	1	9	50	..	
	1	13	5	..	
500	1	..	..	..	Lived at least 9 hrs., 15 min.*
	1	28	50	..	
	1	30	5	..	
	1	32	40	..	
200	1	..	..	..	Lived at least 37 hrs., 5 min.*
	1	62	10	..	
100	1	..	..	..	Lived at least 9 hrs., 15 min.*
	1	45	50	..	
	1	55	50	..	
	1	72	6	..	
90	2	96+	..	..	Experiment terminated; fish alive.
85	2	96+	..	..	Experiment terminated; fish alive.
80	2	96+	..	..	Experiment terminated; fish alive.
75	1	84	45	..	Lived at least 84 hrs., 45 min.*
	1	84	45	..	Lived at least 84 hrs., 45 min.*
70	2	96+	..	..	Experiment terminated; fish alive.
65	1	..	..	..	Lived at least 11 hrs., 35 min.*
	1	96+	..	..	Experiment terminated; fish alive.
60	1	57	31	..	
	1	96+	..	..	Experiment terminated; fish alive.
55	2	96+	..	..	Experiment terminated; fish alive.
50	1	71	30	..	
	1	74	5	..	
40	2	96+	..	..	Experiment terminated; fish alive.
Control	2	..	..	..	Both lived at least 60 hrs., 10 min.*
Control	2	..	..	..	Both lived at least 82 hrs., 37 min.*
Control	2	96+	..	..	Experiment terminated; fish alive

*Fish died during the night, and may have lived a few hours longer than the time indicated

Interpretation of the data in Table 1. —High concentrations are quickly lethal; concentrations between 100 and 500 p.p.m., only moderately so. At strengths 0 to 100 p.p.m. some fish died while others lived. Due to the fact that some of the fish died in the control jars, it is unsafe to attribute the death of the fish to any toxic materials present in these low concentrations. Tests with the same salt on other fish revealed that these low concentrations were not toxic, or only slightly so. For this reason, the bluntnose minnow was discontinued as a test fish and no definite threshold for it is stated.

EXPERIMENTAL RUN WITH THE CREEK CHUB, SEMOTILUS ATROMACULATUS

A very uniform stock of 2 - to 3- inch chubs, of identical age and culture, was obtained from an experimental pond near Saline, Michigan. These fish proved to be very satisfactory as a test fish, as not a single fish died in the control jars or the holding tanks during the entire run of the experiments, and as very consistent results were obtained. The results of the tests conducted with this fish are presented in Tables 2 to 8.

Interpretation of the data in Table 2. —High concentrations are quickly lethal; concentrations between 600 and 1,000 p.p.m. are only moderately so, requiring 20 to 70 hours to produce death. Concentrations below 600 p.p.m. failed to kill these fish in 96 hours. The approximate lethal threshold is regarded, for the purposes of this paper, as 600 p.p.m. of NaCNS. This is the concentration which would be produced by treating 363 p.p.m. of NaCN with polysulphide. The lethal dosage of NaCN is less than 1 p.p.m.

Interpretation of the data in Table 3. — Concentrations between 10,000 and 500,000 p.p.m. are quickly lethal; the average time required to produce death was 4 minutes and 30 seconds. Solutions ranging in concentrations from 1,800 to 3,000 p.p.m. were only moderately toxic. Some of the fish died within a few hours and others were still alive at the end of the run. None of the concentrations below 1,800 p.p.m. were toxic enough to produce death in the allotted period of time (96 hours). The strength established as the toxicity threshold for the experimental conditions was 1,700 p.p.m. of the stock solution, equivalent to 8.5 p.p.m. of NaCNS. In order to attain this concentration from the basic stock solution, a dilution of 1 to 588 parts of water was required. In view of the previous tests on NaCNS, for which the threshold was determined to be 600 p.p.m., the treated, copper-plating solution used should have been non-toxic at a concentration of 120,000 p.p.m., rather than 1,700 p.p.m. The difference between the observed and the theoretical thresholds indicate the presence in the stock solution of toxic substances other than NaCNS. This problem will be discussed later.

TABLE 2

SURVIVAL TIMES OF CREEK CHUBS IN DIFFERENT CONCENTRATIONS OF NaCNS

Experiments started July 2, 1942

P.p.m. of NaCNS	No. of fish	Survival time Hrs.	Min.	Remarks
10,000	1	0	41	
	1	0	44	
	1	0	48	
	1	0	62	
5,000	1	1	33	
	1	1	58	
	1	2	10	
	1	2	50	
2,000	1	8	55	
	1	9	30	
1,000	1	20	10	
	1	..	..	Died between 8 and 19 hrs.
800	1	70	20	
	1	..	..	Died between 80 and 89 hrs.
700	1	65	15	
	1	71	5	
600	2	96+	..	Fish still alive
500	2	96+	..	" " "
400	2	96+	..	" " "
300	2	96+	..	" " "
250	2	96+	..	" " "
200	2	96+	..	" " "
150	2	96+	..	" " "
140	2	96+	..	" " "
130	2	96+	..	" " "
120	2	96+	..	" " "
110	2	96+	..	" " "
100	2	96+	..	" " "
90	2	96+	..	" " "
80	2	96+	..	" " "
Control	4	96+	..	" " "

