INTERNATIONAL PACIFIC SALMON FISHERIES COMMISSION

PROGRESS REPORT

No. 33

ACUTE TOXICITY AT THREE PRIMARY SEWAGE TREATMENT PLANTS

by

D. W. MARTENS and J. A. SERVIZI

NEW WESTMINSTER, B. C.
CANADA
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ABSTRACT

Continuous flow bioassays were conducted at three primary sewage treatment plants in the Greater Vancouver area using fingerling sockeye salmon (Oncorhynchus nerka). Geometric mean survival time (GMST) in undiluted primary effluent and survival during 96 hr exposure to a range of dilutions was measured. Acute toxicity varied from one treatment plant to the other. Measurements were made during dry and wet weather conditions at one treatment plant and acute toxicity was greater during dry weather.

Multiple regression analysis was used to obtain a relationship between GMST and constituents of primary sewage at one treatment plant. Anionic surfactants and un-ionized ammonia were positively correlated with acute toxicity but iron and alkalinity were negatively correlated. However, these substances did not account for all the acute toxicity measured. Although copper was present at significant concentration, it was not correlated with acute toxicity.

INTRODUCTION

Bioassays of sewage at Lulu Island Sewage Treatment Plant (LSTP)[1] quantified the acute toxicity of primary treated sewage at this installation of Greater Vancouver Sewerage and Drainage District (GVSDD) (Martens and Servizi 1974, 1975). Primary sewage was acutely toxic at 17% v/v and the 96 hr LC50 was frequently between 17 and 25% v/v. GVSDD indicated that effluent from LSTP may be atypical owing to the presence of metals and cyanide discharged to sewer by an electroplating industry. Therefore, sewage from Iona (ISTP)[2] and Lions Gate Sewage Treatment Plants (LGSTP)[3] were bioassayed to extend knowledge of the range of acute toxicity of municipal sewage in the Greater Vancouver area.

This report is the result of studies performed at ISTP and LGSTP during dry weather conditions, except for wet weather flow conditions for some bioassays at the latter. Some of the results of studies at LSTP (Martens and Servizi 1974, 1975) are included in this report for purposes of comparison.

METHODS

Bioassay Sites

ISTP and LGSTP are primary plants treating a mixture of domestic, commercial and miscellaneous small industrial effluents. Flows averaged 55.9 IMGPD and 9.2 IMGPD, respectively at ISTP and LGSTP during the study periods. Bioassays were conducted at ISTP during late July to early September 1974 and at LGSTP in the period mid-September to mid-November, 1974. Treatment included prechlorination, preaeration, settling and chlorine disinfection. Although prechlorination dosages were small, they were halted at both plants during the study periods to eliminate any possible interference in measuring the toxicity of primary treated sewage.

1, 2, 3 - Lulu Island, Iona Island and Lions Gate Sewage Treatment Plants will be abbreviated LSTP, ISTP and LGSTP, respectively.

Dilution Water

Fraser River water was used for acclimation and dilution during toxicity studies at LSTP (Martens and Servizi 1974, 1975). However it was not available at ISTP or LGSTP and Vancouver city water was used in its place. Since city water was chlorinated and low in hardness and alkalinity (12 mg/l and 4.0 mg/l respectively, measured at ISTP), it was pretreated to remove chlorine residual and to increase hardness and alkalinity to levels approximating those of Fraser River water. Residual chlorine determinations of city water at ISTP and LGSTP indicated chlorine was absent at ISTP while 0.05 mg/l occurred at LSTP. To assure water used for acclimation and dilution was free of chlorine, 1 mg/l sodium thiosulfate ($Na_2S_2O_3$) was added at both treatment plants.

Analyses of lower Fraser River water during August 1965 indicated an average hardness and alkalinity of 53 mg/l and 43 mg/l, respectively (Servizi and Burkhalter 1970). Increase in hardness and alkalinity of city water was achieved by adding calcium chloride ($CaCl_2$) and sodium bicarbonate ($NaHCO_3$). These were added from stock solutions by precalibrated metering tubes into mixing chambers before flow into a 100 gal reservoir.

