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SITE-SPECIFIC ACUTE AND CHRONIC AQUATIC TOXICITY
TESTING--WESTERN LAKE SUPERIOR SANITARY
DISTRICT TREATMENT PLANT EFFLUENT STUDIES

By

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INTRODUCTION

A need exists for the characterization of natural waters regarding their capacities for reducing the toxic effects of discharged wastes to aquatic organisms. An understanding of effects of naturally occurring ligands in reducing toxicities of various wastes would facilitate the issuance of variances for discharge permit limits upon an environmentally sound site-specific basis.

A study was conducted by the University of Wisconsin-Superior, Superior, WI, to determine the toxicity to aquatic organisms of effluent from the Western Lake Superior Sanitary District (WLSSD) treatment facility in Duluth, MN. Exposures were conducted on site and at the University of Wisconsin-Superior campus with organisms native to the area and thought to have a high level of sensitivity to potentially toxic effluent. Water from the St. Louis River upstream from the WLSSD discharge point and University of Wisconsin laboratory water were used as the control water.

Bioassays were conducted using static and flowing conditions with grab and composited samples of two processed waters within the WLSSD treatment plant and seven species of aquatic organisms. Water samples were collected at various times throughout the summer of 1982.

METHODS

Test Water Description

WLSSD treatment plant processed waters and St. Louis River water were employed as test waters. WLSSD water was examined for suspected toxicity while the river water was used as a 'clean' water control for comparison.

Two types of WLSSD treatment plant processed waters were tested - filtered and final effluent. Filtered effluent was water which had been fully treated except for chlorination. This water was called filtered effluent because it is filtered immediately prior to chlorination. Final effluent was filtered effluent that had been chlorinated and subsequently dechlorinated before discharge to the harbor. Filtered effluent was not released to the harbor during this study without chlorination and dechlorination.

St. Louis River water was collected by submerging 5 gal polypropylene carboys at a site located at the end of the City of Superior, WI boat landing pier on the downstream side of the Arrowhead bridge.

Water Chemistry Determinations

Measurements of pH (Method 424), total alkalinity (Method 403), total hardness (Method 309 B) and dissolved oxygen (Method 422 B) were made on exposure water samples according to Standard Methods for the Examination of Water and Wastewater (APHA, 1975). Water analysis was performed once either at the start or during the exposure period for static bioassays. For the flow-through tests, samples for pH, alkalinity, and hardness were taken every day while dissolved oxygen was measured at least every other day. Alkalinity and pH determinations were completed on the same day they were taken or stored at 3 C overnight before the measurements were made. Samples from the WLSSD treatment plant for hardness determination required fivefold dilution prior to analysis. Without dilution,

the hardness endpoint was not clear. Chlorine content and pH of the tested effluents at the time of discharge are available from WLSSD records and not reported in this report.

Results of final effluent exposure water chemistry measurements made during the study period were quite variable. The geometric mean pH was 7.76 and ranged from 6.7 to 8.6. The mean total alkalinity was $214 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3 with a range from 147 to $362 \text{ mg}\cdot\text{L}^{-1}$. The mean value for total hardness was $210 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3 , ranging from 121 to $252 \text{ mg}\cdot\text{L}^{-1}$. The mean and standard deviation of dissolved oxygen concentrations were $6.8 \pm 1.0 \text{ mg}\cdot\text{L}^{-1}$ ($n=118$).

Results of filtered effluent exposure water chemistry determinations were also highly variable. The geometric mean pH was 7.95 with a range of 6.5 to 8.6. The mean total alkalinity was $193 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3 , ranging from 142 to $278 \text{ mg}\cdot\text{L}^{-1}$. Mean total hardness was $235 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3 and ranged from 208 to $267 \text{ mg}\cdot\text{L}^{-1}$. The mean and standard deviation of dissolved oxygen concentrations were $6.8 \pm 0.9 \text{ mg}\cdot\text{L}^{-1}$ ($n=88$).

