INTERNATIONAL PACIFIC SALMON FISHERIES COMMISSION

PROGRESS REPORT

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NEW WESTMINSTER, B.C.
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ABSTRACT

Continuous flow and static bioassays of dechlorinated primary sewage were conducted at Annacis Island sewage treatment plant using fingerling sockeye salmon (Oncorhynchus nerka). Geometric mean survival time (GMST) was determined using undiluted effluent and survival during 96 hr exposure to a range of dilutions was measured. Acute toxicity was greater during continuous flow than during static bioassays. In addition, acute toxicity was greater during dry weather than during wet weather flow conditions. Mortalities were usually 100% during bioassays of 65% v/v dechlorinated sewage but no mortalities occurred at 10% v/v.

Results were compared with acute toxicities measured at three other primary sewage treatment plants in the Greater Vancouver Sewerage and Drainage District, plus primary plants in San Francisco and Seattle.

Summation of toxic units attributed to anionic surfactants, un-ionized ammonia, cyanide, nitrite and metals measured failed to account for all the acute toxicity measured.

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INTRODUCTION

Acute toxicity to salmon of primary treated municipal sewage at the Lulu Island[1], Iona Island[2] and Lions Gate[3] sewage treatment plants of the Greater Vancouver Sewage and Drainage District varied from one plant to another as quantified by continuous flow bioassays (Martens and Servizi, 1976).

The Annacis Island[4] primary sewage treatment plant was the most recently constructed treatment facility in the Greater Vancouver Regional District, commencing operation in July 1975 at a dry weather flow of about 27 IMGD (122,000 m^3/d) with a projected flow of 54 IMGD (244,000 m^3/d) in 1986. Sewage consisted of a mixture of domestic, commercial and industrial wastewaters, plus stormwater during wet weather. Treatment included prechlorination, pre-aeration, sedimentation, disinfection by chlorination and dechlorination using sulfur dioxide. Effluent was discharged to the South Arm of the Fraser River through submerged diffusers.

Bioassays of dechlorinated effluent at ASTP were conducted between September 27 and November 18, and from December 6 to 16, 1976, to document acute toxicity. Since provincial objectives (Anon. 1975) did not specify static or continuous flow 96 hour bioassays of effluent toxicity, the study included both.

Some data obtained during earlier studies at three other treatment plants in the GVSDD are included in this report for comparison.

METHODS

Continuous flow and static bioassays were conducted simultaneously using Cultus Lake sockeye salmon (Oncorhynchus nerka) fingerlings reared from eggs at the Sweltzer Creek Laboratory. Bioassays consisted of five to six sewage concentrations (100, 65, 40, 25, 17 and 10% v/v), and a control. Acute toxicity was quantified by measuring percent mortality at each concentration during 96 hours and calculating Geometric Mean Survival Time (GMST) at 100% v/v

1, 2, 3, 4 - Lulu Island, Iona Island, Lions Gate and Annacis Island sewage treatment plants will be abbreviated LSTP, ISTP, LGSTP and ASTP, respectively.

dechlorinated sewage (Davis and Mason, 1973). To further quantify acute toxicity, sewage concentration was plotted versus mean mortality on semi-log paper to estimate 96 hour LC50 values (Standard Methods, 1975). Toxic units were calculated by dividing 100 by the LC50 (Brown, 1968).

Fraser River water pumped continuously from the South Arm, outside the influence of the diffuser, was used for control, dilution, fish acclimation and cooling water. Test fish were acclimated to Fraser River water for at least seven days prior to bioassays. Bioassays were conducted by placing ten fish in each 30 l glass aquarium for 96 hours. Flow and exchange rates exceeded those recommended for continuous flow bioassays (Sprague, 1969). The mean weight of fish per unit volume in static bioassays was 0.31 gm/l and ranged from 0.22 to 0.53 gm/l.