TABLE 3

SURVIVAL TIMES OF CREEK CHUBS
IN DIFFERENT CONCENTRATIONS OF TREATED COPPER-PLATING SOLUTION
WITH NORMAL AERATION

Experiment started July 6, 1942

P.p.m. of solution	NaCNS, p.p.m.	NaCN equivalent p.p.m.*	No. of fish	Survival time Hrs.	Min.	Sec.	Remarks
500,000	2,500	1,500	1	0	0	45	
	2,500	1,500	1	0	1	00	
100,000	500	300	1	0	2	00	
	500	300	1	0	2	30	
50,000	250	150	1	0	2	5	
	250	150	1	0	2	20	
10,000	50	30	1	0	5	..	
	50	30	1	0	22	..	
3,000	15-	9	1	0	25	..	
	15	9	1	4	37	..	
2,500	12.5	7.5	2	96+	..	..	Fish still alive
2,300	11.5	6.9	1	4	12	..	
	11.5	6.9	1	23	11	..	
2,200	11.0	6.6	1	2	12	..	
	11.0	6.6	1	96+	..	..	Fish still alive
2,100	10.5	6.3	1	5	37	..	
	10.5	6.3	1	10	23	..	
2,000	10.0	6.0	2	96+	..	..	Fish still alive
1,900	9.5	5.7	1	2	35	..	
	9.5	5.7	1	9	11	..	
1,800	9.0	5.4	1	2	48	..	
	9.0	5.4	1	96+	..	..	Fish still alive
1,700	8.5	5.1	2	96+	..	..	Fish still alive
1,600	8.0	4.8	2	96+	..	..	" " "
1,500	7.5	4.5	2	96+	..	..	" " "
1,400	7.0	4.2	2	96+	..	..	" " "
1,300	6.5	3.9	2	96+	..	..	" " "
1,200	6.0	3.6	2	96+	..	..	" " "
1,100	5.5	3.3	2	96+	..	..	" " "
1,000	5.0	3.0	2	96+	..	..	" " "
500	4.5	2.7	2	96+	..	..	" " "
100	4.0	2.4	2	96+	..	..	" " "
Control	.0	.0	2	96+	..	..	" " "

* This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

TABLE 4

SURVIVAL TIMES OF CREEK CHUBS IN DIFFERENT CONCENTRATIONS OF TREATED COPPER-PLATING SOLUTION, WITH PREAERATION

Experiment started July 12, 1942

P.p.m. of solution	NaCNS, p.p.m.	NaCN equivalent p.p.m.*	No. of fish	Survival time Hrs.	Survival time Min.	Remarks
250,000	1,250	750	1	0	16	
	1,250	750	1	0	22	
100,000	500	300	1	1	2	
	500	300	1	1	22	
75,000	375	225	1	2	10	
	375	225	1	2	45	
50,000	250	150	1	2	25	
	250	150	1	5	13	
25,000	125	75	1	4	12	
	125	75	1	6	22	
18,000	90	54	1	..	..	Died at night; lived at least 30 hours.
	90	54	1	30	25	
16,000	80	48	1	60	15	
	80	48	1	72	12	
15,000	75	45	2	96+	..	Fish still alive
14,000	70	42	2	96+	..	" " "
12,000	60	36	2	96+	..	" " "
10,000	50	30	2	96+	..	" " "
8,000	40	24	2	96+	..	" " "
6,000	30	18	2	96+	..	" " "
Control	0	0	2	96+	..	" " "

* This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

Interpretation of the data in Table 4. — Concentrations above 100,000 p.p.m. of the treated copper-plating stock solution are highly toxic. Those ranging between 25,000 and 50,000 are only moderately so, requiring several hours to produce lethal effects. The approximate lethal threshold is 15,000 p.p.m., as no fish died at this or any lower concentration. This threshold corresponds to a dilution of 1 to 66 parts of water. By preaerating this solution the toxicity was reduced 8.8 times over that established by the normally aerated solution. At this threshold point the solution is computed to have contained only 75 p.p.m. of NaCNS. As the lethal dose of the thiocyanide salt is fixed at 600 p.p.m. for the creek chub, it is obvious that other toxic substances were present in the treated copper-plating solution. This unexplained toxicity was mentioned in the Institute Report No. 806, in which the possible presence of copper was considered. In a recent conversation Doctor Vollertsen reported that further analysis on the treated copper-plating solution revealed, contrary to his previous expectations, that it contained copper, in the amount of .327 grams of copper per liter of solution. This concentration of copper in the stock solution explains the observed toxicity. Copper is strongly toxic to fish life, even in low concentrations. Powers*/ (1917) indicated that 0.0188 p.p.m. of $CuCl_2$ in distilled water killed goldfish in from 3 hours and 30 minutes to 7 hours. Moore and Kellerman (1905) found that the maximum strength of $CuSO_4$ tolerated in hatchery water was 0.143 p.p.m. for brook trout, 0.33 p.p.m. for carp and suckers, and 0.5 p.p.m. for goldfish. At the lethal threshold concentration of 15,000 p.p.m. the treated copper-plating solution contained, according to the data furnished, about 4.5 p.p.m. of copper.