Bioassays

Continuous flow bioassays were conducted with sewage from ISTP and LGSTP using sockeye salmon fingerlings. Test fish were from the Cultus Lake race, reared from eggs at Sweltzer Creek Laboratory. Test fish were acclimated for two weeks or longer to 14°C temperatures before transfer to the bioassay site for acclimation to dilution water. Acclimation of test fish to dilution water was intended to be a minimum of seven days. However owing to technical problems this was not always achieved (TABLE 1).

Bioassays were conducted in 30 liter glass aquaria with flow rates (500 ml/min) sufficient for 99% replacement in about 2.8 hours (Sprague 1973). Ten sockeye fingerlings (maximum mean wet weight 0.8 gms) were exposed up to 96 hours in sewage, sewage-water mixtures and in

TABLE 1 - Acclimation of test fish to dilution water.

Bioassay No.	Acclimation (days) ISTP	Acclimation (days) LGSTP	Bioassay No.	Acclimation (days) LGSTP
1	7	0	16	0
2	8	2	17	2
3		3	18	3
4	16	6	19	7
5	4	7	20	8
6	7	8	21	9
7	11	9	22	10
8	13	7	23	7
9	18	8	24	8
10	9	9	25	9
11		10	26	10
12		7	27	11
13		8	28	12
14		9	29	13
15		10	30	7
			31	8
			32	9
			33	10

control (dilution) water. The daily inflow to aquariums averaged 148 liters per gram of fish per day, well in excess of the recommended value of two to three liters per gram of fish per day (Sprague 1973).

Bioassays consisted of five sewage concentrations and a control. Since test fish in 100% sewage usually died within 24 hours, additional bioassays were conducted at this concentration when possible.

Sewage and dilution water were supplied to each aquarium from head troughs by precalibrated metering tubes. Flows from the tubes were checked at the outset of each bioassay and tubes were cleaned daily to insure constant flow.

Test temperatures varied as much as 3.3°C between control aquaria and those containing 100% sewage in spite of the use of a refrigerated bath. Dissolved Oxygen (D.O.) was supplied by compressed oxygen.

Aquariums were checked almost continuously for mortalities or moribund fish during the initial 8 to 12 hours of each bioassay.

Observations of test fish were restricted by opacity of sewage. Thus a net was used to move test fish near the surface for inspection.

Acute toxicity was quantified by determining the 96 hr LC50 or the Geometric Mean Survival Time (GMST) from observations of mortality (Davis and Mason 1973). Acute toxicity was also summarized in terms of mean mortality at each concentration.

Analyses of Sewage and Dilution Water

Temperature and dissolved oxygen were measured daily in each aquarium, the latter by the alkali-iodide-azide modification of the Winkler method (Standard Methods 1971). Total residual chlorine was measured in 100% sewage and dechlorinated dilution water daily by the iodometric method with an amperometric end point (Standard Methods 1971). The minimum detectable concentration was 0.02 mg/l.

Twenty-four hour composite sewage samples were analyzed on weekdays at the treatment plant for hardness, alkalinity, pH, and ammonia nitrogen. Hardness and alkalinity were measured according to Standard Methods (1971), and ammonia nitrogen was measured by the phenol-hypochlorite method (Harwood and Kühn 1970). Un-ionized ammonia concentrations were calculated after Trussell (1972).

Sub-samples from the aforementioned composite samples were submitted to the West Vancouver Laboratory, Department of the Environment for measurement of extractable (ext) and filtered (fil) metals (cadmium, calcium, copper, chromium, iron, lead, magnesium, manganese, mercury and zinc) using atomic absorption spectrophotometry. Samples for measurement of extractable metals were acidified with 5 mls conc. HNO_3/l. Filtered metals were measured in filtrate from 0.45 micron membrane filters. Additional subsamples were submitted for analysis of anionic surfactants. Nitrite was determined on a grab sample upon delivery using an automated procedure (Kamphake, Hannah and Cohen 1967) of the diazotization method (Standard Methods 1971). Anionic surfactants were determined by the Azure A method (Van Stevenick and Reimersma 1966). Daily grab samples were submitted to the GVSDD laboratory for immediate analysis of cyanide content (Standard Methods 1971).

