Environment Canada
Conservation and Protection
Environmental Protection

Environnement Canada
Conservation et la Protection
Protection de l'environnement



# CHEMICAL CHARACTERIZATION, AQUATIC TOXICITY AND ENVIRONMENTAL IMPACT OF UNTREATED EFFLUENT DISCHARGES FROM THREE TEXTILE MILLS IN THE ATLANTIC REGION



Surveillance Report EPS-5-AR-93-1
Atlantic Region

TD
172
C3352
NO. 93-1
c. 1

Canada

## ENVIRONMENTAL PROTECTION REPORT SERIES

Surveillance reports present the result of monitoring programs carried out by Environmental Protection. These reports will usually be published on a regular basis.

Other categories in the EP series include such groups as Regulations, Codes and Protocols; Policy and Planning; Economic and Technical Appraisal; Technology Development; Briefs and Submissions to Public Inquiries; and Environmental Impact and Assessment.

Inquiries pertaining to Environmental Protection Reports should be directed to Environmental Protection, Conservation and Protection, Environment Canada, Dartmouth, Nova Scotia B2Y 2N6.

# CHEMICAL CHARACTERIZATION, AQUATIC TOXICITY AND ENVIRONMENTAL IMPACT OF UNTREATED EFFLUENT DISCHARGES FROM THREE TEXTILE MILLS IN THE ATLANTIC REGION

L.A. Rutherford, P.A. Hennigar, K.G. Doe, M.L. Nicol,
M.M.E. Holmes, B.C. MacDonald and W.H. Horne

Environmental Protection
Conservation and Protection
Environment Canada
Atlantic Region

1992

EPS-5-AR-93-1

## ABSTRACT

The untreated effluent from three textile mills in the Atlantic Region was chemically characterized and evaluated for acute and sub-lethal aquatic toxicity. In addition, the ecological impact of untreated effluent discharges on aquatic environments was determined. Organic chemicals identified in effluent samples generally fell into one of five groups: detergents/surfactants; plasticizers; dye carriers; mineral oils; and miscellaneous chemicals. The greatest number of organic compounds identified in the effluent samples were typical of auxiliary chemicals used for satisfactory dyeing. Conventional pollutants identified in high concentrations in the effluents were BOD, COD, oil and grease and ammonia in the effluent from one mill. The results of a battery of toxicity tests showed that: all samples were acutely toxic to all organisms tested (except one sample for Microtox); all samples had sub-lethal toxic effects to all species tested, including reproductive impairment in Ceriodaphnia dubia and growth impairment in the alga Selenastrum capricornutum; and all samples were mutagenic. A statistically significant decrease in abundance and diversity of benthic macroinvertebrates was observed at sampling stations in the effluent plume at one mill discharging to a freshwater river. This ecological impact was observed during sampling programs in the fall and spring and was not specific to one group of aquatic organisms. Similar decreases in abundance and diversity of benthic macroinvertebrates were not observed within the influence of the other mill discharges due to unsuitable field sampling conditions.

## RÉSUMÉ

Les effluents non traités de trois usines de textile de la région de l'Atlantique ont été caractérisés et évalués chimiquement en termes de toxicité aquatique aiguë et sublétale. En outre, l'effet écologique des effluents non traités sur les milieux aquatiques a été déterminé. Les produits chimiques organiques identifiés dans les échantillons d'effluents se répartissent en général dans cinq groupes: les détergents/agents tensio-actifs; les plastifiants; les véhiculeurs de colorants; les huiles minérales; et les composés chimiques divers. La plupart des composés organiques identifiés dans les échantillons d'effluents étaient typiques des produits chimiques auxiliaires utilisés pour obtenir une coloration satisfaisante. On a observé dans les effluents de fortes concentrations des polluants classiques suivants: DBO, DCO, huiles et graisses et ammoniaque dans les effluents d'une usine. Les résultats d'une batterie de tests de toxicité ont révélé que: tous les échantillons présentaient une toxicité aiguë pour tous les organismes étudiés (sauf un échantillon, pour le Microtox); tous les échantillons avaient des effets toxiques sublétaux sur toutes les espèces étudiées, notamment des altérations de la reproduction de Ceriodaphnia dubia et de la croissance de l'algue Selenastrum capricornutum; et tous les échantillons étaient mutagènes. Une diminution statistiquement significative de l'abondance et de la diversité des macroinvertébrés benthiques a été observée dans les stations d'échantillonnage du panache des effluents qu'une des usines rejette dans un cours d'eau douce. Cet effet écologique a été observé durant les programmes d'échantillonnage d'automne et de printemps, et n'était pas spécifique d'un groupe d'organismes aquatiques. Des diminutions semblables d'abondance et de diversité des macroinvertébrés benthiques n'ont pas été observées dans la zone d'influence des effluents des autres usines à cause de conditions non propices d'échantillonnage sur le terrain.

## ACKNOWLEDGEMENTS

The authors wish to thank: D. McBain, R. Chenier and D. Moore, Commercial Chemicals Branch, Environmental Protection, Ottawa; E.C. Chen, Industrial Programs Branch, Environmental Protection, Ottawa; Dr. R.J. Maguire, J.L. Metcalfe-Smith, K. Day and B. Dutka, National Water Research Institute, Burlington, Ontario; and J.R. MacDonald, Dr. F.C. Duerden, W.R. Ernst and J.R. Machell, Environmental Protection, Atlantic Region, for their valuable review of this report.

The assistance of: W.R. Ernst in study design; Dr. G. Atkinson, Office of the Science Advisor, Environment Canada, Ottawa in statistical support; G.R.J. Julien, K.M. Kieley, P.J. Hennebury and C.M. Hutchinson, Environmental Protection, Atlantic Region and V. Spencer, A. Hennigar and A. Shepard in effluent collection and field sampling; S. Wade and D. Vaughan, Environmental Protection, Atlantic Region in toxicity testing; and J. Keating, Environmental Protection, Atlantic Region in word processing, is gratefully acknowledged.

Funding for this study was provided by Environment Canada's CONFUND and Priority Substances Assessment Program.

Special thanks are extended to the staff of the textile mills for their cooperation in this study.

# TABLE OF CONTENTS

## TABLE OF CONTENTS (CONT'D)

## TABLE OF CONTENTS (CONT'D)

## TABLE OF CONTENTS (CONT'D)

## LIST OF FIGURES

# LIST OF TABLES

## LIST OF TABLES (CONT'D)

## LIST OF TABLES (CONT'D)

## 1.0 INTRODUCTION

Textile mills use a wide variety of dyes and chemicals. Many of these are not retained in the final product and are discarded in liquid effluent discharges. Textile wastewaters are generally characterized by: aesthetically objectionable colours; high concentrations of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), suspended solids, heavy metals; extreme pH; and elevated temperatures. Furthermore, some dyes, dyeing assistants and finishing compounds are toxic and possibly carcinogenic (Thompson 1974; Netzer and Beszedits 1975; Chen 1989; Maguire and Tkacz 1991). Textile wastewaters contain a variety of chemicals, depending on the processes and raw materials used. The effluents could be toxic to aquatic organisms due to their chemical constituents as well as their physical properties, such as BOD, COD, temperature, pH, solids content (U.S. EPA 1978; Chen 1989).

The acute toxicity of untreated, undiluted textile mill effluent to aquatic organisms has been known for some time (U.S. EPA 1978). Toxicity assessments of textile effluents in Canada have generally focussed on acute toxicity to fish at mills with some form of wastewater treatment. Measured impacts on aquatic systems, particularly those associated with chronic discharges of persistent chemicals, have not been well established.

This project was conducted to assess the toxicity and ecological impact of textile mill effluent discharges, in relation to the definition of a "toxic" substance under the Canadian Environmental Protection Act (CEPA). Under CEPA, a substance is toxic if it has harmful effects to human health or the environment.

The objectives of the study were:

- to chemically characterize significant components of textile mill effluents;
- to determine the environmental concentrations of selected substances in environmental matrices near textile mill effluent discharges;
- to determine the toxicity of textile mill effluents to a variety of aquatic organisms;
- and to measure changes in structure of benthic macroinvertebrate communities related to textile mill discharges.

## 2.0 DESCRIPTION OF TEXTILE MILLS SURVEYED

Site visits were made by Environmental Protection, Atlantic Region staff to six regional textile mills in July 1990. The objective of the site visits was to evaluate the suitability of the mills for study. Criteria used to judge suitability were: the representativeness of their processes (e.g. an attempt was made to include several categories of wet processing textile mills); the mill discharged treated or untreated effluent directly to the receiving environment (watercourse); and the receiving environment was amenable to investigations of the abundance of benthic macroinvertebrates upstream and downstream of the effluent outlet.

Based on the above criteria, three regional textile mills were selected for study: Stanfields Ltd., Truro, N.S.; Britex Ltd., Bridgetown, N.S.; and Tandem Fabrics Inc., Moncton, N.B.

### 2.1 Stanfields

Stanfields Ltd. is a knit fabric dyeing and finishing operation that produces underwear, knitted sportswear and hosiery. The company was established in 1930 and employed approximately 680 people at the Truro plant in 1988-89 (N.S. Dept. of Industry, Trade and Technology 1988).

Atmospheric batch dyeing (in large vats) was used to dye broad, knitted fabric using disperse or reactive dyes. Other chemicals used in large quantities included caustic soda, hydrogen peroxide, sodium chloride, acetic acid and petroleum distillates (carriers). Rag solids were removed from the final effluent before being passed through a heat exchanger. The untreated final effluent was discharged to a municipal sewer line about 200 m from the plant, where it mixed with town sewage before being directly discharged to the Salmon River about 30 m later. The average daily process water discharge from the plant was approximately 180,000 litres (Environmental Protection, unpublished data).

## 2.2 Britex

Britex Ltd. is a woven fabric dyeing and finishing operation specializing in the production of elasticized materials such as knitted, woven and netted elastic fabrics. The company was established in 1980 and employed 200 people in 1988-89 (N.S. Dept. of Industry, Trade and Technology 1988).

Pressurized batch dyeing of knitted, broad cloth and continuous dyeing of narrow, knitted cloth was conducted at the plant. Along with dyes, acetic acid, citric acid and aliphatic alcohols were used in significant quantities at the plant. The process and sanitary waste streams were combined in the plant and discharged untreated into the Annapolis River (approximately 500 m from the plant) via a small wetland and tidally influenced brook. The average daily process water discharge from the plant was approximately 375,000 litres (Environmental Protection, unpublished data).

## 2.3 Tandem Fabrics

Tandem Fabrics Inc. is a woven fabric dyeing and finishing operation that produces vertical surface fabrics for office wall systems, fabrics for upholstery and spun yarn. The company was established in 1974 and employed 120 people in 1990 (N.B. Dept. of Commerce and Technology 1990).

Wet processes conducted at Tandem Fabrics included desizing, scouring, bleaching and dyeing of raw wool and polyester fibres and dyeing of woven fabric. The Upper Dyehouse at Tandem conducted stock dyeing of raw fibre in kettles, the Lower Dyehouse conducted atmospheric batch dyeing of open width, woven fabric in vats. Along with dyes, significant quantities of acetic acid, formic acid, ammonium sulphate and ethylene glycol were used at the plant. The untreated process water from the plant was discharged in batches to Humphrey's Brook. Based on the average number of batches discharged daily from the plant (14 during this survey) and the size of the vats and kettles used for dyeing and treating raw fibre and fabric, the average daily process water discharge from the plant was approximately 84,000 litres.

## 3.0 METHODS

### 3.1 Effluent Collection

At four times during the fall of 1990, final effluent samples were collected for chemical characterization and toxicity assessment from the three textile mills surveyed. The collection method used was dependent on the nature of the discharge (i.e. continuous or batch) and process equipment used at the mill.

#### 3.1.1 Stanfields

A twenty-four hour final effluent sample was collected from Stanfields on October 9-10, October 29-30, November 20-21 and December 3-4, 1990.

Effluent samples were collected from the drainage valve on the heat exchanger using 2.5 cm ID stainless steel tubing to direct the effluent to a 9 L stainless steel pail positioned outside the building that housed the heat exchanger. Two Sigmamotor Model 6200 automatic samplers were used to collect the samples from the stainless steel pail. To minimize the potential for contamination from the medical grade silicone rubber tubing used in the pump head, lengths of silicone tubing were restricted to 30 cm.

For the samples for chemical characterization (excluding metals), a uniform volume of effluent was collected every 15 minutes over a 24 hour period in a 20 L glass carboy that had been detergent washed and rinsed with distilled water, acetone and hexane. Stainless steel tubing (1 cm ID) was used to transport the effluent from the pail to the automatic sampler and from the sampler to the carboy. The composite sample was kept on ice during the period it was collected. After the 24 hour period, the composite sample was vigorously mixed then subdivided into the appropriate container for the various parameters to be measured. The subsamples were transported to the Environmental Protection (EP) Lab on ice and were preserved in accordance with EP Atlantic Region's established protocol.

For the samples for toxicity assessment and metals analyses, a uniform volume of effluent was collected every 15 minutes over a 24 hour period in a 208 L

heavy duty, high density polyethylene drum, that had been detergent washed and rinsed with distilled water. Food grade, vinyl tubing (8 mm ID) was used to transport the effluent from the pail to the automatic sampler and from the sampler to the drum. After the 24 hour period, the composite sample was vigorously mixed and a subsample was collected for metals analyses. Then eight 20 L food grade, polyethylene buckets were filled for toxicity assessment using rainbow trout, Daphnia and Microtox. Those samples were transported to the EP lab within 1 1/2 hours of being collected. During the October 9-10 and October 29-30 sampling runs, three 4 L amber glass ring jugs were filled for Ceriodaphnia (2 jugs), Ames and Selenastrum capricornutum toxicity testing. Those subsamples were shipped on ice to two private laboratories for testing within 24 hours of collection.

### 3.1.2 Britex

A twenty-four hour final effluent sample was collected from Britex on September 17-18, October 1-2, November 14-15 and November 26-27, 1990.

Effluent samples were collected from a sump inside the plant where the process and domestic waste streams were combined. Two Sigmamotor Model 6200 automatic samplers were used to collect the samples from the sump.

The collection methods used at Britex were the same as those described above for Stanfields. The subsamples for Ceriodaphnia, Ames and Selenastrum testing were collected on September 17-18 and October 1-2, 1990 and shipped to private laboratories within 24 hours of collection. All other samples were transported to the EP Lab within 2 1/2 hours of being collected.

### 3.1.3 Tandem Fabrics

A composite final effluent sample was collected from Tandem Fabrics on September 11, September 24, October 23 and November 6, 1990. Since this mill does not have a continuous effluent discharge, grab samples were collected from each batch that was discharged from the mill during the above days.

Grab samples were collected from the dyeing vats in the Upper and Lower Dyehouses in the plant just prior to discharge. One litre stainless steel ladels, which had been detergent washed and rinsed with distilled water, acetone and hexane, were used to collect the grab samples.

For the samples for chemical characterization, one litre of effluent was collected in a clean, one litre glass bottle from the dyeing vats for each batch discharged from the mill. This grab sample was then immediately transferred to a 20 L glass carboy that had been detergent washed and rinsed with distilled water, acetone and hexane. The composite sample in the carboy was kept on ice during the period it was collected. At the end of the production day, the composite sample was vigorously mixed then subdivided into the appropriate containers for the various parameters to be measured. The subsamples were transported to the EP Lab on ice and were preserved in accordance with EP Atlantic Region's established protocol.

For the samples for toxicity assessment, a uniform volume of effluent was collected by grab sampling from the dyeing vats. The grab samples were collected in clean, 20 L food grade, polyethylene buckets. Over the course of a production day, 7 to 9 buckets of effluent were collected. The samples were transported to the EP Lab within 24 hours of being collected. At the lab, the samples were mixed in a 208 L heavy duty, high density, polyethylene drum. Subsamples for Ceriodaphnia, Ames and Selenastrum capricornutum testing were collected from the drum on September 12 and September 25 and immediately shipped to private laboratories for testing.

## 3.2 Chemical Characterization

All effluent samples were analyzed by the Laboratory Division (BIO), Environmental Control Branch, Environmental Protection, Conservation and Protection, Atlantic Region.

### 3.2.1 Organic Analyses

Organic analyses had two objectives: to characterize the more significant organic constituents in untreated effluents from three textile mills in the

Atlantic Region; and to identify and quantify a limited number of target compounds in environmental samples collected near textile mill effluent outfalls. High resolution gas chromatography/mass spectrometry/computer (GC/MS/Comp.) techniques were used to chemically characterize mill effluents and to analyze environmental samples.

### 3.2.1.1 Effluent Analyses

Composite effluent samples (4 liters) were delivered to the laboratory in amber glass ring jugs. The jugs had been washed previously with detergent and rinsed with distilled water, then heat treated at 350°C for 3 hours and solvent rinsed prior to use. All samples were extracted within 24 hours of delivery. Samples were shaken well and one liter removed and stored for surfactant analyses. The pH of the remaining 3 litres was adjusted to 12 with 10% KOH and 300 mL of dichloromethane added. Samples were extracted by rotating bottles on a cell production roller apparatus (Wheaton Instruments, N.J.) at high speed for 17 hours. The dichloromethane layer was removed by drawing the solvent into 100 mL volumetric pipette with a large suction bulb. The solvent was filtered through 80 g of anhydrous $Na_2SO_4$ into a 500 mL glass evaporation flask and concentrated at ambient temperature to 2 or 3 mL. The remaining aqueous sample was carefully adjusted to pH 2 with $H_2SO_4$ (1+1) and further extracted for 18 hours with a second 300 mL of dichloromethane. The solvent was collected, dried and concentrated as previously described.

Analyses of the concentrated base/neutral and acidic extracts were conducted on a Hewlett Packard 5890 gas chromatograph coupled to a HP5970 MSD quadupole mass spectrometer. Chemical components in each extract were separated on a DB-5 30 meter X 0.25 mm id (1 um film thickness), fused-silica capillary column (J&W Scientific). The mass spectrometer was operated in full scan mode (EI, 70EV). The scan range was 35 to 500 amu and the scan rate was 0.92 scans per second. The gas chromatograph oven was held at 40° for 4 minutes and then programmed at 10°C/minute to a final temperature of 280°C and held for 20 minutes. Each day, prior to analyses of samples, the system was tuned with a perfluoro-tetabutylamine (PFTBA) calibration standard as per manufacturer's specifications.

### 3.2.1.2 Sediment Analyses

Sediment core samples were frozen at -14°C and stored until processing for GC-MS analyses. Sediment collection procedures are presented in Section 3.4.3.

Core samples were thawed and thoroughly mixed. An exact amount of sediment (15 g) was weighed into a 150 mL glass beaker. An equal portion was also taken for subsequent dry weight calculations. Anhydrous sodium sulphate (15 g) was mixed thoroughly with the sediment sample. The mixture was packaged in aluminum foil and placed overnight in a refrigerator at 4°C. The following morning, the sediment-sodium sulphate mixture was ground to a fine consistency in a glass mortar and pestle. The ground material was transferred to a 250 mL round bottom flask to which 100 mL of dichloromethane was added. The flask was shaken on a wrist action shaker for 30 minutes after which the flask was centrifuged for 20 minutes. The solvent was decanted and filtered into a 250 mL evaporation flask. The sediment was re-extracted with a second 100 mL of dichloromethane, centrifuged, filtered and the two solvent volumes combined. The solvent was then concentrated to 1 or 2 mL and treated with metallic mercury to remove any elemental sulphur present in the samples. The extract was analyzed by GC-mass spectrometry without further cleanup.

Gas chromatographic equipment and conditions used for the analyses of sediment samples were the same as described in Section 3.2.1.1, except that data acquisition was performed in selective ion mode rather than full scan. Relative retention times, primary (base) and secondary confirmatory ions were used for peak identification. Quantitation of target compounds was conducted using base ion peak areas. Fluorene served as an internal standard and eluted from the capillary column in a region free of interfering peaks.

The following mass ions were used for mass spectrometric identification and quantitation of target compounds:

| Target Compound | Base ion | Confirmatory ion |
|---|---|---|
| Methyl-2-pyrolidinone | 99 | - |
| Caprolactam | 113 | 85 |
| 4-tetramethylbutyl phenol | 135 | 206 |

Dwell time for individual ions was 100 m sec. while the scan time for the MS analysis was 1.2 cycles/second.

### 3.2.1.3 Clam Tissue Analyses

Clam collection procedures are presented in Section 3.4.2.

Clams collected for target compound analysis were shucked, the meats pooled and homogenized in a Waring blender. Approximately 10 g of homogenized tissue were accurately weighed into a 200 mL Berzelius beaker to which 100 mL of acetone were added. The mixture was homogenized at high speed with a Polytron blender for one minute after which the homogenate was vacuum filtered through a millipore gf/c glass fibre filter. The beaker and filter cake were rinsed with two additional 10 mL volumes of acetone that were combined with the first 100 mL of acetone. The filtrate was transferred to a separatory funnel containing 200 mL of dichloromethane/hexane (1:1). The funnel was shaken for 2 minutes and the liquid allowed to separate over 30 minutes into two phases. The bottom aqueous phase (about 10 mL) was drawn off into a 50 mL centifuge tube, saturated with sodium chloride and set aside. The remaining 300 mL of the acetone/hexane/dichloromethane mixture was drawn off into a 500 mL Erlenmeyer flask. The sodium chloride saturated aqueous phase was then returned to the separatory funnel and extracted twice with 30 mL of dichloromethane. The extracts were combined with the acetone/hexane/dichloromethane mixture. The combined solvent was dried over anhydrous sodium sulphate, concentrated and solvent exchanged to 0.5 ml dichloromethane and then made up to 1 mL with cyclohexane/methanol/dichloromethane (6:4:3). The concentrated extract was applied to a glass column (2 cm id) containing Sephadex LH-20 GPC beads (20 g) which had been previously swelled. The column was eluted with cyclohexane/ methanol/dichloromethane (6:4:3). The first 45 mL was discarded and the following 22 mL, containing the target compounds, were collected. The column eluate was transferred to a 125 mL separatory funnel and shaken with 60 mL distilled water. The dichloromethane was drawn off and saved. The water remaining in the separatory funnel was extracted twice with 25 mL dichloromethane. The solvent extracts were combined, dried over anhydrous sodium sulphate and concentrated to a small volume suitable for GC/MS analysis (3-10 mL).

Gas chromatographic equipment and conditions for the analyses of clam tissue were described previously (Section 3.2.1.2), except phenanthrene-d10 was used as an internal standard. The following mass ions were used for mass spectometric identification and quantitation of target compounds:

| Target Compound | Base | Confirmatory |
|---|---|---|
| 2-butoxy ethanol | 87 | 57 |
| 2-ethyl-1-hexanol | 83 | 57 |
| naphthalene | 128 | 102 |
| butoxyethoxy ethanol | 57 | 75 |
| 2-methylnaphthalene | 142 | 115 |
| 1-methylnaphthalene | 142 | 115 |
| biphenyl | 154 | 76 |
| 1,6-dimethylnaphthalene | 156 | 141 |
| 1,4-dimethylnaphthalene | 156 | 141 |
| 1,2-dimethylnaphthalene | 156 | 141 |
| 2,3,5-trimethylnaphthalene | 170 | 152 |
| tetramethylbutyl phenol | 135 | 107 |

### 3.2.1.4 Surfactant Analyses

The method is a modified cobalt thiocyanate active substances analysis similar to that described in American Public Health Association et al. (1989). Composite effluent samples were subsampled and filtered through a medium porosity qualitative filter paper. Exactly 100 mL of filtrate was placed in a modified 125 mL glass sublation apparatus. Sodium bicarbonate (0.5 g) and sodium chloride (10 g) were added, and 10 mL ethyl acetate was layered on top of the aqueous solution by carefully running it down the walls of a sublation tube. A stream of nitrogen gas was bubbled through the two layers at a rate of 100 mL/minute for 5 minutes, after which the ethyl acetate layer was drawn off and collected. The sample was sublated a second time with fresh ethyl acetate. The solvent was drawn off again and combined with the first 10 mL. The sublation cylinder was emptied and rinsed with an additional 10 mL of ethyl acetate which was added to the combined solvent extracts. The combined solvent

was filtered through anhydrous sodium sulphate and vacuum evaporated to remove the ethyl acetate. The residue was dissolved in 10 mL of dichloromethane and transferred to a separatory funnel containing 5 mL of a cobalt thiocyanate reagent. The separatory funnel was shaken and the lower organic layer containing the colored surfactant cobalt complex was drawn off and filtered through glass wool. Absorbances of the filtrate were obtained spectrophotometically at 620 mm.

Surfactant sample concentrations were determined from a standard calibration curve of absorbance readings and known concentrations of Triton X-100 standard solutions.

### 3.2.1.5 Computerized Mass Spectra Search

The mass spectra reference library used in this study to identify chemical compounds in textile mill effluent was the revised NBS/NIH/EPA/MSDC database (p/n HP59988). The library contains over 42,000 mass spectra. Later in the study, a larger Wiley/NBS merged database (p/n HP59983K) was used to supplement chemical identification. The Wiley database contains more than 130,000 mass spectra of 113,000 compounds.

The search technique used to match mass spectra of unknown compounds in effluent samples to mass spectra in the reference database was a probability-based matching algorithm (Hewlet Packard Company 1986). Using the computer's probability-based search technique to manually identify each peak in a chromatogram of a textile effluent, required up to one or two days of the analyst's time. Appendix A contains a macroprogramme devised to automatically perform a peak by peak search of a GC/MS total ion chromatogram. The macro runs unattended and reduces search time to at least one quarter. The macro prints sample information; integrates the total ion chromatogram of a base/neutral or acid extract; and for each peak in the chromatogram, performs a background correction; prints the mass spectrum of the unknown and carries out the algorithm search. If the probability of a match is greater than 65%, the three best reference spectra matches are printed for visual comparison to the unknown spectrum; otherwise, a "<65% probability" or "no match" message is printed. In addition, the names of all other reference spectra matches, match

purity, probability, molecular weight, chemical number and other numerical data related to the search are printed.

GC/MS chromatograms of textile mill effluents contained integrated peaks that were not identified by the computer assisted identification technique. In many cases, unidentified peaks were relatively small and low signal to noise background played a part in non-identification. In other cases, the mass spectra of unknown compounds either were not in the NBS (or Wiley) database or match probabilities were less than 65 percent.

### 3.2.2 Inorganic Analyses

Effluent samples for metal analyses were preserved in the field with 2 mL/L nitric acid. Samples were analyzed for total metals under standard operating conditions using a simultaneous Inductively Coupled Plasma (ICP) instrumentation. The instrument used was the Thermo Jarrell-Ash Model ICAP-61E.

All chemical analyses including: pH, alkalinity as $CaCO_3$ by inflection point titration (using a pH meter); oil and grease (petroleum ether as the solvent); Biochemical Oxygen Demand (BOD); Chemical Oxygen Demand (COD); solids; and ammonia using specific ion electrode, followed the recommended methods prescribed in American Public Health Association et al. (1989).

## 3.3 Aquatic Toxicity Testing

Toxicity assessment of the untreated effluents using rainbow trout, Daphnia, luminescent bacteria (Microtox$^R$) and threespine stickleback was conducted by the Laboratory Division (BIO), Environmental Control Branch, Environmental Protection, Conservation and Protection, Atlantic Region. The samples were mixed thoroughly and sub-sampled for the various tests immediately upon receipt of the samples. Tests were conducted within 48 hours of collection. All tests were performed in accordance with the Environment Canada Biological Test Methods (Environment Canada 1990a, b, c, d and e). Any deviations from these procedures are noted.

Sub-samples were also sent to two private laboratories for testing. Samples were tested for toxicity with algae and for mutagenicity with the Ames test by Bioquest International Inc., Winnipeg, Manitoba. Samples were tested for survival and reproductive impairment with Ceriodaphnia dubia by Beak Consultants Ltd., Brampton, Ontario.

### 3.3.1 Rainbow Trout

Rainbow trout (Oncorhynchus mykiss) tested during the study were from the EP laboratory stock 11 September 1990 (purchased from SPA Co-op, St. Peters, Nova Scotia). Holding and testing occurred at the Bedford Institute of Oceanography (BIO). The fish were held in fibreglass tanks with a continuous flow of clean water at 15 $\pm$ 2°C. A 16-hour light/8-hour dark photoperiod was maintained during holding and testing. The fish were acclimated to these conditions for a minimum of one week before testing. Fish were fed a commercial pelleted fish food daily up to 24 hours before testing.

Rainbow trout were exposed to the effluent in 96-hour static tests. A 50% dilution series was set up with a range from 6.25% to 100% effluent (v/v) plus a control. Control and dilution water was Dartmouth tap water which was sand and carbon filtered, and then UV sterilized to remove chlorine. Ten fish were exposed to each effluent concentration at a maximum loading rate of 0.5 g/L. In one case, only 8 fish were exposed per concentration due to effluent volume restrictions. Fish were not fed during the test. Daily observations were made for mortalities, temperature, dissolved oxygen, and pH. An initial conductivity reading was also taken in each concentration.

Aeration rates were as recommended in the Environment Canada procedure (1990 a, d). This calls for a 30-minute aeration before addition of the fish. If dissolved oxygen levels were acceptable (>70% and <100% saturation) the test was started. If not, pre-aeration was continued for a maximum of 1.5 hours more or until acceptable levels were reached. At that time the dissolved oxygen was recorded and the fish were added. In only two cases was the dissolved oxygen in the 100% test concentration below acceptable levels (Tandem Fabrics sample on September 12, 1990, and Britex sample on November 14, 1990). In those cases where dissolved oxygen concentrations were below 70% saturation

after the maximum pre-aeration period, a replicate test tank of 100% effluent was set up. This replicate tank was aerated vigorously until 70% saturation was reached, at which point the fish were added. An attempt was made to maintain acceptable oxygen levels during the test to determine whether mortality was due only to low dissolved oxygen levels, or if there were other toxicants present.

In some cases supersaturation (i.e. >100%) existed in the 100% effluent test tanks upon set-up. In these cases the test tanks were aerated for the maximum aeration period and then the fish were added regardless of the dissolved oxygen concentration. It was not clear whether the supersaturation actually existed or if there was an interference with the meter reading. Checks made to determine this indicated an interference with the meter reading.

No compensation was made for extreme pH levels (outside the range 5.5 to 8.5) in these tests. This was, however, addressed in the tests with Daphnia magna, showing no significant differences between the pH adjusted and the pH non-adjusted tests.

Calculations were made to determine the 96-hour LC50 (the effluent concentration that causes 50% mortality) and the 95% confidence limits using the recommended program of C.E. Stephan (Environment Canada 1990a, d). This program uses probit, moving average, and binomial methods. If there are two or more partial mortalities in the set of data all three methods are calculated but the probit result is reported. In the case of fewer than two partial responses the binomial method is the only one that will function and it only gives a best estimate of the LC50 with conservative confidence limits. The three methods for calculating an LC50 are discussed in more detail in Stephan (1977).