Temperatures in continuous flow and static bioassays were maintained by immersing aquariums in water baths supplied with Fraser River water. Since the study was conducted during autumn, decreasing Fraser River temperatures caused bioassay temperatures to decline as the study progressed. Initially temperatures in bioassays of undiluted sewage were 14.4^{o}C and 16.8^{o}C respectively, under static and continuous flow conditions. In the final bioassay, temperatures had decreased to 9.7^{o}C and 11.3^{o}C respectively, in static and continuous flow tests. Temperatures also varied with sewage concentration. Maximum mean temperature differential between 100 and 17% v/v sewage was 4.2^{o}C in continuous flow bioassays and 3.6^{o}C in static bioassays.

Dissolved oxygen (D.O.) was maintained near saturation with compressed air or a mixture of compressed air and oxygen.

Aquariums were checked frequently for mortalities during the initial 6 hours of fish exposure. Observations of test fish were restricted by opacity of sewage, necessitating periodic netting of fish for observations.

Dechlorinated effluent for bioassays was obtained immediately upon entering the outfall pipe. Effluent for static bioassays was composited continuously for 24 hours prior to the bioassay. Since test fish in 100% v/v sewage died in less than 24 hours, additional continuous flow and static bioassays were conducted in undiluted sewage.

Analyses of Sewage and Dilution Water

Temperatures and D.O. were measured daily in each aquarium and in dilution water. Oxygen was measured polarographically with a YSI Model 54 oxygen meter. Dilution water D.O. was measured both polarographically and by the alkali-iodide-azide modification of the Winkler method (Standard Methods, 1975) to ensure correct D.O. meter calibration. Dilution water and daily composite sewage samples were analyzed for hardness, alkalinity, pH and ammonia nitrogen. Hardness and alkalinity were measured according to Standard Methods (1975). The phenolhypochlorite method (Harwood and Kühn, 1970) was used to determine ammonia nitrogen. Un-ionized ammonia concentrations were computed from Trussell (1972).

Subsamples of the aforementioned composite samples were submitted to Fisheries and Environment Canada, West Vancouver Laboratory, for measurement of total extractable (ext.) and filtered (fil.) metals (aluminum, cadmium, copper, iron, lead, manganese, mercury, nickel and zinc) by atomic absorption spectophotometry. Nitrite and anionic surfactants were also measured by Fisheries and Environment Canada. Cyanide determinations were made by the GVSDD Laboratory or by Fisheries and Environment Canada on the same day the samples were taken. Methods used for analyses by Fisheries and Environment Canada and by GVSDD were the same as those described by Martens and Servizi (1976). A grab sample of dilution water was obtained once per week for measurement of the aforementioned characteristics.

Residual chlorine or sulfur dioxide in ASTP effluent was measured twice daily by sewage treatment plant staff using the amperometric titration method.

RESULTS

Characteristics of Dilution Water

Hardness, alkalinity and pH of dilution water were in the normal range for Fraser River water (Servizi and Burkhalter, 1970) (Table 1). Chlorine, anionic surfactants, cyanide and nitrite were less than their detection limits. Ammonia nitrogen averaged 0.035 mg/l resulting in an average 0.0002 mg/l un-ionized ammonia at existing pH and temperature. Dilution water was generally low in metal content. The single high values for total extractable copper and zinc, 0.13 and 1.9 mg/l respectively, may have resulted from particulates since filtered copper and zinc concentrations were only 0.01 mg/l.

Characteristics of Sewage

Hardness and alkalinity of dechlorinated sewage averaged 53.3 and 85.4 mg/l as $CaCO_3$ respectively (Table 1). Surfactants and un-ionized ammonia averaged 3.2 mg/l and 0.016 mg/l respectively. Cyanide was generally present at 0.03 mg/l and nitrite averaged 0.027 mg/l. Chlorine was not detected since sewage used for bioassays was pumped from a point downstream of dechlorination.

Several metals were commonly detected in sewage and their mean total extractable concentrations were: iron (1.26 mg/l), zinc (0.17 mg/l), copper (0.11 mg/l) and manganese (0.10 mg/l). The mode for chromium was 0.04 mg/l. Cadmium, lead and nickel were generally not detected.