Dilution water was analyzed each weekday for alkalinity, hardness, pH and ammonia nitrogen.

RESULTS

Characteristics of Dilution Water and Sewage

Metals, nitrite, chlorine, anionic surfactants and ammonia nitrogen were commonly less than the limit of detection in dechlorinated and artificially hardened dilution water (TABLE 2). Hardness averaged 50 mg/l and 54 mg/l respectively at ISTP and LGSTP while alkalinity averaged 52 mg/l and 56 mg/l, respectively.

Sewages contained measurable concentrations of several of the metals tested (TABLES 3 and 4). Zinc, chromium, iron, lead and manganese were highest in LSTP sewage. Copper was highest at LGSTP. Zinc, lead, manganese, cadmium and nickel were at similar concentrations at ISTP and LSTP. Nitrite was similar at LGSTP and LSTP. Cyanide, ammonia nitrogen and anionic surfactants were highest at LSTP and except for cyanide, lowest at ISTP. Cyanide reached 0.34 mg/l in one sample but all other cyanide concentrations at ISTP were less than detectable. The average hardness and alkalinity were greatest at LSTP and least at ISTP. The pH of sewage at all three treatment plants was near neutral.

Samples at LGSTP included dry and wet weather flow conditions. The collection system is primarily of the separate type but some stormwater is included. Thus during wet weather sewage was diluted by stormwater and infiltration. As a consequence most characteristics of sewage at LGSTP were lowest during wet weather, with the exception of dissolved iron, which was highest during wet weather.

Bioassay Conditions

As noted in the METHODS, test temperatures varied as much as 3.3°C between control and aquariums containing 100% sewage within specific bioassays. In addition bioassay temperatures changed with time owing to variation in dilution water and sewage temperatures. This resulted in a temperature range at each bioassay site (TABLE 5) but temperatures were within the range normally tolerated by sockeye salmon (Brett 1952).

Dissolved oxygen also varied during the studies but was always in the range consistent with normal physiological functioning (Davis 1975).

TABLE 2 - Characteristics of dilution water at ISTP and LGSTP.[a]

Characteristic	ISTP Average mg/l	ISTP Range mg/l	LGSTP Average mg/l	LGSTP Range mg/l
Hardness as $CaCO_3$	50	46-59	54	42-66
Alkalinity as $CaCO_3$	52	16-72	56	49-67
pH[b]	7.7	7.3-8.5	7.6	7.2-8.1
Anionic Surfactants	.02	.02 - .02	.05	.05 - .05
Ammonia Nitrogen	.01	.01 - .01	.01	.01 - .01
Temperature °C	15.0	13.3-17.2		
Nitrite	.005	.005- .005	.005	.005- .005
Chlorine	0.02	0.02 -0.02	0.02	0.02 -0.02
Cadmium[c]	.01/.01	.01/.01 - .01/.01	.01/.01[b]	.01/.02 - .03/.03
Copper	.01/.01[b]	.01/.01 - .01/.02	.01/.01[b]	.01/.01 - .07/.06
Chromium	.03/.03	.02/.02 - .03/.03	.03/.03[b]	.02/.02 - .03/.03
Iron	.03/.03	.03/.03 - .52/.06	.20/.10	.07/.03 - .86/.42
Lead	.02/.02	.02/.02 - .02/.02	.02/.02	.02/.02 - .02/.02
Manganese	.03/.03	.03/.03 - .03/.03	.03/.03	.03/.02 - .03/.03
Zinc	.01/.01[b]	.01/.01 - .02/.025	.01/.01[b]	.01/.01 - .02/.02
Mercury	.0008/	.0008/ - .0008	.0008	.0008 - .0008
Nickel	.1/.1	.1/.1 - .1/.1	.1/.1	.1/.1 - .1/.1

[a] Concentrations underscored were less than value shown.
[b] Mode
[c] Metals ext./fil.

