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FINAL REPORT OF THE TOXICITY TESTS ON WASTES FROM THE STYRENE PROCESS OF MANUFACTURING SYNTHETIC RUBBER

by
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At the request of Milton P. Adams, Director of the Michigan Stream Control Commission, and of Mr. T. J. Powers of the Dow Chemical Company, the Institute for Fisheries Research has conducted a series of toxicity tests on waste materials from the styrene manufacturing process. This process is to be used in Texas by the Dow Chemical Co. in the manufacture of synthetic rubber.

The purpose of these tests was to determine the toxicity to fish life of the wastes from the styrene manufacturing process, as it is contemplated to discharge these wastes in streams which might contain fish.

Five 5-gallon samples of the waste products were delivered to the Institute at Ann Arbor, on August 1, 1942. These samples were described as follows:

"1. Mixed Waste Waters-Styrene Development includes all wastes except Nos. 2 and 5, as described below, plus all condenser waters.
2. Ethylene Waste-2B Cracking is the effluent from recovery unit. Might contain small traces of alcohol and benzene.
3. Styrene Still-Condensate Decanter is water containing small amounts of styrene.
4. Ethyl Benzene Plant HCl Scrubber-might contain up to 15% HCl with traces of hydrocarbons.
5. Ethyl Benzene Plant-Composite Wash Water-might contain hydrocarbons and is caustic in reaction."

On September 1, 1942, the Institute for Fisheries Research received the additional information pertaining to the chemical nature of these wastes which is quoted as such:

"Analyses or Test:

Date	8-5-42 Mixed Waste Water Sty. Dev. #1	8-5-42 Ethylene Waste 2-B Cracking #2	8-6-42 Styrene Still Condensate Decanter #3	8-6-42 Ethyl Benzene Plant HCl Scrubber #4	8-6-42 Ethyl Benzene Plant Comp. Wash Water #5

"Sp. Gr. at 20° C.	0.999	0.997	0.999	1.039	1.065
Alkalinity as cc.					
N/10 HCl/100 cc.	2.85	0.30	3.30	...	...
%HCl	...	...	...	7.66	...
% Carbon (organic)	0.02	0.53	0.01	0.03	0.11
% CO_2	0.064	0.0029	0.025	12.3	8.6
% Al	...	...	...	...	1.4
"Hardness as P.P.M.					
$CaCO_3$	127	9	290	201	58,000
P.P.M. Cl	640	29	250	122,700	8,500
P.P.M. Na	268	1	46	152	289
P.P.M. K .....nil		nil	nil	nil	nil
P.P.M. Ca	62	18	379	42	2.1
P.P.M. Mg	83	6.6	114	22.7	11.8
P.P.M.SO_4	43	7	31	48	51
"P.P.M. Solids					
Total	975	45	450	649	32,288
Dissolved	795	27	430	634	32,156
Suspended	180	18	20	15	132
pH (glass electrode)	8.0	4.2	7.0	...	2.8

"Total Solids by evaporation will not include organics which steam-distill such as alcohol or benzene. Organic carbon is by wet combustion method."

On September 16, 1942, the Institute for Fisheries Research received the following additional information pertaining to the chemical nature of these wastes:

"Analyses or Test

Sample No.	1	2	3	4	5
		-Parts per Million-			
$Ca(HCO_3)_2$	65	24	267	...	...
$Ca(SO_4)$	...	9	44	68	7
$MgSO_4$	54	...	...	...	57
$CaCl_2$	...	26	17	61	...
$MgCl_2$	55	26	113	89	...
NaCl 682		2	117	387	735
Solids					
Total 975		45	450	649	32,288
Dissolved ...795		27	430	634	32,156
Suspended ...180		18	20	15	132
HCl	...	...	...	76,600	...
$AlCl_3$	...	...	...	...	69,200
pH	8.0	4.2	7.0	...	2.8
Organic Constituents:					
Benzene	+	700	...	...	700
Ethanol	-	8,850	...	...	...
Styrene	+	...	108	...	...
Ethyl Benzene	+	...	...	330	464

#1 - Mixed Waste Waters.
#2 - Ethylene Waste - 2B Cracking.
#3 - Styrene Still - Condensate Decanter.
#4 - Ethyl Benzene HCl Scrubber.
#5 - Ethyl Benzene Composite Wash Water."

Two species of fish were used in testing the toxicity of these wastes, the smallmouth bass, Micropterus d. dolomieu, and the black bullhead, Ameiurus m. melas. The former is a moderately sensitive fish while the latter is an extremely hardy one. It can be expected that a few species of fresh-water fish would be more sensitive than the smallmouth bass and likewise other species may be more tolerant than the black bullhead. Investigations in fish toleration by other workers with these same species of fish tend to confirm the above statement.

The bass were obtained from the federal fish hatchery at Northville on August 1, and on September 3. They were 2 to 3 inches in length and of this year's hatch.

The black bullheads were obtained by seine from the Susterka Pond, near Rawsonville, Michigan. They were about 1 1/2 inches long when taken. They were collected from one school of fish, which would indicate that they were all of the same parentage, and therefore of relatively uniform constitution.

The tests were performed in the Experimental Aquarium of the University of Michigan Museum of Zoology.

The fish were first conditioned in holding tanks. Both species of fish held well in these tanks and the mortality was remarkably low, less than 2%. The fish were fed at regular intervals and grew considerably during the lapse of time involved during the test period.

Filtered, circulating water was used for dilutions in these tests. This water is the same as that which is used for other Aquarium fishes and has proven to be satisfactory for fish life. The average temperature of the aquarium room was 80° F. The pH varied from 7.0 to 7.9 depending on the frequency of regeneration of the water filtering system. Likewise the D. O. varied somewhat, ranging from 6.9 to 7.4 p.p.m. The experimental equipment consisted of wide-mouth two-quart glass jars, each equipped with an air-line. A range of various concentrations of each stock solution was prepared during the exploratory testing. Each concentration tested was made up to 1 liter and 2 fish were used in each test. Aeration was started in each solution 20 minutes before the introduction of fish and continued as long as the fish remained alive. The time was recorded when the fish were introduced into the solution and again when they died. An arbitrary time limit of 96 hours was set as the duration for a test. If at the end of this period the fish were still alive, the test was concluded.