Organisms

Test organisms used during the series of exposures included Daphnia magna, (water flea), Gammarus pseudolimnaeus, Hyalella azteca (amphipods), Pimephales promelas (fathead minnow), Diplectrona sp. (caddis fly), Hexagenia sp. and Stenonema sp. (mayflies). D. magna were cultured at the University of Wisconsin-Superior with 30 mg of yeast and trout chow (4:1 wt:wt) per liter of water. G. pseudolimnaeus were collected from the Eau Claire River upstream of the city of Gordon, WI on 17 March, 1982. On the same date, H. azteca were collected from the St. Croix River immediately below the St. Croix Flowage dam near Gordon, WI. The amphipods were held at 15 C and fed ground, dried, maple leaves prior to exposure. Fathead minnows were reared in the University of Wisconsin-

Superior culture facilities and fed 48 hr old Artemia sp. (brine shrimp) until testing. Diplectrona sp., Hexagenia sp. and Stenonema sp. were all collected on 9 August 1982 from Mission Creek at Fond du Lac, MN, the Brule River at the Wisconsin Ranger Station, Brule, WI and from Interfalls Lake at Pattison Park, WI, respectively.

During all tests observation for mortalities were made at least once daily.

Static Bioassays

Static tests were conducted on grab samples and on one day and seven day composite samples of WLSSD effluent collected at different times throughout the summer. The tests were run at the University at room temperature which ranged from 21-23 C for the exposures.

The 14 June, 1982, effluent was screened for toxicity with five 30 day-old P. promelas, six G. pseudolimnaeus and eleven H. azteca. They were all tested in 1 L beakers containing 900 mL of the grab sample. In addition, five young P. promelas and five D. magna were tested in 250 mL beakers containing 200 mL of grab sample.

Static exposures performed from 23 June through 2 August, 1982 used grab and one or seven day composite samples of WLSSD effluent. Quadruplicate exposures of five P. promelas and five D. magna in 200 mL of 100% effluent were made in 250 mL beakers. For each exposure, quadruplicate control exposures were performed using laboratory water from the University of Wisconsin-Superior.

Grab samples of WLSSD effluent taken on 8, 9, and 10 September, 1982, were used to expose P. promelas. The samples were diluted with St. Louis River water to provide 0, 25, 50, 75, and 100 percent solutions in duplicate. Ten fish total were exposed to each concentration.

Flow-Through System Description

A continuous flow system was designed to expose various types of organisms to the WLSSD treatment plant final effluent and to St. Louis River water. Temperature control and aeration were provided to the test waters. Organisms were exposed to 100% final effluent and 100% St. Louis River water. An identical exposure apparatus was used for each water type. The apparatus consisted of an exposure tank within a larger tank (water bath). Two types of exposure tanks were employed throughout the three month test period: a large tank into which two small compartmental inserts were placed and a smaller exposure tank with no compartments. Each exposure tank was equipped with a stand pipe and drain tube. Metering pumps delivered test waters to the exposure tanks via stainless steel tubing. The point of inflow was always opposite the tank stand pipe. The WLSSD final effluent diverted to a sampling box was the source of treatment plant test water. St. Louis River water that was collected periodically was pumped from a 56 L polypropylene reservoir into the other exposure tank. To facilitate temperature control, a flow-splitting tank distributed cold tap water equally to each of the two water bath tanks. Adequate oxygenation was supplied by a dual outlet air pump. An air stone was placed in each exposure tank in the area of test water inflow.