TABLE 3 - Characteristics of primary sewage at ISTP and LGSTP.[a]

Characteristic (mg/l except pH)	ISTP Average mg/l	ISTP Range mg/l	LGSTP Average mg/l	LGSTP Range mg/l
Hardness as $CaCO_3$	20	16-25	45	33-90
Alakalinity as $CaCO_3$	76	64-107	128	52-171
pH[b]	7.0	6.7-7.5	7.1	6.8-8.0
Anionic Surfactants	1.10	.08 -2.40	3.75	.26 -5.4
Ammonia Nitrogen	10.7	4.3-20.1	16.1	3.5-23.5
Un-ionized NH_3-N[b]	.04	.01 -.13	.09	0.00- .46
Cyanide CN^-	.03[b]	.03 -.34[c]	.03	.03 - .03
Nitrite	.015	.005-.085	0.039	.005- .24
Cadmium[d]	.01/	.01/-.01/	.03/.03[b]	.01/.01 - .03/.03
Copper	.11/.09	.07/.04 -.17/.15	.22/.14	.07/.06 - .37/.27
Chromium	.08/	.02/.02 -.60/.48	.03/.03[b]	.02/.02 - .07/.04
Iron	.65/.42	.38/.28 -.91/.54	1.04/.58	.64/.25 -2.0/ .76
Lead	.02/.02[b]	.02/.02 -.03/.02	.02/.02[b]	.02/.02 - .04/.02
Manganese	.06/.06	.03/.03 -.09/.08	.04/.03	.03/.03 - .09/.09
Zinc	.10/.08	.01/.01 -.12/.15	.11/.10	.03/.02 - .19/.16
Nickel	.1/ .1	.1/ .1 -.1/ .1	.1/ .1	.1/ .1 - .1/ .1
Mercury	.0008/	.0008/ -.0008/	.0008	.0008 - .0008

a Concentrations underscored were less than value shown.
b Mode
c 0.34 mg/l occurred on one occasion, all other values .03.
d Metals ext./fil.

TABLE 4 - Characteristics of dechlorinated sewage at Lulu Island Sewage Treatment Plant,[a] (Martens and Servizi, 1975).

Characteristic (mg/l except pH and temp.)	Average	Range
Hardness as $CaCO_3$	65.8	44.0 - 78.0
Alkalinity as $CaCO_3$	117.	107. - 130.
pH	7.0[b]	7.0 - 7.3
Anionic Surfactants	4.47	2.2 - 8.8
Ammonia Nitrogen	20.34	12.25 - 23.50
Un-ionized Ammonia	0.06	0.03 - 0.08
Temperature °C	11.1	7.8 - 13.9
Cyanide CN^-	0.11	0.03 - 0.36
Nitrite Nitrogen	0.04	0.005 - 0.08
BOD[b]	131.	80. - 163.
Cadmium[c]	.01/.01	.01/.01 - .04/.02
Calcium	14.1 /-	5.2/- - 16/ -
Copper	.16/.14	.12/.08 - .19/.19
Chromium	.18/.05	.1/.02 - .26/.10
Iron	3.40/1.27	.99/.17 - 4.2/2.3
Lead	.05/ .02	.02/.02 - .13/.02
Magnesium	7.37/ -	4.1 - 11.0/ -
Manganese	.23/ .13	.14/.07 - .34/.19
Zinc	.22/ .17	.08/.07 - .80/.26

[a] Concentrations underscored were less than value shown.
[b] Mode
[c] Metals ext./fil.

TABLE 5 - Dissolved oxygen and temperature in bioassays at ISTP, LGSTP and LSTP.

	Temperature °C		Dissolved Oxygen mg/l	
Sewage Treatment Plant	Average	Range	Average	Range
Iona Island	15.0	12.4-20.7	9.7	6.7-15.3
Lions Gate	12.8	8.2-17.4	9.6	6.7-14.6
Lulu Island				
primary sewage	10.1	6.1-13.3	11.0	6.7-15.0
dechlorinated sewage	9.8	4.7-14.4	10.5	7.0-14.2

Acute Toxicity

Acute toxicity of sewage at each of the three treatment plants was variable as shown by GMST which ranged from 306 to 640 min at ISTP and from 125 to 518 min at LGSTP (TABLE 6). At LSTP, GMST of primary and dechlorinated sewage ranged from 109 to 487 min and from 198 to 603 min, respectively. Comparison using Student's "t" indicated GMST was significantly greater ($p = 0.02$) in dechlorinated than primary sewage at LSTP. There was no tendency in the data to indicate that GMST varied with day of the week (Monday through Friday). Data were obtained at ISTP during essentially dry summer weather, except for some light showers which caused only a small increase in sewage flow. The testing period at ISTP occurred during late winter-early spring when wet weather was common. However, stormwater was largely excluded although some infiltration was believed to occur.