### 3.3.2 Daphnia

Daphnia magna used in toxicity tests were from the EP laboratory cultures at BIO. Culture beakers of adult daphnids were held in an incubator at 20 $\pm$ 2°C with a photoperiod of 16 hours light/8 hours dark. The culture and test water was moderately hard reconstituted water made from the U.S. EPA recipe (Peltier

and Weber 1985) to give a hardness of 80 to 100 mg/L. The daphnids were fed a laboratory culture of Chlorella vulgaris three times a week, supplemented with a suspension of Tetramin (a tropical fish food). Supplements of selenium (0.5 ppb) and vitamin B12 (1 ppb) were used as required.

Adult D. magna of known age (2-4 weeks) provide the neonates (young daphnids) for testing. The day preceding a test, all neonates were removed from the culture beakers to ensure that all neonates in the beakers on the test day were less than 24-hours old.

The daphnids were exposed to the effluent in 48-hour static tests (Environment Canada 1990b, e). Duplicate dilution series were set up with a range from 6.25% to 100% effluent (v/v) plus a control. Control and dilution water was the U.S EPA reconstituted water (Peltier and Weber 1985). Ten neonates were exposed in each test jar giving a loading rate of 15 mL/daphnid. They were not fed during the test.

At the test start and on termination, observations were made for temperature, dissolved oxygen, and pH. Tests were checked daily for mortalities. An initial conductivity reading was also taken in each concentration. Hardness levels were measured in the initial effluent sample.

Where pH levels were extreme (outside the range of 5.5 to 8.5) the replicate concentration series was tested with an adjusted pH to determine the effect of extreme pH values on the toxicity of the samples. The exception to this was the Stanfields sample from October 9-10 which was not adjusted. The decision to adjust the samples was made after reviewing the results of the Stanfields sample.

Calculations were made to determine the 48-hour LC50 and the 95% confidence limits using the recommended program of C.E. Stephan (Environment Canada 1990a, d).

### 3.3.3 Microtox

Freeze-dried luminescent bacteria (Photobacterium phosphoreum) as supplied by Microbics were tested using the Microtox Toxicity Assessment System (Microbics, 1989a, b). The Microtox system measures the difference in the light emitted by the luminescent bacteria before and after exposure to the effluent. Exposure times were 5 and 15 minutes, with the standard duplicate concentration series of 5.625% to 45% effluent (v/v) plus a control. If the EC50 was outside this concentration series either a lower or a higher series was tested to confirm the extrapolated result calculated by the Microtox program. Control and dilution water was the Microtox Diluent. Microtox Osmotic Adjustment Solution (MOAS) was used to osmotically adjust the samples to an acceptable salinity for the bacteria.

Calculations were made to determine the 5 and 15 minute EC50 (the effluent concentration that causes a 50% reduction in light emission) and the 95% confidence limits using the Microtox program. After calculation of the EC50, if an appreciable colour existed in the EC50 concentration, a colour correction test was conducted to eliminate colour as the source of light reduction.

### 3.3.4 Threespine Stickleback

In the case of Tandem Fabrics, where the discharge was to an estuarine receiving water, the threespine stickleback (Gasterosteus aculeatus) was used for testing. The stickleback tested during the study were from the EP laboratory stock 16 November 1989 collected from Grand Desert Beach, Nova Scotia, and held at BIO. They were held in fibreglass tanks with a continuous flow of sand filtered Bedford Basin seawater at 15 $\pm$ 2°C. A photoperiod of 16-hours light/8-hours dark was maintained during holding and testing. The fish were acclimated to these conditions for a minimum of 1 week before testing. Fish were fed frozen brine shrimp daily up to 24 hours before testing. The tests with the threespine stickleback were conducted at 15 $\pm$ 2°C, not 10°C as recommended by Environment Canada 1990c). Otherwise, the tests were conducted as in Environment Canada (1990c).

Threespine stickleback were exposed to the effluent in 96-hour static tests. A 50% dilution series was set up with a range from 6.25% to 100% effluent (v/v) plus a control. Control and dilution water was Bedford Basin seawater which was sand filtered. Ten fish were exposed in each concentration at a maximum loading rate of 0.5 g/L. Fish were not fed during the test. Daily observations were made for mortalities, temperature, dissolved oxygen, and pH. An initial conductivity reading was also taken in each concentration.

Calculations were made to determine the 96-hour LC50 and the 95% confidence limits using the recommended program of C.E. Stephan (Environment Canada 1990a, d).

### 3.3.5 Algae

Six samples of effluent were sent to Bioquest International Inc., Winnipeg, Manitoba for testing using the Selenastrum capricornutum test. Two samples from each mill were tested over 7 days to determine effects on algal growth. Samples were stored at 2°C and bioassays were performed within 48 hours of receipt of samples.

Tests were performed using standard methods according to U.S. EPA protocols (Green et al. 1988). Positive and negative controls were performed with each test. Usually tests of these samples were done in batches with other samples. In these batches, all samples were re-labelled so that the individual performing the test lacked prior knowledge of the origin of the samples.

The alga Selenastrum capricornutum was cultured at 20°C in 500 mL stock cultures in algal salt solution. For tests, rapidly growing cultures were harvested by centrifugation, and resuspended in fresh salt solution to population levels of 3,000,000 cells per mL.

The algal test measures toxicity as a decrease in algal growth. Tests were done by placing 1 mL of fresh algal suspension in each of a series of 500 mL flasks containing 200 mL of a concentration of test material diluted in algal salt solution. This provides for initial algal populations of approximately

15,000 cells per mL. Population levels were determined at the start and end of each test by hemocytometer counts of aliquots from each sample at the beginning and end of each test.

Range-finding tests were done to establish the range of concentrations of test material to be used in definitive toxicity tests. Those tests were carried out by placing 1 mL of algal suspension (approximately 30,000) cells in 200 mL of test sample diluted in algal salt solution. Initial range-finding tests were performed by permitting 7 day growth at 50%, 25%, 12%, 6% and 2% concentrations of test sample. Higher or lower concentrations were used in further tests if needed.

Definitive toxicity tests were carried out over an arithmetic series of concentrations ranging from the "No growth" concentration to the "100% growth" concentration established by the range-finding tests. Three replicates of each sample concentration were run concurrently.

The results of the definitive tests are analyzed by a weighted-trimmed linear regression analysis to calculate the concentration at which 50% growth (the EC50) and 80% growth (the EC20) occured. The regression coefficient ($r^2$), which shows the linearity of the relationship between dose and effect is also calculated. An $r^2$ value near unity shows a tight dose-response relationship.

The results of alga bioassays are expressed as EC50 values in this report, while in the report by Bioquest (see Appendix B) they are referred to as LC50 values.

### 3.3.6 Ames

Ames testing using the bacterium Salmonella typhimurium was completed on six samples. Two samples from each mill were tested using both the spot test and the plate incorporation test to determine sample mutagenicity. Samples were stored at 2°C and bioassays were performed within 48 hours of receipt of samples.

Ames tests were performed according to Maron and Ames (1983). Positive and negative controls were performed with each test. Tests were usually done in

batches with other samples. In those batches, all samples were relabelled so that the individual performing the test lacked prior knowledge of the origin of the samples.

The Ames test uses histidine-requiring mutants to test for the presence of mutagenic materials. Each of 4 tester strains was developed by a specific mutation from a parent stock capable of synthesizing the amino acid, histidine. In the presence of mutagens, a rare mutation may correct the original mutation in the histidine gene, making the new mutant capable of synthesizing histidine. The greater the number of revertants after exposure to test material, the more mutagenic is the test material.

The four tester strains used were TA97, TA98, TA100, TA102. While each tester stain carries a unique mutation, there is some degree of overlap between what is detected. The strains TA97 and 98 detect similar types of events; the addition or removal of bases to DNA. Strains TA100 and TA102 detect changes to individual bases, with a limited potential for overlap.

Each of the mutant test strains also has a reduced capacity to repair damage to genetic material, thus rendering the strain more sensitive to mutagenesis. This increased sensitivity also creates the problem of spontaneous mutation occurring, resulting in some revertants even without mutagens. Negative controls are used for each test set to determine this background revertant frequency.

Some chemicals are not themselves mutagenic, but can be converted to mutagenic compounds by metabolic activity, especially oxidative detoxification reactions. The detection of promutagens by the Ames test is done by adding S9 extract, obtained from the liver of rats that have had their oxidative detoxification system induced by injection with phenobarbitol or polychlorinated biphenyl. The tests reported here used PCB-induced S9 extract obtained from B.C. Research. Tests were done without S9 to detect direct-acting mutagens, and with S9 added to detect promutagens.

The spot test version of the Ames test is performed by placing a spot of test material in the center of a Petri plate containing a uniform distribution of tester strain and a limited amount of histidine. The bacteria have sufficient

histidine for a limited number of cell divisions. The test material will diffuse through the agar forming a concentration gradient, with decreasing concentration of test material at increasing distance from the test spot.

Typically, there is a zone around the test spot where no bacterial growth occurs due to cytotoxicity of the test sample. Beyond this zone of cytotoxicity is an area where the bacteria grow to form a thin uniform growth "lawn". Within the lawn there will be distinct colonies, small areas where extensive growth occurs. These colonies are derived from spontaneous or induced mutations where the bacteria have reverted to being capable of synthesizing histidine.

In the analysis of Ames spot test data, BioQuest uses the following criteria for mutagenesis:

1) Most reversions should occur at the highest level in a more-or-less concentric region from the source of the test material, with decreasing reversion in more distal areas.

2) Levels of reversion should exceed those observed in negative controls.

The plate incorporation version of the Ames test uses test strains and a specific concentration of test material, and S9 (where applicable) uniformly distributed in an agar matrix, which is spread over an agar plate. This test examines reversion of bacteria exposed to a single concentration of test material. The test is performed over a series of concentrations to establish a dose-response pattern.

Prior to performing the plate evaluation test, a series of concentrations of test material was tested to find the maximum concentration of the sample that would support uniform growth of the test strains.

In analysis of the plate incorporation test, several criteria are applied in the determination of mutagenicity:

1) For a sample to be evaluated as mutagenic, the following criteria must be met:

   A) There must be an increasing number of revertants with increasing concentration over the tested series of three concentrations. Because only three concentrations are chosen, based on the maximum concentration giving 10% growth, there is no test for linearity of the dose-response relationship.
   B) The number of reversions at each tested concentration must be greater than those appearing in negative controls.
   C) The rate of reversion in positive controls must be within previously defined parameters.

2) For a sample to be evaluated as non-mutagenic, the following criteria must be met:

   A) The number of reversions at any tested concentration cannot exceed 1.5 times the number of reversions observed in negative controls.
   B) The number of reversions does not increase with increasing concentration over the range of three test concentrations.
   C) The rate of reversion in positive and negative controls must be within previously defined parameters.

A test of a sample with a particular tester strain must meet one of the above sets of criteria, or the test is repeated.

Bioquest's report on bioassessment using Ames tests is presented in Appendix B.

### 3.3.7 Ceriodaphnia dubia

Six samples of effluent were sent to Beak Consultants Ltd., Brampton, Ontario for testing using Ceriodaphnia dubia. Two samples from each mill were tested to determine acute and chronic survival, and reproductive impairment.

Ceriodaphnia dubia stocks were maintained in a 25-L glass aquarium containing laboratory dilution water at 25 $\pm$ 1°C. The photoperiod was 16 hours light, 8

hours dark at 100 ft-C light intensity. Organism density was maintained at a loading rate of less than 40 animals/L to prevent crowding and to discourage gametogeneis and ephippia.

C. dubia stocks were fed a suspension of combined solution of fermented trout chow, Fleischman's yeast and Cerophyl[R] (dried, powdered cereal leaves) daily at a rate of 5 mL/L.

Primary brood animals containing eggs were selected from the stock culture and isolated in 50-mL beakers containing 15 mL dilution water and 0.1 mL food suspension for 24 hours. Brood organisms were transferred daily to fresh solution, and the young produced in the first three broods of each female were discarded. Neonates from the fourth broods were used as parental stock. The young produced in the third or subsequent broods of the parental stock were used in the test, provided 15 or more total neonates were achieved within the first three broods. All neonates were less than 12 hours old and within four to eight hours of age when the test was begun.

Preliminary 24-hour tests were initiated on each sample on the same day the sample was received in order to establish an appropriate testing concentration range. The definitive chronic test was initiated the following day.

Three-brood Ceriodaphnia dubia survival and reproduction tests were performed according to the U.S. EPA method (Weber et al. 1989).

Ten animals were exposed to each effluent concentration and only one animal was placed in each exposure vessel. The tests were conducted in 40-mL polyethylene vessels with 15 mL of test solution. Each test was accompanied by a control containing dilution water only, but subjected to the same test conditions as the effluent concentrations.

All tests were conducted at 25 $\pm$ 1°C under the same photoperiod as the stock culture. Dissolved oxygen, pH, temperature, and conductivity were recorded at the beginning and end of each 24-hour exposure period in each test concentration and control. Alkalinity and hardness were measured at the beginning of each 24-hour exposure time in the highest test concentration and control.

Within about three days, individual C. dubia had matured and began to produce young. Each surviving adult test organism was transferred daily into a new test vessel containing freshly prepared test solution and 0.1 mL food suspension. The young were sacrificed with two drops of 1N HCl and counted.

The tests were terminated if at least 60% of control females had produced three broods after 7 days. One test (sample #1) was continued for an extra day in order to meet the three-brood criterion.

Reproductive data were expressed as cumulative number of young produced per female for each observation time.

Acute (2-day) and chronic (7-day) LC50s were calculated using standard estimation techniques available in the form of a computer program developed by C.E. Stephan, U.S. EPA, Duluth, MN. The moving average method was preferred for data sets which included concentrations causing 0% and 100% mortality, plus a minimum of two concentrations resulting in partial mortality (Bennett, 1952). The binomial method was used when only 0% and 100% responses were observed (Stephan, 1977). The trimmed Spearman-Karber method is not subject to some of the deficiencies of probit and logit models (Hamilton, 1977), and is thus preferred for some data sets, particularly those with only one partial kill and/or, those with a slightly anomalous (although valid) dose-response relationship.

Adult survival was evaluated using Fisher's exact test (computer software was Toxstat, Version 3.0, University of Wyoming). The highest concentration that resulted in no significant reduction on survival (NOEC) and the lowest concentration that resulted in a significant reduction in survival (LOEC) were identified.

Reproduction data were then evaluated for those organisms exposed to concentrations that did not cause a significant reduction in survival. The data (number of young produced per female at each concentration) were tested for normality (Shapiro-Wilks Test) and homogeneity of variance (Bartlett's Test). If the data within each treatment were normally distributed and the variance of each treatment was not significantly different from that of the control group, analysis of variance (ANOVA) was performed, followed by

Dunnett's procedure (Dunnett, 1955), to identify treatments (concentrations) that were statistically significantly different ($p<0.05$) from the control. In cases of non-normality and/or non-homogeneous variances Steel's Many One Rank Test (Steel, 1959) was used following ANOVA. In either case the NOEC and LOEC for neonate production were identified.

If the LOEC for reproduction occurred at a lower concentration than did the LOEC for survival, than the LOEC and NOEC for reproduction were used to estimate the chronic value (Ch.V.). Otherwise the NOEC and LOEC for survival were used. The Ch.V. was then estimated as the geometric mean of the NOEC and LOEC.

Chronic toxicity test data were also analyzed using an interpolation approach (Norberg-King, 1988), to give the inhibition concentration percentage (ICp). This procedure involves a non-parametric monotonic smoothing method. An IC50 (concentration which causes 50% inhibition relative to a control) is comparable to the Ch.V., but, unlike the discrete variable end-points generated by hypothesis testing, the ICp procedure gives a point estimate with an associated confidence interval.

Beak's report on "Assessment of Textile Mill Effluent Toxicity using Ceriodaphnia dubia" is presented in Appendix C.

## 3.4 Environmental and Ecological Investigations

### 3.4.1 Benthic Macroinvertebrates

Benthic macroinvertebrate abundance was determined at five stations (1 control, 4 impacted stations) in the Salmon River (Stanfields) and Humphrey's Brook (Tandem Fabrics) using gravel bag artificial substrate samplers. The samplers were constructed of 3/4" washed crushed stone in 1/2" knotless nylon mesh enclosures (15 x 10 x 3 cm). Benthic macroinvertebrate abundance was determined in the Annapolis River (Britex) by means of Hester-Dendy type artificial substrate samplers which were constructed of twelve 75 x 75 mm pieces of 3 mm masonite, separated by 3 mm spacers and bolted to an iron-bar base.

Figures 1, 2 and 3 present maps that show the location of the sampling stations at the textile mills studied. Table 1 presents information on the distance from the effluent outfall and georeference information for each station at each mill surveyed. Surveys were conducted at Stanfields in September-October, 1990 and May-June 1991, at Britex in September-October, 1990 and at Tandem Fabrics in August-September, 1990.

Ten samplers were deployed at each station. Stations had similar bottom types, depth of water (25 to 50 cm) and proximity to shore. At impacted stations, samplers were deployed in the effluent plume, where visible.

Samplers were retrieved at least one month after being deployed, a time period observed to be suitable for colonization by endemic benthic invertebrates (Coleman and Hynes 1970). Twenty-five samplers, out of a total of 200, were not recovered. Samplers were collected using a 710 $\mu$m mesh sieve and placed in a one litre container with sufficient isopropanol to cover the sampler. The invertebrates were sorted by hand under a dissecting microscope and identified to the lowest convenient taxon and enumerated.

### 3.4.2 Caged Biota

In order to measure environmental contamination in the aquatic environment at Stanfields, ten caged freshwater clams (Anodonta implicata) were deployed at each of the five sampling stations during the benthic macroinvertebrate surveys in the fall of 1990 and spring of 1991. Surviving clams were collected one month after being deployed, wrapped in aluminum foil that had been rinsed with acetone and hexane, and frozen until organic analyses could be conducted.

### 3.4.3 Sediment

At Britex in August 1990, duplicate sediment samples were collected at the five sampling stations using teflon core tubes, and frozen until analyzed for organic contaminants.

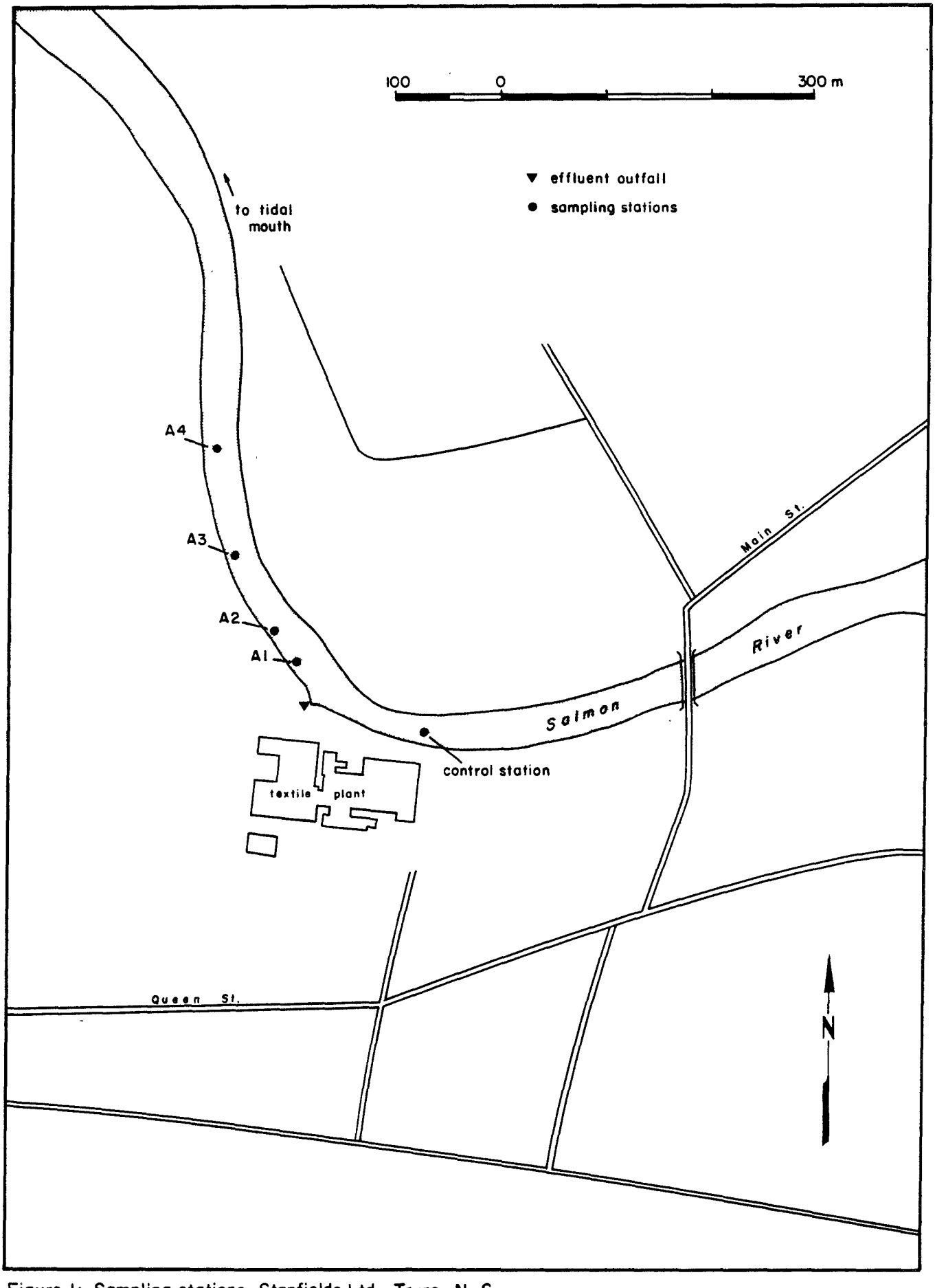



Figure 1: Sampling stations, Stanfields Ltd., Truro, N. S.

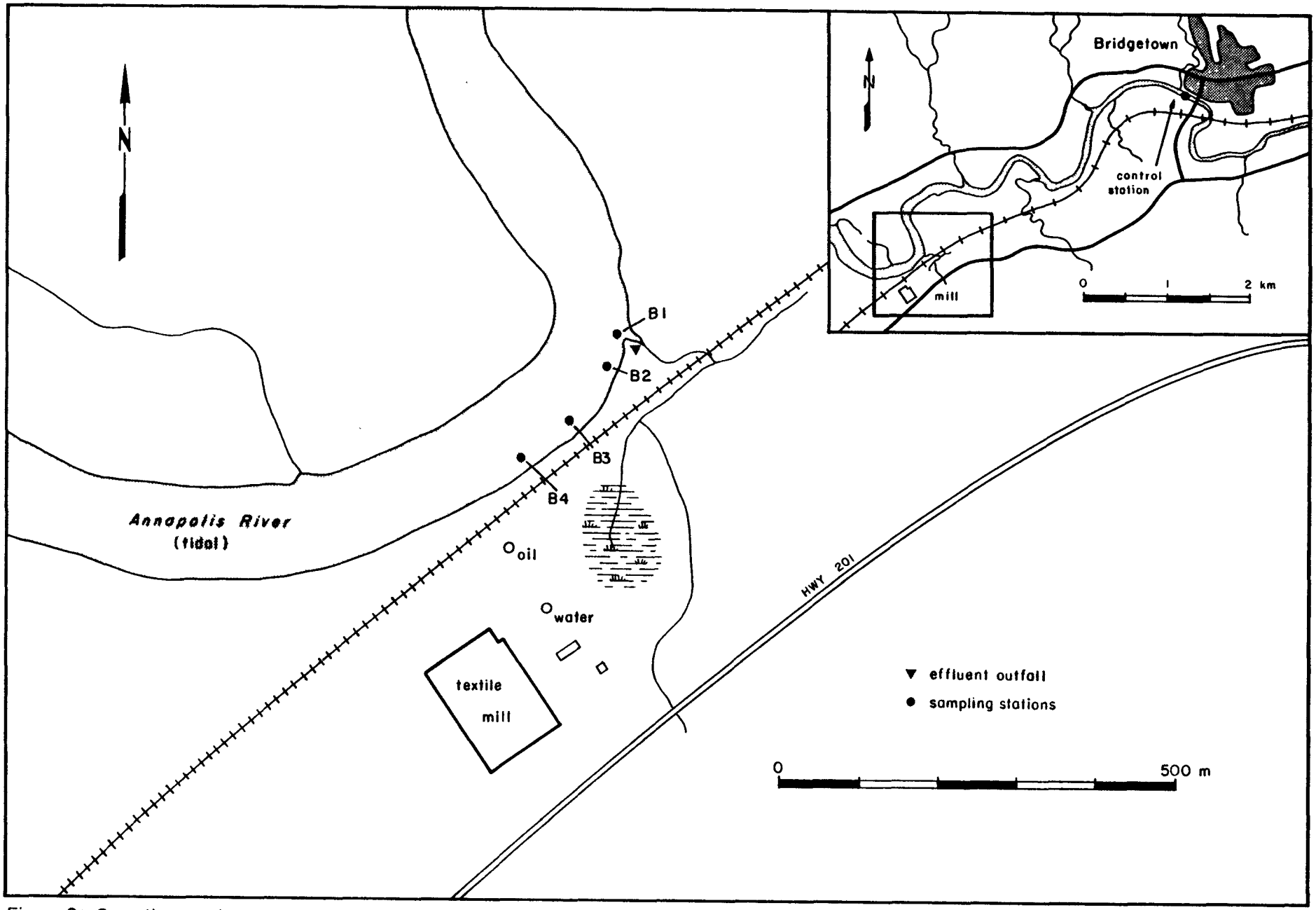



Figure 2: Sampling stations, Britex Ltd., Bridgetown, N. S.

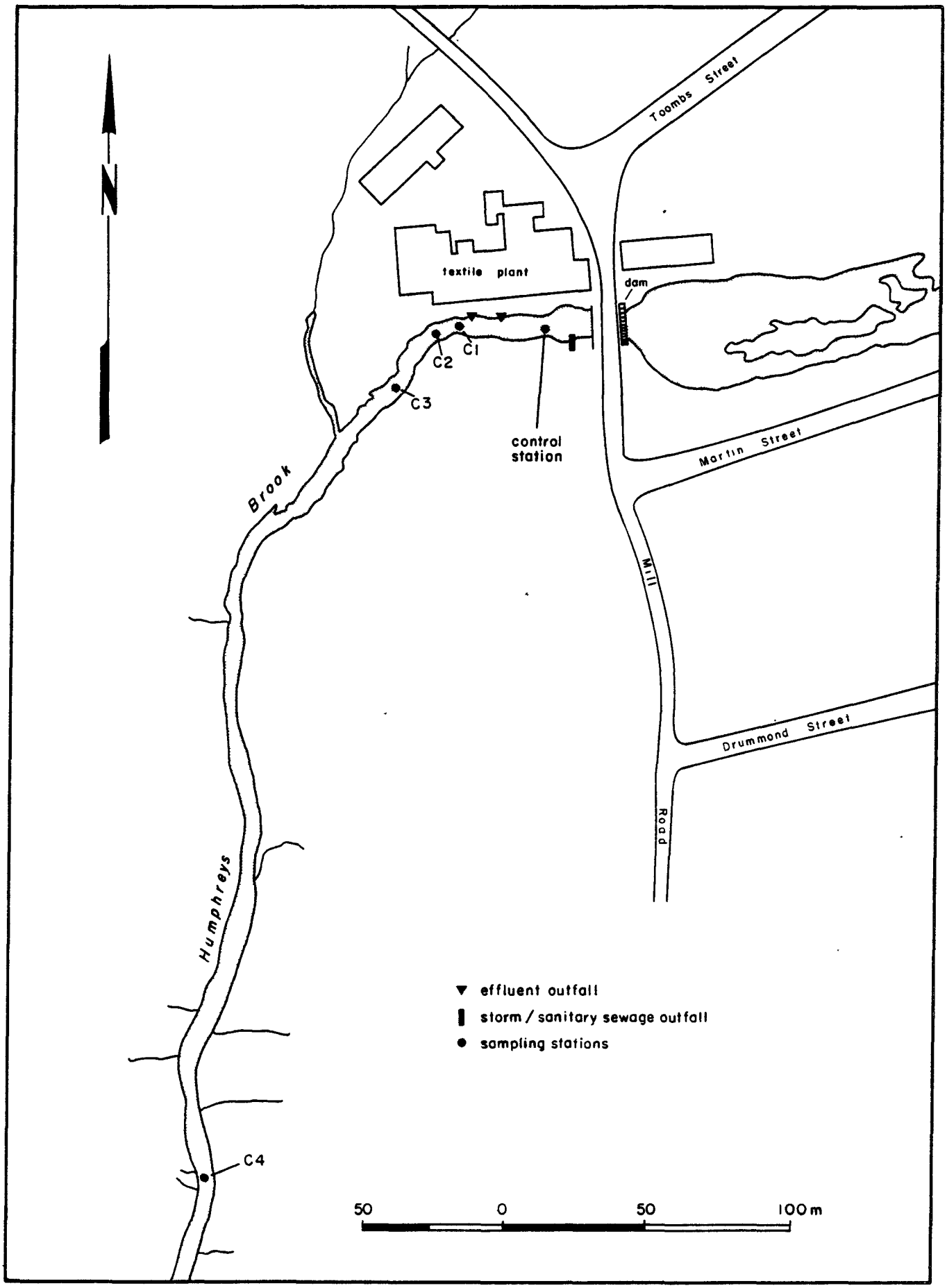



Figure 3. Sampling stations, Tandem Fabrics Inc., Moncton, N.B.