Flow-Through Bioassays

The first flow-through exposures were conducted with Diplectrona sp., Hexagenia sp., P. promelas, and Stenonema sp. Five each of Diplectrona sp. and Hexagenia sp., and ten each of P. promelas and Stenonema sp., were exposed in duplicate. To separate the organisms and expose them in duplicate, two 3 compartment inserts and two stainless steel mesh cages were used within a large exposure tank that measured 34.5 x 20.5 x 10.5 cm. The inserts measured

15.0 x 15.4 x 13.8 cm and had two ends covered with 505 μm Nyltex[®] mesh to allow water exchange. The cages were 9.4 cm long by 4.0 cm in diameter and closed with a neoprene stopper. Ten Stenonema sp. were loaded in each of the cages and positioned in the center of insert compartments. The other organisms were exposed separately in insert compartments. The tank volume exchange rate with final effluent was 5.8 volumes $\cdot\text{day}^{-1}$.

In the second flow-through exposure, twenty fathead minnows were placed into a tank 9.8 x 23 x 11.8 cm containing 2.65 L of water. Flow was increased to provide 20 tank volume exchanges $\cdot\text{day}^{-1}$.

In both exposures a duplicate system was operating using St. Louis River water, collected daily, as a reference. All chambers were covered with a glass plate to prevent contamination and evaporation.

RESULTS

Bioassays of WLSSD effluents (prior to chlorination and immediately after dechlorination) were conducted with waters sampled 14 June through 10 September 1982. Both static and flow-through assays were conducted, with the static tests performed at the UW-Superior testing facility and the flow-through testing done at the WLSSD treatment facility.

Static Bioassays

Twenty-three tests were run with WLSSD effluent and St. Louis River reference water. Four species of aquatic organisms (P. promelas, G. pseudolimnaeus, H. azteca, and D. magna) were included in the tests. Water samples were collected on thirteen occasions from three sites (St. Louis River, WLSSD effluent after final filtering prior to chlorination, and final processed water after chlorination and dechlorination). Some deaths occurred in St. Louis River

reference water in 26.1% of the tests. Similar numbers of deaths in reference and processed waters precluded the use of bioassay data with Daphnia for the composited water samples collected on July 12 and 19 and with P. promelas for water samples collected on July 13 and September 9. Filtered effluent from WLSSD had increased toxicity when compared to St. Louis River water in 46.2% of the remaining tests (Table 1) and the final WLSSD effluent showed increased toxicity when compared to St. Louis River water in 38.9% of the remaining tests. However, mortalities of 5-10% represented the deaths of only 1-2 organisms.

The greatest differences in mortalities of organisms in reference and processed waters were with P. promelas and grab samples of the final effluent collected on 29 July, 8 September, and 10 September. On these three dates (23.1% of sampling dates), mortalities ranged from 33 to 100% in the undiluted final effluent. A portion of the samples collected on 29 July and 10 September were held seven days with aeration and retested with P. promelas. Toxicity was reduced from the initial tests. Mortalities decreased from 40 to 5% for the 29 July grab sample and from 100 to 0% for the 10 September grab sample (Table 1).

Toxicity of the 10 September final effluent was estimated by diluting the effluent with St. Louis River water and exposing P. promelas for 96 hr. The dilutions were 25, 50, and 75% of the processed water. A Spearman-Kärber (trimmed) estimate was made of the median lethal concentration (LC_{50}) for this water sample (Hamilton, et al. 1977). The 96 hr LC_{50} was 55.4% of pure processed water.

Flow-Through Bioassays

Initially, four species of aquatic organisms (P. promelas, Hexagenia sp., Stenonema sp., and Diplectrona sp.) were exposed to St. Louis River water and WLSSD final effluent for 96 hr starting on August 16. The St. Louis River

TABLE 1. Effects upon Survival of Several Species of Aquatic Organisms Exposed to St. Louis River Water (Reference) and Two Processed Waters of the Western Lake Superior Sanitary District (WLSSD) for 96 hr. Processed Waters were Sampled at the Stated Times by Instantaneous Grabs or by Compositing with a Pump for 1 or 7 Days. Reference Waters were all Grab Samples. Exposure Water Temperatures were 21-23 C.