This procedure was followed throughout in testing the 5 waste samples. The only alteration was in dealing with samples No. 4 (Ethyl Benzene HCl Scrubber) and No. 5 (Ethyl Benzene Composite Wash). After the initial tests on wastes Nos. 4 and 5 had been run, these two wastes were treated with sodium hydroxide, to neutralize the acidity which was indicated in the chemical analyses of these wastes. The treated wastes were then tested to determine their toxicities. The reasons for so doing and the chemical

Reactions involved are discussed on pages 16 to 19 inclusive. Because a heavy, toxic precipitate was formed by treating waste No. 5 with NaOH, another method of neutralizing this waste was tried out, involving the use of citric acid in addition to the hydroxide (see p. 23-25).

The toxicity threshold tests were started August 11, 1942, and concluded in the latter part of October, 1942. On August 25, 1942, a preliminary progress report (No. 810) was submitted, covering the entire data received up to that time. Following that report, additional tests were conducted and the total results are recorded in this report.

EXPERIMENTAL RUN WITH THE SMALLMOUTH BASS, *Micropterus d. dolomieu*

Table 1. Survival Times of Smallmouth Bass in Different Concentrations of Waste No. 1 (Mixed Waste Waters)

Experiment started August 11, 1942.

p.p.m. of Stock Solution	No. of Fish Tested	Survival Time Hrs.	Min.	Remarks
1,000,000	2	96+		Fish still alive at end of test
700,000	2	96+		" " " " " " "
300,000	2	96+		" " " " " " "
200,000	2	96+		" " " " " " "
100,000	2	96+		" " " " " " "
50,000	2	96+		" " " " " " "
Control	2	96+		" " " " " " "

INTERPRETATION OF DATA IN TABLE 1.- Of the 14 fish used in this test, not a single fish died during the 96-hour period. The experimental evidence, therefore, indicates that this waste product was non-toxic to these fish in 96 hours. Nothing in the analysis of the Mixed Waste (p. 2) indicates that it would be lethal.

Table 2. Survival Times of Smallmouth Bass in Different Concentrations of Waste No. 2 (Ethylene Waste)

Experiment started August 11, 1942.

P.P.M. OF Stock Solution	No. of Fish Tested	Survival Time Hrs.	Survival Time Min.	Remarks
500,000	1	..	23	
500,000	1	..	25	
500,000	1	..	27	
500,000	1	..	31	
400,000	1	1	25	
400,000	1	1	48	
300,000	1	3	45	
300,000	1	5	20	
200,000	1	23	40	
200,000	1	24	27	
125,000	2	..	..	Lived at least 33 hrs.; died at night
100,000	1	26	..	
100,000	1	56	..	
75,000	1	46	55	
75,000	1	61	25	
60,000	1	66	15	
60,000	1	..	..	Lived at least 68 hrs.;died at night
55,000	1	82	..	
55,000	1	96+		Fish still alive at end of test
50,000	2	96+		" " " " " " "
40,000	2	96+		" " " " " " "
35,000	2	96+		" " " " " " "
30,000	2	96+		" " " " " " "
20,000	2	96+		" " " " " " "
10,000	2	96+		" " " " " " "
Control	2	96+		" " " " " " "

INTERPRETATION OF DATA IN TABLE 2.-Concentrations above 300,000 p.p.m. of Waste No. 2 was highly toxic, requiring a period of less than 6 hours to produce lethal effects. Concetrations below 55,000 p.p.m. gave no indications of being toxic. The approximate lethal threshold established by the Ethylene Waste was 50,000 p.p.m. This concentration was produced by mixing 1 part of the waste with 19 parts of water. When first prepared, the diluted Ethylene Waste is a clear, colorless liquid, but after the introduction of fish, it becomes milky within 24 hours. The cloudy appearance was probably due to a heavy bacterial growth. Upon removal of the liquid at the termination of the test, it was found that a considerable slime deposit was left. The greater the concentration of Ethylene Waste present in test sample, the heavier was the slime deposit.

Table 3. Survival Times of Smallmouth Bass in Different Concentrations of Waste No. 3 (Styrene Still)

Experiment started August 11, 1942.

p.p.m. of Stock Solution	No. of Fish Tested	Survival Time Hrs.	Survival Time Min.	Remarks
1,000,000	1	..	3	
1,600,000	1	..	3.5	
700,000	1	1	40	
700,000	1	2	5	
500,000	1	..	..	Lived 8 to 15 hrs.; died at night
500,000	1	96+	..	Fish still alive at end of test
300,000	1	23	15	
300,000	1	96+	..	Fish still alive at end of test
250,000	1	..	..	Lived 8 to 15 hrs.; died at night
250,000	1	96+	..	Fish still alive at end of test
200,000	1	42	..	
200,000	1	96+	..	Fish still alive at end of test
150,000	1	81	..	
150,000	1	96+	..	Fish still alive at end of test
100,000	1	26	..	
100,000	1	62	..	
75,000	1	49	..	
75,000	1	56	..	
75,000	2	96+	..	Fish still alive at end of test
60,000	1	28	..	
60,000	1	96+	..	Fish still alive at end of test
50,000	2	96+	..	" " " " " " "
40,000	2	96+	..	" " " " " " "
30,000	2	96+	..	" " " " " " "
20,000	2	96+	..	" " " " " " "
Control	2	96+	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 3.-Concentrations above 500,000 p.p.m. of waste No. 3. were highly toxic, producing death in less than 3 hours. Concentrations ranging from 60,000 to 500,000 p.p.m. were quite variable in their effect; in some tests 1 of the 2 fish in the same concentration would die in less than 24 hours, while the other fish would still be alive at the termination of the 96 hour run. It is possible that differences in susceptability among the bass account for this fluctuation, but seem improbable as no other waste tested exhibited any comparable variability in effect.

