RRB-87-65

FRASER RIVER SEDIMENTS AND WATERS EVALUATED BY THE BATTERY OF SCREENING TESTS TECHNIQUE

by

B.J. Dutka[1], T. Tuominen[2], L. Churchland[2], and K.K. Kwan[1]

[1]Rivers Research Branch
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario, L7R 4A6

[2]Water Quality Branch
Environment Canada
502-1001 West Pender Street
Vancouver, B.C. V6E 2M9

February 1988
NWRI Contribution #88-84

ABSTRACT

The suitability of a variety of microbiological, biochemical and toxicant screening tests to become part of a battery of test procedures to identify degraded or degrading water bodies are evaluated in this report. Data were collected from 40 sampling sites within the Fraser River Basin in British Columbia. These data re-emphasize that individual toxicant, biochemical or microbiological screening tests do not provide a sufficient data base upon which realistic management decisions can be made. This study also confirms that the fecal sterol tests do not seem amenable to a "battery of tests" approach and that the Daphnia magna test continues to be the most sensitive procedure for indicating the presence of contaminants with toxicant activity.

RÉSUMÉ

Ce rapport fait état de l'évaluation de divers tests de dépistage des produits toxiques, essais microbiologiques, et essais biochmiques en vue de leur inclusion dans une batterie de tests visant à identifier les cours d'eau dégradés ou en cours de dégradation. Les données recueillies dans 40 sites d'échantillonnage du bassin hydrographique du Fraser en Colombie-Britannique font ressortir encore une fois que ces tests de dépistage de produits toxiques, essais biochmiques ou microbiologiques, ne fournissent pas une base de données suffisante pour prendre des décisions administratives réalistes. Cette étude confirme également que les essais des stérols fécaux semblent ne pas pouvoir être inclus dans une approche de "batterie de tests" et que l'essai Daphnia magna est toujours la méthode la plus sensible pour indiquer la présence de contaminants ayant une activité toxique.

MANAGEMENT PERSPECTIVE

The goal of this ongoing series of studies is to identify degraded or degrading water bodies by using a variety of microbiological, biochemical and bioassay tests. These tests, fecal coliform, fecal streptococci, enterococci, Clostridium, coliphage, coprostanol, cholesterol, ATP-TOX System, SOS Chromotest for genotoxicity, Microtox, algal-ATP and Daphnia magna tests, are being evaluated as potential candidates for a battery of tests procedure which can be used nationally to prioritize water bodies and sediments or selected areas within water bodies for remedial action or further investigations. The battery approach should make it possible to establish "hot spots", areas for immediate concern which were not previously suspected due to inappropriate or one-dimensional testing procedures. Tests which can be performed on refrigerated or frozen samples, 24-96 hours after collection, will be given priority when the selection of the final recommended battery of microbiological, biochemical and bioassay tests is made. The coliphage test, one of the parameters being investigated for the test battery, is of particular importance as it provides information on the potential presence of indicator organisms and bacterial and viral pathogens. The coliphage data from these studies will be related to data from an eight-country, three continent study (S.E. Asia, South America and Northern Africa) monitored by B.J. Dutka through the sponsorship of the International Development Research Centre (IDRC), Ottawa, Canada

Fraser River data suggest the need for fecal coliform isolate identifications to help interpret bacteriological data and the need for the inclusion of more stringent sediment extraction procedures to test for more firmly bound toxicants.

PERSPECTIVE DE GESTION

L'objet de cette série d'études est de déterminer les cours d'eau dégradés ou en cours de dégradation à l'aide de divers essais microbiologiques, biochmiques et bioessais. Ces essais, soit l'essai des coliformes fécaux, l'essai des streptocoques fécaux, l'essai des entérocoques, du chloestérol, le système ATP-TOX, le Chromotest SOS pour la génotoxicité, l'essai Microtox, l'essai sur les algues-ATP et l'essai Daphnia magna, sont évalués en fonction de leur éventuelle inclusion dans une batterie de tests qui pourrait être utilisée à l'échelle nationale pour déterminer les cours d'eau et les sédiments prioritaires ou certaines zones choisies dans ces cours d'eau qui doivent faire l'objet de mesures correctives ou d'études ultérieures. L'approche par batterie devrait permettre de déterminer les "points chauds", les régions qui soulèvent des préoccupations immédiates et qui n'étaient pas soupçonnées auparavant à cause de méthodes d'essai unidimensionnelles ou inappropriées. Les essais qu peuvent être effectués sur des échantillons réfrigérés ou congelés entre 24 et 96 heures suivant leur prélèvement se verront accorder la priorité lors du choix de la batterie finale recommandée pour les essais microbiologiques, les essais biochmiques et les bioessais. L'essai des coliphages, un des paramètres qui fait l'objet de l'évaluation, présente une importance particulière étant donné qu'il donne des informations sur la présence éventuelle d'organismes indicateurs et de pathogènes bactériens et viraux. Les données sur les coliphages

obtenues grâce à ces études seront reliées aux données d'une étude mise en oeuvre dans huit pays et sur trois continents (le Sud-est asiatique, l'Amérique du Sud et le Nord de l'Afrique) sous la direction de B.J. Dutka et sous le parrainage du Centre de recherche et de développement international (CRDI), Ottawa, Canada.

D'après les données recueillies dans le Fraser, il semble nécessaire de déterminer de façon isolée les coiformes fécaux, afin de mieux intercepter les données bactériologiques, et d'inclure des méthodes d'extraction des sédiments plus rigoureuses pour déceler la présence de produits toxiques plus fermement liés.

INTRODUCTION

In previous publications and reports, Dutka et al. (1986, 1986a, 1987, 1987a) described the results of studies to evaluate the suitability of various microbiological, biochemical and bioassay tests to become part of a "battery of test procedures" which could be used to designate, nationally and internationally, water bodies or sediments that are degraded or are being degraded due to toxic chemical discharges, excessive nutrient inputs or microbiological contamination. This "battery of tests" could also be used to monitor the effectiveness of remedial actions or the effect of specific discharges on ambient riverine or lacustrine ecology.

In this paper, we examine waters and sediments and conditions very different from those previously used to evaluate the tests which might be included in the "battery of tests". The sampled waters in this study are those of the Fraser River in the Canadian province of British Columbia. The Fraser River drains an area of 230,000 sq km and has a length of approximately 1400 km from its headwaters in the Rocky Mountains to the Strait of Georgia (Fig. 1 and 1a). The Fraser River estuary receives municipal effluent and storm water originating from the largest population centre in the province (Vancouver) and points upstream. The river is also subject to a multiplicity of industrial discharges. The Fraser is used for commercial shipping, recreational boating and for transporting log booms, which are stored along much of its shoreline. The Fraser also supports a large

commercial salmon fishery and is known worldwide for its sports fishing. The river is a migration route for juvenile and adult salmon and a rearing area for various salmon and trout. The estuary is one of the world's most productive fish, wildlife and agricultural areas. The wetlands support an annual catch of eight million adult salmon and over one million migratory birds on the Pacific Flyway. Farmland in the Fraser flood plain provides most of western Canada's fresh vegetable and berry crops. The waters of the Fraser River are not used for public water supplies but do influence swimming areas in the outer estuary (Kwiatkowski, 1986). Data from 40 water and sediment samples collected from the Fraser River and its estuary are presented and the results discussed.