Processed Water Collection			Exposed Species	Age	Percent Mortality		
Date	Time	Type			Reference	Filter ^{a/}	Final ^{a/}
6/14	1430	Grab	<u>Pimephales promelas</u>	3 day	0	0	0
				30 day	0	0	0
			<u>Gammarus pseudolimnaeus</u>	adult	0	0	0
			<u>Hyalella azteca</u>	adult	0	9.1	0
			<u>Daphnia magna</u>	adult	0	0	0
6/23	1455	Grab	<u>Pimephales promelas</u>	9 day	0	10	0
			<u>Daphnia magna</u>	adult	0	20	5
6/29	0838	Grab	<u>Pimephales promelas</u>	3 day	0	10	0
6/30	1115	Grab	<u>Pimephales promelas</u>	5 day	0	8.3	9.8
7/12		7-day Composite	<u>Pimephales promelas</u>	9 day	0	0	N.T. ^{b/}
			<u>Daphnia magna</u>	adult	11.8	5.0	N.T.
7/13		1 day Composite	<u>Pimephales promelas</u>	10 day	5	4.8	5
7/19		1 day Composite	<u>Pimephales promelas</u>	11 day	0	0	0
			<u>Daphnia magna</u>	adult	11.8	20	10
7/19		7 day Composite	<u>Daphnia magna</u> ^{c/}	adult	0	5	0
7/29	1430	Grab	<u>Pimephales promelas</u>	5 day	10	N.T.	40
7/29 ^{d/}	1430	Grab	<u>Pimephales promelas</u> ^{e/}	13 day	0	N.T.	5
			<u>Pimephales promelas</u>	13 day	0	N.T.	5
8/2	a.m.	Grab ^{f/}	<u>Pimephales promelas</u> ^{e/}	5 day	0	0	5
9/8	p.m.	Grab	<u>Pimephales promelas</u> ^{g/}	0-5 day	10	N.T.	33
9/9	a.m.	Grab	<u>Pimephales promelas</u> ^{g/}	1-6 day	10	N.T.	9.1
9/10	a.m.	Grab	<u>Pimephales promelas</u> ^{g/}	2-7 day	0	N.T.	100
9/10 ^{h/}	a.m.	Grab	<u>Pimephales promelas</u> ^{g/}	8-13 day	0	N.T.	0

a/ Filter is processed water immediately prior to chlorination. Final is the chlorinated and dechlorinated processed water as it leaves the treatment facility. Filter water was aerated during testing, final water was aerated only at stated times (see footnotes e & g).

b/ N.T. = not tested.

c/ 48 h exposure.

d/ Water collected on 7/29 was held and retested on 8/6.

e/ Continuous aeration during exposure to final processed water.

f/ Water collected on 8/2 was aerated and held for testing until 8/7.

g/ Daily 15 minute aeration during exposure.

h/ Water collected on 9/10 held at room temperature and retested on 9/17.

reference water consisted of daily grab samples collected upstream at the Arrowhead bridge and WLSSD final effluent pumped continuously from the treatment plant discharge line. No significant ($p < 0.05$) differences were observed in mortalities between the two tested waters. However, high mortalities in the controls occurred with the exception of the Stenonema bioassay, where no control organisms died. Ten percent mortality occurred in the final effluent test water with Stenonema.

An extended acute flow-through exposure of P. promelas to WLSSD final effluent was then conducted between 1 September and 8 September. The test was designed to proceed for 30 days to observe fish for possible growth differences. However, at 192 hr the test was terminated due to mortality of all organisms in the final effluent test water (Table 2). The reference or control water was St. Louis River water in which 95% of the fish survived the 192 hr exposure.

TABLE 2. On-Site Flow-Through Extended Acute Bioassay of Western Lake Superior Sanitary District (WLSSD) Final Process Water with Pimephales promelas^{a/}

Date	Duration	Percent Mortality	
		St. Louis River	Final Effluent
9/1	24 h	0	0
9/2	48 h	0	0
9/3-6	72-144 h	5	<20 ^{b/}
9/7	168 h	5	<50 ^{b/}
9/8	192 h	5	100

^{a/} Fathead minnows were 15 days old at the start of the extended acute exposure.