**TABLE 1 LOCATION OF SAMPLING STATIONS FOR FIELD INVESTIGATIONS**

| Textile Mill | Station | Distance from Effluent Outfall | Georeference (Z) Zone, (E) Easting, (N) Northing | | |
|---|---|---|---|---|---|
| Stanfields | Control | 85 m upstream | Z20 | E0478300 | N5023700 |
| | 1 | 30 m downstream | Z20 | E0478200 | N5023800 |
| | 2 | 50 m downstream | Z20 | E0478200 | N5023850 |
| | 3 | 100 m downstream | Z20 | E0478200 | N5023900 |
| | 4 | 120 m downstream | Z20 | E0478200 | N5023950 |
| Britex | Control | 4 km upstream | Z20 | E0318600 | N4967500 |
| | 1 | 10 m downstream | Z20 | E0315650 | N4965500 |
| | 2 | 50 m downstream | Z20 | E0315650 | N4965450 |
| | 3 | 100 m downstream | Z20 | E0315600 | N4965400 |
| | 4 | 150 m downstream | Z20 | E0315500 | N4965400 |
| Tandem Fabrics | Control | 20 m upstream | Z20 | E0363350 | N5107500 |
| | 1 | 5 m downstream | Z20 | E0363325 | N5107500 |
| | 2 | 10 m downstream | Z20 | E0363320 | N5107500 |
| | 3 | 50 m downstream | Z20 | E0363300 | N5107450 |
| | 4 | 250 m downstream | Z20 | E0363200 | N5107300 |

# 4.0 RESULTS

## 4.1 Chemical Characterization

Past experience in the EP Atlantic Region organic laboratory indicated that analyses of textile mill effluent is challenging. Traditional separatory funnel solvent-partition techniques result in intractable emulsions, in part, caused by high concentrations of detergent and surfactants, (in the form of long chain linear alcohol ethoxylates and alkylated ethoxyphenols) as well as a myriad of other chemical compounds. The U.S. EPA developed a continuous underflow extraction apparatus for problematic effluents. The customized extractor is very fragile, easily broken, and requires close supervision during the lengthy extraction procedure. In the present study, a procedure was devised that employs a roller apparatus that allows the extraction of effluent samples by gently rotating the sample container (4 liter ring jug) with the liquid sample layered over a large volume of solvent. This creates a relatively large sample-volume interphase area. The formation of emulsions is prevented since the sample-solvent interphase is never broken. The extraction step takes 18 hours; however, several samples can be accommodated at one time and the extraction apparatus can be operated unattended overnight.

The extraction procedure also must be capable of isolating the wide variety of organic chemical substances that are derived from various scouring, bleaching, dying and finishing processes used in textile operations. This study employed basic and acid extraction steps similar to EPA Method 3510 (U.S. EPA 1986) to obtain one base/neutral and one acid fraction per sample.

Separate analyses of the base/neutral and acid extracts helped reduce the complexity of chromatograms and facilitated the automated computer search and identification analysis. Even after acid-base separation, the GC-MS analysis still produced very complex chromatograms of textile mill effluents.

The organic chemical compounds identified by GC/MS/computer analysis of composite effluent samples in this study were only those compounds that were

identified with a high computer-search probability and whose mass spectra also showed a high correlation when compared visually with reference spectra from the computer's database library.

Control samples of the raw intake water for the plant were collected from each mill and analyzed by GC-mass spectrometry. No significant chemical content was detected in those samples.

### 4.1.1 Stanfields

Tables 2 to 5 list organic chemical compounds identified in the effluent samples from Stanfields Ltd., Truro, N.S. The major chemical constituents identified in its effluents were alkylated benzenes and naphthalenes, typical of carriers in dye processes, and mineral oil in the form of n-alkanes.

An estimation of surfactant concentrations in Stanfield effluent samples is represented Table 6. Stanfield surfactant effluent concentrations remained basically the same over the two month sampling period with concentrations ranging from 8.5 mg/L in October to 5.4 mg/L in December. Of the three mills sampled in the study, Stanfield effluent surfactant concentrations were the lowest at roughly 1/6 that of the highest reported value (50 mg/L) from Tandem Fabrics.

Surfactant concentrations in textile mill effluents as determined in this study, could be an underestimate of the actual concentrations in samples. The method used to determine surfactant concentrations (see Section 3.2.1.4), measures only cobalt thiocyanate reactive substances. Non-reactive surfactants would not have been measured.

Quality control data associated with the measurement of surfactant concentrations in effluent samples are provided in Appendix D.

In order to determine whether chemical contaminants from the Stanfield textile waste stream were measurably accumulated in the receiving environment, several contaminants were targeted for quantitation in clams held within the influence

**TABLE 2: GC-MS EFFLUENT ANALYSIS - STANFIELDS - OCTOBER 9-10, 1990**

Sample: Stanfields, Truro , N.S., Composite Effluent Date: 9-10 Oct./90

Laboratory No: 90LR005 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUNDS | PROBABILITY | % PURITY |
|---|---|---|
| 2-Ethyl-1-hexanol | 70 | 77 |
| 2,3-Dihydro-1-methyl-1H-indene | 70 | 86 |
| Naphthalene | 96 | 70 |
| 1,3-Dimethy 5-(1-methylethyl) benzene | 74 | 87 |
| 2,3-Dihydro-4,7-dimethyl-1H-indene | 94 | 83 |
| 2,3-Dihydro-4,7-dimethyl-1H-indene | 95 | 76 |
| 1,4-Dimethyl-2-(1-methylethyl) benzene | 73 | 83 |
| 1-methylnaphthalene | 60 | 100 |
| 2-methylnaphthalene | 60 | 100 |
| 1,1'-Biphenyl | 68 | 60 |
| 2-Ethylnaphthalene | 86 | 93 |
| 1,8-Dimethylnaphthalene | 97 | 95 |
| 1,2,3,4-Tetrahydro-1,8-dimethylnaphthalene | 84 | 100 |
| 1,8-Dimethylnaphthalene | 97 | 97 |
| 1,2-Dimethylnaphthalene | 96 | 93 |
| 1,8-Dimethylnaphthalene | 96 | 79 |
| 1-Dodecanol | 89 | 88 |
| 1,4,6-Trimethylnaphthalene | 71 | 51 |
| 1,4,6-Trimethylnaphthalene | 92 | 53 |
| 1,4,6-Trimethylnaphthalene | 89 | 71 |
| 1,4,6-Trimethylnaphthalene | 94 | 68 |
| 1,4,6-Trimethylnaphthalene | 96 | 70 |
| 1,4,6-Trimethylnaphthalene | 86 | 65 |
| 1,6,7-Trimethylnaphthalene | 82 | 68 |
| 1,4,6-Trimethylnaphthalene | 96 | 67 |
| 1,4,6-Trimethylnaphthalene | 99 | 72 |
| Fluorene | 81 | 100 |
| 1,2-Benzenedicarboxylic acid, butyl 2-methylpropylester | 87 | 76 |
| 2,4-Dichloro-1-[(4-chlorophenyl)thio] benzene | 77 | 69 |
| n-Docosane * | - | - |
| n-Tricosane * | - | - |
| 7-(Diethylamino)-4-methyl-2H-1-benzopyran-2-one | 97 | 74 |
| n-tetracosane * | 93 | 78 |
| n-Pentacosane * | - | - |
| n-Hexacosane * | - | - |
| n-Heptacosane * | - | - |
| n-Octacosane * | - | - |
| n-Nonacosane * | - | - |
| n-Triacosane * | - | - |
| n-Untriacosane * | - | - |
| n-Dotriacosane * | - | - |
| n-Tritriacosane * | - | - |
| Benzoic acid | 87 | 84 |
| 3-Chlorophenol | 96 | 94 |
| Hexadecanoic acid | 85 | 82 |
| Octadecanoic acid | 93 | 71 |
| 2,3-Dichlorobenzoic acid | 87 | 84 |

* Identification made with n-alkane reference standards

TABLE 3: GC-MS EFFLUENT ANALYSIS - STANFIELDS - OCTOBER 29-30, 1990

Sample: Stanfields,Truro, N.S., Composite Effluent Date: 29-30 Oct./90

Laboratory No. 90LR007 Library: NBS-REV X Wiley

| IDENTIFIED COMPOUNDS | PROBABILITY | % PURITY |
|---|---|---|
| 2-Ethyl-1-hexanol | 70 | 74 |
| Naphthalene | 97 | 88 |
| 1,3-Dimethyl-5-(1-methylethyl) benzene | 89 | 94 |
| 1-methylnaphthalene | 71 | 88 |
| 2-methylnaphthalene | 74 | 89 |
| 1,1'-Biphenyl | 93 | 69 |
| 1-Ethylnaphthalene | 96 | 86 |
| 1,7-Dimethylnaphthalene | 97 | 92 |
| 1,8-Dimethylnaphthalene | 96 | 100 |
| 1,4-Dimethylnaphthalene | 97 | 90 |
| 1,8-Dimethylnaphthalene | 93 | 72 |
| 1-Dodecanol | 88 | 70 |
| Acenaphthene | 66 | 93 |
| 1,4,6-Trimethylnaphthalene | 86 | 53 |
| 1,4,6-Trimethylnaphthalene | 70 | 77 |
| 1,4,5-Trimethylnaphthalene | 95 | 68 |
| 1,6,7-Trimethylnaphthalene | 86 | 89 |
| 1,4,6-Trimethylnaphthalene | 96 | 86 |
| 1,4,6-Trimethylnaphthalene | 93 | 73 |
| 2,4-Dichloro-1-[(4-chlorophenyl)thio] benzene | 86 | 76 |
| n-Docosane * | - | - |
| n-Tricosane * | - | - |
| 7-(Diethylamino)-4-methyl-2H-1-benzopyran-2-one | 99 | 89 |
| n-Tetracosane * | | |
| n-Pentrcosane * | - | - |
| n-Hexacosane * | - | - |
| n-Heptacosane * | - | - |
| n-Octacosane * | - | - |
| n-Nonacosane * | - | - |
| n-Triacosane * | - | - |
| n-Untriacosane * | - | - |
| n-Dotriacosane * | - | - |
| Benzoic acid | 67 | 91 |
| Dodecanoic acid | 87 | 92 |
| Tetradecanoic acid | 92 | 71 |
| Hexadecanoic acid | 82 | 90 |
| Octadecanoic acid | 90 | 82 |

* Identification made with n-alkane reference standards

**TABLE 4: GC-MS EFFLUENT ANALYSIS - STANFIELDS - NOVEMBER 20-21, 1990**

Sample: Stanfields, Truro, N.S., Composite Effluent Date: 20-21 Nov./90

Laboratory No: 90LR010 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUNDS | PROBABILITY | % PURITY |
|---|---|---|
| 2, Ethyl-1-hexanol | 70 | 75 |
| Naphthalene | 96 | 77 |
| 1,4-Dimethyl-2-(1-methylethyl) benzene | 79 | 100 |
| 1,3-Dimethyl-5-(1-methylethyl) benzene | 84 | 99 |
| 2,3-Dihydro-4,7-dimethyl-1H-indene | 93 | 83 |
| 2,3-Dihydro-1,3-dimethyl-1H-indene | 96 | 85 |
| 1,4-Dimethyl-2-(1-methylethyl) benzene | 64 | 96 |
| 1-methylnaphthalene | 60 | 99 |
| 2-methylnaphthalene | 60 | 100 |
| N-Ethyl-N-phenylacetamide | 81 | 81 |
| 1,1'-Biphenyl | 80 | 79 |
| 2-Ethylnaphthalene | 96 | 69 |
| 1,8-Dimethylnaphthalene | 97 | 100 |
| 1,3-Dimethylnaphthalene | 96 | 97 |
| 1,4-Dimethylnaphthalene | 96 | 87 |
| 1,2-Dimethylnaphthalene | 93 | 68 |
| 1,4,6-Trimethylnaphthalene | 92 | 60 |
| 1,4,5-Trimethylnaphthalene | 88 | 67 |
| 1,4,6-Trimethylnaphthalene | 95 | 74 |
| 1,6,7-Trimethylnaphthalene | 94 | 77 |
| 2,3,6-Trimethylnaphthalene | 73 | 100 |
| 1,4,6-Trimethylnaphthalene | 95 | 77 |
| 1,4,5-Trimethylnaphthalene | 95 | 63 |
| 1,4,6-Trimethylnaphthalene | 87 | 79 |
| 7-Ethyl-1,4-dimethylazulene | 78 | 97 |
| n-Heneicosane * | - | - |
| 2,4-Dichloro-1-[(4-chlorophenyl)thio] benzene | 69 | 71 |
| n-Docosane * | - | - |
| n-Tricosane * | - | - |
| 7-(Diethylamino)-4-methyl-2H-1-benzopyran-2-one | 99 | 94 |
| n-Tetracosane * | - | - |
| n-Pentacosane * | - | - |
| n-Hexacosane * | - | - |
| n-Heptacosane * | - | - |
| n-Octacosane * | - | - |
| n-Nonacosane * | - | - |
| n-Triacosane * | - | - |
| n-Untriacosane * | - | - |
| n-Dotriacosane * | - | - |
| n-Tritriacosane * | - | - |
| Benzoic acid | 81 | 92 |
| Decanoic acid | 67 | 77 |
| Hexadecanoic acid | 82 | 84 |
| Octadecanoic acid | 92 | 71 |
| Bis (2-ethylhexyl) phthalate | 83 | 89 |

* Identification made with n-alkane reference standards

**TABLE 5: GC-MS EFFLUENT ANALYSIS - STANFIELDS - DECEMBER 3-4, 1990**

Sample: Stanfields, Truro, N.S., Composite Effluent Date: 3-4 Dec./90

Laboratory No: 90LR012 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUND | PROBABILITY | % PURITY |
|---|---|---|
| Naphthalene | 96 | 95 |
| 2,3-Dihydro-1,2-dimethyl-1H-indene | 88 | 100 |
| 2,3-Dihydro-1,3-dimethyl-1H-indene | 87 | 95 |
| 1,4-Dimethyl-2-(1-methylethyl)benzene | 83 | 96 |
| 1-methylnapthalene | 60 | 100 |
| 2-methylnapthalene | 60 | 100 |
| 1,1'-Biphenyl | 73 | 86 |
| 1-Ethylnaphthalene | 95 | 82 |
| 1,5-Dimethylnaphthalene | 99 | 96 |
| 1,2-Dimethylnaphthalene | 97 | 100 |
| 1,4-Dimethylnaphthalene | 96 | 87 |
| 1,8-Dimethylnaphthalene | 91 | 69 |
| 1,4,6-Trimethylnaphthalene | 92 | 63 |
| 1,4,6-Trimethylnaphthalene | 71 | 66 |
| 1,4,6-Trimethylnaphthalene | 97 | 89 |
| 1,6,7-Trimethylnaphthalene | 94 | 77 |
| 1,4,6-Trimethylnaphthalene | 93 | 68 |
| 1,4,5-Trimethylnaphthalene | 96 | 75 |
| 1,4,6-Trimethylnaphthalene | 95 | 87 |
| Fluorene | 76 | 89 |
| N-(3-Chlorophenyl) acetamide | 75 | 71 |
| 1-Bromo-2,4-dinitrobenzene | 88 | 87 |
| 1-Tetradecanol | 81 | 68 |
| 1,2-Benzene dicarboxylic acid, Bis(2-methoxyethyl) ester | 79 | 93 |
| 2,4-Dichloro-1-[(4 chlorophenyl) thio] benzene | 86 | 68 |
| n-Heneicosane * | - | - |
| n-Docosane * | - | - |
| n-Tricosane * | - | - |
| 7-(Diethylamino)-4-methyl-2H-1-benzopyran-2-one | 99 | 95 |
| n-Tetracosane * | - | - |
| n-Pentacosane * | - | - |
| m-Hexacosane * | - | - |
| m-Heptacosane * | - | - |
| n-Octacosane * | - | - |
| n-Nonacosane * | - | - |
| n-Triacosane * | - | - |
| n-Untriacosane * | - | - |
| n-Dotriacosane * | - | - |
| n-Tritriacosane * | - | - |
| Benzoic acid | 89 | 74 |
| Dodecanoic acid | 67 | 86 |
| Hexadecanoic acid | 91 | 93 |
| Octadecanoic acid | 86 | 88 |

* Identification made with n-alkane reference standards

TABLE 6: SURFACTANT CONCENTRATIONS IN EFFLUENT SAMPLES FROM STANFIELDS

| Sampling Date | Concentration (mg/L) |
|---|---|
| October 9-10, 1990 | 6.5 |
| October 29-30, 1990 | 8.5 |
| November 20-21, 1990 | 8.0 |
| December 3-4, 1990 | 5.4 |

TABLE 7: Concentrations of Target Compounds in Clam Tissue (Stanfields)

| Target Compounds | 91LR013 µg/g | 91LR014 * µg/g | Spike Amt. µg/g | % Recovery** |
|---|---|---|---|---|
| 2-butoxyethanol | <0.1 | <0.1 | 2.5 | 71 ± 2.3 |
| 2-ethyl-1-hexanol | <0.1 | <0.1 | 2.3 | 33 ± 5.2 |
| naphthalene | <0.1 | <0.1 | 2.5 | 54 ± 7.8 |
| 2(2-butoxyethoxy)ethanol | <0.1 | <0.1 | 6.8 | 67 ± 6.4 |
| 2-methylnaphthalene | 1.2 | <0.1 | 2.5 | 56 ± 6.0 |
| 1-methylnaphthalene | 1.0 | <0.1 | 2.4 | 53 ± 6.1 |
| biphenyl | 2.8 | <0.1 | 2.2 | 57 ± 6.0 |
| 1,6-dimethylnaphthalene | 2.1 | <0.1 | 2.5 | 58 ± 5.9 |
| 1,4-dimethylnaphthalene | 0.7 | <0.1 | 2.5 | 59 ± 5.7 |
| 1,2-dimethylnaphthalene | 0.2 | <0.1 | 2.5 | 58 ± 6.1 |
| 2,3,5-trimethylnaphthalene | 0.1 | <0.1 | 2.3 | 67 ± 6.1 |
| tetramethylbutyl phenol | <0.1 | <0.1 | 2.9 | 126 ± 11 |

* Control
** Triplicate Analyses

of the mill's outfall. Table 7 presents data from the GC/MS analysis of clam tissue for twelve selected contaminants. The analytes were chosen from the list of chemicals identified by GC/MS computer analysis of the mill's waste effluent. Biphenyl and alkylated naphthalenes are representative dye carriers and 2-ethylhexanol representative of the surfactant group. Although butoxyethanol, butoxyethoxyethanol and tetramethylbutylphenol were not detected specifically in Stanfield effluent, they frequently are present in surfactant mixtures and were included as target compounds.

Of the twelve chemicals monitored, biphenyl; mono-; di-; and trinaphthalenes were detected above the method detection limit of 0.1 ug/g (wet weight). Concentrations in clam tissue ranged from 0.1 ug/g 2,3,5-trimethylnaphthalene to 2.8 ug/g biphenyl. No surfactants were detected in the clam sample. No target compounds were detected in the control sample of uncontaminated clams.

For quality control purposes, the twelve target compounds were spiked into uncontaminated clam samples and analyzed according to the method. Recovery and method precision data also are provided in Table 7.

Table 8 present the analytical results for conventional pollutants and metals for the four effluent samples from Stanfields. Generally, concentrations of pollutants and metals did not vary substantially between sampling dates.

### 4.1.2 Britex

Tables 9 to 12 list the organic chemical compounds identified in waste effluent samples from Britex Ltd., Bridgetown N.S.

GC/MS/computer analyses of Britex effluent using both the NBS revised database and the Wiley database, identified approximately 30% of the chemical compounds detected (i.e. integrated chromatographic peaks). Unlike other mill effluents in this study, effluents from Britex contained significantly more chemical compounds whose mass spectra were not contained in the two reference libraries employed.

## TABLE 8: ANALYTICAL RESULTS (CONVENTIONAL POLLUTANTS AND METALS) FOR FINAL EFFLUENT SAMPLES FROM STANFIELDS (mg/L)

| Parameter | October 9-10 | October 29-30 | November 20-21 | December 3-4 |
|---|---|---|---|---|
| pH (units) | 9.6 | 7.6 | 8.0 | 7.4 |
| Alkalinity | 290 | 320 | 300 | 330 |
| COD | 430 | 440 | 300 | 310 |
| BOD | 60 | 100 | 85 | 110 |
| Suspended Solids | 30 | 87 | 18 | 40 |
| Total Dissolved Solids | 1490 | 1240 | 1090 | 2080 |
| Total Solids | 1500 | 1320 | 1100 | 2120 |
| Ammonia-N | <0.14 | 2.9 | 0.6 | 1.2 |
| Oil and Grease (Pet. Ether) | 0 | 15.0 | 18.6 | 10.2 |
| Calcium | 29.65 | 38.98 | 23.51 | 23.95 |
| Arsenic | 0.08 | 0.09 | <0.05 | <0.05 |
| Cadmium | <0.01 | <0.01 | <0.01 | <0.01 |
| Chromium | <0.01 | <0.01 | <0.01 | <0.01 |
| Copper | 0.01 | <0.01 | <0.01 | 0.03 |
| Iron | 0.05 | 0.02 | 0.07 | 0.04 |
| Lead | <0.02 | <0.02 | <0.02 | <0.02 |
| Nickel | <0.01 | <0.01 | <0.01 | <0.01 |
| Manganese | 0.01 | 0.01 | 0.01 | 0.01 |
| Zinc | 0.04 | 0.03 | 0.07 | 0.03 |

**TABLE 9: GC-MS EFFLUENT ANALYSIS - BRITEX - SEPTEMBER 17-18, 1990**

Sample: Britex, Bridgetown, N.S., Composite Effluent Date: 17-18 Sept./90

Laboratory No: 90LR002 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUND | PROBABILITY | % PURITY |
|---|---|---|
| 2-Ethoxy-1-propanol | 65 | 65 |
| Benzaldehyde | 67 | 87 |
| 2-(2-ethoxyethoxy)ethanol | 70 | 73 |
| 2-ethyl-1-hexanol | 76 | 97 |
| 4, 4-Dimethy-1-pentene | 69 | 90 |
| 1-Methyl-2-pyrrolidinone | 88 | 80 |
| 3, 5, 5-Trimethyl-1-hexanol | 71 | 77 |
| 2-(2-butoxyethoxy)ethanol | 93 | 73 |
| 2-Phenoxyethanol | 68 | 82 |
| Caprolactam | 86 | 78 |
| 4-(2,2,3,3-Tetramethylbutyl)phenol | 83 | 94 |
| 4-Nonylphenol | 70 | 100 |
| N-(phenylmethylene)benzenemethanamine | 92 | 89 |
| 2-(2-(4-(1,1,3,3-tetramethylbutyl) phenoxy) ethoxy) ethanol | 75 | - |
| 2-(2-(2-(4-(1,1,3,3-Tetramethylbutyl)phenoxy) ethoxy)ethoxy)ethanol | 65 | 65 |
| 4,4'-Butylidenebis [2-(1,1-dimethylethyl) phenol | 78 | 100 |
| 2-(2-(2-(2-(4-(1,1,3,3-tetramethylbutyl) phenoxy) ethoxy)ethoxy) ethoxy)ethanol | 70 | 100 |
| Benzoic Acid | 74 | 90 |
| 2-Hydroxybenzoic acid | 97 | 81 |
| 2-Naphthalenol | 86 | 70 |
| 2,4-Dichlorobenzoic acid | 79 | 84 |
| Hexadecanol | 67 | 59 |
| 9-octadecen-1-ol | 89 | 97 |
| Octadecanoic acid, 2-methylpropyl ester | 81 | 107 |
| Bis(2-ethylhexyl)phthalate | 67 | 68 |

* Identification made with n-alkane reference standards

**TABLE 10: GC-MS EFFLUENT ANALYSIS - BRITEX - OCTOBER 1-2, 1990**

Sample: Britex, Bridgetown, N.S., Composite Effluent Date: 1-2 Oct./90

Laboratory No: 90LR004 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUND | PROBABILITY | % PURITY |
|---|---|---|
| 2-Butoxyethanol | 65 | 68 |
| 2-Ethoxy-1-propanol | 65 | 65 |
| 2-Ethyl-1-hexanol | 70 | 83 |
| 1-Methyl-2-pyrrolidinone | 83 | 82 |
| 3,5,5-Trimethyl-1-hexanol | 78 | 70 |
| 2-Phenoxyethanol | 67 | 83 |
| 4-(2,2,3,3-tetramethylbutyl)phenol | 88 | 78 |
| N-(phenylmethyl) benzenemethanamine | 67 | 92 |
| N-Hydroxy-N-(phenylmethyl) benzenemethanamine | 65 | 100 |
| 2-[4-(1,1-Dimethylethyl) phenoxy]ethanol | 67 | 77 |
| 2-[2-[4-(1,1,3,3-Tetramethylbutyl) phenoxy]ethoxy]-ethanol | 75 | 97 |
| 4,4'-Butylidenebis [2-(1,1-dimethylethyl) phenol | 89 | 73 |
| 2-[2-[2-[2-[p-(1,1,3,3-tetramethylbutyl) phenoxy) ethoxyOethoxyOethoxyOethanol | 70 | 100 |
| 2,3-Dichlorobenzoic acid | 91 | 61 |
| 9-Octadecen-1-ol | 60 | 41 |

* Identification made with n-alkane reference standards

**TABLE 11: GC-MS EFFLUENT ANALYSIS - BRITEX - NOVEMBER 14-15, 1990**

Sample: Britex, Bridgetown, N.S., Composite Effluent Date: 14-15 Nov./90

Laboratory No: 90LR009 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUND | PROBABILITY | % PURITY |
|---|---|---|
| 2-ethoxy-1-propanol | 65 | 65 |
| 2-Ethyl-1-hexanol | 70 | 65 |
| 4-(2,2,3,3-Tetramethylbutyl)phenol | 88 | 83 |
| 2-[4-(1,1-Dimethylethyl)phenoxy] ethanol | 70 | 82 |
| 2,2-(4(1,1,3,3) Tetrabutyl)Phenoxy)ethoxy)ethanol | 75 | 100 |
| 4,4'-Butylidenebis[2-(1,1-dimethylethyl) phenol | 83 | 98 |
| 2-[2-[2-[2-[p-(1,1,3,3-Tetramethylbutyl)phenoxy) ethoxy)ethoxy)ethoxy)ethanol | 78 | 98 |
| 2,3-Dichlorobenzoic acid | 91 | 61 |
| 9-Octadecen-1-ol | 60 | 41 |

* Identification made with n-alkane reference standards

**TABLE 12: GC-MS EFFLUENT ANALYSIS - BRITEX - NOVEMBER 26-27, 1990**

Sample: Britex, Bridgetown, N.S., Composite Effluent Date: 26-27 Nov./90

Laboratory No: 90LR011 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUND | PROBABILITY | % PURITY |
|---|---|---|
| 2-Butoxyethanol | 65 | 68 |
| 2-Ethoxy-1-propanol | 65 | 65 |
| 2-Ethyl-1-hexanol | 70 | 76 |
| 1-Methyl-2-pyrrolidinone | 89 | 79 |
| Decamethylcyclopentasiloxane | 86 | 146 |
| 2-(2-Butoxyethoxy) ethanol | 88 | 71 |
| 1-Decanol | 65 | 94 |
| Dodecamethylcyclohexasiloxane | 83 | 108 |
| Methylcyclodecane | 70 | 77 |
| n-Pentadecane * | - | - |
| n-Hexadecane * | - | - |
| 4-(2,2,3,3-Tetramethylbutyl) phenol | 89 | 87 |
| n-Heptadecane * | - | - |
| n-Octadecane * | - | - |
| 4-Nonylphenol | 78 | 100 |
| n-(Phenylmethylene) benzenemethanamine | 87 | 98 |
| n-Nonadecane * | - | - |
| Bis(2-ethylhexyl) phthalate | 81 | 96 |
| 4,4'-Butylidenebis[2-(1,1-dimethyl-ethyl)]phenol | 76 | 97 |
| N,N-Dimethylformamide | 71 | 79 |
| 3,5,5-Trimethyl-1-hexanol | 67 | 69 |
| 2-[2-[4-(1,1,3,3-Tetramethylbutyl)phenoxy ethoxy-ethanol | 87 | 81 |

* Identification made with n-alkane reference standards

The predominant chemicals identified in effluents from the Britex mill were of the surfactant type e.g. alkylated alcohols and phenols and alkylated ethoxylated phenols. Acid extraction of one effluent sample isolated several acidic compounds (benzoic acid, hydroxybenzoic acid and dichlorobenzoic acid) that may have originated in the mill's dyeing operation. Of interest also was the presence of caprolactam, a known contaminant in industrial wastewaters where synthetic fabrics are produced (Tocksteinova and Kopanica 1987), and methyl pyrolidinone which had been reported in the wastestream of a North Carolina polyester fabric finishing and dyeing plant (Gordon and Gordon 1981).

Surfactant concentrations in Britex effluent samples are presented in Table 13 and range from 12 mg/L to 27 mg/L over the three month collection period.

Table 14 presents data from the GC/MS analysis of a control and six sediment samples collected at various distances from the outfall of the Britex plant. Three target compounds were selected for GC/MS analysis. Caprolactam and methyl pyrolidinone were identified in effluent samples from Britex and have been reported previously in waste streams of textile operations (Tocksteinova and Kopanica 1987; Gordon and Gordon 1981). Tetramethylbutyl phenol was selected as representative of surfactants which were the major group of chemical compounds identified in Britex effluents.

As indicated in Table 14, caprolactam and methyl pyrolidinone were not detected in any samples (method detection 0.5 ug/g) collected within the influence of the effluent outfall. Tetramethylbutyl phenol was detected in all but one sample. Concentrations ranged from a low of 0.5 ug/g to a high of 120 ug/g. No target compounds were detected in control sediment collected outside the influence of the effluent outfall.

Spiked sediment recoveries and method precision quality control data associated with the sediment analyses are provided in Appendix E.

Table 15 presents the analytical results for conventional pollutants and metals for the four final effluent samples from Britex. Compared to the effluent samples from Stanfields (Table 8), the Britex effluent samples had markedly

**TABLE 13: SURFACTANT CONCENTRATIONS IN EFFLUENT SAMPLES FROM BRITEX**

| Sampling Date | Concentration (mg/L) |
|---|---|
| September 17-18, 1990 | 27 |
| October 1-2, 1990 | 18 |
| November 14-15, 1990 | 12 |
| November 26-27, 1990 | 21 |

* Quality control data associated with the measurement of surfactant concentrations in effluent samples are provided in Appendix D.