METHODS

Sampling Site

During June 1987 a total of 40 samples, water and sediment, were collected from sites where fine sediment deposits were expected (Fig. 1, 1a). Twenty of the samples were from sloughs and arms of the lower Fraser River in or near Vancouver. Some of the estuarine samples were affected by salt water intrusion. Sample site latitudes and longitudes and sediment descriptions are shown in Table 1.

Sample Collection

Sediments were collected with an Ekman dredge or shovel. Frequently, it was necessary to use the dredge many times before sufficient surface (1-3 cm layer) sediment was collected. At each site, the surface layers were pooled, well mixed, dispensed into aliquots for each testing procedure and refrigerated. To obtain sediment extract for toxicant screening tests, sediments were extracted with Milli Q water (four cartridge system - one Super C carbon cartridge, two Ion-Ex^{tm} cartridges, one Organet-Q^{u} cartridge and a Milli-Stak^{tm} filter, with a glass distilled water feed) by mixing sediment and Milli Q water in a 1:1 ratio and shaking vigorously for two minutes, then centrifuging at 10,000 rpm in a refrigerated centrifuge for 20 minutes. The supernatant was used in toxicity screening tests.

A second portion of the sediment was sieved for size distribution, following the procedure outlined by Duncan and LaHaie (1979). The sample was sieved at 1/2 or 1/4 PHI scale intervals (Krumbein and Pettijohn, 1938). The size distribution was determined with SIZDIST, a programme used in conjunction with the IBM PC computer (Sandilands and Duncan, 1980).

Surface water samples (1L) were collected at each site for fecal coliform, fecal streptococci and coliphage tests. These tests were usually processed within eight hours of collection. Also at each site another 1 L sample of water was collected and preserved at 4°C for toxicant screening tests. Toxicant screening test samples were tested after being concentrated 10x by flash evaporation at 45°C.

A one-litre surface water sample was also collected at each site, for coprostanol and cholesterol analyses. The sample was preserved with 1 mL concentrated H_2SO_4 and refrigerated at 4°C.

Microorganism Tests

Fecal coliform MF, fecal streptococci MF and coliphage tests were performed on all water samples as described by Dutka et al. (1986). Enterococci population estimates were also performed on water samples using the 48 hour, 35°C incubation Azide Dextrose Broth and the five-tube MPN technique with positive tubes being confirmed on Bile Esculin Agar (35°C for 24 hours).

Fecal coliform populations in sediments were estimated using A-1 broth and the five tube MPN technique with 24 hours incubation at 44.5°C. Sediment Clostridium perfringens populations were estimated by using the MPN technique described by Bonde (1963) and Dutka et al. (1986a).

Biochemical and Toxicity Screening Tests

Coprostanol and cholesterol analyses were performed on water samples and the Microtox test was performed on water and sediment extracts as described by Dutka et al. (1986). SOS genotoxicity tests on water and sediment extracts were performed as described by Xu et al. (1987) without S-9 addition. ATP-TOX system, a new toxicity screening test based on toxicant inhibition of bacterial growth and luciferase activity, was applied to water and sediment extracts (Xu and Dutka, 1987). Spirillum volutans, a large aquatic bacterium with a rotating fascicle of flagella at each pole was also used to test water and sediment extract samples for toxicity following procedures described by Dutka and Kwan (1982). An algal-ATP toxicant screening test was also performed on water and sediment extracts. This test is based on the inhibition of ATP production in cultures of the green alga Selenastrum capricornutum (Blaise et al., 1984). The ATP content of the stressed Selenastrum was measured by the procedure described in Luminescence Review (1983). The results are reported as a percentage of Relative Light Units (RLU) output by the tested sample compared to

the non-stressed control which is 100%. A 48-hour Daphnia magna test using ten organisms per sample and sample dilution was also carried out to assess toxicant activity (APHA, 1985) on natural water samples and sediment extracts.

RESULTS

Latitudes, longitudes and brief sampling site descriptions are presented in Table 1. Also shown in this Table is the composition of each sediment sample based on particle size distribution by sieve analyses (Salisbury 1987) and sediment sample classifications (Shepard 1954). The majority of lower Fraser River sediments (sites 1-25) with few exceptions (sites #2, #11, #21 and #23), were composed mainly of silt while the sediments of the upper reaches of the Fraser were composed predominantly of sand with organic material.

The format used to award points for specific data values, in order to rank the sampled waters and sediments from those of most concern to least, is presented in Table 2. The point allocation scheme is biased and not scientifically defensible, but it reflects the author's evolving experience with data accumulated from the application of a variety of toxicant screening tests to waters and sediments throughout Canada, as well as the distribution patterns of health related indicator bacteria in Canadian waters, sediments and effluent discharges.

The present point allocation scheme has evolved over a three year period and is an ongoing viable process which may change with increased data accumulations.

Samples with the most points are deemed to contain the greatest potential hazard to man and organisms found in the aquatic ecosystem. High toxicant levels may have reduced microbial levels/activity in some sediment samples, however, cause and effect relationships were not investigated.

Table 3 is a complex table which presents all of the microbiological, biochemical and toxicological data obtained from the water samples. Examination of the microbiological data in this table reveals that ten sampling sites had fecal coliform densities greater than 200/100 mL, four sites had fecal streptococci densities greater than 100/100 mL and 12 sites had enterococci counts greater than 100/100 mL. Only three sites had these elevated indicator levels in all three tests, #2(Tilbury Slough), #19 (mouth of Brunette River) and #32 (near old Alexandria Ferry). The highest fecal coliform counts (>3000/100 mL) were found in samples 12, 13, 14 and 15, around Mitchell Island and the North Arm of the Fraser River.

Only nine samples were found to contain coliphage, with the highest count being 15 plaque forming units per 100 mL at sampling sites #17 (southwest shore of Annacis Island) and #24 (upstream of Pitt River mouth). The majority of the sites positive for coliphage (eight out of nine) were located downstream of site #27 (south end of Yaalstrick Indian Reserve 1).

Coprostanol and cholesterol levels were generally negative with only three sites positive, for each test. Coprostanol positive sites were #5 (Steveston), #17 (southwest shore of Annacis Island) and #19 (mouth of Brunette River) and cholesterol positive sites were #5, #19 and #39 (Moose Lake, north).

The toxicological screening tests using 10x concentrated water samples generally indicated that toxicant levels at most sites were below the sensitivity level of the tests applied. Both the Spirillum volutans and Algal-ATP screening tests were negative in all samples tested. The ATP-TOX System indicated that only 13 samples were completely negative for toxicant activity; however, the samples which produced a positive effect were only slightly above background and none were found to produce and EC_{50} effect (50% inhibition). The most toxic sample by this test was sample #13 (Mitchell Island, north shore) with 35% inhibition.

The SOS Chromotest which was performed without S-9 addition indicated that only three samples, #21 (Fraser River at Hatzic), #28 (Chilliwack Mountain) and #34 (downstream of Stone Creek) produced a genotoxic effect. Only six water samples were positive in the Microtox test [#6 (North Arm jetty), #8 (upstream of Richmond Island), (#15 Mitchell Island, south shore), #26 (Nicomen Slough), #29 (Bristol Island, Hope) and #35 (Prince George, west bank)] with sample #35 showing the highest degree of toxicant activity in this test.

The Daphnia magna test using natural water samples proved to be the most sensitive screening test for toxicant activity.