Bioassays at LGSTP commenced during dry weather flow but wet weather occurred at end of the test period. The collection system was primarily of the separate type but some stormwater was included. Thus during wet weather, sewage was diluted by stormwater plus infiltration and GMST averaged 370 min, which was significantly longer than the average GMST for dry weather flow (220 min) according to Student's "t" test ($p < 0.001$).

Comparison of average GMST at the three treatment plants indicated that primary sewage at ISTP was significantly less acutely toxic than primary sewage at LSTP ($p < 0.02$) or LGSTP ($p < 0.01$) during dry weather flow (DWF) when compared using Student's "t" test. However, acute toxicity of sewage at ISTP and at LGSTP during wet weather was not significantly different. Furthermore, acute toxicity of sewage at LGSTP during dry weather flow was significantly greater than at LSTP during bioassays at 100% v/v ($p < 0.05$).

Mortality during 96 hr bioassays ranged from zero at 10% v/v to 100% in all bioassays at 80% v/v and greater (TABLE 7). Owing to the nature of the data it was possible to calculate 96 hr LC50's for only a few bioassays using criteria of Standard Methods (1971). Thus in single bioassays at ISTP and LGSTP, LC50's were 45% v/v and 28% v/v, respectively. Three LC50's were calculated for primary sewage at LSTP; 19, 32 and 21% v/v. No mortalities occurred at 25% v/v at ISTP or at LGSTP during wet weather but mean mortalities were 38% at LGSTP during dry weather and 74% at LSTP (TABLE 7). Mean mortality was slightly less in dechlorinated than in primary sewage at LSTP, but mortalities occurred at 17% v/v in both cases. No mortalities occurred at 17% v/v at LGSTP. These comparisons suggest the relative toxicity of the three sewages was ISTP < LGSTP < LSTP.

TABLE 6 - Geometric mean survival time (GMST) of sockeye fingerlings exposed to sewage without dilution.

	G M S T (min)		
Treatment Plant	Average	Range	No. Tests
Iona Island			
primary	445	306-640	9
Lions Gate			
primary, DWF	220	125-370	18
primary, WWF	370	281-518	9
Lulu Island			
primary	302	109-487	12
dechlorinated	449	198-603	11

TABLE 7 - Mean mortality of sockeye fingerlings exposed to various mixtures of primary sewage and dilution water.

Treatment Plant	Mean Mortality, Percent[a]										
	Percent Sewage										
	10	17	25	30	40	45	50	60	65	80	100
ISTP	-	-	0	30[b]	37	90[b]	88	90[b]	100	100[b]	100
LGSTP											
DWF	-	0	38	-	93	-	-	100	-	100[b]	100
WWF	-	0	0	-	80	-	-	100	-	-	100
LSTP											
primary	0	28	74	-	97	-	-	-	-	-	100
dechlorinated	0	21	56	-	93	-	-	-	99	-	100

[a] Three to seven tests, except where noted.
[b] Single test.

Acute Toxicity Related to Constituents

Multiple regression analysis was used to determine which constituents were statistically related to acute toxicity. Geometric Mean Survival Time of sockeye in 100% v/v primary sewage was compared with constituent analysis of the composite sample of the same day. This comparison was possible only with data from LGSTP since there were not enough bioassay tests at ISTP and LSTP to suit the number of constituents measured. The regression equation indicated acute toxicity was negatively correlated with filtered iron and alkalinity but positively correlated with anionic surfactants and un-ionized ammonia:

$$GMST = -249 + 927(Fe) - 143(\text{Anionic Surf.}) - 348(NH_3un) + 4.8(\text{Alk.})$$

Negative correlation with iron appeared related to the fact that iron concentrations were highest during wet weather flow when acute toxicity was lowest. It is suspected that iron increased during wet weather owing to contribution from high flows in the stormwater collection system. Thus iron was an indirect correlation with dilution by stormwater. Although alkalinity was negatively correlated with acute toxicity, it was not related to flow conditions as was iron. The role of alkalinity may have been antagonistic toward acute toxicity of copper (Pagenkopf, Russo, Thurston 1974, Stiff 1971). The positive correlation with anionic surfactants and un-ionized ammonia corresponded with high concentrations during DWF and low concentrations during WWF owing to dilution by stormwater and infiltration.