Interpretation of the data in Table 5. —Concentrations of the normally aerated, treated sodium cyanide solution above 10,000 p.p.m. of stock solution are highly toxic, for an exposure of only a few minutes is fatal to fish. Concentrations below 8,400 p.p.m. were not toxic enough to kill these fish in 96 hours. This lethal threshold would represent 420 p.p.m. of NaCNS, and is produced by a dilution of 1 part of solution to 119 parts of water. Other toxic substances appear to be limited, for the toxicity threshold of pure NaCNS was fixed as 600 p.p.m.

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*/Ellis, M. M. 1937. Detection and Measurement of Stream Pollution. U. S. Bureau of Fisheries, Bulletin, No. 22, pp. 365-437.

TABLE 5

SURVIVAL TIMES OF CREEK CHUBS IN DIFFERENT CONCENTRATIONS OF TREATED SODIUM CYANIDE SOLUTION, WITH NORMAL AERATION

Experiment started July 8, 1942

P.p.m. of solution	NaCNS, p.p.m.	NaCN equivalent p.p.m.✓*	No. of fish	Survival Hrs.	time Min.	Sec.	Remarks
500,000	25,000	21,000	1	0	1	27	Both lost equili-
	25,000	21,000	1	0	1	57	brium in 11 seconds
100,000	5,000	4,200	1	0	2	31	Both lost equili-
	5,000	4,200	1	0	2	47	brium in 28 seconds
50,000	2,500	2,100	1	0	3	28	Lost equilibrium in
	2,500	2,100	1	0	3	57	22 and 42 sec., resp.
10,000	500	420	1	0	5	30	
	500	420	1	0	7	..	
9,000	450	378	1	4	25	..	
	450	378	1	6	30	..	
8,800	440	370	1	44	23	..	
	440	370	1	96+	..	..	Fish still alive
8,400	420	353	2	96+	..	..	" " "
8,000	400	336	2	96+	..	..	" " "
7,000	350	294	2	96+	..	..	" " "
6,000	300	252	2	96+	..	..	" " "
5,000	250	210	2	96+	..	..	" " "
2,000	100	84	2	96+	..	..	" " "
1,000	50	42	2	96+	..	..	" " "
500	25	21	2	96+	..	..	" " "
100	5	4.2	2	96+	..	..	" " "
Control	0	0	2	96+	..	..	" " "

✓* This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

TABLE 6

SURVIVAL TIMES OF CREEK CHUBS IN DIFFERENT CONCENTRATIONS OF TREATED SODIUM CYANIDE SOLUTION WITH PREAERATION

Experiment started July 14, 1942

P.p.m. of solution	NaCNS p.p.m.	NaCN equivalent p.p.m.*	No. of fish	Survival time Hrs.	Min.	Sec.	Remarks
500,000	25,000	21,000	1	0	1	10	
	25,000	21,000	1	0	1	22	
250,000	12,500	10,500	1	0	8	..	
	12,500	10,500	1	0	11	..	
100,000	5,000	4,200	1	0	7	..	
	5,000	4,200	1	0	11	..	
20,000	1,000	840	1	0	48	..	
	1,000	840	1	1	23	..	
15,000	750	630	1	5	30	..	
	750	630	1	22	15	..	
13,000	650	546	1	81	40	..	
	650	546	1	96+	..	..	Fish still alive
12,000	600	504	2	96+	..	..	" " "
10,000	500	420	2	96+	..	..	" " "
Control	0	0	2	96+	..	..	" " "

* This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

<u>Interpretation of the data in Table 6.</u> —Concentrations above 20,000 p.p.m. of the preaerated treated sodium cyanide solution proved highly toxic; the survival time varied from about a minute to about an hour and a half. All of the fish survived in tests below 13,000 p.p.m. The lethal threshold established at 12,000 would contain 600 p.p.m. of NaCNS. This threshold is identical with the one previously established for these salts and can be attained by a dilution of 1 part of stock solution to 83 parts of water. Apparently the difference between the two thresholds for the normally aerated and the preaerated solutions was due to the incomplete removal of the polysulfide by the normal aeration.

TABLE 7

SURVIVAL TIMES OF CREEK CHUBS IN DIFFERENT CONCENTRATIONS OF TREATED CARBURIZING SALTS SOLUTION, NORMAL AERATION

Experiment started July 5, 1942

P.p.m. of solution	NaCNS p.p.m.	NaCN equivalent p.p.m.*	No. of fish	Survival time Hrs.	Min.	Sec.	Remarks
500,000	5,500	4,500	1	0	2	5	Lost equilibrium in 12 sec.
	5,500	4,500	1	0	2	53	Lost equilibrium in 8 sec.
100,000	1,100	900	1	0	3	37	Lost equilibrium in 20 sec.
	1,100	900	1	0	3	58	Lost equilibrium in 20 sec.
50,000	550	450	1	0	3	46	Lost equilibrium in 48 sec.
	550	450	1	0	4	12	Lost equilibrium in 51 sec.
15,000	165	135	1	5	20	..	
	165	135	1	6	11	..	
12,000	132	108	1	2	5	..	
	132	108	1	2	35	..	
11,000	121	99	1	2	15	..	
	121	99	1	2	50	..	
10,000	110	90	1	0	45	..	
	110	90	1	5	20	..	
9,000	99	81	1	5	46	..	
	99	81	1	6	20	..	
8,000	88	72	1	8	12	..	Died at night; lived
	88	72	1	..	..	..	at least 11 hrs., 35 min.
7,000	77	63	1	56	5	..	Died at night; lived
	77	63	1	..	..	..	at least 58 hrs.
6,500	71.5	58.5	2	96+	..	..	Fish still alive
6,000	66	54	2	96+	..	..	" " "
5,500	60.5	49.5	2	96+	..	..	" " "
2,000	22	18	2	96+	..	..	" " "
5,000	55	45	2	96+	..	..	" " "
1,000	11	9	2	96+	..	..	" " "
500	5.5	4.5	2	96+	..	..	" " "
Control	0	0	2	96+	..	..	" " "