Only 11 samples were found to be completely free of toxicant effects as measured by the death of Daphnia magna organisms over a 48 hr period. Within these negative samples, there were seven that had EC_{10} values, but were considered to be borderline values and for evaluation purposes were reported as negative. The samples with the highest concentration of toxicants as measured by Daphnia magna reactions were #12 (North Arm by MacMillan Bloedel, White Pine Division) and #26 (Nicomen Slough).

Microbiological examination of Fraser River sediments (Table 4) produced an interesting set of observations. Only four sites were found to have very low concentrations (<100/10 gm) of fecal coliforms, #2 (Tilbury Slough), #20 (Tree Island Slough), #29 (Bristol Island, Hope) and #40 (Moose Lake, south), while another three had moderate numbers (<500/10 gm), #3 (Deas Slough), #31 (by Chilcotin Highway bridge) and #39 (Moose Lake, north). All the other sediment samples contained more than 2300 fecal coliforms per 10 gm wet weight of sediment with stations #11 (North Arm by Burnaby Bend), #18 (Gundersen Slough), #26 (south end of Yaalstrick Indian Reserve), #33 (Quesnel, east side of Fraser River), #34 (downstream of Stone Creek) and #35 (Prince George, west bank) having 160,000 or more per 10 gm sediment. The Cl. perfringens data indicate that the whole length of the Fraser River has been subjected to fecal pollution at one time or another with sampling sites #26 (Nicomen Slough), #31 (by Chilcotin Highway bridge), #36 (Prince George, east bank), #37 (north shore Fraser downstream of Willow River), #38 (McBride) and #40 (Moose Lake, south) being impacted the least by fecal pollution.

Microtox and Spirillum volutans toxicity tests were found to be negative for toxicant activity with the 1:1 sediment water extracts. The majority of the ATP-TOX System results were negative. However, a gradation of effects was noted from background noise levels to levels indicating the presence of low grade toxicants (site 9, Middle Arm; site 17, southwest shore of Annacis Island; site 32, near old Alexandria Ferry). The SOS Chromotest indicated that sample #13 (Mitchell Island, north shore) produced a genotoxic effect and sample #12 (North Arm by MacMillan Bloedel, White Pine Division) produced a borderline genotoxic effect.

The Algal-ATP test indicated that the majority of the sediment extracts (35/40) contained a chemical/nutrient balance which was stimulating to the growth of algae. Only one sample #31 (by Chilcotin Highway bridge) contained sufficient contaminants to produce a toxic effect in the Algal ATP toxicant screening test.

Similar to tests on the water samples, the Daphnia magna tests on sediment extract indicated that this procedure was the most sensitive indicator of toxicant activity with 50% of the sediment samples showing some toxicant activity, with samples #33 (Quesnel, east side of Fraser) and #30 (Hope behind Croft Island) having the highest toxicant levels.

DISCUSSION

Microbiological studies on Fraser River water samples were generally indicative that the further upstream the samples were

collected the better the microbiological water quality with few exceptions; #32 (Alexandria Ferry), #33 (Quesnel) and #28 (Chilliwack Mountain). Sites #32 and #33 are believed to be responding to bacteria originating in Quesnel sewerage discharges and lumber mill wastes. Similarly site #28 may also be impacted by bacteria originating from Chilliwack sewerage discharges. Cattle herds were noted at and upstream of site #32 and their droppings may also play a role in the elevated bacterial population found here.

At sites #12, #13, #14 and #15 (North Arm of Fraser and Mitchell Island), extremely high fecal coliform counts were found in conjunction with very low fecal streptococci/enterococci counts; a finding suggesting that these organisms do not primarily originate from human fecal material. Unfortunately, isolates were not collected for identification procedures. It is surmised that if this extra step had been carried out, combined with the knowledge of the heavy concentration of forestry related industries in this area, the majority of these fecal coliforms would have been shown to be Klebsiella species and other non-E. coli which respond to organic pollution. In fecal coliform enumeration procedures, Klebsiella respond similarly to E. coli, however health hazard implications are different.

The fecal streptococci counts (MF using KF agar) were generally very low with only three sites having fecal coliform counts greater than 100/100 mL. Enterococci counts which usually paralleled fecal streptococci counts, were higher with 12 water samples having counts

greater than 100/100 mL. Again as no isolates were collected for identification procedures, it is not certain which of the Streptococci species were actually enumerated by the two procedures and also whether the differences in counts are real or merely reflect normal variations in microbiological population estimates.

Fecal sterol test results, as noted in earlier reports (Dutka et al. 1986, 1986a) were low and not associated with predicted sources of fecal material. Sites positive for coprostanol, #5 (Steveston), #17 (southwest shore of Annacis Island) and #19 (mouth of Brunette River), also showed high fecal coliform and Clostridium perfringens in their sediments. Sites 5 and 19 are noted as mooring sites for fishing boats and site 17 is directly downstream of the Annacis sewage treatment plant outfall. Thus at these sites coprostanol and bacteriological concentrations suggest the presence of fecal contamination. Not withstanding the above, it is suspected that preservation procedures were inadequate and the coprostanol was biodegraded, thus perhaps accounting for the low number of positive findings.

Of all the toxicant screening tests used on the water samples, the Daphnia magna 48 hr test proved to be the most responsive to contaminants in the Fraser. The Spirillum volutans and Algal-ATP tests responded the least with all results indicating that no toxicants were present. The Algal-ATP test results indicated a stimulatory effect on algal growth by the Fraser River water samples. In 29 of the samples, the Daphnia test indicated the presence of

toxicant activity. In contrast only seven samples were positive for toxicant effects by the Microtox test, of which four samples, #26 (Nicomen slough), #29 (Bristol Island, Hope), #35 (Prince George, west bank) and #37 (north shore Fraser, downstream of Willow River), were also positive by the Daphnia test.

Only three water samples, #21 (Fraser River at Hatzic), #28 (Chilliwack Mountain) and #34 (downstream of Stone Creek) could be considered positive for genotoxic activity as measured by the SOS Chromotest. These samples were also positive by the Daphnia test but were negative by the Microtox test. In 27 water samples some degree of toxicant activity was indicated by the ATP-TOX system and there also appears to be a relationship between ATP-TOX System values greater than 20% inhibition and the finding of a toxic response with the Daphnia test. However, none of the water samples contained sufficient toxic contaminants to produce an EC_{50} value with the ATP-TOX system.

Based on the point scheme proposed in Table 2, the nine water sample sites of the greatest potential concern are:

1. Sample #26; Nicomen Slough, ranking due mainly to toxicant load,
2. Sample #35; Prince George, west bank, ranking due mainly to toxicant load,
3. Sample #12; North Arm, near MacMillan Bloedel, White Pine Division, ranking due to bacteria and Daphnia test,

   Sample #13; Mitchell Island across channel from Aero Trading, ranking due to bacteria and toxicant loads,

Sample #19; Brunette River mouth, ranking due to bacteria and toxicant load,

4. Sample #17; southwest shore of Annacis Island, ranking due to bacteria and toxicant load,

Sample #28; Chilliwack Mountain, ranking due to toxicant load,

Sample #32; Alexandria Ferry site, ranking due to bacteria and toxicant load.