Because such great variation in the toxicity of the ethylene wastes was indicated, a supplementary test was run. Two bass were placed in each of two concentrations, 1,000,000 and 600,000p.p.m., of the same waste after the removal of some bullheads which had survived these concentrations for 96 hours. One of the bass survived the 1,000,000 p.p.m. sample and both survived the 600,000 p.p.m. solutions. It was apparent that the toxicity of these solutions had been reduced. It was concluded that some highly volatile toxic substance was driven off by continued aeration, or that the

Presence of bullheads had absorbed and removed the toxic substance. Concentrations below 50,000 p.p.m. of Styrene Still Waste were not toxic enough to kill the bass in 96 hours. The approximate lethal threshold established was 50,000 p.p.m. This concentration was produced by mixing 1 part of the Styrene Still Waste with 19 parts of water.

Table 4. Survival Times of Smallmouth Bass in Different Concentrations of Waste No. 4 (Ethyl Benzene HCl Scrubber)

Experiment started August 11, 1942.

p.p.m. of Stock Solution	No. of Fish Tested	Survival Time Hrs.	Min.	Sec.	Remarks
100,000	2	..	..	30	
50,000	1	..	1	..	
50,000	1	..	1	30	
10,000	1	..	2	..	
10,000	1	..	3	..	
5,000	1	..	5	..	
5,000	1	..	7	..	
1,000	1	..	15	..	
1,000	1	..	21	..	
800	1	1	40	..	
800	1	3	10	..	
700	1	10	55	..	
600	1	18	30	..	
600	1	96+	..	..	Fish still alive at end of test
500	2	96+	..	..	" " " " " " "
400	2	96+	..	..	" " " " " " "
300	2	96+	..	..	" " " " " " "
Control	2	96+	..	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 4.-Concentrations above 800 p.p.m. of waste No. 4 were extremely toxic, producing death in a period of less than 30 minutes. The approximate lethal threshold was 500 p.p.m., as all of the fish tested at this and lower concentrations survived. This concentration was produced by a dilution of 1 part of waste No. 4 to 1,999 parts of water. The high toxicity of this waste was probably due to the large percentage of HCl which it contained, as the analysis furnished with these wastes indicated that as much as 15% may be present. Dilutions as low as 1,000 p.p.m. were definitely acid in reaction, but the concentartions ranging near the approximate threshold were neutral or within a pH range tolerable to fishes.

Table 5. Survival Times of Smallmouth Bass in Different Concentrations of Waste No. 5 (Ethyl Benzene Composite Wash)

Experiment started August 11, 1942.

p.p.m. of Stock Solution	No. of Fish Tested	Survival Time Hrs.	Survival Time Min.	Remarks
100,000	1	..	15	
100,000	1	..	21	
50,000	1	..	16	
50,000	1	..	18	
25,000	1	2	20	
25,000	1	4	35	
15,000	1	3	..	
15,000	1	3	10	
10,000	1	3	5	
10,000	1	4	20	
5,000	1	4	..	
3,000	1	3	45	
3,000	1	4	25	
1,000	1	3	10	
1,000	1	3	35	
900	1	76	..	
900	1	87	..	
800	2	96+	..	Fish Still alive at end of test
600	2	96+	..	" " " " " " "
400	2	96+	..	" " " " " " "
Control	2	96+	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 5.-Concentrations of waste No. 5 above 1,000 p.p.m. were highly toxic, causing lethal effects in less than 5 hours. As no fish died in concentrations below 800 p.p.m. this was established as the lethal threshold. This threshold concentration was produced by a dilution of 1 part of waste No. 5 to 1,249 parts of water. Waste no. 5 was definitely acid in reaction, having a pH of 2.8. It was evident, however, that death produced at low concentrations, was not caused by this factor, as the pH in concentrations up to 1,500 p.p.m. was definitely within the toleration ranges of fishes. This waste contained a relative high concentration of aluminum chloride, $AlCl_3$, which was probably the chief toxic substance.

EXPERIMENTAL RUN WITH THE BLACK BULLHEAD, Ameiurus m. melas

Table 6. Survival Times of Black Bullheads in Different Concentrations of Waste No. 1 (Mixed Waste Waters)

Experiment started August 15, 1942.

p.p.m. of Stock Solution	No. of Fish Tested	Survival Time Hours	Remarks
1,000,000	2	96+	Fish still alive at end of test
800,000	2	96+	" " " " " " "
600,000	2	96+	" " " " " " "
400,000	2	96+	" " " " " " "
200,000	2	96+	" " " " " " "
Control	2	96+	" " " " " " "

INTERPRETATION OF DATA IN TABLE 6.-Experimentally, this waste product was not toxic enough to kill bullheads. This was the expected result, since the same waste did not kill bass (Table 1). This Mixed Waste solution remained clear at all the concentrations tested, and no sludge deposit was observed in the bottom of the jars.