**TABLE 14: Concentrations of Selected Target Compounds in Sediment Samples for the Annapolis River (Britex)**

| Station* | 1-methylpyrrolidinone | caprolactam | tetramethylbutylphenol |
|---|---|---|---|
| Control | <0.5 µg/g | <0.5 µg/g | <0.5 µg/g |
| 1** | <0.5, <0.5 µg/g | <0.5, <0.5 µg/g | 0.56, 0.61 µg/g |
| 2 | <0.5 µg/g | <0.5 µg/g | 1.5 µg/g |
| 3 | <0.5 µg/g | <0.5 µg/g | <0.5 µg/g |
| 4 | <0.5 µg/g | <0.5 µg/g | 120 |
| Drainage Ditch Between Plant and Outfall | <0.5, <0.5 µg/g | <0.5, <0.5 µg/g | 36, 39 µg/g |

* See Table 1 for location of sampling stations
** duplicate analysis

## TABLE 15: ANALYTICAL RESULTS (CONVENTIONAL POLLUTANTS AND METALS) FOR FINAL EFFLUENT SAMPLES FROM BRITEX (mg/L)

| Parameter | September 17-18 | October 1-2 | November 14-15 | November 26-27 |
|---|---|---|---|---|
| pH (units) | 6.8 | 6.4 | 6.6 | 6.8 |
| Alkalinity | 70 | 70 | 70 | 70 |
| COD | 385 | 450 | 490 | 890 |
| BOD | 15 | 13 | 220 | 36 |
| Suspended Solid | 10 | 10 | 70 | 10 |
| Total Dissolved Solids | 330 | 170 | 160 | 150 |
| Total Solids | 340 | 180 | 230 | 160 |
| Ammonia-N | 2.6 | 1.0 | 2.6 | 3.9 |
| Oil and Grease (Pet. Ether) | 14 | 9.5 | 10.2 | 13.4 |
| Calcium | 34.19 | 18.57 | 24.66 | 19.52 |
| Arsenic | <0.05 | 0.17 | 0.17 | <0.05 |
| Cadmium | <0.01 | <0.01 | <0.01 | <0.01 |
| Chromium | 0.04 | 0.01 | <0.01 | 0.01 |
| Copper | <0.01 | <0.01 | <0.01 | <0.01 |
| Iron | 0.16 | 0.07 | 0.08 | 0.25 |
| Lead | <0.02 | <0.02 | <0.02 | <0.02 |
| Nickel | <0.01 | <0.01 | <0.01 | <0.01 |
| Manganese | <0.01 | <0.01 | <0.01 | <0.01 |
| Zinc | 0.08 | 0.08 | 0.08 | 0.17 |

lower concentrations of alkalinity, total dissolved solids and total solids. The other parameters measured in the effluent samples from the two textile mills were present in similar concentrations.

### 4.1.3 Tandem Fabrics

Tables 16 to 19 list the chemicals identified by GC/MS computer analysis in effluent from the Tandem Fabrics plant.

Effluent from Tandem Fabrics contained a complex chemical mixture that included dye carriers, detergents/ surfactants, mineral oil and other miscellaneous chemicals. As with other textile effluent samples in this study, the bulk of the organic compounds identified in the Tandem effluent were likely auxiliary chemicals associated with dyeing operations. Unlike Stanfield effluent, however, which contained dye carriers primarily in the form of alkylated naphthalenes, the compounds identified in Tandem Fabrics effluent were alkylated benzenes, biphenyl, alkylated biphenyls as well as naphthalene and methylated naphthalenes derivatives. Few nonionic surfactant compounds were detected even though Tandem effluents had the highest nonspecific surfactant concentrations(Table 20) of the three textile mills studied. This may reflect the use of ionic surfactants rather than nonionic surfactants in Tandem operations. The other major types of chemicals identified were n-alkanes (C21-C31), fatty acids and alcohols.

Table 21 presents the analytical results for conventional pollutants and metals for the four final effluent samples from Tandem Fabrics. Compared to the effluent samples from Stanfields and Britex, COD, BOD and ammonia concentrations were markedly higher in Tandem effluent. Total dissolved solids and total solids concentrations were lower than Stanfields effluent, but higher than Britex effluent.

## 4.2 Aquatic Toxicity

Toxicity data are summarized in Figure 4. Results are provided in Table 22 and Appendix F. Mutagenicity data are presented in Figure 5.

TABLE 16: GC-MS EFFLUENT ANALYSIS - TANDEM FABRICS - SEPTEMBER 11, 1990

Sample: Tandem Fabrics, Moncton, N.B., Composite Effluent Date: 11 Sept./90

Laboratory No: 90LR001 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUND | PROBABILITY | % PURITY |
|---|---|---|
| 1, 2-Dimethylbenzene | 95 | 82 |
| Propylbenzene | 86 | 100 |
| 1-Ethyl-2-methylbenzene | 96 | 100 |
| 1,2,3-Trimethylbenzene | 84 | 83 |
| 1-Ethyl-2-methylbenzene | 89 | 92 |
| 1,2,4-Trimethylbenzene | 93 | 76 |
| 1-Ethyl-4-methylbenzene | 83 | 97 |
| 2,3-Dihydro-Indene | 67 | 70 |
| 1-Methyl-3-propylbenzene | 83 | 97 |
| 1-Methyl-2-(1-methylethyl)benzene | 67 | 100 |
| Methyl (1-methylethyl)benzene | 79 | 100 |
| 1,2,4,5-Tetramethylbenzene | 83 | 83 |
| 1-Methyl-4-(1-methylethyl)-3-cyclohexen-1-ol | 86 | 82 |
| 1-Methyl-4-(1-methylethyl)-cyclohexanol | 87 | 58 |
| 4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol | 91 | 63 |
| Naphthalene | 97 | 86 |
| Isoquinoline | 92 | 87 |
| [1, 1'-Bicyclopentyl] -2-one | 83 | 70 |
| 2-Methylnaphthalene | 84 | 88 |
| 1-Methylnaphthalene | 76 | 86 |
| 1,1'-Biphenyl | 79 | 70 |
| 2-Methyl-1,1'-biphenyl | 66 | 92 |
| 1,4-Dimethylnaphthalene | 96 | 100 |
| 1,6-Dimethylnaphthalene | 97 | 100 |
| 2-Methyl-1,1'-biphenyl | 70 | 89 |
| 1-Dodecanol | 88 | 66 |
| 3-Methyl-1, 1'-biphenyl | 94 | 90 |
| 2-Methyl-1, 1'-biphenyl | 83 | 66 |
| Hexadecanol | 95 | 52 |
| Dodecanoic acid, 2,3-dihydroxypropyl ester | 83 | 57 |
| Tetradecanoic acid, 2,3-dihydroxypropyl ester | 79 | 90 |
| Octanoic acid | 75 | 75 |
| Nonanoic acid | 69 | 99 |
| 3-Hydroxy-1-methoxybenzaldehyde | 94 | 100 |
| 1-(4-hydroxy-3-methoxyphenyl)ethanone | 81 | 91 |
| Triethylene glycol | 78 | 100 |
| 1,1'-Biphenyl-2-ol | 81 | 85 |
| Dodecanoic acid | 67 | 73 |
| Tetradecanoic acid | 80 | 73 |
| Hexadecanoic acid | 86 | 82 |
| Octadecanoic acid | 89 | 58 |

* Identification made with n-alkane reference standards

TABLE 17: GC-MS EFFLUENT SAMPLES - TANDEM FABRICS - SEPTEMBER 24, 1990

Sample: Tandem Fabrics, Moncton, N.B., Composite Effluent Date: 24 Sept./90

Laboratory No: 90LR003 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUND | PROBABILITY | % PURITY |
|---|---|---|
| 1-Ethyl-2-methylbenzene | 94 | 77 |
| 1,2,3-Trimethylbenzene | 93 | 83 |
| 1,2,4-Tetramethylbenzene | 95 | 76 |
| 1-Ethyl-4-methylbenzene | 95 | 100 |
| 3,5,5-Trimethyl-2-cyclohexen-1-one | 71 | 75 |
| [1,1-Bicyclopentyl]-2-one | 74 | 69 |
| 2-Methoxy-5-(1-propenyl) phenol | 92 | 100 |
| 1,1'-Biphenyl | 93 | 86 |
| 2-Cyclopentylidene-cyclopentanone | 74 | 88 |
| Diethylphthalate | 90 | 83 |
| Dodecanoic acid, 2,3-dihydroxypropyl ester | 94 | 67 |
| n-Docosane | 99 | 73 |
| n-Tricosane * | - | - |
| n-Tetracosane | 91 | 66 |
| n-Pentacosane * | - | - |
| 9-Hexylheptadecane | 73 | 76 |
| n-Hexacosane * | - | - |
| n-Heptacosane | 74 | 74 |
| n-Octacosane * | - | - |
| n-Nonacosane * | - | - |
| n-Triacosane * | - | - |
| n-Untriacosane * | - | - |
| n-Dotriacosane * | - | - |
| n-Tritriacosane * | - | - |
| n-Tetratriacosane * | - | - |
| Hexanoic acid | 55 | 82 |
| Octanoic acid | 67 | 79 |
| Nonanoic acid | 87 | 100 |
| Triethylene glycol | 70 | 100 |
| Dodecanoic acid | 89 | 77 |
| Tetradecanoic acid | 92 | 71 |
| Triethylene glycol | 70 | 100 |
| Methyl hexadecanoic acid ester | 82 | 87 |
| Hexadecanoic acid | 75 | 66 |

* Identification made with n-alkane reference standards

TABLE 18: GC-MS EFFLUENT SAMPLES - TANDEM FABRICS - OCTOBER 23, 1990

Sample: Tandem Fabrics, Moncton, N.B., Composite Effluent Date: 23 Oct./90

Laboratory No: 90LR006 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUND | PROBABILITY | % PURITY |
|---|---|---|
| 1,2-Dimethylbenzene | 81 | 91 |
| 1,2-Dimethylbenzene | 89 | 85 |
| (1-methylethyl)benzene | 76 | 100 |
| Propylcyclohexane | 75 | 81 |
| 3,6-Dimethyloctane | 79 | 71 |
| Propylbenzene | 71 | 100 |
| 1-Ethyl-2-methylbenzene | 96 | 96 |
| 1,2,3-Trimethylbenzene | 89 | 79 |
| 1-Ethyl-2-methylbenzene | 84 | 100 |
| 1,2,4-Trimethylbenzene | 93 | 73 |
| n-Decane | 94 | 94 |
| 1-Ethyl-2-methylbenzene | 66 | 100 |
| 2,7,10-Trimethyldodecane | 78 | 97 |
| 2,6-Dimethylnonane | 73 | 71 |
| 3,7-Dimethylnonane | 87 | 85 |
| 1-Methyl-3-propylbenzene | 96 | 99 |
| 2-Methyldecane | 78 | 100 |
| 3-Methyldecane | | |
| 1-Methyl-2-(1-methylethyl) benzene | 89 | 100 |
| 1-Methyl-3-(1-methylethyl) benzene | 88 | 99 |
| 1-Methyl-3-(1-methylethyl) benzene | 88 | 99 |
| Undecane | 96 | 81 |
| 1-Methyl-3-(1-methylethyl) benzene | 84 | 100 |
| 2-Ethyl-1,4-dimethylbenzene | 70 | 100 |
| 1,2,3,4-Tetramethylbenzene | 89 | 72 |
| 3,8-dimethylandecane | 76 | 82 |
| Pentylcyclohexane | 76 | 97 |
| 1-Ethyl-3,5-dimethylbenzene | 89 | 97 |
| Naphthalene | 97 | 77 |
| 1,1'-Biphenyl | 93 | 74 |
| 2-Cyclopentylidene cyclopentanone | 78 | 65 |
| 1-Dodecanol | 76 | 69 |
| 3-Methyl-1-1'-biphenyl | 83 | 98 |
| n-Nonadecane | 84 | 89 |
| n-Eicosane * | - | - |
| 1-Dodecanol | 76 | 75 |
| Triethylene glycol | 78 | 100 |
| 2-Methyl-1-dodecanol | 76 | 72 |
| Dodecanoic acid | 67 | 64 |
| 1-Tetradecanol | 87 | 63 |
| 1-Pentadecanol | 89 | 72 |
| Hexadecanoic acid | 82 | 83 |
| 9-Octaden-1-ol | 79 | 72 |
| Octadecanoic acid | 68 | 63 |

* Identification made with n-alkane reference standards

**TABLE 19: GC-MS EFFLUENT SAMPLES - TANDEM FABRICS - NOVEMBER 6, 1990**

Sample: Tandem Fabrics, Moncton, N.B., Composite Effluent Date: 6 Nov./90

Laboratory No: 90LR008 Library: NBS_REV x Wiley

| IDENTIFIED COMPOUND | PROBABILITY | % PURITY |
|---|---|---|
| 1,2-Dimethylbenzene | 95 | 92 |
| Propylbenzene | 89 | 74 |
| 1-Ethyl-2-methylbenzene | 96 | 91 |
| 1,2,3-Trimethylbenzene | 89 | 67 |
| 1-Ethyl-2-methylbenzene | 94 | 93 |
| 1,2,4-Trimethylbenzene | 89 | 67 |
| n-Decane | 83 | 73 |
| 1,2,3-Trimethylbenzene | 89 | 69 |
| 2-Ethyl-1-hexanol | 67 | 78 |
| 2,3-Dihydro-1H-indene | 86 | 77 |
| 1-Methyl-3-propylbenzene | 97 | 98 |
| 1-Methyl-3-(1-methylethyl) benzene | 87 | 83 |
| 1-Methyl-2-(1-methylethyl) benzene | 86 | 84 |
| Methyl (1-methylethyl) benzene | 91 | 95 |
| Undecane | 83 | 82 |
| 1,2,3,4-Tetramethylbenzene | 88 | 76 |
| 2-Ethyl-1,4-dimethylbenzene | 86 | 89 |
| 1-(4-Methylphenyl) ethanone | 93 | 87 |
| Naphthalene | 95 | 100 |
| Isoquinoline | 83 | 100 |
| [1,1'-Bicyclopentyl]-2-one | 76 | 84 |
| 1,1'-Biphenyl | 92 | 68 |
| 2-Methyl-1,1'-biphenyl | 89 | 93 |
| 1,1'-Methylenebisbenzene | 86 | 81 |
| 1-Dodecanol | 65 | 73 |
| 3-Methyl-1,1'-biphenyl | 88 | 71 |
| 2-Methyl-1,1'-biphenyl | 89 | 69 |
| 5-octadecene | 89 | 94 |
| 3-Chloro-4-nitrobenzenamine | 78 | 52 |
| n-Eicosane * | - | - |
| n-Heneicosane * | - | - |
| n-Docosane * | - | - |
| n-Tricosane * | - | - |
| n-Tetracosane * | - | - |
| n-Pentacosane * | - | - |
| n-Hexacosane * | - | - |
| n-Heptacosane * | - | - |
| n-Octacosane * | - | - |
| n-Nonacosane * | - | - |
| n-Triacosane * | - | - |
| n-Untriacosane * | - | - |
| Nonanoic acid | 62 | 64 |
| 2-Methoxy-5-(1-propenyl) phenol | 93 | 91 |
| 4-Hydroxy-3-methoxybenzaldehyde | 96 | 89 |
| Dodecanoic acid | 87 | 100 |
| Tetradecanoic acid | 95 | 78 |
| 1,1'-Biphenyl-2-ol | 84 | 100 |
| Hexadecanoic acid | 73 | 82 |
| Octadecanoic acid | 82 | 66 |

* Identification made with n-alkane reference standards

TABLE 20: SURFACTANT CONCENTRATIONS IN EFFLUENT SAMPLES FROM TANDEM FABRICS

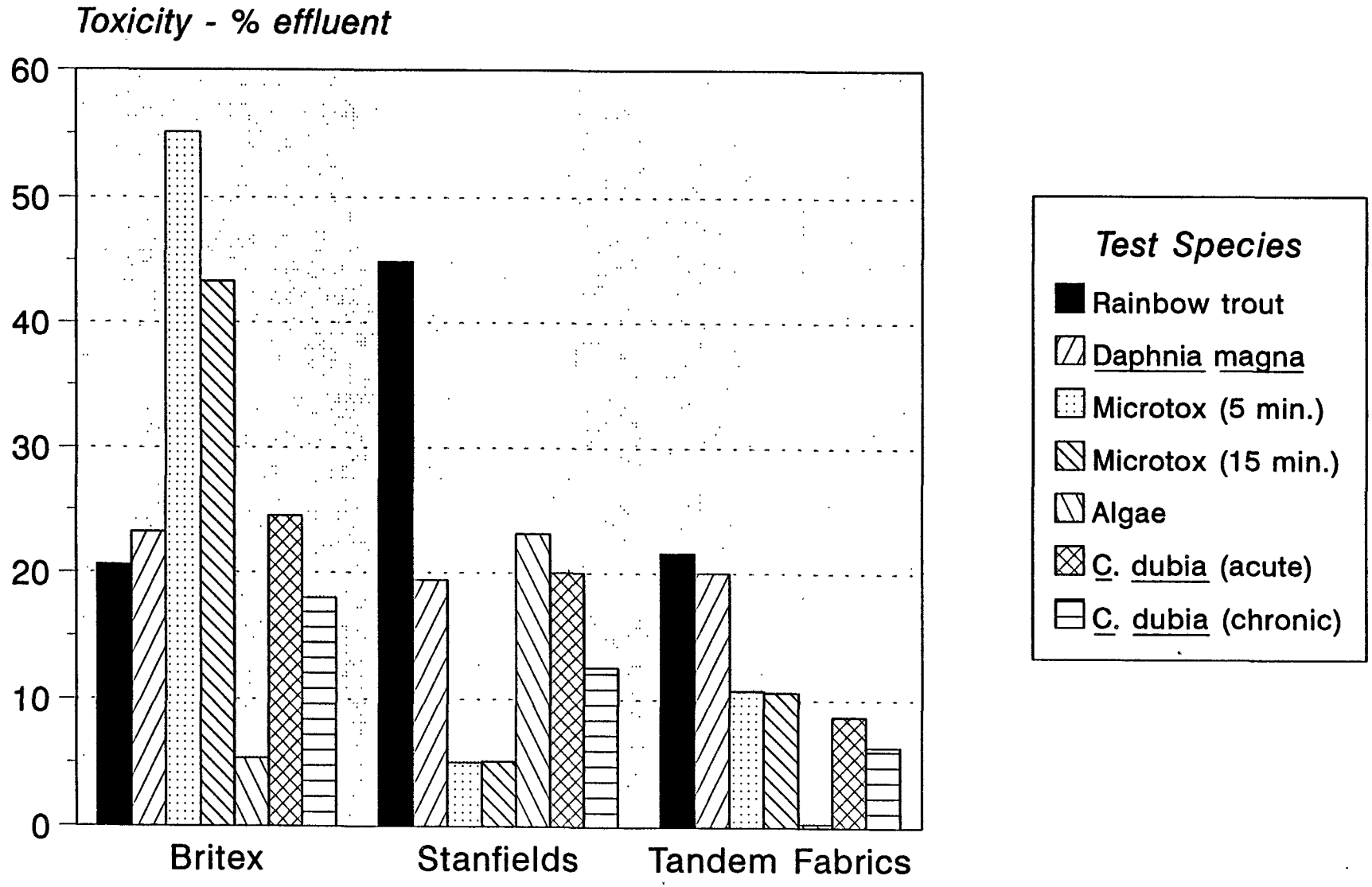
| Sampling Date | Concentration (mg/L) |
|---|---|
| September 11, 1990 | 50 |
| September 24, 1990 | 37 |
| October 23, 1990 | 24 |
| November 6, 1990 | 17 |

* Quality control data associated with the measurement of surfactant concentrations in effluent smaples are provided in Appendix D.

TABLE 21: ANALYTICAL RESULTS (CONVENTIONAL POLLUTANTS AND METALS) FOR FINAL EFFLUENT SAMPLES FROM TANDEM FABRICS (mg/L)

| Parameter | September 11 | September 24 | October 23 | November 6 |
|---|---|---|---|---|
| pH (units) | 6.3 | 5.1 | 6.5 | 5.9 |
| Alkalinity | 30 | 17 | 70 | 18 |
| COD | 1080 | 965 | 730 | 590 |
| BOD | 100 | 280 | 350 | 210 |
| Suspended Solids | 8 | 17 | <5 | <5 |
| Total Dissolved Solids | 520 | 640 | 900 | 590 |
| Total Solids | 520 | 660 | 900 | 600 |
| Ammonia-N | 27.4 | 38.3 | 23.8 | 25.2 |
| Oil and Grease (Pet. Ether) | 42 | 36 | <5 | 28.7 |
| Calcium | 10.22 | 17.03 | 24.71 | 9.81 |
| Arsenic | <0.05 | 0.12 | 0.12 | <0.05 |
| Cadmium | <0.01 | <0.01 | <0.01 | <0.01 |
| Chromium | 0.02 | 0.04 | <0.01 | <0.01 |
| Copper | <0.01 | 0.03 | 0.01 | <0.01 |
| Iron | 0.15 | 0.17 | 0.21 | 0.17 |
| Lead | <0.02 | <0.02 | <0.02 | <0.02 |
| Nickel | <0.01 | <0.01 | <0.01 | <0.01 |
| Manganese | 0.10 | 0.21 | 0.27 | 0.07 |
| Zinc | 0.05 | 0.05 | 0.06 | 0.04 |

# Fig. 4 - Toxicity of Untreated Effluent from Three Textile Mills




*(Toxicity - mean of LC50 or EC50 data for all dates)*

**TABLE 22 TOXICITY DATA FOR THE THREE TEXTILE MILLS SURVEYED**

| Sample Location | Sample Collection Date | Rainbow Trout | *Daphnia magna* | Microtox-EC50 5 min. | Microtox-EC50 15 min. | Stickle-back | Algae EC50 | *C. dubia* (acute) | *C. dubia* (chronic) |
|---|---|---|---|---|---|---|---|---|---|
| Britex | Sept. 17-18/90 | 17.7 | 32.9 | 54.0 | 58.0 | | 0.1 | 31.0 | 23.0 |
| | | | 29.3 | 54.5 | 61.2 | | | | |
| | Oct. 1-2/90 | 11.5 | 20.0 | >100.0 | | | 10.4 | 18.0 | 13.0 |
| | | | 20.0 | >100.0 | | | | | |
| | Nov. 14-15/90 | 35.5 | 24.4 | 22.0 | 18.9 | | | | |
| | | | 25.8 | 21.9 | 19.9 | | | | |
| | Nov. 26-27/90 | 17.7 | 16.5 | 44.3 | 51.9 | | | | |
| | | | 16.4 | 44.4 | 49.6 | | | | |
| Stanfields | Oct. 9-10/90 | 35.4 | 20.0 | 3.9 | | | 19.0 | 20.0 | 14.0 |
| | | | 18.9 | 3.7 | | | | | |
| | | | | 3.6 | 6.2 | | | | |
| | Oct. 29-30/90 | 38.0 | 9.5 | 4.8 | 5.5 | | 27.2 | 20.0 | 11.0 |
| | | | 8.8 | 4.5 | 5.2 | | | | |
| | Nov. 20-21/90 | 35.4 | 35.4 | 2.3 | 2.9 | | | | |
| | | | 27.0 | 2.5 | 3.1 | | | | |
| | | | | 3.1 | | | | | |
| | Dec. 3-4/90 | 70.7 | 17.7 | 5.6 | 6.0 | | | | |
| | | | 17.7 | 6.3 | 6.6 | | | | |
| Tanden Fabrics | Sept. 11/90 | 21.3 | 16.0 | 14.0 | 10.9 | 31.8 | 0.4 | 12.0 | 9.3 |
| | | | 23.8 | 12.5 | 12.1 | | | | |
| | Sept. 24/90 | 8.2 | 12.5 | 11.3 | 9.8 | <12.5 | 0.2 | 5.4 | 3.3 |
| | | | 13.6 | 12.0 | 10.7 | | | | |
| | Oct. 23/90 | 21.3 | 8.3 | 9.3 | 14.3 | <6.25 | | | |
| | | | 6.8 | 7.3 | 8.7 | | | | |
| | Nov. 6/90 | 35.4 | 33.0 | 9.6 | 9.3 | 62.2 | | | |
| | | | 46.0 | 9.6 | 8.8 | | | | |

(All data expressed as an LC50 value [% effluent (v/v)] unless otherwise stated.)

## *Fig. 5 - Mutagenicity of Untreated Effluent from Three Textile Mills*

| *Sample Location and Date* | *Tester Strain TA97* | *Tester Strain TA98* | *Tester Strain TA100* | *Tester Strain TA102* |
|---|---|---|---|---|
| *Tandem - Sept. 11/90* | *S - P* | | *S - P* | |
| *Britex - Sept. 17-18/90* | | | | *S - P* |
| *Tandem - Sept. 24/90* | | *P* | | *S - P* |
| *Britex - Oct. 1-2/90* | *P* | *S - P* | | *S* |
| *Stanfields - Oct. 9-10/90* | | *P* | | *S - P* |
| *Stanfields - Oct. 29-30/90* | *S - P* | | | *S - P* |

*Mutagenisis detected using the Ames Spot test (S) and the Plate Incorporation test (P).*

Each mill tested had variable toxicity to each organism, dependent on date of sampling. As well, each sample of effluent varied in toxicity to each organism tested.

All effluent samples tested were found to be mutagenic. There were differences in the apparent types of mutagenicity associated with each sample suggesting that more than one mutagen was present in the samples.

All of the samples were toxic to all of the organisms tested with the exception of one sample from Britex that showed no decrease in light emission at 100% effluent using the Microtox, but which was toxic to the four other species tested with this sample.

In the two samples where dissolved oxygen concentrations were below 70% saturation after the maximum pre-aeration period, it was found that dissolved oxygen was not the sole factor contributing to toxicity of the effluent. In the extra tests where the dissolved oxygen was kept at acceptable levels by strong aeration, the fish died, indicating the presence of other toxic components in these samples.

The pH adjustment of the replicate Daphnia tests showed no significant effect of extreme pH, indicating the presence of other toxic components.

The Microtox colour correction test showed no significant loss of light due to colour at the EC50 concentrations.

The effluent toxicity was rated from non-detectable toxicity to highly toxic using the classification scheme of Sandhu (1979) (Appendix G).

### 4.2.1 Stanfields

All effluent samples from Stanfields were moderately toxic to rainbow trout with LC50 values ranging from 35.4% to 70.7%. The average LC50 value for rainbow trout was 44.9%. These results were comparable to the LC50 values from the other mills surveyed.

Three effluent samples were moderately toxic to Daphnia magna with LC50 values ranging from 17.7% to 35.4%. The October 29-30, 1990 sample was highly toxic with LC50 values of 9.5% and 8.8%. The average LC50 value for D. magna was 19.4%

All effluent samples were highly toxic to Microtox. EC50 values ranged from 2.3% to 6.6%. The average 5-minute EC50 value was 5.0%; the average 15-minute EC50 value was 5.1%. The effluent samples from Stanfields exhibited the highest toxicity to Microtox of the three mills surveyed in this study.

The effluent samples were moderately toxic to Selenastrum capricornutum with an average EC50 value of 23.1%. The effluent samples from Stanfields exhibited the lowest toxicity to algae of the three mills surveyed.

The effluent samples were moderately toxic to Ceriodaphnia dubia with an average acute LC50 of 20.0% and an average chronic LC50 of 12.5%. The IC50 values, the concentration which would cause a significant effect (reproductive impairment) on 50% of exposed organisms, for the effluent samples ranged from 6.2% to 8.7%.

Ames testing indicated mutagenic properties in the effluent samples. The October 9-10, 1990 sample showed high mutagenicity with strain TA 102 and slight mutagenicity with strain TA 98 in the plate incorporation test. That sample showed slight mutagenicity with strain TA 102 in the spot test. The October 29-30, 1990 sample showed moderate mutagenicity with strains TA 97 and TA 102 (plate incorporation test) and slight mutagenicity with TA 102 (following S9 activation) and TA 97 (spot test).

### 4.2.2 Britex

The effluent samples from Britex were moderately toxic to rainbow trout with LC50 values ranging from 11.5% to 35.4%. The average LC50 value for rainbow trout was 20.6%.

The effluent samples were also moderately toxic to D. magna with LC50 values ranging from 16.4% to 32.9%. The average LC50 value for D. magna was 23.2% which was comparable to the other textile mills surveyed.

The effluent samples ranged in toxicity to Microtox from moderately toxic to non-detectable (EC50 values from 18.9% to <100%). The average 5-minute and 15-minute EC50 values were 55.1% and 43.3% respectively. These average values were significantly higher than the average EC50 values fro the other two mills surveyed, indicating that the Britex effluent was generally less toxic to Microtox.

The effluent samples from Britex were highly to moderately toxic to the alga Selenastrum capricornutum with EC50 values ranging from 0.1% to 10.4%. The highest toxicity encountered in the study was the September 17-18, 1990 sample from Britex (EC50 0.1%). The average EC50 value was 5.3%.

Samples from Britex were moderately toxic to Ceriodaphnia dubia with an average acute LC50 of 24.5% and an average chronic LC50 of 18.0%. The IC50 values ranged from 5.3% to 7.8%.

Both samples from Britex showed mutagenic properties. The September 17-18, 1990 sample showed high mutagenicity with tester strain TA 102 in the plate incorporation test and no mutagenicity in the spot test. The October 1-2, 1990 sample showed moderate mutagenicity with tester strain TA 97 and high mutagenicity with TA 98 in the plate incorporation test. That samples showed moderate mutagenicity with tester strains TA 98 and TA 102 in the spot test.

### 4.2.3 Tandem Fabrics

Three effluent samples from Tandem Fabrics were moderately toxic to rainbow trout with LC50 values ranging from 21.3% to 35.4%. The September 24, 1990 sample was highly toxic to rainbow trout with an LC50 value of 8.2%. The average LC50 value for rainbow trout was 21.6%.

Three of four effluent samples were moderately toxic to D. magna with LC50 values ranging from 12.5% to 46.0%. The September 24, 1990 was highly toxic to D. magna with LC50 values of 6.8% and 8.3%. The average LC50 value was 20.0%.

Microtox testing indicated that the effluent samples ranged from highly toxic to moderately toxic. EC50 values ranged from 7.3% to 14.3%. The average 5-minute EC50 value was 10.7%; the average 15-minute EC50 value was 10.6%.

LC50 values for the threespine stickleback ranged from <6.25% (high toxicity) to 62.2% (low toxicity) for effluent samples from Tandem Fabrics. The October 23, 1990 sample exhibited the highest toxicity.

Both samples from Tandem Fabrics were highly toxic to Selenastrum capricornutum with EC50 values of 0.4% to 0.2%.

The effluent samples were highly to moderately toxic to Ceriodaphnia dubia. The average acute LC50 was 8.7%; the average chronic LC50 was 6.3%. The IC50 values for the effluent samples ranged from 1.8% to 7.0%.

Ames testing indicated mutagenic properties in the effluent samples. The September 11, 1990 sample showed moderate mutagenicity with the tester strain TA 100 and slight mutagenicity with strain TA97 in the plate incorporation test. That sample showed moderate mutagenicity with strain TA 97 and slight mutagenicity with TA 100 in the spot test. The September 24, 1990 sample showed high mutagenicity to strain TA 98 and TA 102 in the plate incorporation test and moderate mutagenicity to strain TA 102 in the spot test.

### 4.2.4 Toxicity Data Correlations

The toxicity data were subjected to statistical tests (Spearman Rank Correlations and Sample Correlations) to determine the relationships between the test results for rainbow trout, Daphnia magna, and Microtox (12 samples for each species). The Spearman Rank Correlation assigns a rank to each data point and correlates the ranked values, while the Sample Correlation correlates the data itself. The data are presented in Appendix H. For both analyses, if the

significance levels (bottom figure) are less than 0.05 they indicate significantly non-zero correlations. If the correlation coefficient (top figure) is positive the assays vary in the same direction, while a negative correlation indicates that the assays vary in the opposite direction. If the coefficient is 1 the assays are in perfect agreement.