Sample #37; Willow River, ranking due to toxicant load,

When water column and sediment microbiological data are examined, some interesting patterns are observed. For instance, based on Clostridium perfringens and fecal coliform counts, site #40 (Moose Lake, south) would be assumed to be a site which is rarely if ever impacted by human fecal pollution and geographically this is borne out, as the lake is in a pristine area near the headwaters of the Fraser. Similarly, it is also believed that site #26 (Nicomen Slough) as well as site #11 (North Arm by Burnaby Bend), #23 (Barnston Island), #31 (by Chilcotin Highway bridge), #36 (Prince George, east bank), #37 (north shore Fraser, downstream of Willow River) and #38 (McBride) are also minimally impacted by human fecal pollution and that the high fecal coliform sediment counts are not related to E. coli levels but rather to Klebsiella and Enterobacter species. However, since no isolates were collected, this can not be proven, although with the level of Clostridium perfringens found here and the very low densities of indicator organisms in the water column, the evidence supports a nonfecal source for these fecal coliforms.

There are other sites, e.g. #5 (Steveston), #25 (mouth of Coquitlam River), #35 (Prince George, west bank) and #39 (Moose Lake, north) where water column data do not reflect the degree of fecal pollution indicated by the sediment data. In making these assumptions with respect to fecal pollution sites versus organic pollution sites based on Clostridium data, one always must be cognisant that C. perfringens spores can survive for years and represent not only fresh pollution but also past pollution patterns which may have changed.

The water sediment extracts used to test for toxicant and genotoxic activity were found, generally, to contain very low levels of compounds promoting these activities. The results seen in Table 4 make it obvious that (a) there are little or no chemicals in the Fraser River samples with toxic or genotoxic activity or (b) that the water extraction procedure used is not able to extract the organic and/or heavy metals contaminants from the sediments.

The Algal-ATP procedure was positive at only one site, #31 (by Chilcotin Highway bridge) while the sediment water extract from 35 other sites showed stimulatory effects, an effect also noted in the 10x water samples. Presence of genotoxic activity at site #13 (Mitchell Island, north shore) and suspected genotoxic activity at site #12 (North Arm by MacMillan Bloedel Mill) were not confirmed or supported by water column results which is not surprising because of the volume and rate of water movement and probable intermittent nature of contaminant imputs.

The Daphnia magna test on the sediment extract was the only toxicant screening test which frequently indicated the presence of toxicant activity in the water extracts. These results were very similar to the water column results. Interestingly, the only sediment extract positive by the Algal-ATP test (#31) was also positive in the Daphnia magna test while the two sites #12 and #13 which indicated the presence of genotoxic activity were both negative when tested by the Daphnia magna test.

Using the point scheme shown in Table 2, the 11 sediments of the greatest concern based on their point score are:

1. Sample #33; Quesnel area, ranking due to bacteria and toxicant load,
2. Sample #30; Hope behind Croft Island, ranking due to bacteria and toxicant load,
3. Sample #25; Coquitlam River mouth, ranking due to bacteria and toxicant load,
4. Sample #32; Alexandria ferry, ranking due to bacteria and toxicant load,
5. Sample #18; Gundersen Slough, ranking due to bacterial load,
   Sample #34; Stoner area, downstream of Stone Creek, ranking due to bacteria and toxicant load,

6. Sample #17; Southwest shore of Annacis Island, ranking due to bacterial load,

   Sample #31; Upstream Chilcotin Highway bridge, ranking due to toxicant load,

   Sample #35; Prince George, west bank, ranking due to bacteria and toxicant load,

7. Sample #5; Steveston Cannery Channel, ranking due to bacterial load,

   Sample #26; Nicomen Slough, ranking due to bacteria and toxicant load.

Surprisingly, the majority of the sediments of potential concern based on their point score totals are upstream of Coquitlam River. These concerns are primarily microbiological in sediments collected in the lower Fraser and both microbiological and toxicological in sediments collected upstream of Coquitlam River.

Examination of the top nine water column and nine sediment sites revealed that there were only three sites common to each list. These are listed below.

Water Column Rank	Sediment Extract Rank	Site
2	6	Site 35, Prince George, west bank
4	4	Site 32, Alexandria Ferry
4	6	Site 17, Southwest shore Annacis Island

The results of this study are very illustrative of and supportive of the need for a battery of tests, the composition of which should be very carefully selected to reflect local conditions. Of the toxicant screening tests evaluated, the Daphnia magna test was the most sensitive procedure for indicating the presence of contaminants with toxicant activity.

Two major shortcomings of this study became obvious as the data were being analyzed. One was the need for fecal coliform isolate identification to clarify the sources of the large bacterial populations found in some water column and sediment samples. The other need was for the testing of solvent and acid extracted sediments for toxicological activity. Testing the water extracted sediments provided information on the toxic effects of contaminants which were likely to be biologically available in the aqueous environment; whereas testing of solvent and acid extracts would have provided information on potential toxic effects of more firmly bound contaminants. Thus, it is believed that these missing features would have produced a much clearer picture of the potential hazardous sites within the Fraser River.

Use of the "battery of tests" approach reemphasizes that individual toxicant, biochemical and microbiological screening tests do not provide a sufficient data base for realistic management decisions to be made. This study also further confirms that the fecal sterol tests are not amenable to a "battery of tests" approach and their cost benefit ratio is very high.

Further refinement of the present "battery of tests" will continue with emphasis on the inclusion of more vigorous extraction procedures and evaluating their effect on acute and chronic test results. The eventual goal will be to select a maximum of two microbiological tests and three toxicant screening tests as a core group. The ranking scheme will be reviewed after each study to ensure the points allocated to various response levels continue to reflect country wide conditions.

ACKNOWLEGEMENTS

We would like to thank A. Jurkovic for technical and ekmaning support in the field, mobile laboratory and home laboratory. Toxicant screening test support from K. Jones and R. McInnis are gratefully acknowledged. We are also indebted to Keith Salisbury for sediment particle size distributions.

REFERENCES

APHA. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Ed. American Public Health Association, Washington, D.C.

Blaise, C., Legault, R., Bermingham, N., Van Coillie, R. and Vasseur, P. 1984. Microtest mesurant l'inhibition de la croissance des algues C150 par le dosage de l'ATP. Sc. et Tech. de l'Eau, 17: 245-250.

Bonde, G.J. 1963. Bacterial Indicators of Water Pollution. A Study of Quantitative Estimation. Tuknisk Furlag, Copenhagen.

Duncan, G.A. and LaHaie, G.G. 1979. Size Analysis Used in the Sedimentology Laboratory. Hydraulics Division Manual, NWRI, CCIW, Burlington, Ontario, Canada, September.

Dutka, B.J. and Kwan, K. 1982. Application of Four Bacterial Screening Procedures to Assess Changes in the Toxicity of Chemicals in Mixtures. Environ. Pollut. Series A, 29: 125-134.

Dutka, B.J., Walsh, K., Kwan, K.K., El-Shaarawi, A., Liu, D.L. and Thompson, K. 1986. Priority Site Selection of Degraded Areas Based on Microbial and Toxicant Screeting Tests. Water Poll. Res.J. Canada, 21: 267-282.

Dutka, B.J., Jones, K., Xu, H., Kwan, K.K. and McInnis, R. 1986(a). Phase II. Priority Site Selection for Degraded Areas Based on Microbial and Toxicant Screening Tests. NWRI Contribution No. 86-174, NWRI, CCIW, Environment Canada, Burlington, Ontario, Canada.

Dutka, B.J., Jones, K., Kwan, K.K., Bailey, H. and McInnis, R. 1987. Use of Microbial and Toxicant Screening Tests for Priority Site Selection of Degraded Areas in Water Bodies. Water Poll. Res. J. Canada, 22: 326-339.