Table 7. Survival Times of Black Bullheads in Different Concentrations of Waste No. 2 (Ethylene Waste)

Experiment started August 15, 1942.

p.p.m. of Stock Solution	No. of Fish Tested	Survival Time Hrs.	Survival Time Min.	Remarks
1,000,000	1	..	10	
1,000,000	1	..	14	
800,000	1	..	15	
800,000	1	..	18	
500,000	1	..	26	
500,000	1	..	40	
300,000	2	96+	..	Fish still alive at end of test
275,000	1	..	..	
275,000	1	23	25	
275,000	1	28	40	
250,000	1	..	..	Lived at least 75 hrs.; died at night
250,000	1	96+	..	Fish still alive at end of test
225,000	1	19	20	
225,000	1	96+	..	Fish still alive at end of test
200,000	1	46	25	
200,000	1	..	..	Lived at least 75 hrs.; died at night
200,000	1	96+	..	Fish still alive at end of test
175,000	2	96+	..	
150,000	1	..	..	Lived at least 75 hrs.; died at night
150,000	1	96+	..	Fish still alive at end of test
125,000	2	96+	..	" " " " " " "
120,000	1	61	..	
120,000	1	96+	..	Fish still alive at end of test
100,000	2	96+	..	" " " " " " "
80,000	2	96+	..	" " " " " " "
60,000	2	96+	..	" " " " " " "
40,000	2	96+	..	" " " " " " "
Control	2	96+	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 7.-Concentrations of the Ethylene Waste at 500,000 p.p.m. and above were highly toxic, killing the bullheads in less than 1 hour. Concentrations between 100,000 and 500,000 p.p.m. in some tests produced death within a few hours, but in other tests failed to kill the fish in the 96 hour period. This variation in toleration may have been due in part to the activity of these fish while in the test jars. A considerable difference in activity was displayed by these fish. Some would immediately settle to the bottom of the test jar and remain quietly for the entire test, while others, when placed in jars, exhibited continual movement. Carpenter (1927) indicated that with the increase of metabolic rate in fishes a corresponding direct increase in susceptability was produced. It was not apparent why these fish differed in their behavior. At and below this concentration of 100,000 p.p.m. no fish died. This indicated lethal threshold concentration was produced by a dilution of 1 part of Ethylene Waste to 9 parts of water. A white milky appearance was evident in these series of tests as in those reported in Table 2 (see p. 6).

Table 8. Survival Times of Black Bullheads in Different Concentrations of Waste No. 3 (Styrene Still)

Experiment started August 15, 1942.

p.p.m. of Stock Solution	No. of Fish Tested	Survival Time (Hours)	Remarks
1,000,000	2	96+	Fish Still alive at end of test
800,000	2	96+	" " " " " " "
600,000	2	96+	" " " " " " "
500,000	2	96+	" " " " " " "
400,000	2	96+	" " " " " " "
350,000	2	96+	" " " " " " "
250,000	2	96+	" " " " " " "
180,000	2	96+	" " " " " " "
100,000	2	96+	" " " " " " "
80,000	2	96+	" " " " " " "
Control	2	96+	" " " " " " "

INTERPRETATION OF DATA IN TABLE 8.-Twenty-two fish were tested in various concentrations ranging from the lowest to full strength and not a single fish died. High concentrations of waste No. 3 did produce a slight initial effect. When introduced into the test jars, the bullheads became quite agitated. This condition, however, was soon lost and the fish in turn became quite passive; in fact, on several occasions the jar had to be tapped in order to determine whether the fish were dead. All of the concentrations of this waste tested were clear throughout the test and no sludge deposit was observed in the bottom of the test jars.

Table 9. Survival Times of Black Bullheads in Different Concentrations of Waste No. 4 (Ethyl Benzene HCl Scrubber)

Experiment started August 15, 1942.

p.p.m.of Stock Solution	No. of Fish Tested	Survival Time Hrs.	Min.	Sec.	Remarks
10,000	1	..	3	30	
10,000	1	..	4	15	
5,000	1	..	11	..	
5,000	1	..	16	..	
2,000	1	..	40	..	
2,000	1	..	51	..	
1,000	1	1	10	..	
1,000	1	1	30	..	
900	1	..	..	..	Lived at least 74 hrs.; died at night
900	1	96+	..	..	Fish still alive at end of test
800	2	96+	..	..	" " " " " " "
700	2	96+	..	..	" " " " " " "
500	2	96+	..	..	" " " " " " "
Control	2	96+	..	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 9.-Concentrations above 1,000 p.p.m. were highly toxic, causing death in less than 1 hour. The approximate lethal threshold established was 900 p.p.m., produced by diluting 1 part of waste No. 4 with 1,110 parts of water. This waste product contained a high concentration of Hcl as was mentioned in the discussion under table 4 (page 9). Apparently the high toxicity of this waste was due to its acidity.

Table 10. Survival Times of Black Bullheads in Different Concentrations of Waste No. 5 (Ethyl Benzene Composite Wash)

Experiment started August 15, 1942.

p.p.m. of Stock Solution	No. of Fish Tested	Survival Time Hrs.	Survival Time Min.	Remarks
50,000	1	..	15	
50,000	1	..	18	
10,000	2	8	..	
5,000	1	11	30	
5,000	1	12	..	
3,000	1	15	..	
3,000	1	24	..	
2,000	1	12	30	
2,000	1	48	..	
1,600	1	26	30	
1,600	1	..	..	Lived at least 69 hrs.; died at night
1,400	1	..	..	" " " 85 " " " "
1,400	1	96+	..	Fish still alive at end of test
1,200	2	96+	..	" " " " " " "
1,000	2	96+	..	" " " " " " "
800	2	96+	..	" " " " " " "
500	2	96+	..	" " " " " " "
Control	2	96+	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 10.-Bullheads were able to survive only 15 and 18 minutes in a concentration of 50,000 p.p.m. of waste No. 5. Higher concentrations would be expected to be more quickly lethal. No fish died in concentrations below 1,200 p.p.m. which was thus established as the approximate lethal threshold. This concentration was produced by a dilution of 1 part of waste No. 5 to 833 parts of water. The probable toxic substance present in this waste is for the most part aluminum chloride which was mentioned on page2.