*This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

<u>Interpretation of the data in Table 7.</u>—Concentrations above 50,000 p.p.m. of the normally aerated treated carburizing salts solution kill fish within a few minutes. Solutions ranging in concentrations from 8,000 to 15,000 p.p.m. require from 2 to 8 hours of exposure to produce death. A 6,500 p.p.m. concentration is the approximate lethal threshold; this contains 71.5 p.p.m. of NaCNS.

A dilution of 1 part of stock solution to 153 parts of water would produce this concentration. The difference between this threshold and the one previously established for the thiocyanide salts is 528.5 p.p.m. This was for the most part accounted for by the removal of the excess polysulfide from this solution (see interpretation of Table 8).

TABLE 8

SURVIVAL TIMES OF CREEK CHUBS IN DIFFERENT CONCENTRATIONS OF TREATED CARBURIZING SALTS SOLUTION WITH PREAERATION

Experiment started July 10, 1942

P.p.m. of solution	NaCNS p.p.m.	NaCN equivalent p.p.m.*	No. of fish	Survival time Hrs.	Survival time Min.	Remarks
250,000	2,750	2,250	1	..	30	
	2,750	2,250	1	..	39	
80,000	880	720	1	47	15	
	880	720	1	..	..	Died at night: no record
60,000	660	540	1	50	35	
	660	540	1	..	..	Died at night; lived at least 57 hrs.
50,000	550	450	1	67	15	
	550	450	1	84	12	
45,000	495	405	2	96+	..	Fish still alive
40,000	440	360	2	96+	..	Fish still alive
35,000	385	315	2	96+	..	" " "
30,000	330	270	2	96+	..	" " "
25,000	275	225	2	96+	..	" " "
20,000	220	180	2	96+	..	" " "
17,000	187	153	2	96+	..	" " "
14,000	154	126	2	96+	..	" " "
10,000	110	90	2	96+	..	" " "
Control	0	0	2	96+	..	" " "

*This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

Interpretation of the data in Table 8. —The only run with preaerated treated carburizing salts solution was tested on a high concentration (250,000 ppm) which proved to be highly toxic. Those concentrations ranging between 50,000 and 80,000 p.p.m. were lethal to fish in from 47 to 84 hours. The test fish were able to tolerate concentrations up to 45,000 p.p.m. of the stock solution. This represented 495 p.p.m. of NaCNS and a dilution of 1 part of solution to 22 parts of water was necessary to attain this concentration. There still remains unaccounted for a difference of 105 p.p.m. between the two thresholds. It is possible that not all of the excess polysulfide was removed by the preaerating process. A longer aeration period was not tried on this solution. Also, it is possible that other toxic substances are present in this solution. In as much as there is only a small difference between the two toleration ranges, it seems that the threshold established for the preaerated solution is satisfactory.

Test of the Precipitate

1,600 cc. of the copper-plating solution (full strength) was preaerated for 24 hours. The solution was then filtered and the precipitate was removed and added to an amount of water equivalent to the original amount of solution present, allowing for the displacement of the sludge.

Two creek chubs were placed in this solution and they remained alive until the run was terminated at 96 hours. Apparently this precipitate is not very toxic.

EXPERIMENTAL RUN WITH THE PUMPKINSEED, *LEPOMIS GIBBOSUS*

A supply of 2- to 3- inch sunfish was obtained from the Huron River, 4 miles west of Ann Arbor, Michigan. They were placed in the Aquarium room and allowed an adequate time to become acclimated to the changed conditions. The fish held fairly well; only a few died in the stock tanks during the experimental run. The results of tests conducted with this fish are presented in Tables 9-12.

GRAPH I

RELATIVE SUSCEPTIBILITY OF CREEK CHUBS TO SODIUM THIOCYANIDE AND TO TREATED CYANIDE WASTES, UNDER DIFFERENT CONDITIONS OF AERATION