Dutka, B.J., Kwan, K.K., Jones, K. and McInnis, R. 1987a. Application of the Battery of Screening Tests Approach to Sediments in the Port Hope Area of Lake Ontario, Canada. NWRI Contribution #87-134, NWRI, CCIW, Environment Canada, Burlington, Ontario, Canada.

Krumbein, W.C. and Pettijohn, F.J.. 1938. Manual of Sedimentary Petrography. Appleton-Century-Crofts. New York.

Kwiatkowski, R.E. 1986. Water Quality in Selected Canadian River Basins - St. Croix, St. Lawrence, Niagara, Souris and the Fraser Estuary. IWD Scientific Series No. 150, IWD, Water Quality Branch, Environment Canada, Ottawa.

Luminescens Review. 1983. Bulletin No. 204, Turner Designs. Mountain View, Cal., U.S.A.

Salisbury, K. 1987. Particle size data report, Fraser River, B.C. NWRI Research on Applications Branch Technical Report #RAB-87-07b. Canada Centre for Inland Waters, Burlington, Ontario, Canada.

Sandilands, R.G. and Duncan, G.A. 1980. SIZDIST - A Computer Programme for Size Analysis. Hydraulics Division Technical Note No. 80-08. CCIW, Burlington, Ontario, Canada.

Shepard, F.P. 1954. Nomenclature Based on Sand-silt Ratios. J. Sed. Petrology 24:151-158.

Xu, H., Dutka, B.J. and Kwan, K.K. 1987. Genotoxicity Studies on Sediments Using a Modified SOS Chromotest. Toxicity Assessment, 2: 79-88.

Xu, H. and Dutka, B.J. 1987. ATP-TOX System - A New Rapid Sensitive Bacterial Toxicity Screening System Based on the Determination of ATP. Toxicity Assessment 2:149-166.

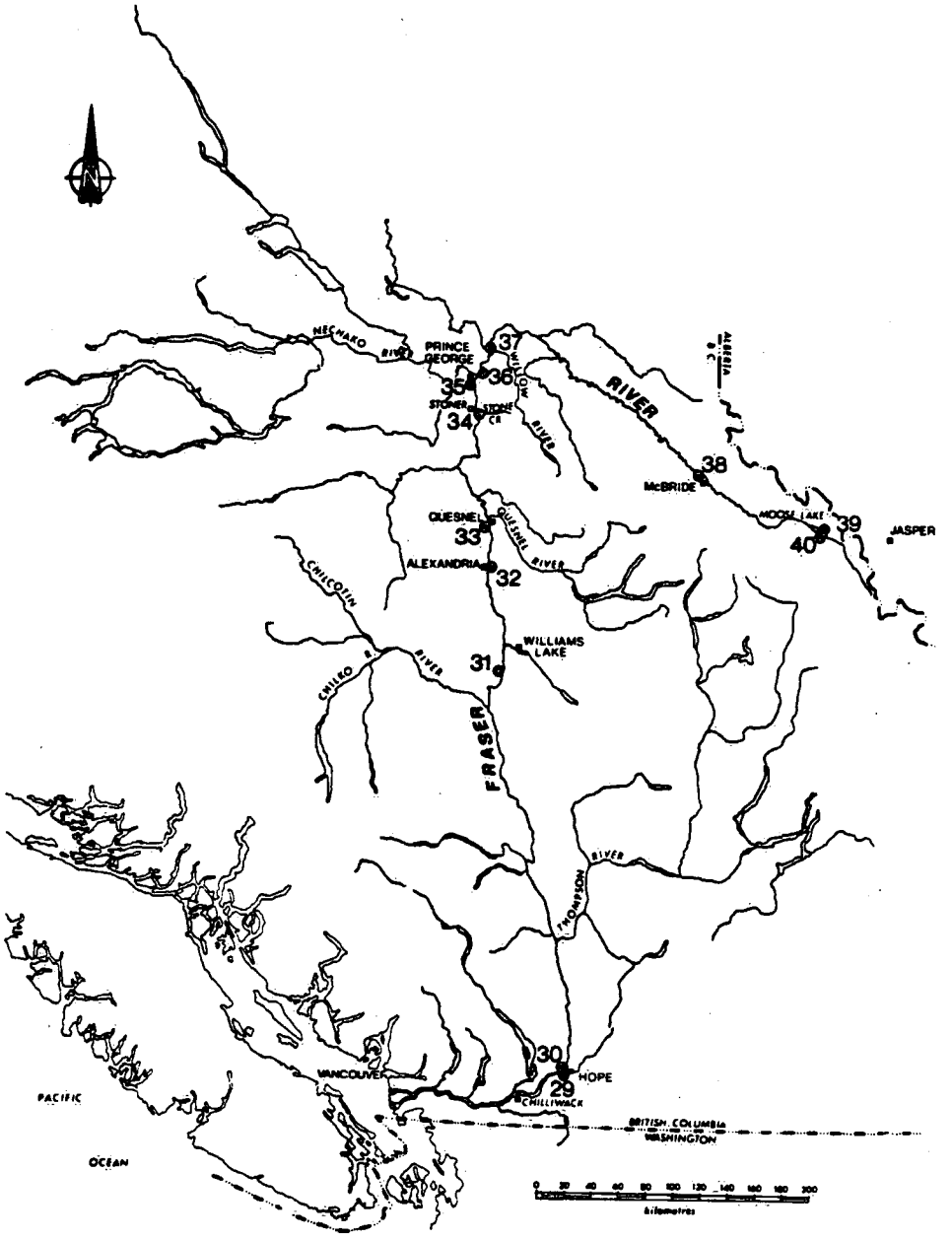

Figure 1. Interior British Columbia Fraser River Sampling Sites using Battery of Tests Approach