With both statistical analyses, using all 12 sample results for Microtox, Daphnia magna, and rainbow trout, only the results for the Microtox 5 and Microtox 15 minute assays showed significant positive correlation. As well, with the ranked correlation, the Microtox 5 and 15 minute assays were negatively correlated with the rainbow trout assays, but the actual sample correlations, using the EC50 data, were not significant (P=0.09). The results for the three species indicated that each species may have responded to different components of these chemically complex effluents. These three tests, therefore, complement each other as a test battery.

Six samples were tested using all five different species (rainbow trout, D. magna, Microtox, algae, and Ceriodaphnia) and these results were also subjected to the statistical tests described above. The data are presented in Appendix I.

With the Spearman Rank Correlations there was a significant correlation between the Microtox 15 minute assay and the D. magna assay but that correlation did not exist for all 12 samples tested. As expected, the Microtox 5 and 15 minute results were positively correlated, as were the Ceriodaphnia acute and chronic results.

With the Sample Correlations the Microtox 5 and 15 minute results correlated, as did the acute and chronic Ceriodaphnia results. The rainbow trout results correlated very positively with the algal test results, but were not of the same magnitude. The Ceriodaphnia chronic assay results indicated a possible positive correlation with the D. magna results, but were not significant. All other test results did not correlate.

The sample size for these correlations was small (n=6). With more samples, other tests may have shown better correlations.

## 4.3 Environmental and Ecological Impact

Benthic macroinvertebrate data collected during field surveys at the three textile mills are presented in Appendix J.

### 4.3.1 Stanfields

The Salmon River in the vicinity of the Stanfield's plant in Truro, N.S. is a fast flowing, freshwater river. The substrate at the five sampling stations was mostly cobble with some medium and coarse gravel. The sampling station furthest downstream from the effluent outlet (Station 4) was approximately 50 m above head of tide during the survey.

The mean daily discharge in the Salmon River during September and October 1990 was 0.896 cubic metres/second and 9.15 cubic metres/second respectively. The mean daily discharge during May and June 1991, when the second field survey was conducted, was 5.43 cubic metres/second and 3.14 cubic metres/second respectively (Environment Canada 1990 and 1991, unpublished data).

The water at the Control station was clear. The gravel bag artificial substrates were clearly visible when deployed and retrieved. The effluent plume from the textile mill was highly coloured, was approximately 5 m wide (covering about one third of the width of the river) and travelled down the south side of the river. The plume was clearly visible at Station 4, 120 m downstream from the effluent outlet. The retrieval of the gravel bags in the effluent plume was dependent upon the colour of dye that was being discharged from the plant, as the bags were not visible when dark colours (e.g. purple, blue, black) were being processed in the mill.

Twenty three taxa were collected at the five sampling stations during the 1990 survey at Stanfields. Aquatic insects, which were dominated by clingers, accounted for thirteen of the taxa, followed by snails (6 species) and leeches (4 species).

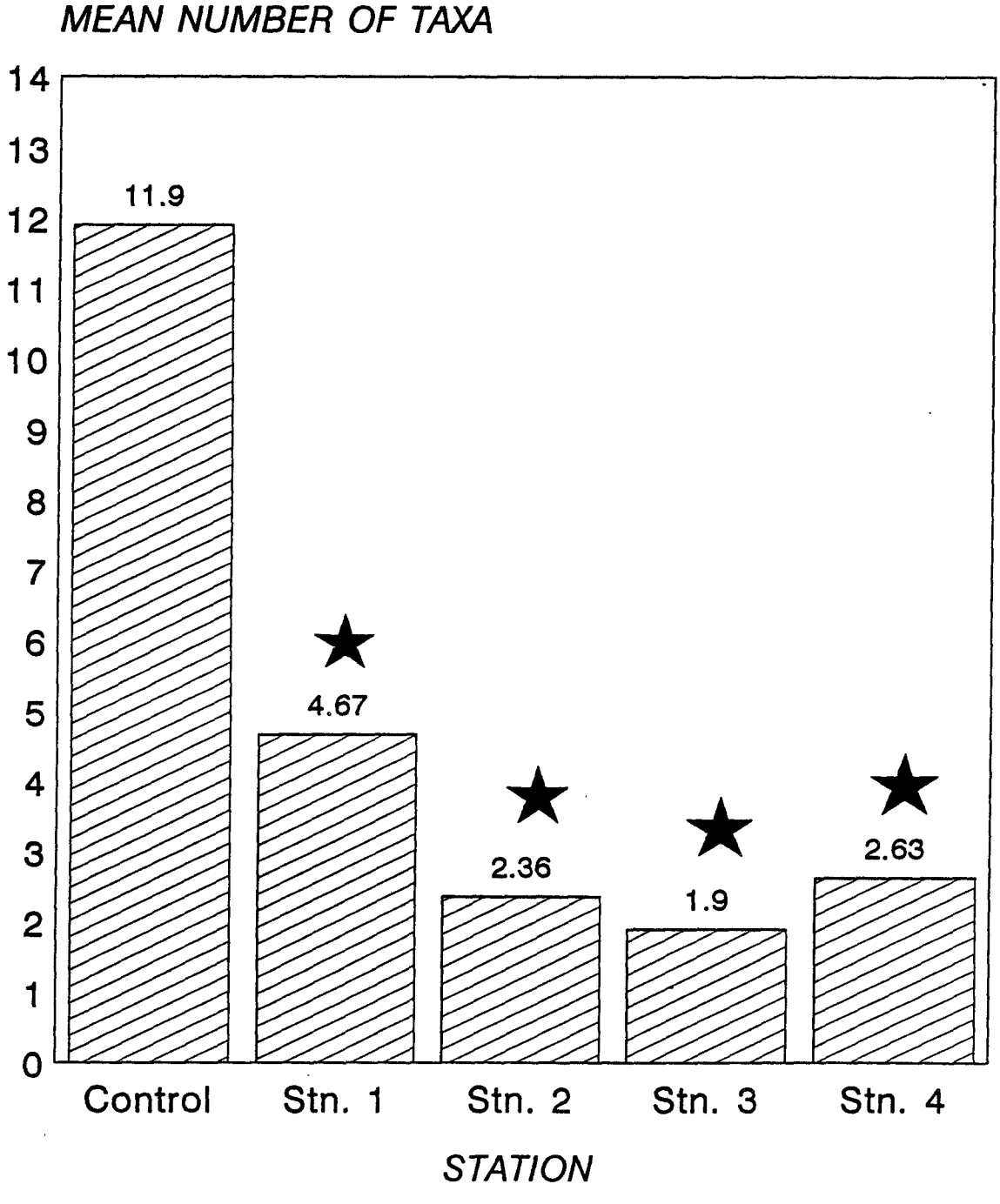
Species composition at the Control station was dominated by Chironomidae (39.9%), Amnicola limosa (27.1%), Physa heterostropha (10.9%), Gyraulus deflectus (7.7%) and Hydropsyche bronta (6.5%). The dominant taxa at the Control station were fairly evenly distributed between aquatic insects (46.4%) and snails (45.7%). The dominant taxa at the Impacted stations were Chironomidae (75.9%), Amnicola limosa (8.0%), Physa heterostropha (6.7%) and Gyraulus deflectus (5.2%). Snails represented a smaller proportion of species composition (19.9%) at the Impacted Stations, compared to the Control.

Figure 6 indicates that species diversity, as measured by the mean number of taxa at the sampling stations, was significantly lower at all of the Impacted stations compared to the Control ($p<0.05$).

Camargo (1990) recently developed an "ecotoxicological index" for assessing the environmental impacts of man's activities on freshwater communities. The index combines a measure of the percent difference between the number of species occurring above and below a disturbance point with a measure of the species substitution between the two sites. The values of the ecotoxicological index, or EI, range from 0 (no impact) to 100 (maximum impact). The EI for the control station always has a value of 0. The EIs for and Impacted stations for the 1990 field survey at Stanfields were 63.2 (Station 1), 68.4 (Station 2), 73.7 (Station 3) and 73.7 (Station 4). These values indicate a significant change in community structure between the Control and Impacted stations.

Table 23 presents the mean number of benthic macroinvertebrates collected at the five sampling stations in the Salmon River during the 1990 field survey. Statistical analysis was conducted when more than five individuals of a taxa were present at one station. For 13 of 14 taxa statistically analyzed, Dunnett's One-tailed T test (Zar 1974) indicated that number of benthic macroinvertebrates at all four impacted stations were significantly lower than the Control ($p<0.05$). For one species of leech, Helobdella stagnalis, numbers were significantly lower than the Control at stations 1, 2 and 3, but not at station 4 ($p<0.05$). These data indicate that the biological impact of the effluent discharge was not specific to one group of organisms as aquatic insects (caddisflies, mayflies, beetles and chironomids), snails and leeches were all negatively impacted by the untreated effluent.

## *Fig. 6 - Mean Number of Taxa Collected at Sampling Stations in the Salmon River (Stanfields) - October 1990*




*Stars represent results of Dunnett's Two-tailed T test such that values covered by a star are significantly lower than the Control station ($p<0.05$).*

TABLE 23 MEAN NUMBER OF BENTHIC MACROINVERTEBRATES COLLECTED AT SAMPLING STATIONS IN THE SALMON RIVER (STANFIELDS) - OCTOBER, 1990

| Invertebrate | | Control | Station 1 | Station 2 | Station 3 | Station 4 |
|---|---|---|---|---|---|---|
| Chironomidae | mean | 262.90 | 11.89* | 13.00* | 18.00* | 47.63* |
| | SD | 108.34 | 5.67 | 9.42 | 9.63 | 20.85 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Amnicola limosa | mean | 178.60 | 6.44* | 2.18* | 0.30* | 0.00* |
| | SD | 65.55 | 6.31 | 4.09 | 0.67 | 0.00 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Ferrissia rivularis | mean | 22.70 | 0.67* | 0.27* | 0.10* | 0.13* |
| | SD | 23.79 | 0.87 | 0.90 | 0.32 | 0.35 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Physa heterostropha | mean | 71.70 | 5.67* | 1.09* | 0.20* | 0.88* |
| | SD | 21.74 | 4.90 | 2.43 | 0.42 | 1.25 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Gyraulus deflectus | mean | 50.40 | 4.11* | 1.27* | 0.40* | 0.13* |
| | SD | 23.03 | 3.33 | 3.58 | 0.52 | 0.35 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Helisoma trivolus | mean | 4.40 | 0.22* | 0.00* | 0.00* | 0.00* |
| | SD | 3.78 | 0.44 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Helobdella stagnalis | mean | 6.50 | 0.00* | 0.00* | 0.00* | 2.75 |
| | SD | 7.07 | 0.00 | 0.00 | 0.00 | 4.27 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Stenelmis sp. | mean | 10.70 | 0.00* | 0.00* | 0.00* | 0.00* |
| | SD | 7.15 | 0.00 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Stenonema vicarium | mean | 0.70 | 0.00* | 0.00* | 0.00* | 0.00* |
| | SD | 1.06 | 0.00 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Paraleptophlebia sp. | mean | 2.60 | 0.11* | 0.00* | 0.00* | 0.00* |
| | SD | 2.59 | 0.33 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Stagnicola elodes | mean | 2.00 | 0.00* | 0.00* | 0.00* | 0.00* |
| | SD | 1.89 | 0.00 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Brachycentrus numerosus | mean | 0.60 | 0.00* | 0.00* | 0.00* | 0.00* |
| | SD | 1.35 | 0.00 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Helicopsyche borealis | mean | 0.50 | 0.00* | 0.00* | 0.00* | 0.00* |
| | SD | 0.97 | 0.00 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 9 | 9 | 9 | 8 |
| Hydropsyche bronta | mean | 42.70 | 0.00* | 0.00* | 0.00* | 0.00* |
| | SD | 29.67 | 0.00 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 9 | 9 | 9 | 8 |

* represent results of Dunnett's One-tailed T test such that values covered by an asterisk are significantly lower than the Control Station ($p < 0.05$) Dunnett's test was conducted on data transformed by deriving the square root of all values. Statistical analysis was conducted when more than five individuals of a species occurred at one station.

Seventeen taxa were collected at the five sampling stations during the 1991 field survey in the Salmon River at Stanfields. This represented a decrease of 6 species from the 1990 survey. Aquatic insects, again dominated by clingers, accounted for eleven of the taxa, followed by snails (4 species) and leeches (2 species).

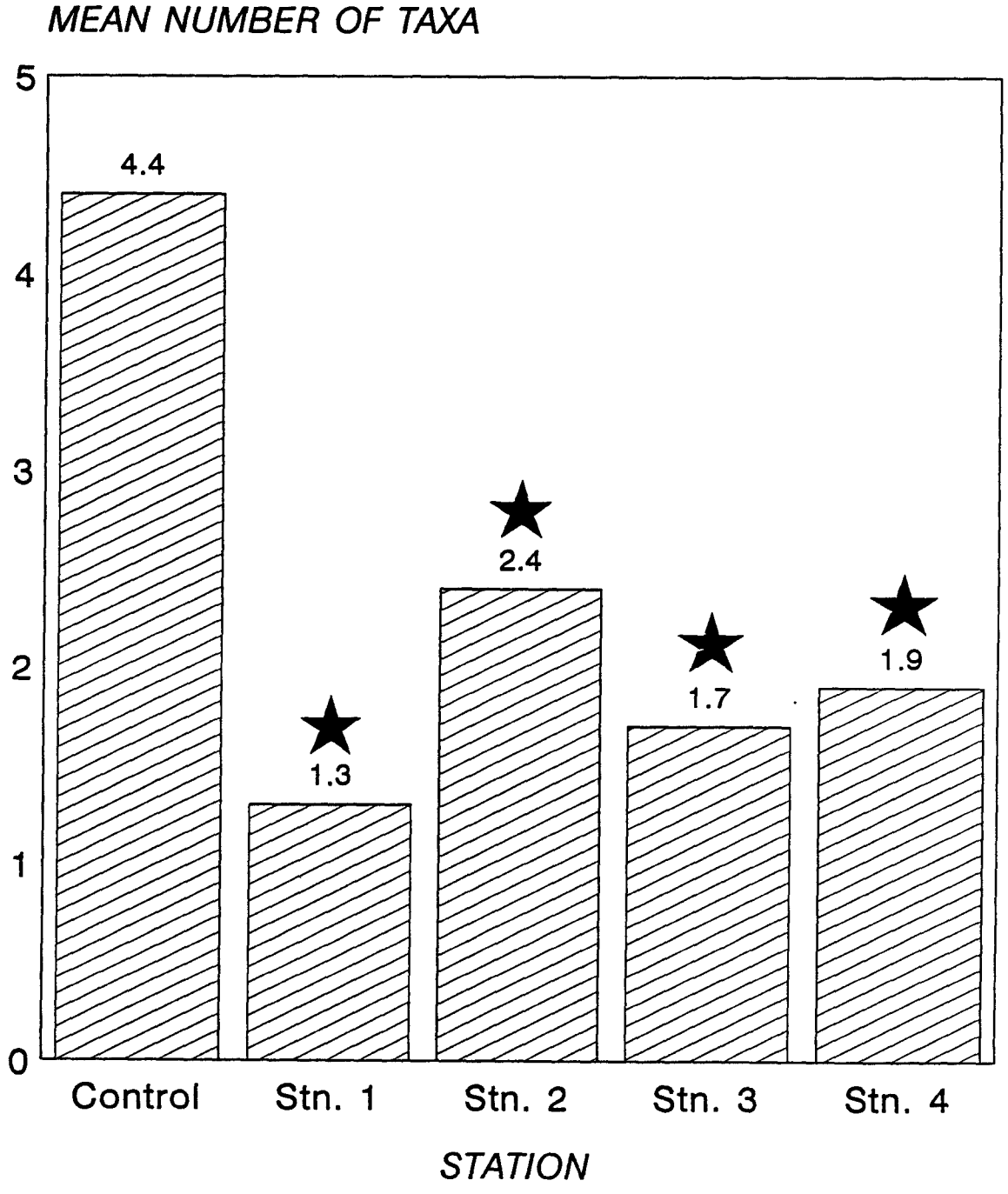
Chironomids dominated the species composition of the Control station (93.5%), with two species of mayflies, Baetis sp. (2.4%) and Ephemerella sp. (1.1%), and one species of snail, Amicola limosa (1.0%), occurring in relatively significant numbers. The Impacted stations were largely dominated by chironomids (98.3%).

Figure 7 indicates that species diversity, as measured by the mean number of taxa at the sampling stations, was significantly lower at all of the Impacted stations compared to the Control ($p<0.05$).

The EIs for the Impacted stations for the 1991 field survey at Stanfields were 80.0 (Station 1), 53.3 (Station 2), 73.3 (Station 3) and 73.3 (Station 4). These values indicate a significant change in community structure between the Control and the Impacted stations.

Table 24 presents the mean number of benthic macroinvertebrates collected at the five sampling stations in the Salmon River during the spring of 1991. For 2 of the 7 taxa statistically analyzed, Dunnett's One-tailed T test indicated that numbers of benthic macroinvertebrates at all four impacted stations were significantly lower than the Control ($p<0.05$). For another two taxa, the numbers of aquatic invertebrates were significantly lower than the Control at stations 1, 2 and 3, but not at Station 4 ($p<0.05$). For one species, the mean number of individuals was significantly lower at stations 1, 3 and 4, but not at Station 2 ($p<0.05$). Numbers of one species of mayfly, Ephemerella bicolor, and one species of beetle, Stenelmis sp., were not significantly different than the Control at any of the impacted stations. Of the five taxa that exhibited decreased numbers at three or more of the impacted stations, three were aquatic insects (2 mayflies, 1 chironomid) and two were snails.

***Fig. 7 - Mean Number of Taxa Collected at Sampling Stations in the Salmon River (Stanfields) - June 1991***




*Stars represent results of Dunnett's Two-tailed T test such that values covered by a star are significantly lower than the Control station ($p<0.05$).*

TABLE 24 MEAN NUMBER OF BENTHIC MACROINVERTEBRATES COLLECTED AT SAMPLING STATIONS IN THE SALMON RIVER (STANFIELDS) - JUNE, 1991

| Invertebrate | | Control | Station 1 | Station 2 | Station 3 | Station 4 |
|---|---|---|---|---|---|---|
| Chironomidae | mean | 157.30 | 7.10* | 4.20* | 115.50* | 140.20 |
| | SD | 56.70 | 2.88 | 1.69 | 50.95 | 46.04 |
| | n | 10 | 10 | 10 | 10 | 10 |
| Amnicola limosa | mean | 1.60 | 0.10* | 0.80 | 0.10* | 0.00* |
| | SD | 2.41 | 0.32 | 1.23 | 0.32 | 0.00 |
| | n | 10 | 10 | 10 | 10 | 10 |
| Stenelmis sp. | mean | 0.40 | 0.30 | 0.50 | 0.20 | 0.70 |
| | SD | 0.97 | 0.67 | 0.71 | 0.42 | 1.06 |
| | n | 10 | 10 | 10 | 10 | 10 |
| Ephemerella bicolor | mean | 0.40 | 0.00 | 0.20 | 0.40 | 0.20 |
| | SD | 0.52 | 0.00 | 0.42 | 0.52 | 0.63 |
| | n | 10 | 10 | 10 | 10 | 10 |
| Ephemerella sp. | mean | 1.80 | 0.00* | 0.00* | 0.00* | 0.00* |
| | SD | 3.05 | 0.00 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 10 | 10 | 10 | 10 |
| Physa heterostropha | mean | 1.00 | 0.00* | 0.10* | 0.00* | 0.50 |
| | SD | 1.63 | 0.00 | 0.32 | 0.00 | 0.71 |
| | n | 10 | 10 | 10 | 10 | 10 |
| Baetis sp. | mean | 4.10 | 0.00* | 0.00* | 0.00* | 0.00* |
| | SD | 4.95 | 0.00 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 10 | 10 | 10 | 10 |

* represent results of Dunnett's One-tailed T test such that values covered by an asterisk are significantly lower than the Control Station ($p < 0.05$) Dunnett's test was conducted on data transformed by deriving the square root of all values. Statistical analysis was conducted when more than five individuals of a species occurred at one station.

Four taxa were collected in sufficient numbers during both the 1990 and 1991 surveys to warrant statistical analyses. Of these four, Chironomidae, Amnicola limosa and Physa heterostropha exhibited significantly lower numbers at the Impacted Stations during both the spring and fall surveys. Only one invertebrate, Stenelmis sp., occurred in significantly lower numbers in the fall, but not in the spring.

The ten caged freshwater clams at each of the four sampling stations in the effluent plume deployed during the September - October 1990 survey, were all dead when retrieved after a period of one month. There was no tissue left for chemical analysis. The ten freshwater clams at the control station all survived.

Four of the ten freshwater clams deployed at Station 4 survived when retrieved after a period of one month during the May - June 1991 survey. Tissue from those clams, along with the ten surviving clams from the control station, were analyzed for selected organic compounds (see Section 4.1.1). The ten freshwater clams at Stations 1, 2 and 3 in the effluent plume all died and had no tissue for chemical analysis.

### 4.3.2 Britex

The Annapolis River near the Britex plant is a slow moving, tidal river. Water levels in the river are controlled at its mouth at a tidal generating plant. The substrate at the five sampling stations was composed of fine sand and silt. At low tide, the multi-plate samplers were in approximately 30 cm of water, at high tide, 150 cm of water.

The mean daily discharge in the Annapolis River during September and October 1990 was 3.65 cubic metres/second and 9.01 cubic metres/second, respectively (Environment Canada 1990, unpublished data).

The water at the Control and Impacted stations was slightly turbid. The effluent outlet discharged to a small tidal brook. The outlet was approximately 10 m from the river. The effluent plume from the textile mill was not clearly visible in the river.

Eight taxa were collected at the five sampling stations during the 1990 survey at Britex. the Control station was dominated by Gammaris tigrinus (98.6%) and Chironomidae (1.2%). Species composition at the Impacted stations was very similar to that exhibited at the Control, Gammaris tigrinus (98.4%) and Chironomidae (1.2%).

Figure 8 indicates that species diversity, as measured by the mean number of taxa at the sampling stations, was significantly lower at stations 2 and 3 compared to the Control ($p<0.05$). The mean number of taxa at stations 1 and 4, however, were not significantly different than the Control ($p<0.05$).

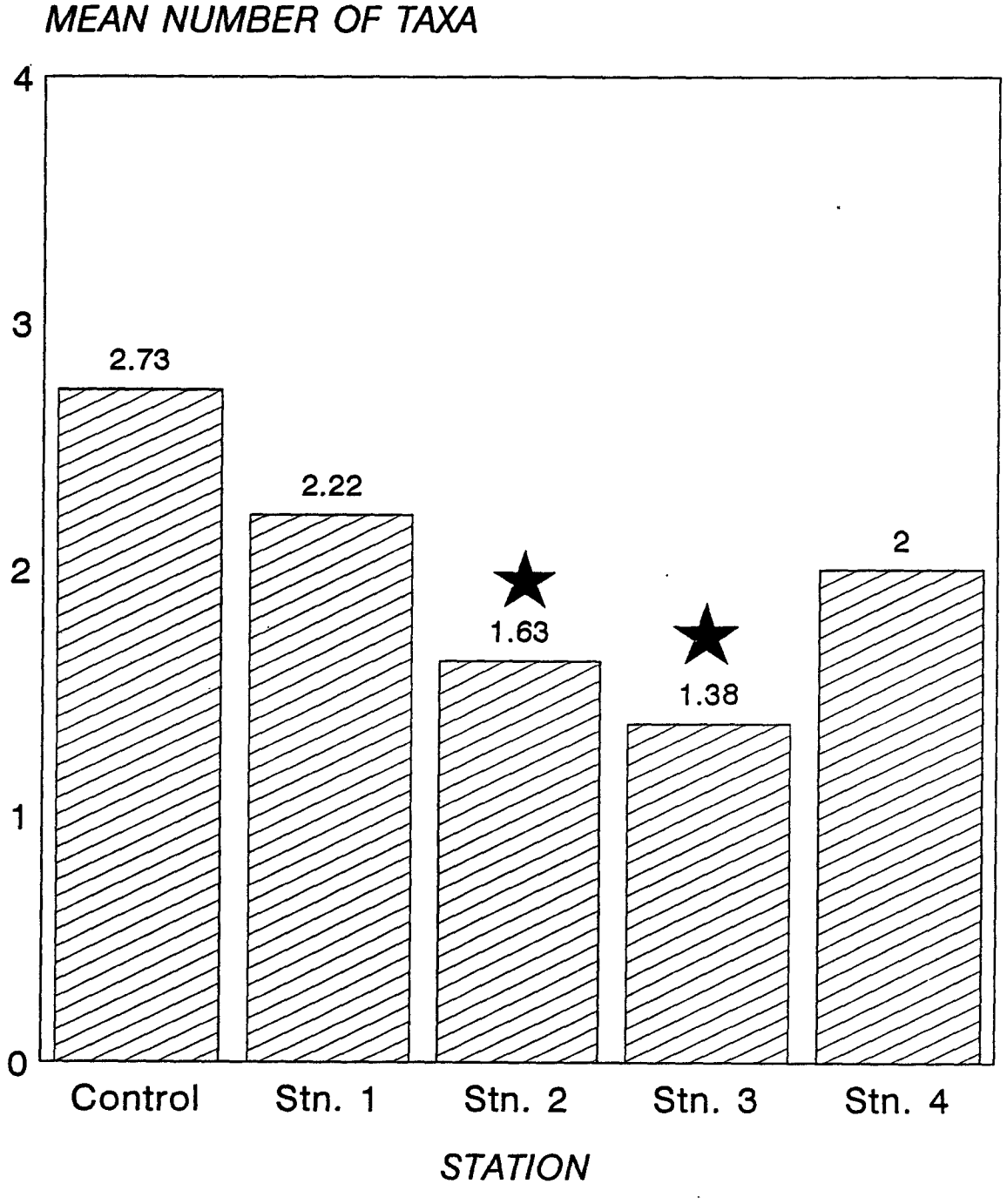
The EIs for the Impacted stations were 64.3 (Station 1) and 71.4 (Station 2, 3 and 4). These values indicate a significant change in community structure between the Control and Impacted stations. Caution should be taken in evaluating these values however. The Control station was 4 km upstream and although still tidally influenced, salinity differences between the Control and Impacted stations may have had an effect on the number of freshwater species at the Control. An examination of the raw data for the survey in Appendix J indicates that small numbers of freshwater snails (Stagnicola elodes, Physa heterostropha, Amnicola limosa) and aquatic insects (Hydropsyche morosa, Stenelmis sp.) were collected at the Control, but not at the Impacted stations.

Table 25 presents the mean number of benthic macroinvertebrates collected at the five sampling stations in the Annapolis River. For the three species statistically analyzed, Dunnett's One-tailed T test indicated that numbers of invertebrates were not significantly lower at the four Impacted stations compared to the Control ($p<0.05$).

### 4.3.3 Tandem Fabrics

Humphrey's Brook in the vicinity of Tandem Fabrics, Moncton, N.B., is a tidally influenced, flow controlled stream. A freshwater reservoir and sluice gate occurs about 100 m upstream of the textile mill's effluent outlet. A combined storm and sanitary sewer discharges untreated sewage into the brook just below

## *Fig. 8 - Mean Number of Taxa Collected at Sampling Stations in the Annapolis River (Britex) - October 1990*




*Stars represent results of Dunnett's Two-tailed T test such that values covered by a star are significantly lower than the Control station ($p<0.05$).*

TABLE 25 MEAN NUMBER OF BENTHIC MACROINVERTEBRATES COLLECTED AT SAMPLING STATIONS IN THE ANNAPOLIS RIVER (BRITEX) - OCTOBER, 1990

| Invertebrate | | Control | Station 1 | Station 2 | Station 3 | Station 4 |
|---|---|---|---|---|---|---|
| Chironomidae | mean | 6.18 | 5.78 | 3.00 | 3.75 | 6.78 |
| | SD | 3.82 | 5.40 | 4.17 | 7.50 | 6.65 |
| | n | 10 | 9 | 8 | 9 | 9 |
| Gammaris tigrinun | mean | 495.64 | 349.56 | 324.38 | 442.25 | 455.56 |
| | SD | 202.03 | 199.89 | 155.39 | 243.53 | 166.14 |
| | n | 10 | 9 | 8 | 9 | 9 |
| Gammaris oceanicus | mean | 0.00 | 5.44 | 0.00 | 0.00 | 0.00 |
| | SD | 0.00 | 7.99 | 0.00 | 0.00 | 0.00 |
| | n | 10 | 9 | 8 | 9 | 9 |

* represent results of Dunnett's One-tailed T test such that values covered by an asterisk are significantly lower than the Control Station ($p < 0.05$) Dunnett's test was conducted on data transformed by deriving the square root of all values. Statistical analysis was conducted when more than five individuals of a species occurred at one station.

the reservoir. The substrate at the five sampling stations was predominantly composed of medium and coarse gravel and fine sand and silt, with some rubble and debris. Station 4 appeared to be more tidally influenced than the other stations.

The water at all of the sampling stations, including the Control, was extremely turbid. The gravel bag artificial substrates were not clearly visible when deployed and retrieved.

Eleven taxa were collected at the five sampling stations during the 1990 survey at Tandem Fabrics. Aquatic insects, dominated by clingers, accounted for six of the taxa, followed by snails (3 species) and leeches (2 species).

Chironomidae dominated the species composition at both the Control and Impacted stations 97.9% and 99.4% respectively. Chironomids are very tolerant of oxygen reduced environments (Kovalak 1979), which appeared to be the case for Humphrey's Brook.

Figure 9 indicates that species diversity, as measured by the mean number of taxa at the sampling stations, was not significantly different at the Impacted stations compared to the Control ($p<0.05$). EIs for the Impacted stations were 41.7 (Station 1), 0 (Station 2) and 50.0 (Station 3 and 4). These values indicate no impact at Station 2, and a minimal change in community structure between the Control and Impacted stations.