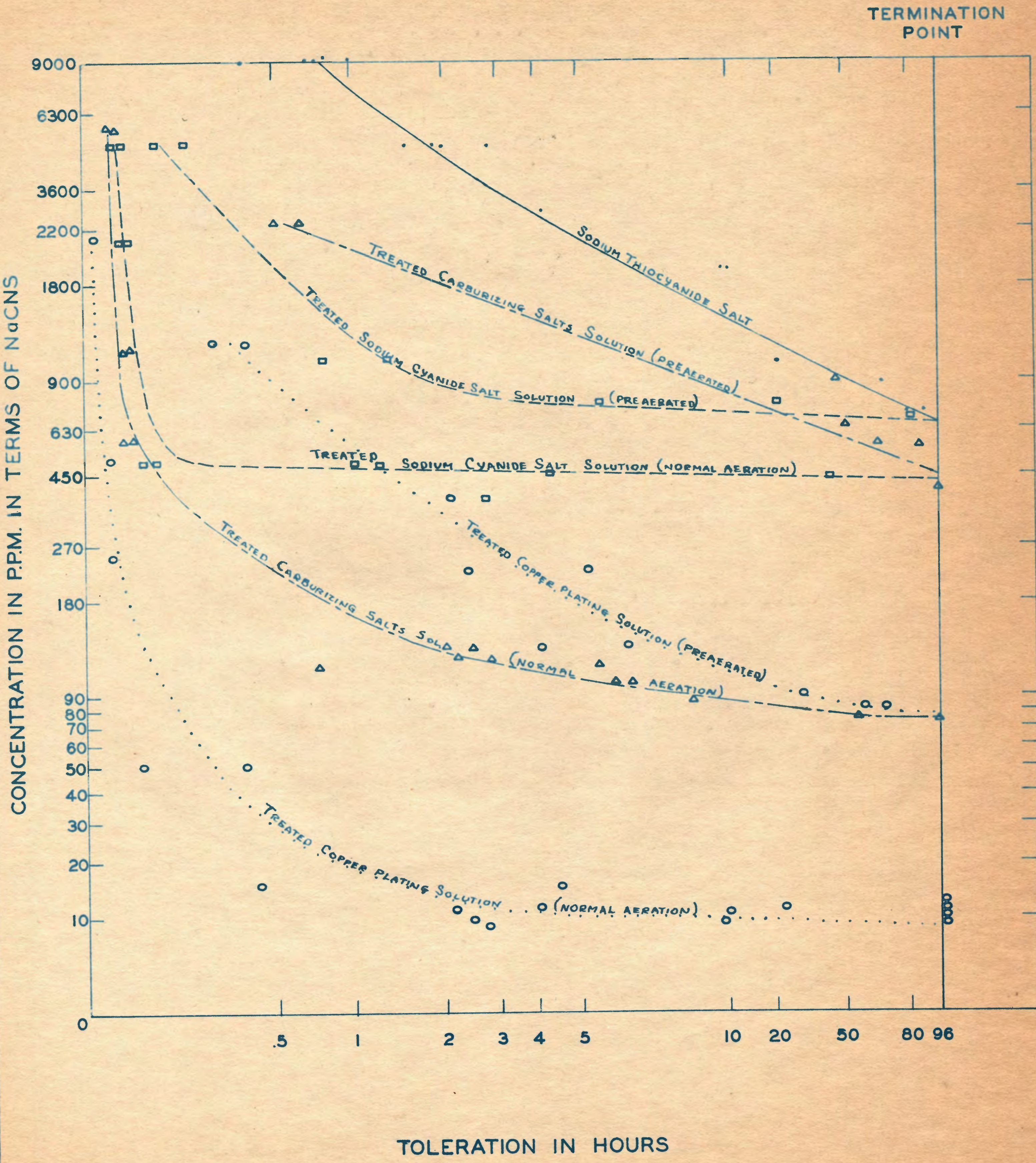


TABLE 9

SURVIVAL TIMES OF PUMPKINSEED WITH DIFFERENT CONCENTRATIONS OF SODIUM THIOCYANIDE SALT

Experiment started July 13, 1942

P.p.m. of solution	No. of fish	Survival time Hrs.	Min.	Remarks
1,000	1	14	30	
	1	18	45	
800	1	24	5	
	1	27	45	
600	1	40	35	
	1	51	20	
	1	62	..	
	1	85	30	
500	2	96+	..	Fish still alive
400	2	96+	..	" " "
300	2	96+	..	" " "
200	2	96+	..	" " "
100	2	96+	..	" " "
Control	2	96+	..	" " "

Interpretation of the data in Table 9.—It was not deemed necessary to test high concentrations on this fish because the tests on the creek chub adequately proved the toxicity of high concentrations. Concentrations above 500 p.p.m. are definitely toxic, requiring only a few hours of exposure to produce death. In all tests, these fish were able to tolerate concentrations as great as 500 p.p.m. The lethal threshold established was 500 p.p.m. of NaCNS. This is the concentration which would be produced by treating 302 p.p.m. of NaCN with polysulfide.

TABLE 10

SURVIVAL TIMES OF PUMPKINSEED WITH DIFFERENT CONCENTRATIONS OF COPPER-PLATING SOLUTION, NORMAL AERATION

Experiment started July 14, 1942

P.p.m. of solution	NaCNS p.p.m.	NaCN equivalent p.p.m.*	No. of fish	Survival time Hrs.	Min.	Remarks
4,000	20	12	1	1	10	
	20	12	1	1	40	
3,500	17.5	10.5	2	96+	..	Fish still alive
3,000	15	9	2	96+	..	" " "
2,300	11.5	6.9	2	96+	..	" " "
2,100	10.5	6.3	2	96+	..	" " "
Control	0	0	2	96+	..	" " "

* This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

Interpretation of the data in Table 10. —A concentration of the treated copper-plating solution, at a strength of 4,000 p.p.m. is highly toxic, producing death in less than two hours. Though no tests at high concentrations were conducted, one would expect that the toxicity would increase as has been shown in the tests conducted on this same solution with the creek chub. Concentrations up to 3,500 p.p.m. were not toxic enough to produce death in a 96 hour period. This threshold was established by a dilution of 1 part of the treated copper-plating solution to 286 parts of water.