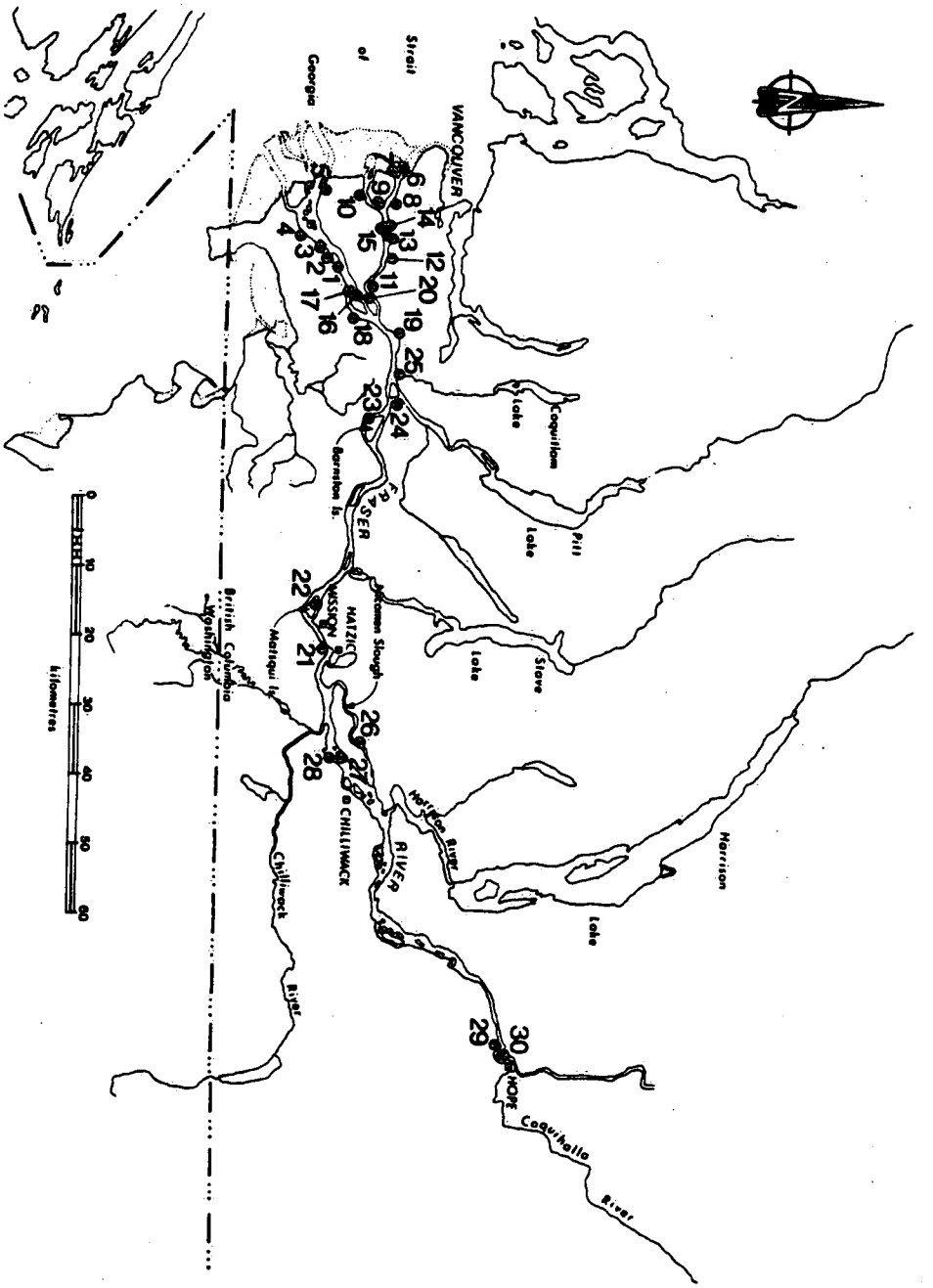

Figure 1a. Lower Fraser River Sampling Sites using Battery of Tests Approach.

Table 1. Fraser River Basin, British Columbia, Canada. Sampling Site Locations and Sediment Description.

Station Name and Number	Latitute	Longitude	Sediment Description and Shepard Classification
1. Cove just upstream of Tilbury Dock	49°08'54"N	123°00'36"W	sand 6.24%, silt 78.05% clay 15.71% SILT
2. Tilbury Slough West side of Hopcott Rd. (off culvert)	49°08'18"N	123°01'30"W	sand 78.73%, silt 16.81%, clay 4.46% SAND
3. Deas Slough	49°07'14"N	123°03'30"W	sand 12.75%, silt 62.66% clay 24.59% CLAYEY SILT
4. Ladner Harbour	49°05'31"N	123°05'24"W	sand 5.29%, silt 68.19%, clay 26.52% CLAYEY SILT
5. Steveston, Cannery Channel	49°07'09"N	123°10'00"W	sand 19.67%, silt 61.05% clay 19.28% SANDY SILT
6. North Arm Jetty, North shore of jetty	49°13'26"N	123°12'54"W	sand 30.37, silt 55.92%, clay 13.71%, organic material present SANDY SILT
7. McDonald Slough	49°12'45"N	123°11'11"W	sand 20.48%, silt 62.03% clay 17.49% SANDY SILT
8. Just upstream of Richmond Island, near mouth of storm sewer	49°12'20"N	123°08'51"W	sand 27.89%, silt 59.28%, clay 12.84% SANDY SILT
9. Middle Arm, West shore downstream of marinas	49°11'21"N	123°08'19"W	sand 12.04%, silt 61.30%, clay 26.65%
10. Middle Arm, downstream of Dinsmore Bridge, south shore	49°10'32"N	123°09'22"W	sand 17.69%, silt 63.92% clay 18.39% CLAYEY SILT

Table 1. continued

Station Name and Number	Latitute	Longitude	Sediment Description and Shepard Classification
11. North Arm, North shore at Burnaby Bend	49°10'52"N	122°58'33"W	sand 51.85%, silt 38.23% clay 9.91% SILTY SAND
12. North Arm, North shore at dock of MacMillan Bloedel, White Pine Division	49°12'11"N	123°02'03"W	sand 14.47%, silt 66.71%, clay 18.82% CLAYEY SILT
13. Mitchell Island North shore across channel from Aero Trading	49°12'18"N	123°05'25"W	sand 23.55%, silt 61.63% clay 14.82%, organic material present SANDY SILT
14. Mitchell Island, North shore ≈400 m upstream of transmission line crossing	49°12'16"N	123°06'33"W	sand 28.93%, silt 58.81%, clay 12.27% SANDY SILT
15. Mitchell Island, South shore at Western Canada Steel	49°11'59"N	123°06'03"W	sand 37.21%, silt 53.08% clay 9.71%, organic material present SANDY SILT
16. Annacis Channel, North shore downstream end near Shelter Island Marina	49°10'04"N	122°58'08"W	sand 30.28, silt 49.15%, clay 20.57% SANDY SILT CLAY
17. Southwest shore of Annacis Island, near Purfleet Pt.	49°09'34"N	122°58'53"W	sand 28.90%, silt 54.22% clay 16.88% SANDY SILT
18. Gundersen Slough	49°10'20"N	122°55'05"W	sand 16.2%, silt 56.15%, clay 27.65% CLAYEY SILT
19. Mouth of Brunette River	49°13'11"N	122°53'26"W	sand 15.27%, silt 66.09%, clay 18.64% CLAYEY SILT
20. Tree Island Slough	49°11'02"N	122°57'44"W	sand 21.23%, silt 58.25% clay 20.51%, organic material present SANDY SILT CLAY

Table 1. continued

Station Name and Number	Latitute	Longitude	Sediment Description and Shepard Classification
21. Across Fraser River from Haztic	49°08'29"N	122°15'46"W	sand 70.44%, silt 27.40% clay 2.16% SILTY SAND
22. Matsqui Island, Northwest shore	49°07'44"N	122°21'05"W	sand 42.76%, silt 53.77%, clay 3.47% SANDY SILT
23. Barnston Island, Parsons Channel at bend in island	49°11'15"N	122°43'00"W	sand 71.81%, silt 25.80% clay 2.38%, organic material present SILTY SAND
24. North Shore of Fraser just upstream of mouth of Pitt River	49°13'31"N	122°45'30"W	sand 31.71%, silt 57.86%, clay 10.43%, organic material present SANDY SILT
25. Mouth of Coquitlam River	49°13'32"N	122°48'13"W	sand 19.56%, silt 60.33% clay 20.11% CLAYEY SILT
26. Nicomen Slough downstream of Highway 7 Bridge at Deroche	49°11'08"N	122°04'03"W	sand 32.24, silt 62.32, clay 5.45%, organic material present SANDY SILT
27. South end of Yaalstrick Indian Reserve 1	49°09'45"N	122°02'53"W	sand 63.50%, silt 33.49% clay 3.01%, organic material present SILTY SAND
28. South shore of river, on down-current base of Chilliwack Mountain	49°08'47"N	122°03'10"W	sand 71.70%, silt 25.27%, clay 3.03%, organic material present SILTY SAND
29. Hope, backwater behind Bristol Island	49°22'24"N	121°28'34"W	sand 30.51%, silt 43.83%, clay 24.66%, organic material present SANDY SILTY CLAY
30. Hope, backwater behind Croft Island	49°22'39"N	121°27'28"W	sand 73.21%, silt 23.48% clay 3.31%, organic material present SILTY SAND