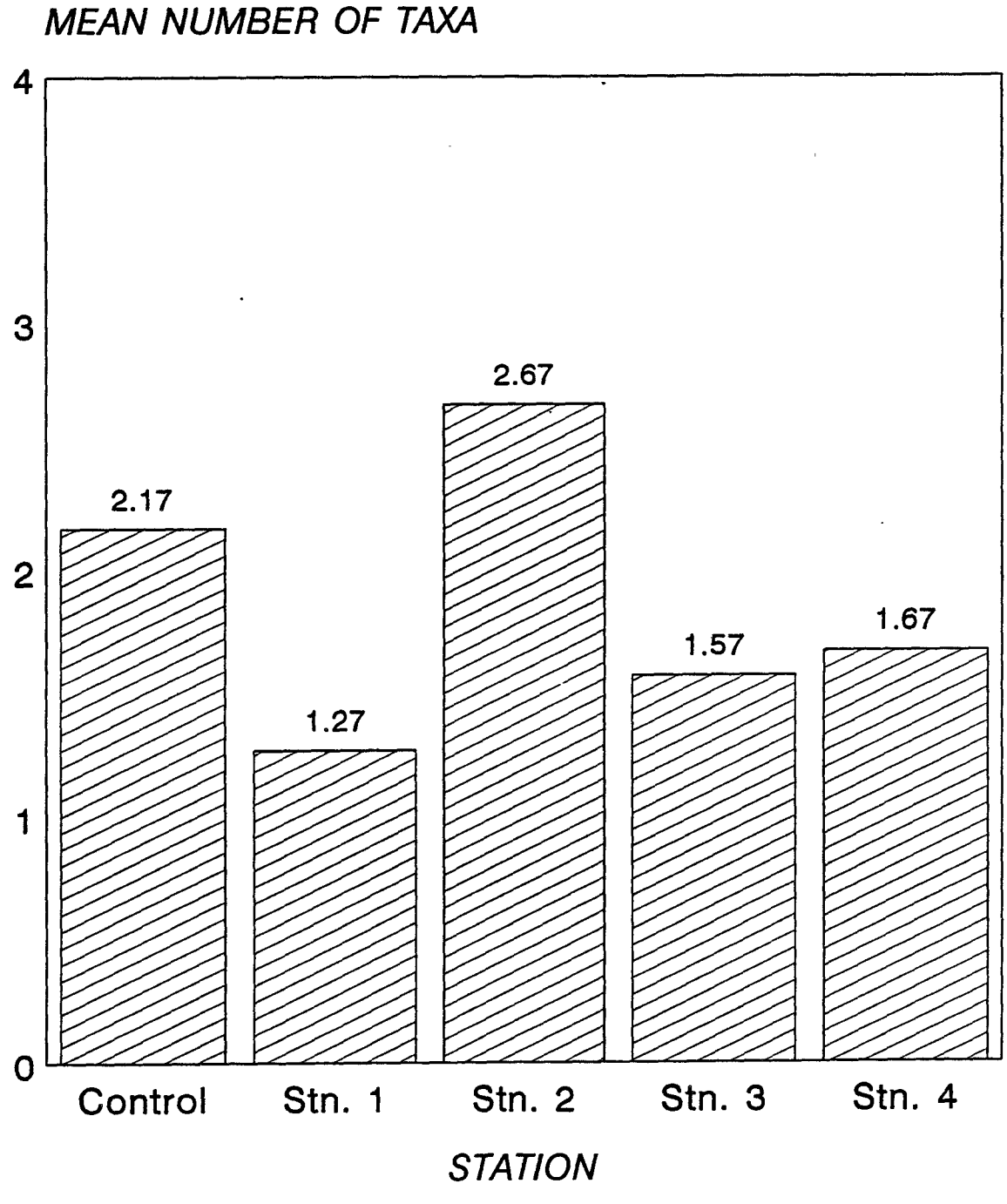
Table 26 presents the mean number of benthic macroinvertebrates collected at the five sampling stations in Humphrey's Brook. For three of the eleven taxa statistically analyzed, Dunnett's One-tailed T test indicated that numbers of aquatic invertebrates at all four Impacted stations were not statistically lower than the Control ($p<0.05$).

**TABLE 26 MEAN NUMBER OF BENTHIC MACROINVERTEBRATES COLLECTED AT SAMPLING STATIONS IN HUMPHREY'S BROOK (TANDEM FABRICS) - SEPTEMBER, 1990**

| Invertebrate | | Control | Station 1 | Station 2 | Station 3 | Station 4 |
|---|---|---|---|---|---|---|
| Chironomidae | mean | 76.67 | 82.18 | 243.83 | 197.43 | 41.0 |
| | SD | 30.90 | 55.59 | 76.97 | 42.39 | 50.41 |
| | n | 6 | 10 | 6 | 7 | 6 |
| Erpobdella punctata | mean | 0.33 | 0.09 | 0.00 | 0.14 | 0.67 |
| | SD | 0.52 | 0.30 | 0.00 | 0.38 | 0.82 |
| | n | 6 | 10 | 6 | 7 | 6 |
| Physa heterostropha | mean | 0.83 | 0.09 | 1.00 | 0.43 | 0.33 |
| | SD | 1.17 | 0.30 | 1.26 | 0.53 | 0.82 |
| | n | 6 | 10 | 6 | 7 | 6 |

* represent results of Dunnett's One-tailed T test such that values covered by an asterisk are significantly lower than the Control Station ($p < 0.05$) Dunnett's test was conducted on data transformed by deriving the square root of all values. Statistical analysis was conducted when more than five individuals of a species occurred at one station.

## *Fig. 9 - Mean Number of Taxa Collected at Sampling Stations in Humphrey's Brook (Tandem Fabrics) - September 1990*




*Stars represent results of Dunnett's Two-tailed T test such that values covered by a star are significantly lower than the Control station ($p<0.05$).*

## 5.0 DISCUSSION

Based on information obtained from Environment Canada surveys conducted in 1973/74, 1981/82 and 1985/86, Chen (1989) reported that there are approximately 1,000 textile manufacturing facilities in Canada. About 55% of those facilities are located in Quebec and 40% in Ontario. Approximately 20% of the mills use wet processing and about half of those wet processing mills belong to the woven fabric and knit fabric dyeing and finishing sub-categories. More than 80% of the wet processing mills discharge their effluents to a municipal system; about 10% of wet processing mills discharge directly to a natural watercourse with no wastewater treatment.

The three textile mills surveyed in this study are representative of the major wet processing sub-categories, however, from a waste treatment perspective, they are not representative of the majority of wet processing facilities in Canada in that they discharge directly to natural watercourses without treatment. In a 1989 survey to determine chemical use in the textile sector in Atlantic Canada, MacGregor (1990) reported that no wet processing mill in the region treated its effluent. However, at that time, the Crossley Karastan wet processing mill in Truro, N.S. used primary treatment (settling pond) before discharging effluent to the municipal sewer system. In 1990 Wink Industries, Caraquet, N.B. opened and utilized tertiary treatment technologies to treat its process effluents.

A number of authors have commented on the variability of textile wastewaters over time and between mills (Thompson 1974; Netzer and Beszedits 1975; Chen 1989). Production at many textile mills, particularly some in the Atlantic Region, is market-driven, thus the nature of effluent discharges could be expected to change over time as different products are produced to meet market demands. Over the course of this study, however, there were relatively few differences in the types of chemical compounds identified in the effluent from the same mill over the collection period. The most notable differences were probably due to dilution factors of final effluents. There were considerable differences, however, in the chemical composition of the waste streams produced by the three mills, which no doubt reflects the different processes used at each mill.

MacGregor (1990) surveyed 41 potential chemical users in the textile sector in the Atlantic Region in 1989-90 to determine general chemical use and use of chemicals on the national and regional Priority Substances Lists. The most common chemicals used by wet processing mills in the Region were: peroxide (126,000 kg); latex (65,000 kg); azo dyes (57,000 kg); sodium hydroxide (16,000 kg); petroleum distillates (10,000 L); acetic acid (9,000 L); detergent (7,000 L); phosphoric acid (7,000 L); sodium and ammonium sulphate (7,000 kg); citric acid (5,000 L); and formic acid (3,000 kg). Dye carriers (petroleum distillates), detergents and plasticizers (components of latex formulations) were found in effluents during this survey.

Priority chemicals used in the textile industry in the region included: 1,1,1 trichloroethane (3,541 L); toluene (1,840 L); trichloroethylene (1,581 L): xylenes (213 L); dichloromethane (120 L); 1,4 dichlorobenzene (23 kg); and chromium (23 kg). MacGregor (1990) indicated that quantities of 1,4 dichlorobenzene and chromium were significantly under-reported by factors of four to ten respectively. MacGregor (1990) reported that most of the priority chemical losses were by evaporation, however, toluene, xylenes and chromium were discharged in liquid effluents. This study did not reveal toluene or xylene in effluents. Chromium was found in concentrations slightly above the 0.02 $\mu$g/L guideline for the protection of fish in freshwater (CCREM 1987) in some samples from Britex and Tandem Fabrics.

A survey of the U.S. National Technical Information Service (NTIS), Environmental Library Automated Service (ELIAS) and Chemical Abstracts databases provided few reported studies that attempted to identify chemicals present in wastes of textile operations. In studies found on the subject, there is a notable similarity in the types of chemical compounds identified in textile mill waste streams and the chemical compounds identified in Maritime textile mill effluents. Gordon and Gordon (1981) examined effluent from a North Carolina textile finishing and dyeing plant employing purge and trap GC/MS computer techniques. Approximately 80 different volatile and semi-volatile compounds were identified. A significant number of the compounds reported in the waste stream of the North Carolina mill are the same compounds or similar compounds identified as dye carriers, surfactants/detergents, plasticizers and

miscellaneous chemicals in Maritime textile mill effluents. Major differences between the two studies, the presence of low molecular weight volatiles and the absence of mineral oil related compounds reported in the 1981 study, are probably due, in part, to the different extraction methods employed in each study.

The U.S. EPA released a report in 1979, identifying organic compounds in secondary effluents from 23 textile mills (U.S. EPA 1979). Again many of the semi-volatile compounds identified in the EPA study were identical or similar to compounds detected in Maritime effluents. The EPA study included volatile as well as semi-volatile compounds. In secondary textile mill effluent, volatile components comprised, however, only a minor portion of all organic compounds identified.

In the context of the U.S. EPA list of priority pollutants, only five base/neutral extractable compounds (naphthalene, acenaphthalene, fluorene, diethylphthalate, bis(2-ethylhexyl) phthalate) and one acid extractable compound (chlorophenol) were identified in Maritime textile mill effluent. The small number of priority pollutants is consistent with earlier analytical work conducted on Quebec and Ontario textile mill effluents in 1982 (Hennigar et al. 1982; Chen 1989) and with the 1979 EPA study in which only 1% to 3% of the total organic mass was attributed to priority pollutants (U.S. EPA 1979). Of the nearly 100 different organic compounds reported by the EPA in 1979, only five base/neutral extractable priority pollutants greater than 10 $\mu g/l$ (naphthalene, diethylphthalate, bis(2-ethylhexyl) phthalate, 1,2,4-trichlorobenzene and n-nitroso-di-n-propylamine) and three acid extractables (2,4-dimethylphenol, chlorocresol and pentachlorophenol) were reported.

Four substances (or group of substances), Bis(2-ethylhexyl)phthalate, PAHs, arsenic and chromium, identified in effluent samples from Maritime textile mills are on the CEPA Priority Substances List. Those substances will be assessed for environmental and human health impacts.

Organic chemicals identified in effluent samples from Maritime textile mills generally fell into one of five groups: detergents/surfactants (ethoxy and

phenoxyethanols, ethylhexanol, nonylphenol, ethoxylated acylphenoxyethanols, etc.); plasticizers (diethylphthalate, bis(hexylethyl)phthalate); dye carriers (alkylated benzenes, mono-.di- and tri-methylnaphthalenes, biphenyl and methylbiphenyls, benzoic acid, naphthalenol, etc.); mineral oils (C10-C32 n-alkanes) and miscellaneous chemicals (methylpyrolidinone, caprolactam, etc.).

The greatest number of compounds identified in the effluent samples were typical of auxiliary chemicals used in textile dyeing. Of these, dye carriers made up the largest portion of all chemical compounds identified. Dye carriers present a major source of pollution in the finishing of synthetic fibers. A U.S. EPA report estimated that 90% of the dye carriers used in the dyeing process are consumed in the operation while up to 10% are rinsed to waste (U.S. EPA 1978).

All dye types whether disperse, reactive, direct, acid, sulphur, vat or developed normally use surfactants (U.S. EPA 1985), in addition to other auxiliary chemicals in the dyeing process. Surfactants, such as the type identified in this study, are known to be deleterious to aquatic organisms at concentrations present in textile mill waste streams (Thompson 1974). Chronic toxicity usually occurs at concentrations greater than 0.1 mg/L (Lewis 1991).

With respect to conventional pollutants, textile wastewaters are typically characterized by extreme pH, elevated temperatures, and high concentrations of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total suspended solids (TSS) and heavy metals (Netzer and Beszedits 1975; Chen 1989). Results of this investigation confirm many of the above conclusions.

In previous Environment Canada surveys, most textile mills had an effluent pH value within the range of 6 to 9 (Chen 1989). Two samples from Tandem Fabrics fell below this range (5.1 and 5.9), and one sample from Stanfields was above this range (9.6), however, the rest of the samples collected from the mills surveyed were within the range of 6 to 9. The N.S. Department of the Environment had developed a proposed Sewer Service Bylaw that sets a maximum permissible discharge to sanitary or combined sewers without treatment, storm sewers or direct to the environment (NSDOE 1989). Using the NSDOE bylaw as a generic guide, most of the samples collected from the mills surveyed were within the pH limits of 5.5 to 9.5. All samples from Tandem Fabrics were more acidic

than the recommended guidelines (pH 6.5 to 9) for the protection of freshwater aquatic life (CCREM 1987). Generally, effluent from Tandem Fabrics was slightly acidic, effluent from Britex was in the neutral range, and effluent from Stanfields was slightly alkaline.

All mills, in previous Environment Canada surveys, discharging to a municipal system met BOD limits set by local municipal sewer bylaws (Chen 1989). None of the mills surveyed in this study met the NSDOE proposed limit of 20 mg/L BOD as the maximum permissible discharge to sanitary or combined sewers without treatment, storm sewers or direct to the environment. Two samples collected from Britex were 1.8 and 11 times higher than the maximum permissible discharge limit, all samples from Stanfields were 3 to 5.5 times higher than the permissible limit, and all samples from Tandem were 5 to 17.5 times higner than the permissible limit. The lack of wastewater treatment was likely the reason why Maritime mills exceeded BOD limits in proposed municipal sewer bylaws.

Suspended solids in effluents from textile mills include fibre, pieces of cloth, and dirt washed from natural fibres (Thompson 1974). Suspended solids may be deposited in the receiving stream where currents are slow. Fibre and cloth were visible in significant quantities in the Salmon River and Humphrey's Brook downstream of Stanfields and Tandem Fabrics respectively. All mills, in previous Environment Canada surveys, discharging to a municipal system met TSS limits set by local municipal sewer bylaws (Chen 1989). Most of the samples collected from Maritime textile mills met the NSDOE proposed maximum permissible discharge limit of 20 mg/L suspended solids. The exceptions were three samples from Stanfields that were 1.5 to 4.4 times higher and one sample from Britex that was 3.5 times higher than the permissible limit.

COD values obtained from eight textile mills in Ontario and Quebec show that COD is generally 2 to 2.6 times as large as BOD but may reach 6 times as great (Thompson 1974). COD values from Maritime mills in this survey were generally 2.1 to 4.4 times BOD values, although three samples from Tandem Fabrics were 24.7 to 34.6 times BOD values. All of the samples collected during the survey were well above the NSDOE proposed maximum permissible discharge limit for COD of 30 mg/L. Samples were 10 to 14.7 times higher, 12.8 to 29.7 times higher and

19.7 to 36 times higher than the proposed limit at Stanfields, Britex and Tandem Fabrics respectively. Again, the lack of wastewater treatment was likely the reason why Maritime mills exceeded the proposed bylaws.

Chen (1989) reported that some mills surveyed by Environment Canada in 1973/74, 1981/82 and 1985/86 were found to discharge in excess of the 15 mg/L oil and grease limit set by local municipal sewer bylaws. Four of the twelve samples collected in this survey exceeded 15 mg/L oil and grease and nine of twelve samples exceeded the NSDOE proposed maximum permissible discharge limit of 10 mg/L. Three samples from Stanfields and Britex were slightly higher than the permissible limit while three samples from Tandem Fabrics were 2.9 to 4.2 times higher than 10 mg/L.

In Environment Canada and EPA surveys, zinc, copper and chromium were the most frequently found metal pollutants in textile mill effluents. The highest concentrations detected for these pollutants were in the 0.1 mg/L to 0.2 mg/L range (Chen 1989). The highest concentration of metals found in effluents in this study was a sample from Britex that had 0.17 mg/L zinc. All metals, with the exception of four arsenic samples, were found in concentrations below the proposed NSDOE permissible discharge limits. Two samples from Britex and Tandem Fabrics were slightly above the permissible limit of 0.1 mg/L. Two samples from each mill studied were slightly above the recommended guideline of 0.05 mg/L arsenic for the protection of freshwater aquatic life. Ten of twelve samples were slightly above the guideline of 0.03 mg/L zinc for the protection of freshwater aquatic life (CCREM 1987).

Colour is a particular problem when dealing with textile effluents. It primarily originates from the dumping of used dye baths. It has been estimated that 90% of dyes applied end up in fabrics, with the remaining 10% discharged to waste streams (Porter 1973). MacGregor (1990) indicated that 57,000 kg of azo dyes were used by Atlantic Region textile mills in 1989, therefore it would be expected that up to 5,700 kg of those dyes were discharged to the environment. There is particular concern with regard to the heavily used disperse azo dyes in aquatic environments. Those dyes are very lipophilic and will absorb strongly to sediments. Under anaerobic conditions, reductive cleavage of the azo linkage can occur, releasing aromatic amines that may be acutely toxic, mutagenic or carcinogenic (Maguire and Tkacz 1991).

Very little is known of the environmental occurrence, persistence and fate of dyes in the environment in Canada. Maguire and Tkacz (1991), in the only published report of the occurrence of dyes in the Canadian environment, found 15 dyes in water, suspended solids and sediment downstream from textile mills in the Yamaska River, Quebec in the period 1985-87. Three dyes were identified, Disperse Red 60, Disperse Blue 26 and Disperse Blue 79 along with 2-bromo-4,6-dinitroaniline, a mutagenic degradation product of Disperse Blue 79. The authors noted that proper treatment of dye-containing wastes may dramatically reduce contamination of the Yamaska River.

The toxicity assessment component of this study indicated that all samples collected from Maritime textile mills were acutely toxic to all organisms tested, except one sample from Britex in the Microtox test. All samples showed sub-lethal toxic effects to all species tested, including reproductive impairment in Ceriodaphnia, growth impairment in algae, and genotoxicity in the Ames test.

Toxicity assessment of textile effluent in Canada has focussed on fish toxicity to date. Chen (1989) reported on fish toxicity data from Environment Canada surveys in 1981/82 and 1985/86. Mills with secondary treatment produced a "non-toxic" effluent with a 96-h LC50 of about 100% for rainbow trout and guppy fry. Mills with primary treatment (screening and equalization) produced effluent that was moderately to highly toxic to rainbow trout (LC50 7%-14%) and moderately to exhibiting low toxicity to guppy fry (42%-75%). Untreated effluents from Maritime mills in this study exhibited moderate toxicity to rainbow trout with LC50 values ranging from 11.5% to 35.4%. One sample was highly toxic with an LC50 of 8.2%. These values are comparable to acute toxicity results for rainbow trout reported by Chen (1989) for mills with screening and equalization.

The high toxicity of the Tandem Fabrics samples and the most toxic Britex sample to the algae Selenastrum capricornutum suggests the possibility of biological effects in the receiving environment at concentrations as low as 0.1% effluent. In this study, algae were found to be the organism most sensitive to textile mill effluents, a conclusion that has also been found by others (Walsh et al. 1980).

The Ceriodaphnia dubia IC50 values, the concentration that would cause a significant effect (reproductive impairment) on 50% of exposed organisms, ranged from 1.8% to 8.7%. With reproductive impairment at such low concentrations of effluent, a disruption of the foodchain would be a possibility in the receiving waters of these mills. A study by Moore et al. (1987) showed that very small amounts (1.0 ppm) of certain surfactants, a common component of textile mill effluents, will cause chronic problems to Ceriodaphnia. Appendix K shows the dilution ratios required to prevent reproductive impairment in Ceriodaphnia dubia for the samples collected in this study.

Ames testing indicated that all samples were mutagenic. There were differences in the apparent types of mutagenicity associated with each sample. For example, only one sample (from Tandem Fabrics) showed mutagenicity to the bacterial strain TA 100, while other samples were mutagenic to one or more other tester strains. The results suggest that more than one mutagen is present in the samples. Other studies have demonstrated the mutagenic activity of textile mill effluents (Brookman 1980a, b).

Most chemical carcinogens have mutagenic activity. While the specific compound(s) responsible for mutagenesis in these samples are unknown, if the receiving waters are used for potable, or perhaps even recreational use, tight controls may be warranted on the release of these effluents. An important question is whether or not the mutagenic compounds in these effluents remain present in the ambient environment outside of a small mixing zone, or whether they quickly degrade.

Toxicity data correlations indicated that effluent toxicity, between different species, was positively correlated with rainbow trout and algae. A negative correlation existed between Microtox and rainbow trout. Positive correlations existed between Microtox 5 and 15 minute results, and also between Ceriodaphnia acute and chronic results. With a larger data base more correlations might exist, as a level of significance was approached by some results. The lack of correlations between tests with different organisms indicates that a battery of tests is more indicative of the overall toxicity of an effluent than an individual test with one organism.

This study confirms the conclusion that effluents from textile mills without wastewater treatment would be toxic to fish (Chen 1989). However, there is insufficient data in this study to determine the organic contaminants responsible for the toxicity of the effluents since chemical characterization was largely limited to determining the presence of organic compounds in the effluents, rather than determining concentrations of those compounds. Certain organic components of textile waste streams such as detergents, dyes, dye carriers, surfactants and other phenolic compounds can be toxic to aquatic organisms at concentrations present in effluents (Thompson 1974; Netzer and Beszedits 1975; Chen 1989).

The high concentrations of ammonia in samples from Tandem Fabrics (23.8 mg/L to 38.3 mg/L) would be toxic to aquatic organisms. The U.S. EPA (1985) found that ammonia was acutely toxic to freshwater organisms at concentrations (uncorrected for pH) ranging from 0.53 to 22.8 mg/L for 19 invertebrate species representing 14 families, and from 0.083 to 4.60 mg/L for 29 fish species from 9 families. Zinc concentrations in effluents at Britex may cause toxicity to aquatic organisms. One sample from Britex (0.17 mg/L zinc) was above the 96-h LC50 value of 0.09 mg/L zinc for rainbow trout. All Britex samples were slightly above the chronic toxicity value of 0.07 mg/L for zinc.

Due to the high concentrations and wide variety of pollutants present in textile mill effluents, the design of effective treatment methods has entailed formidable difficulties. Chen (1989) reviewed the various primary, secondary and tertiary treatment technologies in use today in the textile sector in Canada. He concluded that biological treatment was able to reduce the effluent pollutant content as well as the toxicity to an acceptable limit and should be adopted at the Best Practical Technology (BPT) model for textile mill effluents. Zaloum (1987) showed the effectiveness of biological treatment of wastewater from a textile mill in Quebec from an acute aquatic toxicity standpoint. The U.S. EPA examined the levels of removal of toxicity to algae and fish of specific toxic pollutants achieved by selected tertiary systems treating secondary effluents from textile mills. It was concluded that multi-media filtration followed by activated carbon treatment provided the best overall removal efficiency for toxic compounds as measured by algal and fish toxicity tests (U.S. EPA 1979).

Thompson (1974) noted that few studies have been performed on the effects of textile mill effluents on aquatic life or the impact of wastes from a particular mill on receiving waters. Thompson recommended determining the toxicity of textile mill waste by assessing the effects of the effluent on bottom-dwelling organisms in the receiving environment. This is the first examination of water quality based on macroinvertebrate communities specifically related to textile mill discharges. Barton and Metcalfe-Smith (1992) examined the responses of macroinvertebrate communities to municipal/industrial versus agricultural pollution in the Yamaska River basin in Quebec, a river basin where ten textile mills operate.

According to DePauw et al. (1986), the optimal particle size range for gravel in artificial substrate samplers is 4.0 - 8.0 cm because that range yields substantially more taxa than the 1.0 - 3.0 or 10.0 - 20.0 cm ranges. Voshell et al. (1989) concluded that by decreasing the mesh size used to sieve samples from 600 to 250 $\mu$m, 400% more organisms could be collected. The combined effects of small-sized substrate pieces (2.0 cm) and large mesh size (710 $\mu$m) used in this study probably accounted for the low diversity of organisms reported. Although the methods used were not ideal to characterize the benthic macroinvertebrate communities in the receiving environments, they were adequate to determine changes in the diversity and number of species present at the control and impacted stations that were amenable to collection using the sampling techniques employed.

Both field surveys at Stanfields indicated that untreated effluent discharged from the mill was causing a biological impact in the Salmon River. The discharge was reducing the numbers of individuals and species of benthic macroinvertebrates in the area of the river in contact with the effluent plume. The impact of the effluent discharge was not specific to one group of organisms, as aquatic insects, snails and leeches were all negatively impacted. In three of four taxa collected during both surveys, impacts of the effluent discharge were observed in more than one season. The observed impacts are a classic community response to toxic pollution as opposed to nutrient enrichment (i.e. sewage). Sewage pollution causes a decrease in diversity as sensitive organisms are lost and an increase in the abundance of tolerant organisms due to nutrient

enrichment. Toxic pollution causes a decrease in both diversity and abundance because there is no additional food source for the tolerant organisms (Kovalak 1981).

The deployment of ten caged freshwater clams at each of the four sampling stations in the effluent plume at Stanfields (and at a control station), during both field surveys, also clearly showed a biological impact from the effluent discharge at the mill. While all clams survived during both surveys at the control station, all clams died in the effluent plume during the September-October 1990 survey and all clams but four (at Station 4) died during the May-June 1991 survey.

The toxicity assessment component of this study indicated that the untreated effluent discharged from the Britex plant was acutely and chronically toxic to aquatic organisms. The field survey data, however, did not indicate a significant decrease in numbers of benthic macroinvertebrates downstream from the effluent outlet at Britex. Although there may be some community structure impacts as indicated by the decrease in mean number of taxa at two stations and the EIs calculated for all four impacted stations, it is uncertain what may be causing the decrease in taxa. Toxicity of the effluent, salinity differences between the Control and Impacted stations, or some other factor may account for the observed differences. Since the effluent plume in the river was not visible, it is quite possible that the placement of the artificial substrates missed the plume. Alternatively, the significant volume of water in the Annapolis River may be diluting the effluent such that its toxic effects on aquatic organisms are being mitigated.

Although the toxicity assessment component of this study indicated that untreated effluent discharged from Tandem Fabrics is acutely and chronically toxic to aquatic organisms, the field study did not indicate significant decreases in numbers of individuals or species of benthic macroinvertebrates downstream from the effluent discharge. In this case, the biological effects of the effluent discharge were almost impossible to measure since Humphrey's Brook was receiving considerable stress from raw sewage that would also negatively impact aquatic community structure.

## 6.0 RECOMMENDATIONS

Based on this study, the following recommendations are made:

1) Untreated textile mill effluents are toxic to a wide variety of aquatic organisms and have a deleterious effect on aquatic biota in freshwater receiving environments. These untreated wastewaters should not be discharged directly to a watercourse or to a municipal system where there is no sewage treatment.

2) Wastewater treatment of textile mill effluents is necessary and should be directed at reducing the effluent pollution content as well as the toxicity to an acceptable level. Given the complex chemical nature of textile wastewaters, and the fact that a number of constituents of textile mill effluents may be toxic to aquatic organisms, a battery of toxicity tests should be used to assess the toxicity of treated textile wastewaters. This study indicated that a battery of tests is more indicative of overall toxicity of textile effluent than an individual test with one organism.

3) Although most textile mills are concentrated in two provinces, the federal government should reassess the need for federal regulatory initiatives in Canada for wastewater discharges from textile mills, particularly in light of the Government of Canada's commitment in the Green Plan to promote pollution prevention in our inland waters and to provide regulatory control of toxic substances in effluents from major industrial sectors. The two main federal legislative and regulatory instruments that could be used to address textile wastewater discharges are the Fisheries Act and the Canadian Environmental Protection Act.

4) Since short-term tests of effluent toxicity do not accurately predict the effects of persistent chemicals that bioaccumulate, the cumulative effects on fish of lifetime effluent exposure, or the influence of environmental conditions on effluent toxicity, the overall adequacy of effluent regulations should be assessed by monitoring for environmental effects at each receiving site. Effects monitoring programs at textile mills should include toxicity assessment using a battery of toxicity tests including chronic tests and the assessment of benthic macroinvertebrate communities.

5) Further studies are required to:
   - evaluate the effluent toxicity and biological impact of treated textile effluent discharges on freshwater ecosystems;
   - identify and quantify organic indicator/target compounds in textile effluents based on assessment of chemicals used in textile processes and chemical characterization of treated effluents;
   - examine the fate and effects of dyes in the aquatic environment downstream from textile mills;
   - identify the mutagenic compounds in textile effluent discharges and determine whether they remain in the receiving environment or whether they quickly degrade.

## 7.0 REFERENCES

American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard methods for the examination of water and wastewater. 17th Edition. L.S. Clesceri, A.E. Greenberg, R.R. Trussell (eds.). American Public Health Association, Washington, D.C.

Barton, D.R. and J.L. Metcalfe-Smith. 1992. A comparison of sampling techniques and summary indices for assessment of water quality in the Yamaska River, Quebec, based on benthic macroinvertebrates. Environ. Monitoring and Assessment 21: 225-244.

Beak Consultants Limited. 1990. Assessment of textile mill effluent toxicity using Ceriodaphnia dubia. Beak Ref: 3080.1 (unpublished report).

Bennett, B.M. 1952. Estimation of LD50 by moving averages. J. Hygiene 50: 157-164.

BioQuest International Inc. 1990. Report on bioassessment - Ames and Selenastrum tests - Textile Mill Effluents 1-6 (unpublished report).

Brookman, D.H. 1980a. Biological characteristics of textile mill effluent. Bio-Research Laboratories Ltd. report for Environment Canada. Report No. 13470 (unpublished report).

Brookman, D.H. 1980b. Biological characteristics of textile mill effluent. Bio-Research Laboratories Ltd. report for Environment Canada. Report No. 13470B (unpublished report).

Camargo, J.A. 1990. New diversity index for assessing structural alterations in aquatic communities. Bull. Environ. Contam. Toxicol. 48: 428-434.

Canadian Council of Resource and Environment Ministers (CCREM). 1987. Canadian Water Quality Guidelines.

Chen, E.C. 1989. Environmental assessment of the Canadian textile industry. Environment Canada, Chemical Industries Division, Industrial Programs Branch. Report EPS 5/TX/1. June 1989. Ottawa, Ontario.