^{b/} Effluent was highly colored and percentages are approximations based on observations of netted fish on 9/7 and 9/8.

DISCUSSION

The processed water discharged from the WLSSD treatment facility is not continuously toxic to aquatic organisms on an acute basis. However, significant toxicity does periodically occur. In 23.1% of the samples collected from 14 June to 10 September, 1982, mortalities to fathead minnows in the undiluted final effluent ranged from 33 to 100%.

The grab sample of final effluent collected on 10 September resulted in 100% mortality. When this effluent was diluted with St. Louis River water, the resultant LC_{50} to fathead minnows was 55.4% of the pure processed water. From this it would appear that the mixture of WLSSD final effluent and harbor water would be acutely toxic at times to organisms in the immediate vicinity of the diffuser pipes where the effluent is discharged. Dependent upon the total volume of St. Louis River water for dilution of the WLSSD effluent, toxicity would be diminished as distance from the diffuser pipes increased. The dilution factor can be determined from other studies.

The observation that acute toxicity of the final processed water from WLSSD was greatly reduced upon holding with aeration for a period of seven days indicated that the chemical agents responsible for the toxic effects were volatile or chemically unstable in the water. One possible cause for the observed periodic toxicity that was examined was the concentration of total residual chlorine in the final effluent. Records of the chlorine feed rate and chlorine concentrations in the mixer and final effluent were obtained from WLSSD (Figure 1). Elevated concentrations of chlorine in the final effluent were recorded on 6 September and 9-10 September. Grab samples of final effluent collected on 8 and 10 September resulted in 33 and 100% mortality, respectively, to fathead minnows in static bioassay. The 96 hr LC_{50} for total residual chlorine with fathead minnows was