RESULTS OBTAINED FROM TESTS CONDUCTED WITH TREATED WASTES NO. 4 (Ethyl Benzene HCl Scrubber) AND WASTE NO. 5 (Ethyl Benzene Composite Wash)

Waste No. 4 (Ethyl Benzene HCl Scrubber) was especially high in HCl content and waste No. 5 (Ethyl Benzene Composite Wash) had a pH of 2.8. It was suggested by T. J. Powers of the Dow Chemical Co. that any samples of wastes showing an excess of alkali or acid be neutralized before testing, "since we have sufficient dilution water to buffer these wastes." Wastes No. 4 and 5 proved to be the most highly toxic of the five samples tested and both wastes were acid in reaction. Sodium hydroxide was used to neutralize the acidity in these wastes. In treating waste No. 4, the chemical reaction which took place regarding the HCl was as follows:

$$NaOH + HCl \quad NaCl + H_2O$$

Enough NaOH was added to satisfy the Cl ion until a pH of 7.2 to 7.6 was attained. There was no evidence of any precipitate and the physical properties appeared to be unchanged. This solution was then tested for its toxicity.

During the treatment of waste No. 5 (Ethyl Benzene Composite Wash) with sodium hydroxide (NaOH) a heavy precipitate was thrown down. The chemical analyses of waste No. 5 indicated that it contained 69,200 p.p.m. of aluminum chloride ($AlCl_3$). The pH of this waste was 2.8. Upon treating this waste with NaOH, the following chemical reactions probably took place:

$$AlCl_3 + 3(Na^+ + OH^-) \longrightarrow Al(OH)_3 + 3(Na^+ + Cl^-)$$

or

$$H_3AlO_3 + 3(Na^+ + OH^-) \rightleftharpoons (3Na^+ + AlO_3) + 3H_2O$$

$$HAlO_2 + (Na^+ + OH^-) \rightleftharpoons (Na^+ + AlO_2^-) + H_2O$$

The first chemical reaction apparently took place as there was a heavy precipitate of aluminum hydroxide ($Al(OH)_3$). The latter 2 chemical reactions take place when an excess of the base is added, the aluminum hydroxide failing the precipitate because it reacts as an acid, H_3AlO_3 or $HAlO_2$ ($Al_2O_3 \cdot H_2O$), with the base. As a result of the chemical reaction obtained, it was necessary to filter the treated waste and conduct toxicity tests on both the supernatant and the precipitate. The pH of the treated waste was 6.9. The concentrations of the precipitate and the supernatant were computed on the basis of the original known volume of the untreated waste. As an example, a given quantity of the waste was neutralized and the precipitate obtained from the known quantity was filtered and washed and enough dilution water added to bring it up to its original volume. Likewise enough dilution water was added to the supernatant to bring it up to the original volume. The supernatant and the precipitate were then considered as full strength solutions.

Table 11. Survival Times of Smallmouth Bass in Different Concentrations of Neutralized Waste No. 4 (Ethyl Benzene HCl Scrubber)

Experiment started September 23, 1942.

p.p.m. of Treated Waste	No. of Fish Tested	Survival Time Hrs.	Min.	Sec.	Remarks
500,000	1	..	..	9	
500,000	1	..	..	11	
100,000	1	..	55	..	
100,000	1	1	3	..	
75,000	1	2	..	..	
75,000	1	2	12	..	
65,000	1	7	..	..	
65,000	1	16	..	..	
55,000	2	..	..	..	Lived at least 73 hrs.; died at night
50,000	2	96+	..	..	Fish still alive at end of test
40,000	2	96+	..	..	" " " " " " "
30,000	2	96+	..	..	" " " " " " "
20,000	2	96+	..	..	" " " " " " "
10,000	1	6	55	..	
10,000	3	96+	..	..	Fish still alive at end of test
Control	2	96+	..	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 11.-Concentrations of neutralized waste No. 4 above 75,000 p.p.m. were highly toxic, requiring only a few hours to produce death. Concentrations below 50,000 p.p.m. were not toxic enough to produce death in the 96 hour test. One fish did die in the 10,000 p.p.m. test, but in consideration of the facts that death occurred in less than 7 hours and that the other 3 fish survived in the same concentration, it was concluded that the fatality was caused by factors other than the toxic substances present in this waste. For this reason, the data from this fish was excluded in computing the toxicity threshold, which was computed as 50,000 p.p.m. This concentration was prepared by a dilution of 1 part of neutralized waste No. 4 (Ethyl Benzene HCl Scrubber) to 19 parts of water.

Table 12. Survival Times of Black Bullheads in Different Concentrations of Neutralized Waste No. 4 (Ethyl Benzene HCl Scrubber)

Experiment started September 19, 1942.

p.p.m. of Treated Waste	No. of Fish Tested	Survival Time Hrs.	Min.	Sec.	Remarks
400,000	1	..	..	18	
400,000	1	..	..	23	
300,000	1	..	..	37	
300,000	1	..	..	42	
250,000	1	..	..	31	
250,000	1	..	..	44	
200,000	1	..	..	41	
200,000	1	..	1	1	
150,000	1	..	46	..	
150,000	1	..	58	..	
120,000	1	1	22	..	
120,000	1	1	56	..	
110,000	2	..	..	..	Lived at least 4 hrs. 35 min.; dead at10 hrs
100,000	2	..	..	..	Lived between 10-18 hrs.; died at night
90,000	1	..	..	..	Lived at least 18 hrs.
90,000	1	20	30	..	
80,000	2	96+	..	..	Fish still alive at end of test
70,000	2	96+	..	..	" " " " " " "
60,000	2	96+	..	..	" " " " " " "
50,000	2	96+	..	..	" " " " " " "
Control	2	96+	..	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 12.-Concentrations above 200,000 p.p.m. of neutralized waste No. 4 were highly toxic, producing death in less than 1 minute. Concentrations ranging between 100,000 and 200,000 p.p.m. were somewhat variable, but in all tests required over an hour to produce lethal effects. All of the fish tested were able to tolerate concentrations of 80,000 p.p.m. and below. The approximate lethal threshold established was 80,000 p.p.m. of neutralized waste No. 4. This concentration was prepared by a dilution of 1 part of neutralized waste No. 4 to 11.5 parts of water.