TABLE 11

SURVIVAL TIMES OF PUMPKINSEED WITH DIFFERENT CONCENTRATIONS OF TREATED-CARBURIZING SALTS SOLUTION, NORMAL AERATION

Experiment started July 15, 1942

P.p.m. of solution	NaCNS p.p.m.	NaCN equivalent p.p.m.*	No. of fish	Survival time Hrs.	Survival time Min.	Remarks
11,000	121	99	1	2	20	
	121	99	1	4	16	
9,000	99	81	1	2	42	
	99	81	1	3	51	
8,000	88	72	1	3	48	
	88	72	1	5	40	
7,000	77	63	2	96+	..	Fish still alive
6,000	66	54	2	96+	..	" " "
5,000	55	45	2	96+	..	" " "
Control	0	0	2	96+	..	" " "

*This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

Interpretation of the data in Table 11. —Concentrations of 8,000 to 11,000 p.p.m. of the normally aerated treated-carburizing salts solution killed the sunfish in a period of 2 to 6 hours. The fish were able to tolerate concentrations of 7,000 and below. This concentration contained 88 p.p.m. of NaCNS. The dilution necessary to reach the 7,000 p.p.m. threshold would be 1 part of stock solution to 143 parts of water.

TABLE 12

SURVIVAL TIMES OF PUMPKINSEED WITH DIFFERENT CONCENTRATIONS OF SODIUM CYANIDE SOLUTION, NORMAL AERATION

Experiment started July 15, 1942

P.p.m. of solution	NaCNS p.p.m.	NaCN equivalent p.p.m.*	No. of fish	Survival time Hrs.	Survival time Min.	Remarks
11,000	550 550	462 462	1 1	3 4	20 12	
9,000	450 450	378 378	1 1	5 6	32 5	
8,000	400 400	336 336	1 1	2 3	14 18	
7,000	350 350	294 294	1 1	9 12	50 35	
6,000	300 300	252 252	1 1	16 19	28 32	
5,000	250 250	210 210	1 1	21 58	10 ..	
4,500	225	189	2	96+	..	Fish still alive
4,000	200	168	2	96+	..	" " "
Control	0	0	2	96+	..	" " "

* This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

Interpretation of the data in Table 12.— The sunfish were able to tolerate concentrations of the treated sodium cyanide solution up to 4,500 p.p.m. Higher concentrations were lethal in a period of a few hours, depending on the concentration. A dilution of 1 part of stock solution to 222 parts of water would be necessary to attain this threshold.

TESTS CONCERNING THE PREAERATED SOLUTIONS

It was necessary to terminate these tests temporarily in July, following the tests with normally aerated solutions. They were resumed again in September. A new supply of pumpkinseed sunfish was obtained, September 3, 1942, and given ample time to become acclimated to Aquarium room conditions before being tested. Before any attempt was made to resume the testing of the preaerated solutions, a re-test on the normally aerated solutions was in order.

There was a possibility that the toxicity of the solutions had changed during the lapse of time between the two tests. A check was made on the normally aerated copper-plating solution to test its toxicity. Concentrations of 4,000, 6,000 and 10,000 p.p.m. of the normally aerated solution was prepared. It required from 48 to 60 hours exposure in the 10,000 p.p.m. of the normally aerated, treated copper-plating solution to produce death. The fish were able to tolerate concentrations of 4,000 and 6,000 p.p.m. for 96 hours. It will be recalled that in the original test on this solution, conducted July 14, the fish died in the 4,000 p.p.m. of the normally aerated treated copper-plating solution in a period of less than 2 hours.

A test was also conducted with the preaerated treated carburizing salts solution. A concentration of 80,000 p.p.m. of this solution was prepared and the fish introduced. No fish died during the 96 hour experimental run. The concentration of NaCNS present in this prepared solution, computed from the original analytical data, was 880 p.p.m. This was 380 p.p.m. of NaCNS above the threshold previously established for this salt (see Table 9). It is apparent that the toxicity of the treated copper-plating solution and the preaerated treated carburizing salts solution had been reduced during the intermittant time of testing, or that the physiological conditions of the fish materially changed in direction of greater toleration. Some of the reduction in toxicity of the normally aerated treated copper-plating solution can be accounted for by the partial removal of the excess polysulfide which took place during the intermittent time of the two tests; which was evidenced by the presence of a grayish precipitate in the full strength treated copper-plating solution. This, however, does not explain the reduction in toxicity of the preaerated treated carburizing salts solution, as, in this case, the excess polysulfide was removed before testing and yet the toxicity was definitely reduced by standing six weeks. Another possibility is that further chemical changes have taken place in both of the solutions, altering the chemical nature of the previous toxic substances.

For this reason, no further attempt was made to run the tests on the preaerated solutions. It was thought that the results, if the tests were conducted, would not be comparable with those made previously on normally aerated solutions.