Since a portion of the collection system tributary to ASTP included storm sewers, sewage flow increased as a consequence of rainfall. Rainfall was light to moderate during October and November, and sewage flows during bioassays ranged from dry weather values between 24 and 29 IMGD (109,000 and 132,000 m^3/d), to wet weather flows between 30 and 37 IMGD (136,000 and 168,000 m^3/d). Rainfall was greater during bioassays in December and sewage flows were in the range 36 to 53 IMGD (163,000 to 251,000 m^3/d).

Table 1. Characteristics of dilution water and dechlorinated sewage[a].

Characteristic (mg/l except pH)	Dilution Water		Sewage	
	Mean	Range	Mean	Range
Hardness as $CaCO_3$	42.6	32.2-50.3	53.3	22.2-88.4
Alkalinity as $CaCO_3$	42.8	30.0-47.3	85.4	47.3-128.0
pH	7.4	7.1-7.8	6.7	6.4-7.1
Anionic Surfactants	0.5	.05-.1	3.2	1.8-4.7
Ammonia Nitrogen	.035	.013-.156	12.6	6.5-17.7
Un-ionized NH_3-N	.0002	.00003-.003	.016	.004-.68
Cyanide CN	.03	.02-.03	.03	.03-.06
Nitrite -N	.005		.027	.008-.080
Cadmium[c]	.01[b]/.01[b]		.01/.01	.01-.01/.01-.01
Copper	.01[b]/.01[b]	.01-.13/.01-.01	.11/.08	.09-.20/.03-.15
Chromium	.02[b]/.02[b]	.02-.03/.02-.02	.04[b]/.02[b]	.02-.34/.02-.03
Iron	.87 /.03[b]	.47-1.6/.03-.60	1.26/.67	1.0-1.8/.33-.99
Lead	.02[b]/.02[b]	.02-.03/.02-.02	.02[b]/.02[b]	.02-.09/.02-.04
Manganese	.03[b]/.03[b]	.03-.11/.03-.09	.10/.07	.07-.12/.06-.12
Zinc	.01[b]/.01[b]	.01-1.9/.01-.11	.17/.16	.07-.64/.06-.12
Nickel	.05 /.05	.05-.05/.05-.05	.05[b]/.05[b]	.05-.12/.05-.11
Mercury	.0002[d]			
Chlorine	- Not Detected -		- Not Detected -	

[a] - Concentrations underscored were less than the value shown.
[b] - Mode.
[c] - Metals, extractable/filterable.
[d] - Single sample.

Acute Toxicity

No mortalities or distress occurred among control fish. Acute toxicity was absent during bioassays at 17% v/v sewage except in continuous flow bioassays under dry weather flow (DWF) conditions (Table 2). In every bioassay at 25 and 40% v/v, mortality was significantly less (student's test, $p = 0.02$ and 0.01 respectively) under static than under continuous flow conditions. Furthermore, mean mortalities were greater during DWF than wet weather flow (WWF) at these two bioassay concentrations. Mortalities were 100% at all bioassays of 65 and 100% v/v dechlorinated sewage, except mortality averaged 99% for static bioassays at the former concentration under WWF conditions. To further quantify acute toxicity, 96 hr LC50 and toxic unit values were estimated. Toxic unit values were 2.7 times greater during DWF continuous flow bioassays than during WWF static bioassays. In each case data in Table 2 show that greatest mortality at ASTP occurred during continuous flow bioassays under DWF, whereas least mortality occurred during static bioassays coincident with WWF.

In previous studies, acute toxicities of effluents free of chlorine residuals were measured at Lions Gate, Iona Island and Lulu Island primary treatment plants (Martens and Servizi, 1976). Toxic units ranged from 2.5 at ISTP to 4.8 at LSTP (Table 2).

Geometric Mean Survival Time in continuous flow bioassays of 100% v/v dechlorinated sewage averaged 114 and 320 minutes during DWF and WWF respectively (Table 3). The GMST was not obtained for 16 of 32 continuous flow bioassays at 100% v/v sewage because fish were not being observed. In four cases the GMST was less than about 200 minutes under DWF conditions, and in 12 cases the GMST was between 300 and 1400 minutes (i.e. overnight) during WWF.