Obviously there are toxic principles other than acidity in the Ethyl Benzene HCl Scrubber waste. Experiments run simultaneously on salt wastes indicate that much if not all of the toxicity to bass and to bullheads of neutralized waste No. 4 can be attributed to the known chloride content.

Table 13. Survival Times of Black Bass in Different Concentrations of Neutralized Waste No. 5 (Ethyl Benzene Composite Wash)

Experiment started October 10, 1942. For method of treatment see p. 3 Part A, Clear Supernatant

P.P.M. of Treated Waste	No. of Fish Tested	Survival Time hrs.	Min.	Sec.	Remarks
100,000	1	..	6	..	
100,000	1	..	8	30	
75,000	1	..	50	..	
75,000	1	1	5	..	
60,000	1	8	28	..	
60,000	1	20	..	..	
50,000	1	52	15	..	
50,000	1	96+	..	..	Fish still alive at end of test
40,000	2	96+	..	..	" " " " " " "
30,000	2	96+	..	..	" " " " " " "
20,000	1	6	..	..	
20,000	3	96+	..	..	Fish still alive at end of test
10,000	2	96+	..	..	" " " " " " "
Control	2	96 +	..	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 13, Part A.-Concentrations above 75,000 p.p.m. of the supernatant from the neutralized stock solution were highly toxic, killing the fish in less than 1 hour. Tests on concentrations ranging between 50,000 and 75,000 p.p.m. indicated only a moderate toxicity. The approximate lethal threshold for the clear supernatant of neutralized waste No. 5 was established at 40,000 p.p.m. This concentration was prepared by a dilution of 1 part of the clear supernatant to 24 parts of water. Two tests were run on the 20,000 p.p.m. concentration as the first test resulted in the death of 1 fish in less than 6 hours, and it was thought that this fish died from causes other than the toxic substances present in this waste. Both of the 2 fish used in the second test survived the 96 hour test period, which offered further evidence that the death of the fish in the first test was caused by other factors. For this reason, the death of the one fish was ignored in computing the approximate lethal threshold.

Table 13, Part B. Washed Precipitate

p.p.m. of Precipitate	No. of Fish Tested	Survival Time Hrs.	Min.	Remarks
1,000,000	1	..	8	
1,000,000	1	..	11	
500,000	1	..	21	
500,000	1	..	25	
400,000	1	..	24	
400,000	1	..	29	
200,000	2	3-5	..	
100,000	2	6-14	..	
50,000	1	14	10	
50,000	1	17	..	
35,000	1	61	..	
35,000	1	77	..	
25,000	2	96+	..	Fish still alive at end of test
Control	2	96+	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 13, Part B.-Concentrations above 200,000 p.p.m. of the washed precipitate were highly toxic, producing death in a few minutes. A concentration of 25,000 p.p.m. was not toxic enough to kill the bass in a 96 hour period. This concentration was prepared by a dilution of 1 part of the precipitate to 39 parts of water. The fish in the concentration of 25,000 p.p.m. exhibited considerable agitation during the entire run. This agitated condition might have been caused by the presence of irritating substances in continuous contact with the respiratory organs of the fish, as the bubbling air in the test jars, created enough current in the solution to keep the precipitate in suspension at all times. Carpenter[1] found that lead salts were not definitely toxic to fish life but that they coagulated the mucus of the gills, forming a colloidal film that caused asphyxiation. Later Carpenter[2] showed that none of the metal ions entered the body. Jones[3] corroborated these findings and extended them to all metals. The possibility that partial asphyxiation existed in these tests is proposed.

[1] K. E. Carpenter. 1925. The Biological factors involved in the destruction of river fisheries by pollution due to lead mining. Ann. Applied Biol., 12: 1-13.

[2] K. E. Carpenter. 1930. Further research on the action of metallic salts on fishes. Journ. Exp. Zool., 56: 407-422.

[3] J. R. E. Jones. 1939. The Relation between the electrolytic solution pressures of the metals and their toxicity to the stickleback (Gasterosteus aculeatus). Jour. Exp. Biol., 16: 425-437.

Table 14. Survival Times of Black Bullheads in Different Concentrations of Neutralized Waste No. 5 (Ethyl Benzene Composite Wash)

Experiment started October 10, 1942.

Part A, Clear Supernatant

p.p.m. of Super-natant	No. of Fish Tested	Survival Time Hrs.	Min.	Sec.	Remarks
200,000	1	..	2	30	
200,000	1	..	3	15	
100,000	1	..	41	..	
100,000	1	..	46	..	
85,000	1	20	15	..	
85,000	1	23	45	..	
75,000	1	74	..	..	
75,000	1	96+	..	..	Fish still alive at end of test
65,000	2	96+	..	..	" " " " " " "
60,000	2	96+	..	..	" " " " " " "
50,000	2	96+	..	..	" " " " " " "
40,000	2	96+	..	..	" " " " " " "
30,000	2	96+	..	..	" " " " " " "
Control	2	96+	..	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 14, Part A.-Concentrations above 100,000 p.p.m. are highly toxic, producing death in less than 1 hour. In all of the tests with concentrations less than 75,000 p.p.m. the fish were able to survive. The approximate lethal threshold established was 65,000 p.p.m. which represents a dilution of 1 part of the clear supernatant to 14.4 parts of water.

Table 14, Part B, Washed Precipitate

p.p.m. of Percipitate	No. of Fish Tested	Survival Time Hrs.	Survival Time Min.	Remarks
1,000,000	1	..	21	
1,000,000	1	..	35	
500,000	1	1	11	
500,000	1	1	58	
400,000	1	1	31	
400,000	1	2	10	
200,000	1	11	55	
100,000	2	30-37	..	
75,000	2	96+	..	Fish still alive at end of test
50,000	2	96+	..	" " " " " " "
Control	2	96+	..	" " " " " " "

INTERPRETATION OF DATA IN TABLE 14, Part B.-Concentrations above 100,000 p.p.m. of the precipitate were highly toxic, producing death in a few hours. The approximate lethal threshold established was 75,000 p.p.m. This concentration was prepared by a dilution of 1 part of precipitate to 12.3 parts of water. The bullheads used in this test on the washed precipitate reacted as did the bass.