Table 1. continued

Station Name and Number	Latitute	Longitude	Sediment Description and Shepard Classification
31. Upstream of Chilcotin Highway Bridge, west bank	51°59'12"N	122°16'30"W	sand 95.03%, silt and clay 4.97% SAND
32. East shore of Fraser River at site of old Alexandria Ferry	52°39'00"N	122°29'22"W	sand 66.60%, silt 32.38%, clay 1.02%, organic material present SILTY SAND
33. Quesnel, East side of river, 2 km downstream of Westply lumber mill	52°54'41"N	122°28'40"W	sand 33.17%, silt 51.38% clay 15.45% SANDY SILT
34. Stoner, just downstream of mouth of Stone Creek	53°38'10.5"N	122°40'07"W	gravel .12%, sand 65.40%, silt, 32.94%, clay 1.55%, organic material present SILTY SAND
35. Prince George, west bank of river, 1.4 km downstream of Hwy 97 Bridge	53°52'53"N	122°45'40"W	sand 54.21%, silt 40.32% clay 5.47% SILTY SAND
36. Prince George, east bank of Fraser, downstream of mouth of Bittner Creek	53°55'48"N	122°39'59"W	sand 58.00, silt 39.43%, clay 2.57%, organic material present SILTY SAND
37. North shore of Fraser, downstream of mouth of Willow River	54°04'56"N	122°32'15"W	sand 59.64%, silt 35.66% clay 4.70% SILTY SAND
38. McBride, west bank of river just downstream of Hwy 16 bridge	53°18'12"N	120°08'27"W	sand 76.48%, silt 21.48%, clay 2.04%, organic material present SAND
39. Moose Lake, North of Fraser River entrance to lake	52°56'11"N	118°50'38"W	sand 61.74%, silt 34.19%, clay 4.07%, organic material present SILTY SAND
40. Moose Lake, South of Fraser River entrance to lake	52°55'56"N	118°51'06"W	sand 69.15%, silt 28.72% clay 2.14%, organic material present SILTY SAND

Table 2. Point Awarding Scheme for Sample Ranking, Based on Suspected Contained Hazards.

Fecal Coliform Fecal Streptococci Enterococci per 10 g/100 mL sediment /100 mL water	Coliphage per 100 mL water	Clostridium Perfringens per 10g/100 mL sediment	Points	Coprostanol ppb	SOS Chromotest Genotoxicity Induction Factor per mL 10x Water Sample 1:1 Milli Q Water	Algal-ATP % Relative Light Units per mL 10x water sample 1:1 Milli Q Water	Points	Cholesterol ppb	Points
1 - 100	5 - 24	1 - 25	1	<1.0	1.0 - 1.29	100 - 50	1	<2.0	1
101 - 500	25 - 100	26 - 100	2	1 - 3.0	1.30 - 1.50	49 - 20	3	2.1 - 4.0	2
501 - 2500	101 - 250	101 - 500	3	3.1 - 5.0	1.51 - 2.0	19 - 1.0	5	4.1 - 6.0	3
2501 - 16000	251 - 1000	501 - 2500	4	5.1 - 7.0	2.1 - 3.0	.9 - .1	7	6.1 - 8.0	4
16001 - 160000	1001 - 5000	2501 - 10000	7	7.1+	3.1+	.09+	10	8.1+	5
160000+	5001+	10000+	10						

ATP-TOX System % Inhibition per mL 10x Water Sample 1:1 Milli Q Water Sediment Extract	Microtox EC_{50} % per mL 10x Water Sample 1:1 Milli Q Water Sediment Extract	Points	Daphnia magna EC % per mL 1:1 Milli Q Water Sediment Extract	Points	Daphnia magna EC % per mL Water Sample	Points	Spirillum volutans 10x Water Sample 1:1 Milli Q Water Sediment Extract	Points
1 - 30	40.0+	1	EC_{20} at 100%	1	EC_{20} at 100%	1	negative	0
31 - 60	40.0 - 25.0	3	EC_{40} at 100%	2	EC_{40} at 100%	2	positive	5
61 - 90	24.0 - 10.0	5	EC_{50} at 100%	4	EC_{50} at 100%	5		
91 - 99	9.0 - 1.0	7	at 75%	5	at 75%	7		
100	.9 +	10	at 50%	6	at 50%	8		
			at 25%	8	at 25%	10		
			at 10%	10				

Table 3. Results of Fraser River Basin Water Analyses by Battery of Tests Approach

Sample Number	Fecal Coliform MF-mFC /100 mL	Fecal Strepto-cocci MF-KF /100 mL	Entero-cocci MPN /100 mL	Coliphage /100 mL	Fecal Sterols		Microtox EC_{50} % mL	Algal-ATP RLU[1] %	Spirillum volutans 120 min test	SOS Chromotest Induction Factor	ATP-TOX % Inhibition[2]	Daphnia[3] Magna EC_{50} % Sample	Points	Rank
					Coprostanol ppb	Cholesterol ppb								
1	150	42	79	<5	<0.05	0.3	NEG	S[2]	NEG	.77	NEG	EC_{20}	6	12
2	400	490	220	<5	<0.05	0.6	NEG	S	NEG	.54	19	NEG	8	10
3	60	18	11	<5	<0.05	0.6	NEG	S	NEG	1.00	16	NEG	6	12
4	198	57	49	<5	<0.05	0.3	NEG	S	NEG	.92	NEG	NEG	5	13
5	43	30	49	<5	0.5	2.2	NEG	S	NEG	.92	NEG	NEG	6	12
6	100	45	110	<5	<0.05	0.4	49.8	S	NEG	.69	17	NEG	7	11
7	200	39	110	<5	<0.05	0.6	NEG	S	NEG	.85	14	NEG	7	11
8	100	34	170	<5	<0.05	0.4	18.1	S	NEG	.92	14	NEG	11	7
9	70	59	49	10	<0.05	0.1	NEG	S	NEG	.92	NEG	EC_{30}	6	12
10	210	30	130	<5	<0.05	0.2	NEG	S	NEG	.92	8.7	EC_{40}	9	9
11	100	30	49	<5	<0.05	0.2	NEG	S	NEG	.92	NEG	NEG	4	10
12	4600	37	79	<5	<0.05	0.2	NEG	S	NEG	.92	NEG	50%	15	3
13	6400	43	49	<5	<0.05	0.1	NEG	S	NEG	.92	35	88%	15	3
14	3100	25	33	<5	<0.05	0.1	NEG	S	NEG	1.15	NEG	NEG	8	10
15	4100	47	31	5	<0.05	0.2	19.8	S	NEG	.92	NEG	NEG	13	5
16	120	220	170	10	<0.05	0.2	NEG	S	NEG	.92	18	EC_{40}	11	7
17	130	76	310	15	0.3	0.9	NEG	S	NEG	.93	1.0	88%	14	4
18	110	89	140	<5	<0.05	0.6	NEG	S	NEG	.86	28	100%	11	7
19	910	227	170	<5	0.2	1.5	NEG	S	NEG	.86	16	100%	15	3
20	20	17	49	10	<0.05	0.3	NEG	S	NEG	1.14	18	NEG	8	10