The GMST was obtained for only four static bioassays at 100% v/v because mortalities usually occurred overnight when observations were not being made. However, in 19 additional static tests at 100% v/v, fish were commonly distressed within 300 minutes and the GMST occurred sometime between 300 and 1400 minutes.

Table 2. Mean mortality of sockeye salmon fingerlings exposed to various mixtures of primary sewage and dilution water under continuous flow bioassay conditions, except where noted.

Treatment Plant	Mean Mortality, Percent[b] Percent Sewage 10	17	25	30	40	45	50	60	65	80	100	96 hr LC50 % v/v	Toxic Units 100/LC50
ASTP													
DWF	-	50	96		100				100		100[d]	17	5.9
DWF[a]	-	0	3		71				100		100[d]	35	2.9
WWF	0[c]	0	7		80				100[d]		100[d]	33	3.0
WWF[a]	0[c]	0	0		37				99		100[d]	45	2.2
ISTP			0	30[c]	37	90[c]	88	90[c]	100	100[c]	100	40	2.5
LGSTP													
DWF		0	38		93			100		100[c]	100	28	3.6
WWF		0	0		80			100			100	33	3.0
LSTP													
Primary	0	28	74		97						100	21	4.8
Dechlor.	0	21	56		93				99		100	23	4.3

a - static bioassay.
b - Three to nine tests, except where noted.
c - One or two tests.
d - Ten or more tests.

Comparison of mortalities in undiluted sewage at ASTP indicates that mortalities usually occurred in a shorter time under continuous flow than under static bioassay conditions.

Measurements of GMST at three primary treatment plants in the GVSDD (Martens and Servizi, 1976) were added (Table 3) where it is seen that the most toxic sewage usually occurred at ASTP during DWF and continuous flow bioassays.

Table 3. Geometric mean survival time (GMST) of sockeye salmon fingerlings exposed to dechlorinated sewage without dilution under continuous flow bioassay conditions, except where noted.

	GMST (min)		
Treatment Plant	Average	Range	No. Tests
ASTP			
DWF	114	26-204	5
DWF[a]	240	152-360	3
WWF	320	279-354	12
WWF[a]	1583	-	1
ISTP	445	306-640	9
LGSTP			
DWF	220	125-370	18
WWF	370	281-518	9
LSTP			
Primary	302	109-486	12
Dechlorinated	449	198-603	11

[a] static bioassay.

DISCUSSION

Bioassays in undiluted sewage demonstrated that mean mortality was greater during continuous flow than static bioassay conditions. Furthermore, during bioassays of undiluted sewage to obtain GMST, mortality occurred within minutes to about five hours in the continuous flow mode, but in static tests mortality usually occurred between five and 24 hours of exposure. Static bioassays were conducted using 24-hour composite samples wherein compositing would have diluted peaks in toxicity, but in continuous flow bioassays, fish would have been exposed to toxic peaks. Since fish may die or be acutely distressed during short term exposure to highly toxic solutions, it is considered likely the difference in mortality between continuous flow and static bioassays reflects temporal changes in toxicity of the sewage. In addition, the results suggest that peaks in sewage toxicity occurred during the day but were diluted by less toxic sewage during collection of a 24-hour composite sample.

Provincial objectives for municipal sewage discharges do not specify static or continuous flow bioassays as the criterion for measuring acute toxicity. However, if the more sensitive result is desired when measuring acute toxicity, comparison indicates on-line continuous flow tests would be preferred over static bioassays.

Toxicity of sewage at ASTP was greater under DWF than WWF conditions. A similar result was observed at LGSTP (Martens and Servizi, 1976). Dilution by stormwater was believed the cause for lower toxicity during WWF.