RESULTS OF TOXICITY TESTS CONDUCTED WITH WASTE NO. 5 (Ethyl Benzene Composite Wash), TREATED WITH CITRIC ACID AND THEN NEUTRALIZED.

It will be recalled, that during the process of neutralizing waste No. 5 (Ethyl Benzene Composite Wash) with sodium hydroxide, a heavy precipitate of aluminum hydroxide was encountered, Mr. T. J. Powers of the Dow Chemical Co. was informed of this reaction and was asked to suggest a possible treatment method which would prevent the formation of this heavy precipitate. His reply is quoted in part:

"The Al may be kept in solution when the solution is neutralized to a pH of 7.0 by addition of citric acid, tartaric acid, or Rochelle Salts (K.Na, tartrate). Other organic compounds may possibly be satisfactory but citrate and tartrate are the most commonly used anions to hold Al or Fe in solution.

"According to Merck Chemical Catalogue April 1942, citric acid is the cheapest compound of the three.

"Potassium and Sodium Tartrate U. S. P. 53 cents per pound in lots of 25 lbs.

"Tartaric acid U. S. P. 84 cents per pound in lots of 50 pounds.

"Citric acid U. S. P. 25 cents per pound in lots of 112 pounds.

"Sodium citrate is more expensive than citric acid.

"25 c/c. sample requires 3.0 g. of citric acid.

"3 grams of citric acid (monohydrate) $H_3C_6H_5O_7 \cdot H_2O$ will hold the Al of 25 c/c. of sample 5 in solution when neutralized to a pH of 7. neutralized with NaOH."

The suggestions furnished by the Dow Chemical Co. were followed in the treatment of waste No. 5. Citric acid was used to prevent the precipitate of Al when waste No. 5 was almost neutralized with NaOH to a pH of 6.9. The chemical reactions which took place are complicated; all that can be stated is that the Al is bound to the citrate and precipitation prevented.

An adequate supply of waste No. 5 (Ethyl Benzene Composite Wash) was treated in this manner and dilutions were prepared from this supply. Following are the results obtained from tests conducted with this waste.

Table 15. Survival Times of Smallmouth Bass in Different Concentrations of Waste No. 5 (Ethyl Benzene Composite Wash), Treated with Citric Acid and Neutralized with NaOH

Experiment started October 26, 1942.

p.p.m. of Treated Solution	No. of Fish Tested	Survival Time			Remarks
		Hrs.	Min.	Sec.	
500,000	1	..	..	51	
500,000	1	..	1	11	
250,000	1	..	1	56	
250,000	1	..	2	15	
100,000	1	..	9	30	
100,000	1	..	11	..	
60,000	1	1	40	..	
60,000	1	2	10	..	
40,000	1	3	10	..	
40,000	1	4	5	..	
30,000	2	5-8	..	..	
20,000	1	47	35	..	
20,000	1	96+	..	..	Fish still alive at end of test
10,000	1	58	..	..	
10,000	1	96+	..	..	Fish still alive at end of test
Control	2	96+	..	..	Fish still alive at end of test

INTERPRETATION OF DATA IN TABLE 15.-All of the tests conducted with concentrations above 30,000 p.p.m. proved to be highly toxic, causing death to bass in less than 8 hours. All of the tests conducted with concentrations of less than 30,000 p.p.m. proved less toxic. The lowest concentration tested (10,000 p.p.m.) proved to be slightly toxic. The approximate lethal threshold was not computed, but would fall in the range of concentrations below 10,000 p.p.m. The reason for not finding the approximate lethal threshold was that it seemed unwarranted as by the other treatment method on waste No. 5 (see table 13) the approximate lethal threshold established was well above 10,000 p.p.m.

Table 16. Survival Times of Black Bullheads in Different Concentrations of Waste No. 5 (Ethyl Benzene Composite Wash), Treated with Citric Acid and Neutralized with NaOh

Experiment started October 26, 1942.

p.p.m. of Treated Solution	No. of Fish Tested	Survival Time			Remarks
		Hrs.	Min.	Sec.	
500,000	1	..	1	36	
500,000	1	..	1	57	
250,000	1	..	3	..	
250,000	1	..	3	45	
100,000	1	..	38	..	
100,000	1	..	46	..	
80,000	1	..	40	..	
80,000	1	..	48	..	
60,000	1	1	2	..	
60,000	1	1	9	..	
50,000	1	23	..	..	
50,000	1	29	..	..	
40,000	2	40-52	..	..	
30,000	2	48-60	..	..	
Control	2	96+	..	..	Fish still alive at end of test

INTERPRETATION OF DATA IN TABLE 16. All of the concentrations tested proved to be toxic. The approximate lethal threshold would be below 30,000 p.p.m. As a higher toleration range was achieved by the other treatment method on waste No. 5 (see table 14, page 21) the additional tests required to attain an approximate lethal threshold seemed unwarranted.

SUMMARY AND DISCUSSION

Four of the five waste products of the styrene process of manufacturing synthetic rubber, tested under laboratory conditions proved to be toxic enough to kill smallmouth bass in less than 96 hours. The only waste which failed in this respect was waste No. 1 (Mixed Waste Waters, without wastes No. 2 and 5). The remaining four wastes are listed below according to their degree of toxicity, from highest to lowest:

No. 4 (Ethyl Benzene HCl Scrubber)
No. 5 (Ethyl Benzene Composite Wash)
No. 3 (Styrene Still Waste)
No. 2 (Ethylene Waste)

The neutralizing of the acid wastes No. 4 and 5, by sodium hydroxide, brought about a reduction in toxicity (see table 17), which changed their sequence (as given above), to the following order:

No. 5 (Ethyl Benzene Composite Wash) precipitate
No. 5 (Ethyl Benzene Composite Wash) supernatant
No. 4 (Ethyl Benzene HCl Scrubber)
No. 3 (Styrene Still Waste)
No. 2 (Ethylene Waste).