Coleman, M.J. and H.B.N. Hynes. 1970. The vertical distribution of the invertebrate fauna in the bed of a stream. Limnol. Oceanog. 15: 31-40.

DePauw, N., D. Roels and A.P. Fontoura. 1986. Use of artificial substrates for standardizing sampling of macroinvertebrates in the assessment of water quality by the Belgian Biotic Index. Hydrobiologia 133: 237-258.

Dunnett, C.W. 1955. Multiple comparison procedure for comparing several treatments with a control. J. Amer. Statist. Assoc. 50: 1096-1121.

Environment Canada. 1990a. Environmental Protection Series. Biological test method: acute lethality test using rainbow trout. Report EPS 1/RM/9.

Environment Canada. 1990b. Environmental Protection Series. Biological test method: acute lethality test using Daphnia spp. Report EPS 1/RM/11.

Environment Canada. 1990c. Environmental Protection Series. Biological test method: acute lethality test using threespine stickleback (Gasterosteus aculeatus). Report EPS 1/RM/10.

Environment Canada. 1990d. Environmental Protection Series. Biological test method: reference method for determining acute lethality of effluents to rainbow trout. Report EPS 1/RM/13.

Environment Canada. 1990e. Environmental Protection Series. Biological test method: reference method for determining acute lethality of effluents to Daphnia magna. Report EPS 1/RM/14.

Gordon, A. and M. Gordon. 1981. Analysis of volatile organic compounds in textile finishing plant effluent. Ky. Acad. Sci. Vol. 43(3-4): 149-157.

Government of Canada. 1990. Canada's Green Plan. Ottawa, Ontario.

Green, J.C., C.L. Bartels, W.J. Warren-Hicks, B.P. Parkhurst, G.L. Linder, S.A. Peterson and W.E. Miller. 1988. Protocols for short-term toxicity screening of hazardous waste sites. U.S. Environmental Protection Agency Corvalis.

Hamilton, M.A., R.C. Russo and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassay. Environ. Sci. Technol. 11: 714-717.

Hennigar, P., V. Miketuk, O. Vaidya, K. Gunn. 1982. Toxic chemicals inventory in textile mill effluents - protocol evaluation/development and environmental measurements. Report prepared by Laboratory Division, EP Atlantic Region for the Abatement Compliance Branch, Water Pollution Control Directorate. Ottawa, Ontario.

Hewlett Packard Company. 1986. PBM Search and Parametric Retrieval Software Handbook. November 1986.

Kovalak, W.P. 1981. Assessment and prediction of impacts of effluents on communities of benthic stream macroinvertebrates. in Ecological assessments of effluent impacts on communities of indigenous aquatic organism. ASTM STP 730. J.M. Bates and C.I. Weber (eds.). American Society for Testing and Materials. 1981. p. 255-263.

Lewis, M. 1991. Chronic and sublethal toxicities of surfactants to aquatic animals: A review and risk assessment. Wat. Res. Vol. 25(1) p. 101-113.

MacGregor, C. 1990. Overview of priority chemicals used by the textile industry in Atlantic Canada. Contract for Environment Canada. Seatech Investigation Services Ltd. Halifax, N.S.

Maguire, R.J. and R.J. Tkacz. 1991. Occurrence of dyes in the Yamaska River, Quebec. Water Poll. Res. J. Canada Vol. 26(2) p. 145-161.

Maron, D.M. and B.N. Ames. 1983. Revised methods for the Salmonella mutagenicity test. Mutation Research. Vol. 113. p. 173-215.

Microbics. 1989a. Microtox data collection system installation/information. Version 2. Microbics Corporation, 2232 Rutherford Road, Carlebad, California, 92008 USA.

Microbics. 1989b. A Microtox manual: How to run toxicity tests using the Microtox Model 500. Microbics Corporation, 2232 Rutherford Road, Carlebad, California, 93008 USA.

Moore, S.b., R.A. Diehl, J.M. Barnhardt, and G. Brooks Avery. 1987. Aquatic toxicities of textile surfactants. Textile Chemist and Colorist.

Netzer, A. and S. Beszedits. 1975. Pollution from textile mills: Problems and solutions. Water and Pollution Control. May 1975. p. 17-20.

New Brunswick Department of Commerce and Technology. 1990. New Brunswick Director of Products and Manufacturers 1990. Fredericton, N.B.

Norberg-King, T. 1988. An interpretation estimate for chronic toxicity: The ICp approach. National Effluent Toxicity Assessment Centre Technical Report 05-88. U.S. Environmental Protection Agency, Duluth, MN.

Nova Scotia Department of Industry, Trade and Technology. 1988. Nova Scotia Directory of Manufacturers 1988-1989. Statistics and Research Services, N.S. Dept. of Industry, Trade and Technology. Halifax, N.S.

Nova Scotia Department of the Environment. 1989. NSDOE proposed sewer service by law, 1989. Halifax, N.S.

Peltier, W.H. and C.I. Weber (Editors). 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. Third Edition. EPA/600/4-85/013. Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, Ohio, 45268. 216 pp.

Porter, J.J. 1973. A study of the photodegradation of commercial dyes. U.S. Environmental Protection Agency Report EPA-R2-73-058. U.S. EPA Office of Research and Monitoring, Washington, D.C. 94 pp.

Sandhu, S.S. 1979. Short term biotesting of environmental effluents. p. 225-230 In: Ayers, F.A. (Compiler). Symposium Proceedings: Textile Industry Technology (December 1978, Williamsberg, Va.). U.S. EPA, EPA0600/2-79-104. NTIS #PB299132. 376 p.

Steel, R.G. 1959. A multiple comparison rank sum test: treatments versus control. Biometrics 15: 560-572.

Stephan, C.E. 1977. Methods for calculating an LC50, pp. 65-84. In: F.L. Mayer and J.L. Hamelink (eds.). Aquatic Toxicology and Hazard Evaluation. American Society for Testing and Materials, ASTM STP 634.

Thompson, B. 1974. The effects of effluent from the Canadian textile industry on aquatic organisms - A literature review. Environment Canada Fisheries and Marine Service. Technical Report No. 489. Winnipeg, Manitoba.

Tocksteinova, Z. and M. Kopanica. 1987. Determination of caprolactam by adsorptive voltammetry after separation by thin layer chromatography. Analytica Chimica Acta 199: 77-83.

U.S. Environmental Protection Agency. 1978. Environmental pollution control: textile processing industry. EPA-625/7-78-002. October 1978.

U.S. Environmental Protection Agency. 1979. Source assessment - textile plant wastewater toxics study, Phase II. EPA-600/2-79-019i.

U.S. Environmental Protection Agency. 1986. Test methods for evaluating solid waste. Volume 1, Section B. EPA Method 3510.

U.S. Environmental Protection Agency. 1985. Textile dyes and dyeing equipment: classification, properties, and environmental aspects. EPA-600/2-85/010.

U.S. Environmental Protection Agency. 1987. Toxic Substances Control Act (TSCA): Good laboratory practice standards. 40 CFR Part 792. Federal Register, Vol. 52, No. 248, Monday, 28 December 1987.

Voshell, J.R. Jr., R.J. Layton and S.W. Hiner. 1989. Field techniques for determining the effects of toxic substances on benthic macroinvertebrates in rocky-bottomed streams. Aquat. Toxicol. and Hazard Assess. 12: 134-155.

Walsh, G.E., L.H. Bahner, and W.B. Horning. 1980. Toxicity of textile mill effluents to freshwater and estuarine algae, crustaceans, and fishes. Environmental Pollution (Series A), 21: 169-179.

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessler, J.R. Menkedick, T.w. Neiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Second edition. U.S. Environmental Protection Agency, EPA/600/4-89/001.

Zaloum, R. 1987. Biological treatment of textile finishing mill effluent. Environment Canada, Environmental Protection, Conservation and Protection. Report EPA 3/PF/3. March 1987.

Zar, J.H. 1974. Biostatistical analysis. Prentice-Hall Biological Sciences Services. Prentice-Hall Inc. Englewood Cliffs, N.J.

# Appendix A

## Macroprogramme for Peak to Peak Search of a GC/MS Total Ion Chromatogram

MACROPROGRAMME FOR PEAK TO PEAK SEARCH OF A GC/MS TOTAL ION CHROMATOGRAM

```
NAME LIBSEARCHP
   WINDOW 3, 0:1, 0:1
   FILE
   INITTHRESH
   STARTSET CFILE
   TAB HEADER, PRINTER:
   TAB EV, PRINTER:
   TIC
   CLEAR
   DR 2
   INTEGRATE
   PA 1.5
   OF
   CLEAR
   DR 3
      DR PRINTER
   N=1
   WHILE N<=NPEAKS
      PEAK N
      PK_RT=RET_TIME
      PEAKNUMBER N, START, Y
      SUBTRACT SUPPRESS
      DR 3
      STRATEGY 1, 10, SMART, SCAN
      PBM LIBVOL:WILEY.L
      GETSCALARS RESULTS, X
         IF NUM_HITS >0
            IF QUALITY >49
            TABULATE RESULTS, PRINTER:
               IF NUM_HITS >3
               MAX_HITS=3
               ELSE
               MAX_HITS=NUM_HITS
               ENDIF
            M=1
               WHILE M<=MAX_HITS
               GETSCALARS RESULTS, X, 1, M
               GETMSREF ENTRY_NUM,,,FULL
               MERGE
               M=M+1
               ENDWHILE
            NORMALIZE
            CLEAR
            DR 3
            TOF
            SCR
            ELSE
            CLEAR
            MESSAGE MATCH PROBABILITY <50 FOR PEAK, PK_RT, 30, 15
            ALPHACOPY
            ENDIF
            ELSE
               CLEAR
            MESSAGE NO MATCHES WERE FOUND FOR PEAK, PK_RT, 30, 15
            ALPHACOPY
            ENDIF
            N=N+1
            EXCHANGE
            ENDWHILE
            END
            CL
            MESSAGE ANALYSIS COMPLETE TOTAL NUMBER OF PEAKS=,N,28,12
            ALPHACOPY
```

# Appendix B

## Report on Bioassessment - Ames and *Selenastrum* Tests

## Textile Mill Effluents 1-6

**BioQuest International Inc.**

#3-2725 Pembina Highway
Winnipeg, Manitoba
*Canada R3T 2H5*

Phone: (204)-269-7264
*Fax: (204)-269-6897*

December 4, 1990

# REPORT ON BIOASSESSMENT
# Ames and *Selenastrum* Tests
# Textile Mill Effluents 1-6

*November 27, 1990*

*Revised 26 December, 1990*

# REPORT ON BIOASSESSMENT
# Ames and Selenastrum Tests
# Textile Mill Effluents 1-6

## Table of Contents

## Executive Summary

1. Six samples of textile mill effluent were tested for toxicity using the *Selenastrum capricornutum* test, and for mutagenicity using the Ames test.

2. Samples 1, 2, and 3 were highly toxic to *Selenastrum*, the concentrations calculated to permit 50% growth of the algae (the $LC_{50}$) were 0.37%, 0.95%, and 0.15% for samples 1, 2, and 3 respectively.

3. Samples 4, 5, and 6 were also toxic to *Selenastrum*, but were less toxic than samples 1, 2, and 3. The $LC_{50}$ for samples 4, 5, and 6 were 10.35%, 18.97%, and 27.21%, respectively.

4. All samples showed mutagenic properties.

5. The mutagenic properties differ among the six samples. Two different variations of the Ames test for mutagenicity were performed. Each method used 4 different tester stains of bacteria (TA97, TA98, TA100, and TA102). Both variations tested the raw effluent and tested the effect of metabolic oxidation on the raw effluent by exposure to activated rat-liver S9 homogenate.

   A. *The Spot Test.* This version of the Ames test involves placing a spot of test material on an agar plate containing a tester strain. The test material diffuses from this spot to form a concentration gradient, along which mutagenesis may occur. The spot test detected mutagenesis in all samples except sample 2. Mutagenesis was detected for the following conditions:

| Sample | TA97 | TA98 | TA100 | TA102 |
|---|---|---|---|---|
| Sample 1: | Yes | | Yes | |
| Sample 2 | | | | Yes |
| Sample 3: | | | | Yes |
| Sample 4: | | Yes | | Yes |
| Sample 5: | | | | Yes |
| Sample 6: | Yes | | | Yes |

   B *The Plate Incorporation Test.* This version of the Ames test involves mixing the bacterial with one concentration of test material. The test is repeated over a series of concentrations. All six samples produced mutagenesis using the plate test. Mutagenesis was detected under the following conditions:

| Sample | TA97 | TA98 | TA100 | TA102 |
|---|---|---|---|---|
| Sample 1 | Yes | | Yes | |
| Sample 2 | | | | YES |
| Sample 3 | | Yes | | Yes |
| Sample 4 | Yes | Yes | | |
| Sample 5 | | Yes | | Yes |
| Sample 6 | Yes | | | Yes |

6. Because of the inconsistencies between the plate and spot tests, the tests were repeated on three different occasions by three different workers. The results were highly reproducible.

## Introduction

Gallon bottles of textile mill effluent were received by BioQuest at 7 day intervals during the months of September and October. A total of six samples, labeled 1-6, were received. Samples were stored at 2° Celsius.

The mutagenicity of each sample was determined by the Ames test, using strains of the bacterium *Salmonella typhimurium*. The acute toxicity of each sample was determined by the *Selenastrum capricornutum* test, using inhibition of growth of algal populations as a measure of toxic effect. Bioassays were performed within 48 hours of receipt of samples.

Tests were performed using standard methods for each test; Selenastrum was performed according to U.S. EPA protocols (1988), Ames tests were performed according to Maron and Ames (1983, Mutation Research, 113, 173-215). Positive and negative controls were performed with each test. Usually tests of these samples were done in batches with other samples. In these batches, all samples were re-labelled so that the individual performing the test lacked prior knowledge of the origin of the samples.

## The *Selenastrum* Test

### General Methods

The algae *Selenastrum capricornutum* was cultured at 20°Celsius in 500 mL stock cultures in algal salt solution. For tests, rapidly growing cultures were harvested by centrifugation, and resuspended in fresh salt solution to population levels of 3,000,000 cells per mL.

The algal test measures toxicity as a decrease in algal growth. Tests were done by placing 1 mL of fresh algal suspension in each of a series of 500 mL flasks containing 200 mL of a concentration of test material diluted in algal salt solution. This provides for initial algal populations of approximately 15,000 cells per mL. Population levels were determined at the start and end of each test by hemocytometer counts of aliquots from each sample at the beginning and end of each test.

## Range-Finding Tests

Range-finding tests were done to establish the range of concentrations of test material to be used in definitive toxicity tests. These tests are carried out by placing 1 mL of algal suspension (approximately 30,000) cells in 200 mL of test sample diluted in algal salt solution. Initial range-finding tests were performed by permitting 7 day growth at 50%, 25%, 12%, 6% and 2% concentrations of test sample. Higher or lower concentrations were in further tests if needed. Table 1 shows results of range-finding tests.

Table 1. Results of *Selenastrum* Range Finding Tests. Numbers are the minimum concentration giving 100% growth and maximum concentration totally inhibiting growth

| Sample | No Growth | 100% Growth |
|---|---|---|
| Sample 1 | 1% | 0.05% |
| Sample 2 | 1% | 0.05% |
| Sample 3 | 1% | 0.05% |
| Sample 4 | 25% | 6% |
| Sample 5 | 25% | 0.5% |
| Sample 6 | 70% | 12% |

## Definitive tests

Definitive toxicity tests were carried out over an arithmetic series of concentrations ranging from the "No growth" concentration to the "100% growth" concentration established by the range-finding tests. Three replicates of each sample concentration were run concurrently.

The results of the definitive tests are analyzed by a weighted-trimmed linear regression analysis to calculate the concentration at which 50% growth (the $LC_{50}$) and 80% growth (the $LC_{50}$) occurs. The regression coefficient ($r^2$), which shows the linearity of the relationship between dose and effect is also calculated. An $r^2$ value near unity shows a tight dose-response relationship. The results of the definitive tests are shown in Table 2.

Table 2. Results of the *Selenastrum capricornutum* bioassay.

| Sample | $LC_{20}$ | $LC_{50}$ | $r^2$ | Comment |
|---|---|---|---|---|
| Sample 1 | 0.03 | 0.37 | 0.93 | Highly Toxic |
| Sample 2 | 0.05 | 0.11 | 0.95 | Highly Toxic |
| Sample 3 | 0.05 | 0.15 | 0.89 | Highly Toxic |
| Sample 4 | 7.33 | 10.35 | 0.92 | Toxic |
| Sample 5 | 2.90 | 18.97 | 0.99 | Toxic |
| Sample 6 | 18.44 | 27.21 | 0.88 | Toxic |

## Conclusions

1. Samples 1, 2, and 3 are all highly toxic. Samples 2 and 3 should be considered slightly more toxic than sample 1.

2. Samples 3, 4, and 5 are toxic, but less toxic than samples 1, 2 and 3. Sample 4 is the most toxic of these three samples, while sample 6 is the least toxic.

# Ames Tests for Mutagenicity

## The Logic of the Ames Test

The Ames test uses histidine-requiring mutants to test for the presence of mutagenic materials. Each of 4 tester strains was developed by a specific mutation from a parent stock capable of synthesizing the amino acid, histidine. In the presence of mutagens, a rare mutation may correct the original mutation in the histidine gene, making the new mutant capable of synthesizing histidine. The greater the number of revertants after exposure to test material, the more mutagenic is the test material.

The four tester strains are TA97, TA98, TA100, and TA102. While each tester strain carries a unique mutation, there is some degree of overlap between what is detected. The strains TA97 and 98 detect similar types of events; the addition or removal of bases to DNA. Strains TA100 and TA102 detect changes to individual bases, with a limited potential for overlap.

Each of the mutant test strains also has a reduced capacity to repair damage to genetic material, thus rendering the strain more sensitive to mutagenesis. This increased sensitivity also creates the problem of spontaneous mutation occurring, resulting in some revertants even in without mutagens. Negative controls are used for each test set to determine this background revertant frequency.

Some chemicals are not themselves mutagenic, but can be converted to mutagenic compounds by metabolic activity, especially oxidative detoxification reactions. The detection of promutagens by the Ames test is done by adding S9 extract, obtained from the liver of rats that have had their oxidative detoxification system induced by injection with phenobarbitol or polychlorinated biphenyl. The tests reported here used PCB-induced S9 extract obtained from B.C. Research. Tests are done without S9 to detect direct-acting mutagens, and with S9 added to detect promutagens.

## The Spot Test

The spot test version of the Ames test is performed by placing a spot of test material in the center of a Petrie plate containing a uniform distribution of tester strain and a limited amount of histidine. The bacteria have sufficient histidine to for a limited number of cell divisions. The test material will diffuse through the agar forming a concentration gradient, with decreasing concentration of test material at increasing distance from the test spot.

Typically, there is a zone around the test spot where no bacterial growth occurs due to cytotoxicity of the test sample. Beyond this zone of cytotoxicity is an area where the bacteria grow to form a thin uniform growth "lawn". Within the lawn there will be distinct colonies, small areas where extensive growth occurs. These colonies are derived from spontaneous or induced mutations where the bacteria have reverted to being capable of synthesizing histidine.

In the analysis of Ames spot test data, BioQuest uses the following criteria for mutagenesis:

1. Most reversions should occur at the highest level in a more-or-less concentric region from the source of the test material, with decreasing reversion in more distal areas.

2. Levels of reversion should exceed those observed in negative controls.

The results of three replicate spot test (3 plates per replicate) are shown in Table 3. Sample 1 shows moderate mutagenesis. Sample 1 shows moderate mutagenicity with tester strains TA97 and TA100. Sample 4 shows moderate mutagenicity with tester strain TA102. Sample 3 shows moderate mutagenicity with tester strains TA98 and

TA102. Sample 5 shows slight mutagenicity with strain TA102. Sample 6 only shows mutagenicity with tester strain TA102 following S9 activation; sample 6 contains a promutagen. Sample 2 shows no mutagenicity.

Table 3. Results of the Ames spot test on textile mill effluents 1-6. The symbols "+", "++", and "+++" refer to detection of 3-5 times the background mutation rate, 5-10 times the background mutation rate, or more than 10 times the background mutation rate, respectively.

| | TA 97 | | TA 98 | | TA 100 | | TA 102 | |
|---|---|---|---|---|---|---|---|---|
| Sample | - S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Sample 1 | ++ | ++ | - | - | + | + | - | - |
| Sample 2 | - | - | - | - | - | - | - | - |
| Sample 3 | - | - | - | - | - | - | ++ | ++ |
| Sample 4 | - | - | ++ | ++ | - | - | ++ | ++ |
| Sample 5 | - | - | - | - | - | - | + | + |
| Sample 6 | + | + | - | - | - | - | - | + |

## Plate Incorporation Test

The plate incorporation version of the Ames test uses test strain and a specific concentration of test material, and S9 (where applicable) uniformly distributed in an agar matrix, which is spread over an agar plate. This test examines reversion of bacteria exposed to a single concentration of test material. The test is performed over a series of concentrations to establish a dose-response pattern.

Prior to performing the plate evaluation test, a series of concentrations of test material is tested to find the maximum concentration of the sample that will support uniform growth of the test strains. The results of this evaluation are shown in Table 4. Samples 1, 2, and 3 were tested at concentrations of 0.05%, 0.01% and 0.005%. Samples 3, 4, and 5 were tested at 1%, 0.5% and 0.1%.

Table 4. Results of range-finding tests for plate incorporation tests.

| Sample | 100 Growth |
|---|---|
| Sample 1 | 0.05% |
| Sample 2 | 0.05% |
| Sample 3 | 0.05% |
| Sample 4 | 1.00% |
| Sample 5 | 1.00% |
| Sample 6 | 1.00% |

In analysis of the plate incorporation test, several criteria are applied in the determination of mutagenicity:

1. For a sample to be evaluated as *mutagenic*, the following criteria must be met:

   A. There must be an increasing number of revertants with increasing concentration over the tested series of three concentrations. Because only three concentrations are chosen, based on the maximum concentration giving 10% growth, there is no test for linearity of the dose-response relationship.

   B. The number of reversions at each tested concentration must be greater than those appearing in negative controls.

   C. The rate of reversion in positive controls must be within previously defined parameters.

2. For a sample to be evaluated as *non-mutagenic*, the following criteria must be met:

   A. The number of reversions at any tested concentration cannot exceed 1.5 times the number of reversions observed in negative controls.

   B. The number of reversions does not increase with increasing concentration over the range of three test concentrations.

   C. The rate of reversion in positive and negative controls must be within previously defined parameters.

A test of a sample with a particular tester strain must meet one of the above sets of criteria, or the test is repeated.

The results of the plate incorporation test are shown in Table 5. Sample 1 shows slight mutagenicity with tester strains TA97 and moderate mutagenicity with test strain TA100. Sample 2 shows high mutagenicity with tester strain TA102. Sample 3 shows high mutagenicity to tester strains TA98 and TA102. Sample 4 shows moderate mutagenicity with tester strain TA97 and high mutagenicity with tester strain TA98. Sample 5 shows light mutagenicity to tester strain TA98 and high mutagenicity with tester strain 102. Sample 6 shows moderate mutagenicity with tester strains TA97 and TA102.

Table 5. Results of the Ames plate incorporation test on textile mill effluents 1-6. The symbols "+", "++", and "+++" refer to detection of 3-5 times the background mutation rate, 5-10 times the background mutation rate, or more than 10 times the background mutation rate, respectively.

| | TA 97 | | TA 98 | | TA 100 | | TA 102 | |
|---|---|---|---|---|---|---|---|---|
| Sample | - S9 | +S9 | -S9 | +S9 | -S9 | +S9 | -S9 | +S9 |
| Sample 1 | + | + | - | - | ++ | ++ | - | - |
| Sample 2 | - | - | - | - | - | - | +++ | +++ |
| Sample 3 | - | - | +++ | +++ | - | - | +++ | +++ |
| Sample 4 | ++ | ++ | +++ | +++ | - | - | - | - |
| Sample 5 | - | - | + | + | - | - | +++ | +++ |
| Sample 6 | ++ | ++ | - | - | - | - | ++ | ++ |

## Interpretation of Ames Test Results

The Ames test revealed moderate to high mutagenicity associated with each sample. However, there were differences in the apparent type of mutagenicity. Samples 2, 3, 5, and 6 were mutagenic to strain TA102 in the more sensitive plate incorporation test. Samples 1, 4, and 6 were mutagenic to TA97 in the plate incorporation test. Samples 3, 4, and 5 were mutagenic to TA98 in the plate incorporation test. Only sample 1 showed mutagenicity to TA100. The results suggest that more than 1 mutagen is present in the samples.

Several inconsistencies were observed between the spot test and the plate test:

A. Sample 2 showed no mutagenesis in the spot test, but was highly mutagenic to TA102 in the plate incorporation test.

B. Sample 3 was highly mutagenic to TA98 in the plate incorporation test, but not mutagenic to this strain in the spot test.

C. Sample 4 showed moderate mutagenicity to strain TA97 in the plate incorporation test, but was not mutagenic to TA97 in the spot test. However, sample 4 was mutagenic to strain TA102 in the spot test, but not in the plate incorporation test.

D. Sample 5 was slightly mutagenic to TA98 in the plate incorporation test, but not in the spot test.

E. Sample 6 showed a promutagen with strain TA102 in the spot test but showed a direct-acting mutagen in the plate incorporation test.

To determine if these inconsistencies were due to operator error, the tests were replicated two further times. Each replicate gave similar results. Positive and negative controls of each set of tests also gave consistent results.

There are two differences between the spot test and the plate incorporation test:

1. in the spot test the various components of the test mixture may diffuse at different rates, resulting in different concentration gradients for different materials, while in the plate test there is a uniform distribution of test sample. Some mutagenicity may be due to the interaction of two or more components of the sample.

2. in the plate incubation test, the agar containing the mix of bacteria and sample is held at 45° Celsius for several minutes. Some potentially mutagenic component could require heat-activation to be mutagenic.

## Conclusions

1. All six samples are mutagenic.

2. Samples 2, 3, 4, and 5 show the highest mutagenicity.

## Environmental Implications of Mutagenesis

As indicated by Table 6, the Ames test is a highly sensitive bioassay for detecting very rare events. Because the Ames test uses very large populations these rare event can be detected. In reality, the risk of mutagenesis is quite low, even in the presence of potent mutagens.

Table 6. Code values for detection of mutagenesis and approximate mutation frequencies.

| Code | Mutation Frequency |
|---|---|
| + | $5 \times 10^{-6}$ |
| ++ | $10^{-5}$ |
| +++ | $2 \times 10^{-5}$ |

However, even this low risk is unacceptable in the context of *human health.* Mutagens are also carcinogens. Humans should not be exposed to the levels of mutagens detected in the six samples. If the nearby receiving waters are utilized for potable or recreational use, tight controls should be exercised on the release of these effluents.

In the context of the *natural environment*, mutagens are less of a problem than acutely toxic materials. Typically mutagens are reactive compounds, which have a short environmental half-life. Given appropriate dilution and time, these materials should have no environmental impact.

For any *natural population*, the death rate due to mutagenesis or associated carcinogenesis is undetectable in the background of predation and competition. However, exposure to mutagens will increase the frequency of tumors in natural populations.

While the real risk associated with mutagens is low, the perceived risk is high. If only because of public fear, the release of mutagenic materials should be prevented whenever possible. In many ways, the detection of mutagens poses more of a political problem than a problem of high toxicological risk. However, this is a statement most easily made by a private consultant.

The detection of mutagens in textile mill effluent is not surprising considering the complexity of treatments in the process. The real question is if these compounds are present in the ambient environment.

## Conclusions

1. All samples are toxic to *Selenastrum*. Samples 1, 2, and 3 are the most toxic.

2. All samples are mutagenic. Samples 2, 3, 4, and 5 are the most mutagenic.

# Appendix C

## Assessment of Textile Mill Effluent Toxicity Using

## *Ceriodaphnia dubia*

# ASSESSMENT OF TEXTILE MILL EFFLUENT TOXICITY USING *CERIODAPHNIA DUBIA*

Prepared for:

ENVIRONMENT CANADA
Conservation and Protection
Dartmouth, Nova Scotia

Prepared by:

BEAK CONSULTANTS LIMITED
14 Abacus Road
Brampton, Ontario
L6T 5B7

Patricia Orr, B.Sc.
Aquatic Toxicologist

January 1991
BEAK Ref: 3080.1

# 1.0 INTRODUCTION

Textile mills utilize a wide varitey of dyes and chemicals. Many of these are not retained in the final textile product and are discarded as liquid effluent into aquatic ecosystems. These effluents may be toxic to aquatic organisms due to chemical contamination as well as their physical properties, such as pH, BOD, COD, temperature, and solids content. Although the acute toxicity of untreated, undiluted textile mill effluent to aquatic organisms has been known for some time (IEC, 1982; Thompson, 1974), measured impacts on aquatic systems have not been well established.

Accordingly, Environment Canada designed a study entitled "Characterization, Aquatic Toxicity and Environmental Impact of Textile Mill Effluents" to determine whether textile mill effluents can be defined as a toxic substance under the Canadian Environmental Protection Act. As part of the toxicity assessment portion of this study, large composite samples of effluent were obtained for toxicity testing. Beak Consultants Limited (BEAK) was retained to conduct the *Ceriodaphnia dubia* chronic survival and reproduction test on each sample.

## 2.0 MATERIALS AND METHODS

### 2.1 Sample Collection

Shipping containers were supplied by Environment Canada. All samples were collected by Environment Canada and each was sent to BEAK in two 4-L glass jugs (each contained in a plastic pail) by overnight courier.

### 2.2 Toxicity Tests

Culture Conditions

*Ceriodaphnia dubia* stocks were maintained in a 25-L glass aquarium containing laboratory dilution water at 25 ± 1°C. The photoperiod was 16 hours light, 8 hours dark at 100 ft-C light intensity. Organism density was maintained at a loading rate of less than 40 animals/L to prevent crowding and to discourage gametogenesis and ephippia.

*C. dubia* stocks were fed a suspenision of combined solution of fermented trout chow, Fleischman's yeast and Cerophyl$^{R}$ (dried, powdered cereal leaves) daily at a rate of 5 mL/L.

Primary brood animals containing eggs were selected from the stock culture and isolated in 50-mL beakers containing 15 mL dilution water and 0.1 mL food suspension for 24 hours. Brood organisms were transferred daily to fresh solution, and the young produced in the first three broods of each female were discarded. Neonates from the fourth broods were used as parental stock. The young produced in the third or subsequent broods of the parental stock were used in the test, provided 15 or more total neonates were achieved within the first three broods. All neonates were less than 12 hours old and within four to eight hours of age when the test was begun.