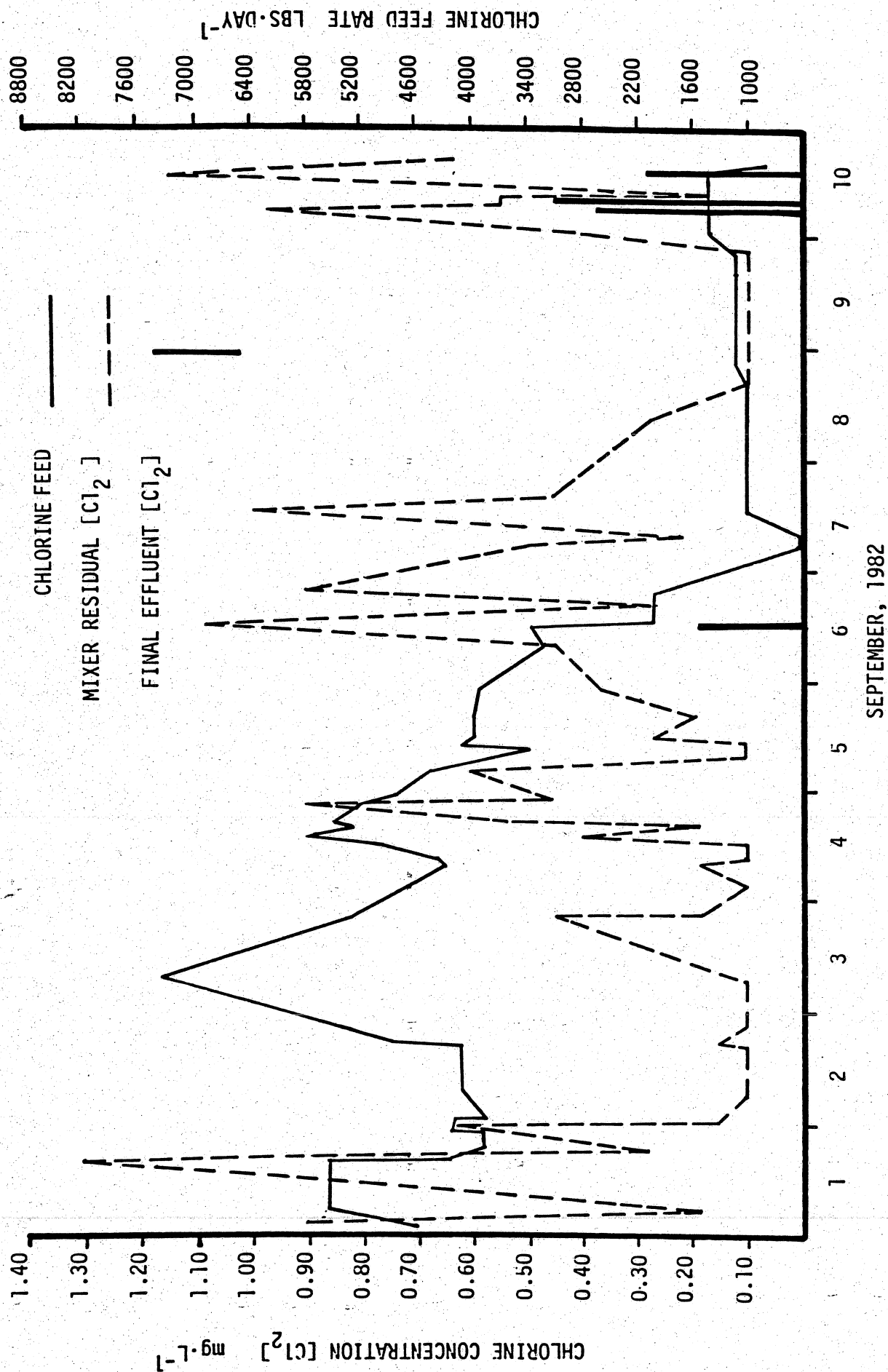


Figure 1. Chlorine feed rate (lbs·day⁻¹) and concentrations of chlorine in the mixer and final effluent (mg·L⁻¹) at the WLSDD wastewater treatment facility from September 1 to September 10, 1982.

reported as 130 and 86 $\mu\text{g}\cdot\text{L}^{-1}$ in two separate tests (Arthur et al., 1975). The 24 hr LC_{50} for fathead minnows was 145 and 140 $\mu\text{g}\cdot\text{L}^{-1}$ as determined by the same authors.

On 6 September, the final effluent chlorine concentration reached a recorded high value of 180 $\mu\text{g}\cdot\text{L}^{-1}$ and on 9-10 September the concentration for the three recorded spikes ranged from 270 to 450 $\mu\text{g}\cdot\text{L}^{-1}$. The toxicity of the final effluent grab sample collected on 10 September may have been caused by elevated concentrations of chlorine on that date. However, possible causes for the mortalities in the static bioassays conducted with grab samples collected on 29 July and 8 September are not known.

In the extended acute flow-through exposure with young fathead minnows that started on 1 September, mortalities began occurring between 3 and 6 September, with complete mortality occurring by 8 September. These mortalities may have resulted from the elevated chlorine concentration on 6 September.

WLSSD personnel indicated that excess carbon dioxide (CO_2) may occasionally be present in sufficient concentrations to cause mortality of fathead minnows. Evidence of high CO_2 content of the final effluent does exist, although no CO_2 measurements were made in this study.

The pH of some grab samples increased with time. Samples taken on 8, 9 and 10 September 1982 had an initial pH of 6.95, 6.81 and 6.61, respectively (WLSSD records). They exhibited a gradual increase of pH to 8.12, 8.55 and 8.45, respectively, when held 1 day for bioassay testing. Five water samples taken during the period, 1 September to 5 September 1982, had a mean pH of 7.13 and ranged from 7.05 to 7.21 when measured within 6 hr after sampling. They exhibited an increase of 0.45 to 0.60 pH units according to plant records of pH for final effluent discharged during this period. It is not known whether these five

samples would have increased to a pH greater than 8.1 as did samples taken during the later sampling period.