SUMMARY AND DISCUSSION

The toxicity of cyanide wastes is greatly reduced by treating them with an excess of calcium polysulfide. By this treatment, the NaCN content is converted to NaCNS which is much less toxic. By comparing our data for NaCNS with those obtained by Wendell H. Krull[1] for KCN, it is estimated that NaCNS is about 600 times less toxic than KCN. In terms of the anion, it is computed that CNS is about 1,000 times less toxic than CN.

The treated wastes, however, retain a considerable toxicity attributed to the excess polysulfide. In all three types of wastes studied, the toxicity due to this chemical was greatly reduced or eliminated by strong aeration (see graphs 1 and 2) over a period of time inversely proportional to the concentration of the polysulfide. By this method, the polysulfide was precipitated out. The sulfur precipitate was indicated by one test as having little toxicity.

TABLE 13

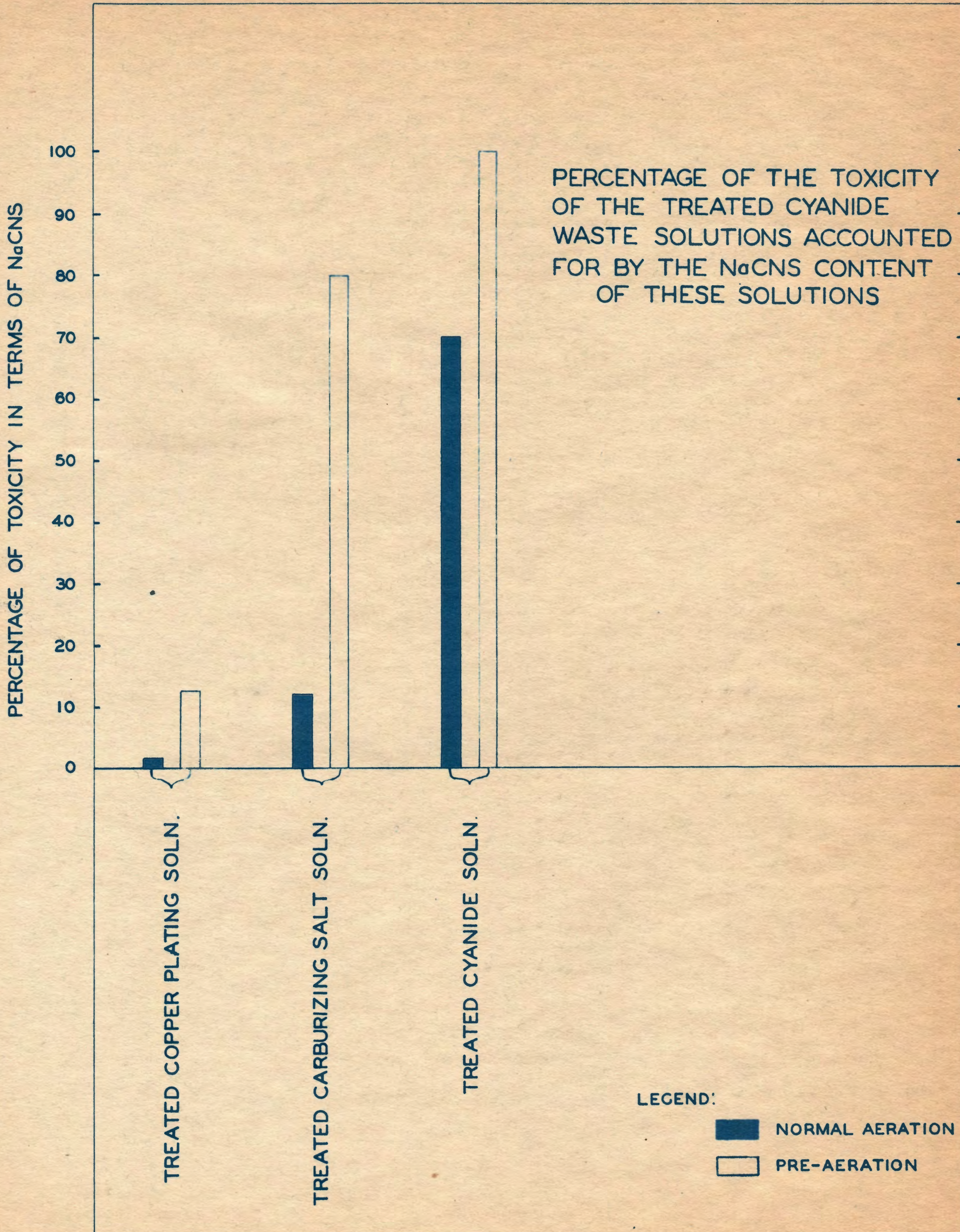
TOXICITY OF SODIUM THIOCYANIDE AND TREATED CYANIDE WASTES: SUMMARY OF DATA

Species and Materials N.A. = Normal aeration P.A. = Preaeration	Lethal Threshold		Dilutions of Stock Solution
	NaCNS p.p.m.	NaCN equivalent p.p.m.*	
Creek chub			
Commercial sodium thiocyanide salts	600	362.6	1 to 1,660
Treated copper-plating solution (N.A.)	8.5	5.1	1 to 588
" " " " (P.A.)	75	45	1 to 66
Treated sodium cyanide solution((N.A.)	420	352.8	1 to 119
" " " " (P.A.)	600	504	1 to 83
Treated carburizing salts sol. (N.A.)	71.5	58.5	1 to 153
" " " " (P.A.)	495	405	1 to 22
Pumpkinseed sunfish			
Commercial sodium thiocyanide salts	500	302.3	1 to 2,000
Treated copper-plating solution (N.A.)	17.5	10.5	1 to 286
Treated sodium cyanide solution (N.A.)	225	189	1 to 222
Treated carburizing salts sol. (N.A.)	77	63	1 to 143

* This is the concentration of NaCN which would have existed, if the cyanide salt had not been treated to form NaCNS.