RLU[1] = Relative Light Units
S[2] = Growth stimulatory
Daphnia[3] = performed on unconcentrated water sample

Table 3. Results of Fraser River Basin Water Analyses by Battery of Tests Approach continued

Sample Number	Fecal Coliform MF-mFC /100 mL	Fecal Strepto-cocci MF-KF /100 mL	Entero-cocci MPN /100 mL	Coliphage /100 mL	Fecal Sterols		Microtox EC_{50} % mL	Algal-ATP RLU[1] %	Spirillum volutans[2] 120 min test	SOS Chromotest[2] Induction Factor	ATP-TOX % Inhibition[2]	Daphnia Magna EC_{50} % Sample	Points	Rank
					Coprostanol ppb	Cholesterol ppb								
21	90	30	49	5	<0.05	0.5	NEG	S	NEG	1.43	29	EC_{40}	11	7
22	40	49	49	<5	<0.05	0.6	NEG	S	NEG	1.14	NEG	EC_{40}	7	11
23	21	27	49	<5	<0.05	0.6	NEG	S	NEG	1.14	3.0	100%	11	7
24	130	42	49	15	<0.05	0.1	NEG	S	NEG	1.14	NEG	EC_{20}	8	10
25	100	16	17	<5	<0.05	0.2	NEG	S	NEG	1.14	NEG	EC_{30}	7	11
26	60	28	17	<5	<0.05	0.5	24.9	S	NEG	1.00	17	50%	19	1
27	120	22	79	5	<0.05	0.1	NEG	S	NEG	1.14	2.4	82%	13	5
28	300	32	70	<5	<0.05	0.1	NEG	S	NEG	1.43	7.1	78%	14	4
29	10	29	12	<5	<0.05	0.1	46.5	S	NEG	1.14	17	84%	12	6
30	110	49	130	<5	<0.05	0.4	NEG	S	NEG	.86	18	100%	12	6
31	150	17	49	<5	<0.05	0.2	NEG	S	NEG	1.29	14	100%	12	6
32	1500	117	280	<5	<0.05	0.1	S	S	NEG	1.00	NEG	82%	14	4
33	310	40	110	<5	<0.05	0.1	NEG	S	NEG	1.14	24	82%	13	5
34	45	10	79	<5	<0.05	0.1	NEG	S	NEG	1.43	NEG	80%	12	6
35	12	4	23	<5	<0.05	0.1	5.7	S	NEG	.14	1.2	100%	18	2
36	15	2 5	43	10	<0.05	0.2	NEG	S	NEG	1.14	1.7	92%	12	6
37	25	4	13	<5	<0.05	0.3	30.8	S	NEG	1.14	1.9	92%	14	4
38	4	1	1 8	<5	<0.05	0.5	NEG	S	NEG	1.08	21	84%	11	7
39	2	<1	1 2	<5	<0.05	1.8	NEG	S	NEG	1.00	19	84%	10	8
40	<1	<1	<2	<5	<0.05	0.8	NEG	S	NEG	1.00	21	84%	8	10

Table 4. Results of Fraser River Basin Sediment Analyses by Battery of Tests Approach

Sample number	Fecal coliform A1 broth 10g/100 mL MPN	*Clostridium perfringens* 10g/100 mL MPN	Microtox EC_{50}/mL Water Extract	Algal-ATP %RLU/mL Water Extract	*Spirillum volutans* 120 min test Water Extract	SOS Chromotest Induction Factor/mL Water Extract	ATP-TOX % Inhibition /mL Water Extract	*Daphnia Magna* EC_{50} Water Extract	Points	Rank
1	7900	170	S	S[1]	NEG	1.00	NEG	75%	13	9
2	9	1,600	NEG	S	NEG	1.00	NEG	EC_{20}	7	15
3	280	240	NEG	S	NEG	.92	NEG	NEG	5	16
4	7,000	430	NEG	S	NEG	1.00	NEG	NEG	8	14
5	7,900	>16,000	NEG	S	NEG	1.08	NEG	NEG	15	7
6	3,300	2,800	NEG	S	NEG	.83	NEG	NEG	12	10
7	3,500	1,600	NEG	S	NEG	.67	NEG	NEG	8	14
8	11,000	16,000	NEG	S	NEG	.83	8.3	EC_{30}	15	7
9	7,900	1,100	NEG	S	NEG	.92	28	NEG	9	13
10	4,900	430	NEG	S	NEG	1.00	NEG	NEG	8	14
11	160,000	95	NEG	S	NEG	.92	NEG	100%	13	9
12	2,300	840	NEG	NEG	NEG	1.34	0.8	NEG	11	11
13	92,000	430	NEG	NEG	NEG	1.47	19	NEG	14	8
14	9,500	2,100	NEG	S	NEG	1.15	21	NEG	10	12
15	4,900	700	NEG	S	NEG	.89	2.5	NEG	9	13
16	7,000	1,200	NEG	S	NEG	1.00	13	NEG	10	12
17	7,000	16,000	NEG	S	NEG	1.00	23	NEG	16	6
18	>160,000	3,500	S	S	NEG	.86	NEG	NEG	17	5
19	3,300	1,200	S	S	NEG	.78	2.7	NEG	12	10
20	95	430	S	S	NEG	1.00	NEG	NEG	5	16

S[1] = Growth stimulatory

Table 4. Results of Fraser River Basin Sediment Analyses by Battery of Tests Approach continued

Sample number	Fecal coliform Al broth 10g/100 mL MPN	Clostridium perfringens 10g/100 mL MPN	Microtox EC_{50}/mL Water Extract	Algal-ATP %RLU/mL Water Extract	Spirillum volutans 120 min test Water Extract	SOS Chromotest Induction Factor/mL Water Extract	ATP-TOX % Inhibition /mL Water Extract	Daphnia Magna EC_{50} Water Extract	Points	Rank
21	92,000	240	S	S	NEG	1.00	NEG	EC_{30}	12	10
22	35,000	280	S	S	NEG	.78	NEG	EC_{20}	11	11
23	4,900	70	S	S	NEG	1.00	NEG	EC_{30}	8	14
24	13,000	140	NEG	S	NEG	.86	1.0	NEG	8	14
25	3,500	16,000	NEG	S	NEG	.93	7.1	31%	21	3
26	7,160,000	40	NEG	S	NEG	1.00	NEG	EC_{40}	15	7
27	4900	540	NEG	S	NEG	.93	NEG	60%	13	9
28	2300	2,200	S	S	NEG	1.00	12	40%	13	9
29	46	1,100	S	S	NEG	1.00	NEG	45%	12	10
30	28,000	700	NEG	S	NEG	1.07	NEG	6.4%	22	2
31	220	11	NEG	25.9	NEG	1.17	9	18%	16	6
32	35,000	950	NEG	NEG	NEG	1.17	25	30%	19	4
33	160,000	3,500	NEG	NEG	NEG	.92	NEG	.24%	24	1
34	>160,000	700	NEG	S	NEG	1.17	NEG	EC_{40}	17	5
35	>160,000	1,400	S	S	NEG	.92	NEG	EC_{40}	16	6
36	35,000	17	S	S	NEG	1.17	NEG	NEG	9	13
37	22,000	39	S	S	NEG	1.17	NEG	NEG	9	13
38	92,000	15	S	S	NEG	1.08	NEG	EC_{20}	10	12
39	490	35,000	NEG	S	NEG	1.17	10	NEG	14	8
40	5	49	NEG	S	NEG	1.00	NEG	EC_{30}	5	16