Comparison of sewage toxicity in the absence of chlorine among the four primary treatment plants in the GVSDD indicates the most toxic conditions occurred during DWF at ASTP, where the mean 96 hr LC50 for dechlorinated sewage was 17% v/v (Table 2). During WWF, sewage at ASTP and LGSTP had mean 96 hr LC50's equal to 33% v/v. The least toxic sewage occurred at ISTP when continuous flow bioassays were the basis for comparison. Measurements of acute toxicity using GMST (Table 3) support the observation that toxicity was usually greatest at ASTP during DWF.

The mean 96 hr TL50 (LC50) for golden shiners (Notomigosus chrysoleucas) at four San Francisco primary STP's was 45% v/v and ranged from 38 to 59% v/v (Esvelt, Kaufman and Selleck, 1971). Rainbow trout (Salmo gairdneri) and chinook salmon (O. tshawytscha) were generally slightly more sensitive.

Dechlorinated primary sewage from the Metro Seattle West Point STP was reported to have 96 hr LC50's of 32 and 28% v/v for English sole (Parophrys vetulus) and shiner perch (Cymotogaster aggregata) respectively, during continuous flow bioassays under saline conditions (Stober et al, 1977).

Although there is no assurance that golden shiners, English sole, shiner perch and sockeye salmon are equally sensitive to sewage toxicity, comparison with results in Table 2 indicates that toxicity of sewage at treatment plants in the GVSDD was similar to, or slightly more than, that measured in Seattle and San Francisco.

Static bioassays of rainbow trout were conducted using 24 hour composited samples of dechlorinated sewage from ASTP during one week of the DWF period of the study reported herein (Higgs, 1977). The mean of three 96 hr LC50 values obtained during the week was 68% v/v compared with 35% v/v reported herein for sockeye salmon. Furthermore, no sockeye salmon survived bioassays at 65% v/v during eight static bioassays under DWF conditions. Reasons for the different results are not known but the fish used were different (rainbow trout versus sockeye salmon) and Higgs reported sewage was preaerated for 19 to 23 hours between collection and commencement of bioassays.

Acute toxicity of primary sewage at LGSTP was correlated with un-ionized ammonia and anionic surfactant concentrations (Martens and Servizi, 1976). In another study the acute toxicity of primary sewage in the San Francisco Bay area was correlated with Methylene Blue Active Substances (MBAS) and un-ionized ammonia. However, in neither case did these correlations account for all of the toxicity measured. Correlation analysis between acute toxicity and constituents of sewage was not possible for the data from ASTP, but certain observations are possible as discussed below.

The acute toxicity of a mixture has been attributed to the sum of acute toxicities of the constituents expressed in toxic units. Toxic units are calculated by dividing the concentration of a substance by its 96 hr LC50 (Brown, 1968). Calculation of toxic units using mean concentrations from Table 1 and 96 hr LC50 data from Martens and Servizi (1976) attributes 0.8 toxic units to anionic surfactants, 0.04 toxic units to un-ionized ammonia and 0.3 toxic units to cyanide. Copper is not included because alkalinity and binding by organic substances may have been antagonistic to copper toxicity (Martens and Servizi, 1976). The other metals were present at less than the 0.2 toxic unit criterion recommended for estimating combined effects of toxicants (Brown, Jordon and Tiller, 1969). Excluding un-ionized ammonia and nitrite for the reason given above leaves a calculated total of 1.1 toxic units in sewage at ASTP, equivalent to a 96 hr LC50 at 91% v/v. Since the sum of toxic units was far less than the acute toxicity measured at ASTP, it is evident that substances besides those measured contributed substantial acute toxicity.

CONCLUSIONS

1. Continuous on-line bioassays indicated greater acute toxicity to sockeye salmon than parallel static bioassays using 24 hour composite samples of sewage.
2. Acute toxicity of sewage was greater during dry weather flow than during wet weather flow.
3. Of the four primary treatment plants tested in the GVSDD, acute toxicity of sewage free of chlorine residual was greatest at the Annacis Island plant during dry weather flow where the 96 hr LC50 averaged 17% v/v.
4. Calculations indicated anionic surfactants, un-ionized ammonia, cyanide, nitrite and the metals failed to account for all the acute toxicity measured.

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