The acute toxicity of mixtures has been attributed to the sum of acute toxicity of the constituents expressed in toxic units (Brown 1968). Toxic units are calculated by dividing the concentration of a substance by its 96 hr LC50. This simplified approach assumes toxic effects are additive whereas there is a possibility of synergism or antagonism. However, calculation of toxic units may give an indication of the importance of various substances in acute toxicity of sewage at each of the three sewage treatment plants. Calculations of toxic units herein are based upon 96 hr LC50 values summarized by Esvelt, Kaufman and Selleck (1971), except for copper and nitrite. Copper and zinc 96 hr LC50 values are from Servizi and Martens (Ms. 1971). The 96 hr LC50 for nitrite was derived from Brown and McLeay (1974).

Multiple regression analysis identified anionic surfactants and un-ionized ammonia as sources of acute toxicity at LGSTP but the total toxic units contributed by these substances was 1.17, equivalent to an LC50 of about 85% v/v (TABLE 8). This calculation substantially underestimated actual toxicity of primary sewage at LGSTP. Although copper was not correlated with acute toxicity in regression analysis, the average concentration corresponded to 1.4 toxic units. Nitrite was calculated to contribute almost as many toxic units as un-ionized ammonia. Similar calculations for sewage at ISTP and LSTP indicated filtered copper and anionic surfactants were associated with major values

TABLE 8 - Estimated toxic units of selected constituents of sewage.[a]

Sewage and Constituent	Concentration, mg/l		96 hr LC50, mg/l		Toxic Units	
	Average	Range	Average	Range	Average	Range
Iona Island						
Anionic Surfactants	1.11	0.008-2.4	4[b]	2-6[b]	0.28	.01- 1.2
Un-ionized Ammonia	0.04	0.01 -0.13	0.4[b]	0.2 -.9[b]	0.01	.01- 0.65
NO^-_2	0.015	0.005-0.085	0.23[d]		0.07	0- 0.37
Cu(fil)	0.09	0.04 -0.15	0.10[c]	0.08 -.11	0.9	.36- 1.88
Lions Gate						
Anionic Surfactants	3.75	0.26 -5.4	4	2-6	0.94	.04- 2.7
Un-ionized Ammonia	0.09	0-0.46	0.4	0.2 -.9	0.23	0- 2.3
NO^-_2	0.04	0.005-0.24	0.23		0.17	.02- 1.04
Cu(fil)	0.14	0.06 -0.27	0.10	0.08 -.11	1.4	.55- 3.4
Lulu Island						
Anionic Surfactants	4.47	2.2 -8.8	4	2-6	1.12	.37- 4.4
Un-ionized Ammonia	0.06	0.03 -0.08	0.4	0.2 -.9	0.15	.03- 0.4
Cyanide	0.11	0.03 -0.36	0.1[b]	0.025-.2	1.1	0-14.4
NO^-_2	0.04	0.005-0.08	0.23		0.17	0- 0.4
Cu(fil)	0.14	0.08 -0.19	0.10	0.08 -.11	1.4	.73- 2.38

[a] Concentrations underscored were less than value shown.
[b] Esvelt, Kaufman and Selleck (1971)
[c] Servizi and Martens (Ms 1971)
[d] Brown and McLeay (1974)

of toxic units. In addition, cyanide was a significant source of toxic units at LSTP. Zinc, chromium, iron, lead, manganese, nickel and mercury were not tabulated since the average toxic units for each was well below the 0.2 T.U. criterion recommended for estimating combined effects of toxicants (Brown, Jordan and Tiller 1969).