Table 17. A SUMMARY OF THE TOXICITY THRESHOLDS ESTABLISHED BY THE SMALLMOUTH BASS AND THE BLACK BULLHEAD WITH TREATED AND UNTREATED WASTE PRODUCTS FROM THE STYRENE MANUFACTURING PROCESS

Species and Wastes	Lethal Threshold		Dilution Used for Making Concentration		Reduction in Toxicity
	untreated	treated	untreated	treated	
Black Bass					
Mixed Waste Waters	1,000,000		0		
Ethylene Waste	50,000		1:19		
Styrene Still Waste	50,000		1:19		
Ethyl Benzene HCl Scrubber	500	50,000	1:1999	1:19	100 X
Ethyl Benzene Composite Wash	800	*50,000	1:1249	1:19	62.5 X
Black Bullheads					
Mixed Waste Waters	1,000,000		0		
Ethylene Waste	100,000		1:9		
Styrene Still Waste	1,000,000		0		
Ethyl Benzene HCl Scrubber	900	80,000	1:1110	1:11.5	89 X
Ethyl Benzene Composite Wash	1,200	*70,000	1:833	1:13.2	59 X

*Lethal Threshold based on the average threshold of the supernatant and the precipitate.

Three of the 5 waste products from the styrene manufacturing process proved to be highly toxic (see table 17) to the black bullhead and are listed in the decreasing order of their toxic strength:

No. 4 (Ethyl Benzene HCl Scrubber)
No. 5 (Ethyl Benzene Composite Wash)
No. 2 (Ethylene Waste)

Further reduction in toxicity was achieved by neutralizing wastes No. 4 and 5 with sodium hydroxide.

The black bullheads were approximately twice as resistant as were the smallmouth bass to the toxic substances in these wastes. Published and original work both indicate the bullheads are very resistant fish, whereas the smallmouth bass is a moderately susceptible species. The sluggishness of the bullheads and the constant activity of the bass, as displayed in the tests here reported, suggest a possible explanation for the differential susceptibility. Carpenter (1927), Cole (1939), and others have shown that degree of susceptibility is correlated with the metabolic rate.

Waste No. 4 (Ethyl Benzene HCl Scrubber) contained a high percentage of HCl and was found to be highly toxic to both species of fish tested. The toxicity of this waste was greatly reduced when this acid condition was corrected. Apparently a great part but not all of the toxicity was due to the presence of HCl.

Waste No. 5 was likewise acid in reaction and its toxicity was also reduced by neutralization. In treating waste No. 5, a heavy precipitate resulted. This precipitate, consisting of aluminum hydroxide, plus materials fluxed down with this chemical, proved to be toxic. As this substance is insoluble it would accumulate as a sludge. It is believed that even with great dilution the aluminum chloride of the unneutralized waste would hydrolize in any natural water to produce the harmful sludge. Considerable accumulations of such sludge might destroy spawning beds, food and other natural conditions essential to fish production, even though the fish themselves would not be directly killed.

SUGGESTIONS REGARDING TREATMENT AND DISPOSAL OF WASTES

1. Though some of the five wastes did not prove to be toxic to fish in the laboratory tests, and others were not highly toxic, it would be advisable to allow a considerable margin of safety in computing dilution requirements from these tests. Other species of fish, or breeding fish, or fish in their young stages, may be much more susceptible to the toxic substances.

2. Waste No. 2 (Ethylene Waste) produced a slimy, perhaps bacterial sludge in the tests, even at non-toxic concentrations. This condition might cause a pollution problem. Further research is needed to determine whether this slimy sludge is harmful to fish life, and to determine the effect of this waste on the dissolved oxygen content of the waters into which the waste is discharged.

3. The treatment of waste No. 4 (Ethyl Benzene HCl Scrubber) involves some problems. The untreated waste required a dilution of about 2,000 times to be rendered non-toxic in 96 hours to bass in the experiments; a much greater dilution would be required to insure that no ill effects would result in routine disposal. The neutralizing of this waste by hydroxide reduced its toxicity under experimental conditions about 100 times, so that only 19 dilutions were required. Whether neutralization would be quickly affected by diluting waters would depend on the buffer capacity of these waters.

4. The toxicity of waste No. 5 (Ethyl Benzene Composite Wash) was also reduced by neutralization, but complications resulted. When NaOH alone was used in the treatment, the $AlCl_3$ was changed to $Al(OH)_3$ which precipitated out as a fine flux, which proved definitely toxic. The probable accumulation of this flux as a harmful sludge is mentioned above (page 27). This sludge would probably be produced by the hydrolysis of the untreated waste, and is therefore a problem to be considered, whether or not the waste is to be neutralized before disposal. Treatment of waste No. 5 with certain chemicals (as citric acid), before neutralization, prevented the precipitation of the aluminum, but proved less effective in decreasing the toxicity.

Mechanical removal of the $Al(OH)_3$ flux would reduce by about 50 times the toxicity of the waste and hence the amount of dilution water required.

Removal of the $Al(OH)_3$ sludge might be effected by treatment with waste No. 4, which contains about 15% HCl, so as to dissolve the precipitate and to produce $AlCl_3$, which might then be reused in the plant. At the same time the toxicity of waste No. 4 would thus be decreased. We are of course not in position to say whether these suggestions are practicable.

Samples, analyses and advice supplied by T. J. Powers, Dow Chemical Co.

Tests performed by George N. Washburn, with cooperation of John T. Greenbank.

Report prepared by Carl L. Hubbs and George N. Washburn.

INSTITUTE FOR FISHERIES RESEARCH

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