[1] Krull, W. H., 1933. Manuscript report, Inst. Fish. Res. Mich., No. 207:1-3

GRAPH II



PERCENTAGE OF THE TOXICITY OF THE TREATED CYANIDE WASTE SOLUTIONS ACCOUNTED FOR BY THE NaCNS CONTENT OF THESE SOLUTIONS
PERCENTAGE OF TOXICITY IN TERMS OF NaCNS
100
90
80
70
60
50
40
30
20
10
0
TREATED COPPER PLATING SOLN.
TREATED CARBURIZING SALT SOLN.
TREATED CYANIDE SOLN.
LEGEND:
NORMAL AERATION
PRE-AERATION

The toxicity threshold established for commercial sodium thiocyanide and for the preaerated polysulfide-treated sodium cyanide waste is 600 p.p.m. (Table 13 and Graph I). After preaeration the treated carburizing salts wastes remain somewhat more toxic (threshold, 495 p.p.m.) than the thiocyanide, and the treated copper-plating waste is very much more toxic (threshold, 75 p.p.m.). It appears, therefore, that other toxic substances are present in the wastes. This is particularly true of the copper-plating waste (Table 4), which is now found to contain enough copper to explain its high toxicity. The NaCNS content of the several wastes accounts for the following proportions of their toxicity:

Polysulfide-treated wastes	Percentage of toxicity of treated cyanide wastes accounted for by the NaCNS content	
	Normal aeration	Preaeration
Copper plating	1.4%	12.5%
Carburizing salts	11.9%	80.2%
Sodium cyanide	70.0%	100.0%

These results are also presented in Graph II. The percentage by which the toxicity of the treated wastes was reduced by preaeration as compared with results obtained under conditions of normal aeration was as follows:

Treated copper-plating waste	89.0%
Treated carburizing salts waste	87.0%
Treated sodium cyanide waste	30.0%

The results summarized above are based on the tests in which the creek chub was used. Essentially confirmatory results for tests under normal aeration were obtained in the trials run with a sunfish (the pumpkinseed): the pumpkinseed was slightly less resistant, but the order of tolerance for the several wastes was similar.

Preliminary trials with copper-plating and carburizing salts wastes which had stood at room temperature for six weeks suggest that the toxicity of those wastes might be reduced by ponding.

The polysulphide treatment of cyanide wastes, supplemented by adequate aeration to break down the residual polysulphide, is indicated as more effective than the permanganate, the sulfuric acid, or the lime treatment.* Comparing our results with those obtained by Hubbs (quoted in part by Eldridge), it is estimated that the preaerated polysulfide-treated sodium cyanide wastes are 200 times less toxic than the product of permanganate treatment; 100 times less toxic than the product of the sulfuric acid treatment, and 20 times less toxic than the product of the lime treatment.

*Eldridge, E. F., 1933. The Removal of Cyanide from Plating Room Wastes. Bull. Mich. Eng. Exp. St., 52:12-16

SUGGESTIONS FOR FURTHER RESEARCH

1. Since the wastes used in these tests were synthetically produced in the chemical laboratory, and might differ in some way from those resulting from commercial processes, it would seem desirable that trials be run with actual effluents from a plant which is treating its cyanide wastes with calcium polysulfide. We note, however, that Dr. B. H. Vollertsen informed the Institute for Fisheries Research that the difference between the synthetid solutions furnished for test and those which would result in industry lies in the amount of sodium or copper cyanide present. Doctor Vollertsen further stated that the solutions furnished represent a maximum concentration that might be encountered.

2. Trials should be run to determine the most practicable method of aerating the wastes to break down the residual polysulfide. Air-releasers, sprays, and baffles might be tried.

3. Experiments should be run on the treated copper-plating wastes to determine a practicable method for the removal of the copper content. Dr. Vollertsen suggested (personal communication) that this might be accomplished by passing the waste through a filter of iron filings, or by electrolysis.

4. The reduction of the toxicity of the several wastes by holding them in ponds might well be determined.

5. For a more complete and reliable estimate of the biological effects of the treated wastes, experiments should be run on other fish species, on the eggs and larvae as well as the adults, and on other aquatic organisms.

6. As suggested by Doctor Vollertsen, it is desirable to determine the probable effects of the treated wastes on the operation of sewage disposal plants, to see to what degree the deleterious effects of cyanide on sewage disposal may be reduced. Preliminary arrangements have been made with the Michigan Stream Control Commission for the running of these tests. Professor Eldridge of Michigan State College has agreed to supervise this phase of the work.

INSTITUTE FOR FISHERIES RESEARCH

By Carl L. Hubbs and George N. Washburn

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