During the second flow-through bioassay the total alkalinity of bioassay water was frequently much greater than hardness. For final effluent grab samples taken on 8, 9, and 10 September and analyzed at the end of acute bioassay exposures, the total alkalinity was 317, 362 and 236 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3 , respectively. Corresponding hardness values were 152, 141, and 121 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3 , respectively. The differences between alkalinity and hardness are greater than that of samples taken earlier in the study. Alkalinity and hardness values for the time period 1 September to 5 September 1982 had a mean of 218 and 174, respectively, with a range of values from 210 to 235 and 165 to 187, respectively. The interaction of high pH influent from the Potlatch Corporation with other influent and process waters already within the plant may result in a temporary buffering of the final effluent.

Discharge water from the Potlatch Corporation passes through the Cloquet pumping station and travels to the WLSSD treatment plant where it mixes with other plant influent waters. Highly alkaline discharge water from the Potlatch Corporation reached the Cloquet station approximately at 9:00 a.m., 6 September and continued until termination of our testing on 10 September, 1982. The mean and range of pH values for Cloquet station effluent was 10.36, and 8.77 to 11.32, respectively. Approximately 21 hrs later, the beginning of the alkaline water arrived at the treatment plant influent point, as indicated by a pH increase from 7.48 to 8.14 in 1 hr. For the period 6 September to 9 September, 1982, final effluent mean pH was 6.82 and the range of pH was 6.45 to 7.17. In comparison, final effluent mean pH was 6.59 and ranged from 6.45 to 6.81 during the period 1 September to 5 September, 1982. Alkaline influent was not present then.

Carbon dioxide is readily dissolved into an alkaline solution and the concentration of HCO_3^- and $\text{CO}_3^{=}$ are allowed to increase, subsequently increasing carbonate alkalinity. This is the condition that existed in the Potlatch effluent from 6 to 10 September. Apparently, in-plant precipitation, complexation, and dilution by other influents are sufficient to maintain a final effluent pH near neutrality with elevated alkalinity during the time highly alkaline Potlatch effluent is present.

During the secondary treatment process microbial respiration produces some CO_2 but an excess of free CO_2 may already have been present in the influent. This would tend to preserve the high alkalinity of alkaline influent; that is, it would allow relatively large amounts of CO_2 to remain in solution as HCO_3^- as $\text{CO}_3^{=}$. When the process waters pass from the region of high free CO_2 concentration to a region of lower concentration, CO_2 is evolved according to the following reactions.



When CO_2 is evolved, hydroxyl ion (OH^-) is produced, raising pH of final effluent. These reactions are probably responsible for the pH increase observed for the grab samples described earlier.

This mechanism, although simplistic, may explain a probable cause for the gradual pH increase, marked difference between alkalinity and hardness of final effluent when alkaline influent was present and possibly, test organism mortalities. All of the factors which influence CO_2 concentrations are not

accounted for. High free CO₂ concentrations may have occurred at other times during the study and may be associated with conditions other than alkaline influent.

REFERENCES

- American Public Health Association. 1975. Standard Methods for the Examination of Water and Wastewater (14th ed.). American Public Health Association, Washington, D.C.
- Arthur, J.W., R.W. Andrew, V.R. Mattson, D.T. Olson, G.E. Glass, B.J. Halligan, and C.T. Walbridge. 1975. Comparative toxicity of sewage-effluent distribution to freshwater aquatic life. U.S. Environmental Protection Agency, Duluth, MN. Ecological Research Series EPA-600/3-75-012.
- Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Kärber Method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11:714-719. Correction 12:417(1978).