Summation of average toxic units in TABLE 8 for LGSTP, including copper and nitrite, equaled 2.74, equivalent to a 96 hr LC50 of 37% v/v. This value falls within the range expected based upon mean mortality data. In the case of ISTP, summation of average toxic units, but excluding values for un-ionized ammonia and nitrite since they were well under the recommended criterion of 0.2 T.U., yields a value of 1.18 T.U., equivalent to a 96 hr LC50 of 85% v/v. Since mean mortality data indicate the 96 hr LC50 would be between 40 and 45% v/v (TABLE 7), summation of toxic units underestimated acute toxicity. For LSTP, the summation of average toxic units equaled 3.94, equivalent to a 96 hr LC50 of 25% v/v. This agrees with mean mortality of dechlorinated sewage from which the constituents were measured. However, in each case a large part of the toxic unit total was associated with filtered copper but since copper was not correlated with acute toxicity as LGSTP, its contribution to acute toxicity is subject to doubt and will be considered under DISCUSSION.

DISCUSSION

Based upon GMST, primary sewage at LGSTP during dry weather flow was the most toxic, but based upon mean mortality during 96 hr exposure, primary sewage at LSTP appeared most toxic. A result of this type is not explainable using the data at hand, but diurnal variation of sewage strength could have contributed to the results observed.

Mean mortality data indicated that 96 hr LC50's would be between 40 and 45% v/v at ISTP and between 25 and 40% at LGSTP, whereas at LSTP, the LC50 would occur between 17 and 25% v/v. For comparison the mean 96 hr LC50 (TLm) to golden shiners (Notemignoses chrysoleucos) for primary sewage from four treatment plants in the San Francisco area was 45% v/v (Esvelt, Kaufman and Selleck 1973). Mean 96 hr LC50's at the four plants ranged from 37.5 to 58.8% v/v. Limited comparison indicated, with only one exception, that salmonids (rainbow trout, Salmo gairdneri and chinook salmon, O. tshwytscha) were slightly more sensitive to primary sewage than golden shiners (Esvelt, Kaufman and Selleck 1971). Although there is no assurance that rainbow trout, chinook and sockeye salmon are equally sensitive to sewage toxicity, the results suggest that acute toxicity of sewage at ISTP and LGSTP was similar to that in the San Francisco area but primary sewage at LSTP was more toxic.

Acute toxicity of primary sewage at LGSTP was positively correlated with surfactants and un-ionized ammonia but was negatively correlated with filtered iron and alkalinity. Part of the acute toxicity of primary sewage in the San Francisco area was correlated with Methylene Blue Active Substance (MBAS) and ammonia nitrogen with the remainder attributed to metals and perhaps reduced substances (Esvelt, Kaufman and Selleck 1973). The MBAS test is a gross measure of detergent content but includes other organic substances sensitive to Methylene Blue. On the other hand, only anionic surfactants were measured by the Azure A test used in this study. Thus the results herein indicate that anionic surfactants (detergents) contributed to acute toxicity of primary sewage.

The average concentration of anionic surfactants was greatest at LSTP (4.47 mg/l), less at LGSTP (3.75 mg/l) and lowest at ISTP (1.10 mg/l). Since acute toxicity was correlated with surfactant concentration at LGSTP, it appears reasonable to assume that surfactants were a significant cause of acute toxicity at LSTP. According to calculations in TABLE 8, surfactants contributed limited acute toxicity at ISTP.

Acute toxicity of primary sewage at LGSTP was correlated with un-ionized ammonia but not with ammonia nitrogen. This agrees with the belief that acute toxicity of ammonia is related to un-ionized ammonia, which is dependent upon pH and temperature (McKee and Wolf 1963). On the other hand, ammonia nitrogen was used in correlating acute toxicity and constituents of sewage in the San Francisco area because the pH range was only 6.6 to 7.6 (Esvelt, Kaufman and Selleck 1971). However, based on recent information, this pH range was great enough to cause a tenfold difference in the calculated concentrations of un-ionized ammonia (Trussell 1972, Emerson et al 1975). Martens and Servizi (1974) recalculated un-ionized ammonia concentrations reported by Esvelt, Kaufman and Selleck (1971), using tables by Trussell (1972) and found concentrations in San Francisco area sewage were much less than the amount reported lethal (0.2 to 0.9 mg/l, TABLE 8). Un-ionized ammonia at LGSTP averaged about 23% of the amount reported lethal but was correlated with acute toxicity of primary sewage by multiple correlation analysis. On the other hand, ammonia nitrogen was not correlated with acute toxicity. It appears that further work is required to redefine the lethal concentrations using current ionization constants to better define the role of ammonia in acute toxicity of sewage.

Nitrite was present at about 17% of its 96 hr LC50 at LSTP and LGSTP, but was not identified by regression analysis as being correlated with acute toxicity. The concentration at ISTP was well below the lethal level. Thus nitrite appeared to play a minor role, if any, in acute toxicity of primary sewage. However, under certain conditions of temperature, time and type of treatment, conversion of ammonia to nitrite may be significant. Thus nitrite should not be overlooked as a cause of toxicity in other sewage toxicity studies.

Filtered iron was negatively correlated with acute toxicity at LGSTP and was present at concentrations well below those reported lethal. Since iron contributes to hardness and since some substances are reported to be less toxic as hardness increases, calculations were made to determine the effect which iron may have had upon hardness. However, calculations indicated that the range of iron concentrations measured would have little effect upon the total hardness at LGSTP. As explained previously, iron concentrations were greatest during wet weather flow and least during dry weather. Thus it appears that iron reflected the dilution and lowered acute toxicity which occurred owing to stormwater and infiltration.

Metals were not correlated with acute toxicity of sewage at LGSTP by multiple regression analysis and all metals measured were present at less than lethal levels, except copper. Average measured copper concentrations in sewage at all three treatment plants were in excess of the lethal concentration for sockeye salmon in pollutant free water at hardness of about 80 mg/l and alkalinity of 63 mg/l (Servizi and Martens, Ms. 1971). Hardness and alkalinity of sewage at LGSTP averaged 45 and 128 mg/l, respectively. It has commonly been held that acute toxicity of copper is inversely related to hardness, but in this case the hardness of sewage was less than that of the water in which lethal levels were measured. This result suggests that some substance other than hardness was antagonistic to the toxic action of copper. The result may be partially explained by recent analyses which have indicated that alkalinity, not hardness, is the major factor controlling the toxic form of copper (Pagenkopf, Russo and Thurston 1974, Stiff 1971). Since acute toxicity of primary sewage at LGSTP was not correlated with either filtered copper or hardness, but was negatively correlated with alkalinity, the latter characteristic may have played an antagonistic role insofar as acute toxicity of copper was concerned.

Binding of metal ions by some types of organic substances present in natural water is another factor which may have reduced toxic action of copper, and other metals (Ramamoorthy and Kushner 1975). According to these authors, metal-organic complexes pass through 0.45 micron membrane filters and thus would have been included in the "filtered" state reported. However, toxic action would have been less than when the metal was in the free ionic form.

In view of the foregoing factors concerning acute toxicity of copper, toxic units calculated in TABLE 7 may overestimate the role which copper played in acute toxicity at each of the treatment plants. If this is the case, summation of toxic units, using the measurements reported, would not reflect the relative importance of the various constituents to total acute toxicity and the summation would substantially underestimate acute toxicity in each case. As a consequence, substances in addition to those measured would seem to play a role in acute toxicity of sewage.

CONCLUSIONS

1. Based upon mean mortality during 96 hr bioassays, primary sewage at LSTP was most toxic, followed by primary sewage from LGSTP and ISTP.
2. Primary sewage was more toxic at LGSTP during dry weather than during wet weather flow conditions.
3. Multiple correlation analysis identified un-ionized ammonia and surfactants as causes of acute toxicity at LGSTP but these constituents did not account for all the toxicity observed.
4. Although copper was present at all three treatment plants in amounts which could be considered lethal, it was not identified by correlation analysis as a source of acute toxicity at LGSTP.
5. Alkalinity was inversely related to acute toxicity at LGSTP by correlation analysis and was possibly antagonistic to acute toxicity of copper.

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