Preliminary Tests

Preliminary 24-hour tests were initiated on each sample on the same day the sample was received in order to establish an appropriate testing concentration range. The definitive chronic test was initiated the following day.

Definitive Tests

Three-brood *Ceriodaphnia dubia* survival and reproduction tests were performed according to the U.S. EPA method (Weber *et al.* 1989).

Ten animals were exposed to each effluent concentration and only one animal was placed in each exposure vessel. The tests were conducted in 40-mL polyethlylene vessels with 15 mL of test solution. Each test was accompanied by a control containing dilution water only, but subjected to the same test conditions as the effluent concentrations.

All tests were conducted at 25 $\pm$ 1°C under the same photoperiod as the stock culture. Dissolved oxygen, pH, temperature, and conductivity were recorded at the beginning and end of each 24-hour exposure period in each test concentration and control. Alkalinity and hardness were measured at the beginning of each 24-hour exposure time in the highest test concentration and control.

Within about three days, individual *C. dubia* had matured and began to produce young. Each surviving adult test organism was transferred daily into a new test vessel containing freshly prepared test solution and 0.1 mL food suspension. The young were sacrificed with two drops of 1N HCl and counted.

The tests were terminated if at least 60% of control females had produced three broods after 7 days. One test (sample #1) was continued for an extra day in order to meet the three-brood criterion.

Reproductive data were expressed as cumulative number of young produced per female for each observation time.

## 2.3 Data analysis

LC50

Acute (2-day) and chronic (7-day) LC50s were calculated using standard estimation techniques available in the form of a computer program developed by C. E. Stephan, U.S. EPA, Duluth, MN. The moving average method was preferred for data sets which included concentrations causing 0% and 100% mortality, plus a minimum of two concentration resulting in partial mortality (Bennett, 1952). The binomial method was used when only 0% and 100% responses were observed (Stephan, 1977). The trimmed Spearman-Karber method is not subject to some of the deficiencies of probit and logit models (Hamilton, 1977), and is thus preferred for some data sets, particularly those with only one partial kill and/or, those with a slightly anomolous (although valid) dose-response.

Chronic Value

Adult survival was evaluated using Fisher's exact test (computer software was Toxstat, Version 3.0, University of Wyoming). The highest concentration which resulted in no significant reduction on survival (NOEC) and the lowest concentration which resulted in a significant reduction in survival (LOEC) were identified.

Reproduction data were then evaluated for those organisms exposed to concentrations which did not cause significant reduction in survival. The data (number of young produced per female at each concentration) were tested for normality (Shapiro-Wilks Test) and homogeneity of variance (Bartlett's Test). If the data within each treatment were normally distributed and the variance of each treatment was not significantly different from that of the control group, analysis of variance (ANOVA) was performed, followed by Dunnett's

procedure (Dunnett, 1955), to identify treatments (concentrations) which were statistically significantly different ($p < 0.05$) from the control. In cases of non-normality and/or non-homogeneous variances Steel's Many One Rank Test (Steel, 1959) was used following ANOVA. In either case the NOEC and LOEC for neonate production were identified.

If the LOEC for reproduction occurred at a lower concentration than did the LOEC for survival, than the LOEC and NOEC for reproduction were used to estimate the chronic value (Ch.V.). Otherwise the NOEC and LOEC for survival were used. The Ch.V. was then estimated as the geometric mean of the NOEC and LOEC.

Inhibition Concentration Percentage

Chronic toxicity test data was also analyzed using an interpolation approach (Norberg-King, 1988), to give the inhibition concentration percentage (ICp). This procedure involves a non-parametric monotonic smoothing method.

An IC50 (concentration which causes 50% inhibition relative to a control) is comparable to the Ch.V., but, unlike the discrete variable end-points generated by hypothesis testing, the ICp procedure gives a point estimate with an associated confidence interval.

# 3.0 RESULTS AND DISCUSSION

## 3.1 Quality Assurance/Quality Control (QA/QC)

During the study period, the toxicity laboratory was in compliance with U.S. EPA standards of Good Laboratory Practise (U.S. EPA, 1987).

Control of test precision was monitored using reference toxicant tests (NaPCP) as outlined in Environment Canada (1990). The results of tests performed during the study period fell within established control limits (Figures 3.1 and 3.2).

All tests met the criteria for test acceptability as outlined in the U.S. EPA *C. dubia* survival and reproduction method (Weber *et al.*, 1989). These included:

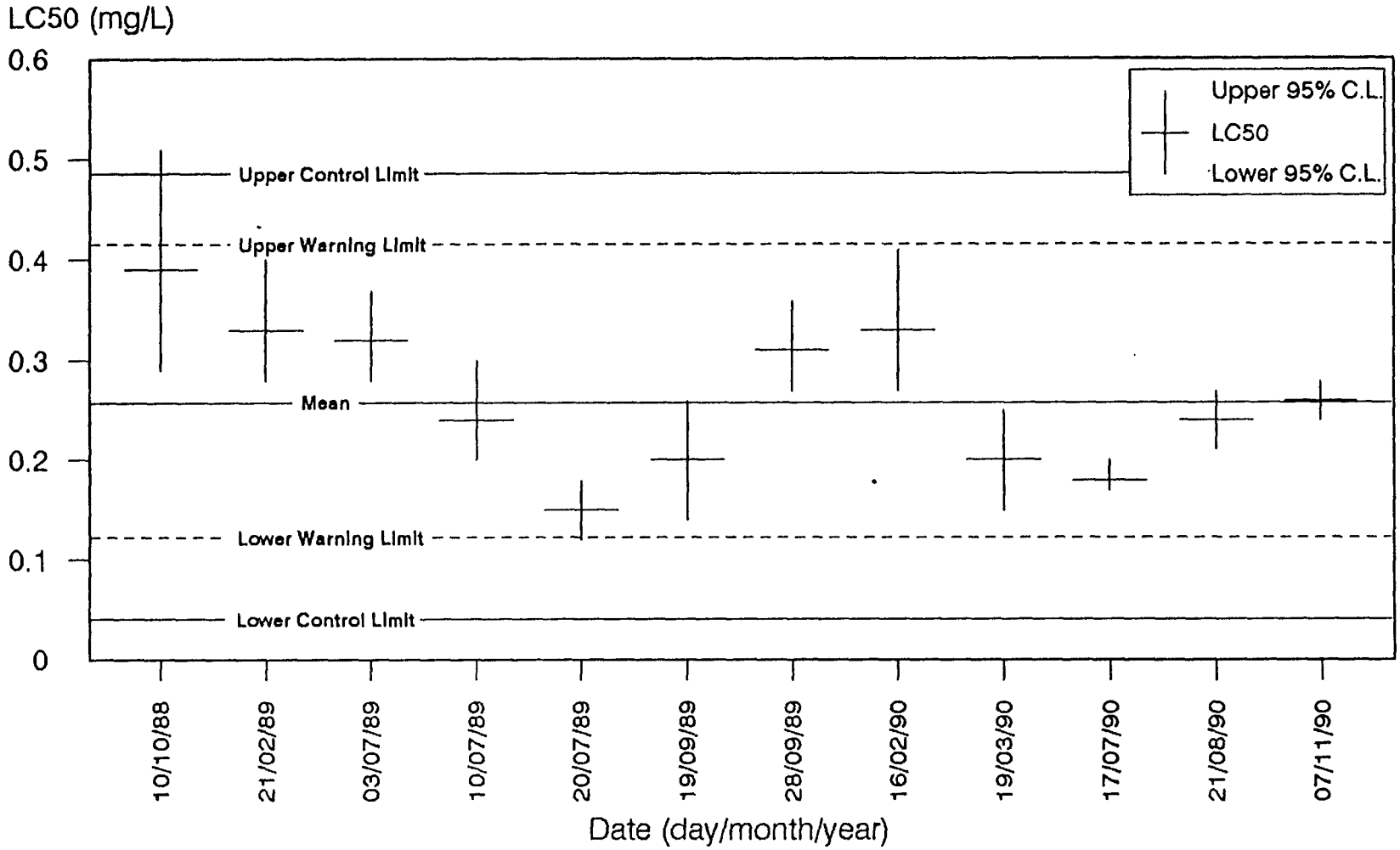
- no more than 20% mortality among control organisms;
- control organisms produced an average of 15 or more young per surviving female;
- at least 60% of control females produced at least three broods; and
- defined dose-response relationship.

## 3.2 Test Results and Discussion

All effluents tested were toxic. Chronic LC50s ranged from 9.3% to 23% effluent (%v/v)(Table 3.1).

Chronic values ranged from 0.89% to 8.8%, and, except for the first sample, reproduction was a more sensitive indicator of toxicity than survival (Table 3.1). That is, for most of

# FIGURE 3.1 CONTROL CHART FOR CERIODAPHNIA 3-BROOD REPRODUCTION TEST USING NaPCP



# FIGURE 3.2 CONTROL CHART FOR CERIODAPHNIA 3-BROOD REPRODUCTION TEST USING NaPCP

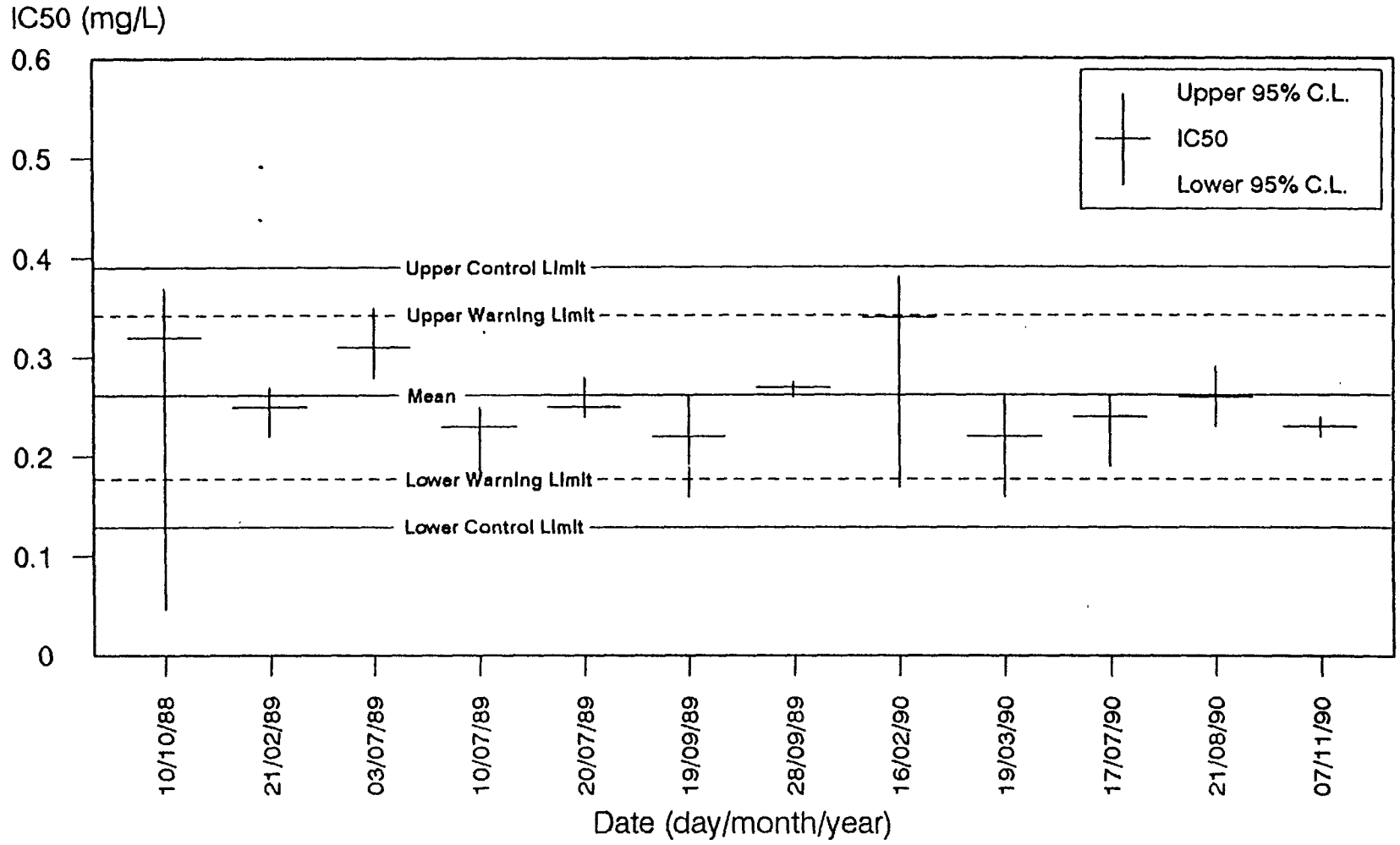


TABLE 3.1: RESULTS OF CHRONIC *C. DUBIA* SURVIVAL AND REPRODUCTION TESTS CONDUCTED ON TEXTILE MILL EFFLUENTS

| Effluent No. | Acute LC50 (% v/v) | 95% Confidence Interval (acute LC50) | Chronic LC50 (% v/v) | 95% Confidence Interval (chronic LC50) | NOEC[a] (%v/v) | Ch.V.[b] (%v/v) | IC50[c] (%v/v) | 95% Confidence Interval (IC50) |
|---|---|---|---|---|---|---|---|---|
| 1 | 12[d] | 10-15 | 9.3[e] | 7.2-13 | 5 | 7.1[g] | 7.0 | 4.8-7.7 |
| 2 | 31[d] | 26-37 | 23[d] | 19-29 | 6.3 | 8.8 | 7.8 | 2.7-8.8 |
| 3 | 5.4[d] | 4.3-6.6 | 3.3[d] | 2.8-3.9 | 0.63 | 0.89 | 1.8 | 1.3-1.9 |
| 4 | 18[f] | 13-25 | 13[d] | 11-16 | 3.1 | 4.4 | 5.3 | 4.8-5.9 |
| 5 | 20[e] | 15-27 | 14[e] | 10-20 | 6.3 | 8.8 | 8.7 | 5.9-9.7 |
| 6 | 20[d] | 17-24 | 11[d] | 8.7-14 | 3.1 | 4.4 | 6.2 | 4.6-7.8 |

[a] NOEC - highest concentration which showed no significant observable effect.

[b] Ch.V. - chronic value; geometric mean between NOEC (defined above) and LOEC (lowest concentration resulting in a significant effect); "effect" was measured in terms of reproductive impairment (number of young) relative to controls except where noted otherwise.

[c] IC50 - concentration which causes a 50% reduction in the reproduction of exposed organisms.

[d] Trimmed Spearman-Karber method, alpha = 0% (trim).

[e] Moving average method.

[f] Binomial method.

[g] "Effect" was based on survival

the effluents tested, *C. dubia* reproduction was affected at a lower concentration than that which impaired survival.

Effluent IC50s ranged from 1.8% to 8.7%, and for all effluents the IC50 showed good agreement with the corresponding Ch.V. (i.e., each supports the other). Given also, that all concurrent measures of laboratory QA/Qc showed good performance, these results can likely be accepted with a high degree of confidence.

The environmental significance of these results is difficult to interpret without knowing whether the effluent samples tested are representative of each mill's effluent quality and without knowledge of the receiving waters into which each effluent discharges.

If the samples are assumed to be representative, the dilution required to prevent toxic effects to *C. dubia* in the receiving environment can be estimated (Table 3.2). The ratios listed in the Ch.V. and IC50 columns indicate the dilutions which, if not met, may result in reproductive impairment of *C. dubia* (ie., the threshold effect concentration for each effluent would be exceeded). In order to provide additional protection, the NOEC can be used to estimate receiving water dilution requirements for each effluent (Table 3.2).

The degree of dilution required for five of the six effluents to prevent sublethal effects among sensitive organisms such as *C. dubia* may be easily achieved in some types of receiving environments (eg., large, fast-flowing rivers). The effluent represented by sample #3, however, probably requires in excess of a 100:1 dilution to protect against sublethal effects in the receiving environment. The potential impact of each effluent should be considered in terms of its specific receiving environment.

If effluent plume dispersion studies have been conducted at each receiving water site, then the location at which a specific dilution is met can be estimated. The area of the receiving water extending from the outfall to that point represents a zone of potential biological impact as predicted by the *C. dubia* test. The results of other laboratory tests (eg., other species

TABLE 3.2 Estimated Receiving Water-To-Effluent Dilution Ratios Required To Prevent Reproductive Impairment of *C. dubia*

| Sample # | NOEC | Ch.V. | IC50 |
|---|---|---|---|
| 1 | 19:1 | 14:1 | 14:1 |
| 2 | 15:1 | 11:1 | 12:1 |
| 3 | 157:1 | 112:1 | 56:1 |
| 4 | 31:1 | 22:1 | 18:1 |
| 5 | 15:1 | 11:1 | 11:1 |
| 6 | 31:1 | 22:1 | 16:1 |

and end-points) can also be used to define potential zones of impact. These predictions can then be tested by conducting surveys of receiving water biological communities in and outside of the estimated zone(s) of impact.

Single tests on individual effluents are useful to identify the relative toxicity of a number of effluents, but must be replicated in order to characterize the variability in quality of each effluent. The lower 95% confidence limit of the mean toxicity value should then be used to describe the mixing zone within which sublethal effects might be expected 95% of the time the effluents discharged. The dilutions estimates presented in this evaluation do not consider effluent variability.

## 4.0 REFERENCES

Bennett, B.M. 1952. Estimation of LD50 by moving averages. J. Hygiene 50: 157-164.

Dunnett, C.W. 1955. Multiple comparison procedure for comparing several treatments with a control. J. Amer. Statist. Assoc. 50: 1096-1121.

Hamilton, M.A., R.C. Russo and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11: 714-717.

IEC (International Environmental Consultants). 1982. Survey of Textile Wet Processing and Pollution Abatement Technology. Report prepared for Water Pollution Control Directorate, Environmental Protection Service, Environment Canada. EPS 3-WP-82-5.

Norberg-King, T. 1988. An Interpretation Estimate for Chronic Toxicity: The ICp Approach. National Effluent Toxicity Assessment Centre Technical Report 05-88. U.S. Environmental Protection Agency, Duluth, MN.

Environment Canada. 1990. Guidance Document on Control of Toxicity Test Precision Using Reference Toxicants. Prepared by Orr, P.L., D.R. Hart and G.R. Craig, Beak Consultants Limited, Brampton, Ontario. Prepared for Environmental Conservation and Protection, Environment Canada, Hull, Quebec. Report EPS 1/RM/12.

Steel, R.G. 1959. A multiple comparison rank sum test: treatments versus control. Biometrics 15: 560-572.

Stephan, C.E. 1977. Methods for calculating an LC50, pp. 65-84. In: F.L. Mayer and J.L. Hamelink (eds.). Aquatic Toxicology and Hazard Evaluation. American Society for Testing and Materials, ASTM STP 634.

Thompson, B. 1974. The Effects of Effluent from the Canadian Textile Industry on Aquatic Organisms - A Literature Review. Technical Report No. 489. Fisheries and Marine Services, Research and Development Directorate, Environment Canada, Ottawa, Ontario.

U.S. EPA (U.S. Environmental Protection Agency). 1987. Toxic Substances Control Act (TSCA): Good Laboratory Practice Standards. 40 CFR Part 792. Federal Register, Vol. 52, No. 248, Monday, 28 December 1987.

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessler, J.R. Menkedick, T.W. Neiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg. 1989. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Second Edition. U.S. Environmental Protection Agency, EPA/600/4-89/001.

# Appendix D

## Surfactant (Triton X-100) Recovery From Spiked Water Samples (60.9 mg/L)

Surfactant (Triton X-100) Recovery From Spiked Water Samples (60.9 mg/l)

| Spike | Amount Recovered | % Recovery |
|---|---|---|
| 1 | 54 mg/l | 89 |
| 2 | 52 mg/l | 86 |
| 3 | 47 mg/l | 78 |
| | | mean = 84% C.V. = 7.0 |

# Appendix E

## Target Compound Recoveries from Spiked Sediments

## (Triplicate Samples)

Target Compound Recoveries from Spiked Sediments (triplicate samples)

| Target Compound | Amount of Spike | Avg. Recovery % | Amount of Spike | Avg. Recovery |
|---|---|---|---|---|
| 1-methylpyrrolidenone | 0.62 μg/g | 118 ± 21 | 10.3 μg/g | 95 ± 3.2 |
| caprolactam | 0.76 μg/g | 100 ± 23 | 12.6 μg/g | 133 ± 3.9 |
| tetramethylbutylphenol | 0.79 μg/g | 98 ± 1.8 | 13.2 μg/g | 72 ± 14 |

# Appendix F

## Textile Mills - Toxicity Data

TEXTILE MILLS - TOXICITY DATA - EXPRESSED AS MEANS*

| Sample Description | RBT | D. magna | Micro5 EC50 | Micro15 EC50 | Algae EC50 | CD.acute | CD.chronic | CD.IC50 |
|---|---|---|---|---|---|---|---|---|
| Britex, Bridgetown, Nova Scotia | 20.6 | 23.2 | 55.1 | 43.3 | 5.3 | 24.5 | 18.0 | 6.6 |
| Stanfields, Truro, Nova Scotia | 44.9 | 19.4 | 5.0 | 5.1 | 23.1 | 20.0 | 12.5 | 7.5 |
| Tandem Fabrics, Moncton, N.B. | 21.6 | 20.0 | 10.7 | 10.6 | 0.3 | 8.7 | 6.3 | 4.4 |

*(data expressed as a mean of all sampling dates - LC50 as % effluent (v/v) unless otherwise stated)

# Appendix G

## Classification Scheme for Acute Lethal Toxicity Results

CLASSIFICATION SCHEME FOR ACUTE LETHAL TOXICITY RESULTS*

| Toxicity Classification | Range of LC50s (%) defining this Toxicity |
|---|---|
| High Toxicity | <10 |
| Moderate Toxicity | 10 - 50 |
| Low Toxicity | 50 - 100 |
| Non-detectable Toxicity | >100 |

* After Sandhu (1979).

# Appendix H

## Toxicity Data - Spearman Rank Correlations and Sample Correlations for Rainbow Trout, *Daphnia magna* and Microtox

Spearman Rank Correlations

| | RBT | D__magna | Micro5 | Micro15 |
|---|---|---|---|---|
| RBT | 1.0000 | .0714 | -.6930 | -.7014 |
| | ( 12) | ( 12) | ( 12) | ( 12) |
| | 1.0000 | .8127 | .0215 | .0200 |
| D__magna | .0714 | 1.0000 | .1608 | .0771 |
| | ( 12) | ( 12) | ( 12) | ( 12) |
| | .8127 | 1.0000 | .5937 | .7983 |
| Micro5 | -.6930 | .1608 | 1.0000 | .9632 |
| | ( 12) | ( 12) | ( 12) | ( 12) |
| | .0215 | .5937 | 1.0000 | .0014 |
| Micro15 | -.7014 | .0771 | .9632 | 1.0000 |
| | ( 12) | ( 12) | ( 12) | ( 12) |
| | .0200 | .7983 | .0014 | 1.0000 |

Coefficient (sample size) significance level

Sample Correlations

| | RBT | D__magna | Micro5 | Micro15 |
|---|---|---|---|---|
| RBT | 1.0000 | .1080 | -.5080 | -.5117 |
| | ( 12) | ( 12) | ( 12) | ( 12) |
| | .0000 | .7382 | .0918 | .0890 |
| D__magna | .1080 | 1.0000 | .0924 | .0792 |
| | ( 12) | ( 12) | ( 12) | ( 12) |
| | .7382 | .0000 | .7753 | .8067 |
| Micro5 | -.5080 | .0924 | 1.0000 | .9959 |
| | ( 12) | ( 12) | ( 12) | ( 12) |
| | .0918 | .7753 | .0000 | .0000 |
| Micro15 | -.5117 | .0792 | .9959 | 1.0000 |
| | ( 12) | ( 12) | ( 12) | ( 12) |
| | .0890 | .8067 | .0000 | .0000 |

Coefficient (sample size) significance level

# Appendix I

## Toxicity Data - Spearman Rank Correlations and Sample Correlations for Rainbow Trout, *Daphnia magna*, Microtox, algae and *Ceriodaphnia dubia*

Spearman Rank Correlations

| | RBT | D__magna | Micro5 | Micro15 | Algae | CD_acute | CD_chronic | CD_IC50 |
|---|---|---|---|---|---|---|---|---|
| RBT | 1.0000 | -.3714 | -.6000 | -.6571 | .7143 | .5218 | .2571 | .6000 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | 1.0000 | .4062 | .1797 | .1417 | .1102 | .2433 | .5653 | .1797 |
| D__magna | -.3714 | 1.0000 | .7714 | .8857 | -.6000 | .3189 | .6000 | .3143 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .4062 | 1.0000 | .0845 | .0476 | .1797 | .4758 | .1797 | .4822 |
| Micro5 | -.6000 | .7714 | 1.0000 | .9429 | -.5429 | -.0580 | .1429 | .3143 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .1797 | .0845 | 1.0000 | .0350 | .2248 | .8968 | .7494 | .4822 |
| Micro15 | -.6571 | .8857 | .9429 | 1.0000 | -.6000 | -.0580 | .2571 | -.1429 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .1417 | .0476 | .0350 | 1.0000 | .1797 | .8968 | .5653 | .7494 |
| Algae | .7143 | -.6000 | -.5429 | -.6000 | 1.0000 | .1160 | -.0286 | .6857 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .1102 | .1797 | .2248 | .1797 | 1.0000 | .7954 | .9491 | .8480 |
| CD_acute | .5218 | .3189 | -.0580 | -.0580 | .1160 | 1.0000 | .8986 | .6957 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .2433 | .4758 | .8968 | .8968 | .7954 | 1.0000 | .0445 | .1198 |
| CD_chronic | .2571 | .6000 | .1429 | .2571 | -.0286 | .8986 | 1.0000 | .7143 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .5653 | .1797 | .7494 | .5653 | .9491 | .0445 | 1.0000 | .1102 |
| CD_IC50 | .6000 | .3143 | -.3143 | -.1429 | .0857 | .6957 | .7143 | 1.0000 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .1797 | .4822 | .4822 | .7494 | .8480 | .1198 | .1102 | 1.0000 |

Coefficient (sample size) significance level

Sample Correlations

| | RBT | D__magna | Micro5 | Micro15 | Algae | CD_acute | CD_chronic | CD_IC50 |
|---|---|---|---|---|---|---|---|---|
| RBT | 1.0000 | -.2871 | -.5627 | -.5368 | .8156 | .3418 | .1883 | .6437 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .0000 | .5811 | .2450 | .2722 | .0479 | .5073 | .7208 | .1678 |
| D__magna | -.2871 | 1.0000 | .4816 | .5198 | -.5454 | .6440 | .8027 | .5096 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .5811 | .0000 | .3335 | .2905 | .2630 | .1675 | .0545 | .3018 |
| Micro5 | -.5627 | .4816 | 1.0000 | .9978 | -.2445 | .3285 | .4106 | -.0473 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .2450 | .3335 | .0000 | .0000 | .6406 | .5250 | .4187 | .9290 |
| Micro15 | -.5368 | .5198 | .9978 | 1.0000 | -.2339 | .3861 | .4664 | -.0050 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .2722 | .2905 | .0000 | .0000 | .6556 | .4497 | .3512 | .9925 |
| Algae | .8156 | -.5454 | -.2445 | -.2339 | 1.0000 | .2115 | .0127 | .2979 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .0479 | .2630 | .6406 | .6556 | .0000 | .6875 | .9809 | .5663 |
| CD_acute | .3418 | .6440 | .3285 | .3861 | .2115 | 1.0000 | .9724 | .7282 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .5073 | .1675 | .5250 | .4497 | .6875 | .0000 | .0011 | .1008 |
| CD_chronic | .1883 | .8027 | .4106 | .4664 | .0127 | .9724 | 1.0000 | .7356 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .7208 | .0545 | .4187 | .3512 | .9809 | .0011 | .0000 | .0956 |
| CD_IC50 | .6437 | .5096 | -.0473 | -.0050 | .2979 | .7282 | .7356 | 1.0000 |
| | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) | ( 6) |
| | .1678 | .3018 | .9290 | .9925 | .5663 | .1008 | .0956 | .0000 |

Coefficient (sample size) significance level

# Appendix J

## Benthic Macroinvertebrate Data

AQUATIC BENTHIC INVERTEBRATE DATA – STANFIELDS – 1990

| STATION | Chironomidae | Fenisola rivulata | Amiocla limosa | Physa heterostropha | Gyraulus deflectus | Helisoma trivolvis | Gloeliphonia complanata | Hydropsyche morosa | Erpobdella punctata | Helobdella stagnalis | Helobdella stagnicola | Stenelmis sp. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1a | 19 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1b | 14 | 1 | 21 | 16 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1c | 14 | 2 | 9 | 8 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1d | 7 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1e | 18 | 1 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1f | 12 | 2 | 8 | 7 | 11 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1g | 5 | 0 | 6 | 4 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1h | 16 | 0 | 5 | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1i | 3 | 0 | 2 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 107 | 6 | 56 | 51 | 37 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MEAN | 11 89 | 0.67 | 6 44 | 5 67 | 4.11 | 0.22 | 0.00 | 0.00 | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 |
| SD | 5 67 | 0.87 | 6.31 | 4 80 | 3.33 | 0 44 | 0 00 | 0 00 | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 |
| 2a | 10 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2b | 29 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2c | 6 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 2d | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2e | 28 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2f | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2g | 16 | 3 | 14 | 6 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2h | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2i | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2j | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2k | 3 | 0 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 143 | 3 | 24 | 12 | 14 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| MEAN | 13.00 | 0.27 | 2.18 | 1.09 | 1 27 | 0 00 | 0 09 | 0 09 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 |
| SD | 9 42 | 0 90 | 4 09 | 2.43 | 3 68 | 0 00 | 0 30 | 0 30 | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 |
| 3a | 32 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3b | 14 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3c | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3d | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3e | 25 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3f | 24 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3g | 15 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3h | 32 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3i | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3j | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 180 | 1 | 3 | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MEAN | 18.00 | 0.10 | 0.30 | 0 20 | 0 40 | 0 00 | 0 00 | 0.00 | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 |
| SD | 9 63 | 0.32 | 0.67 | 0 42 | 0.52 | 0.00 | 0 00 | 0 00 | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 |
| 4a | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 |
| 4b | 76 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| 4c | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| 4d | 44 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 0 |
| 4e | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 4f | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 |
| 4g | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4h | 43 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| TOTAL | 381 | 1 | 0 | 7 | 1 | 0 | 0 | 0 | 1 | 22 | 5 | 0 | 0 |
| MEAN | 47.63 | 0.13 | 0.00 | 0 88 | 0 13 | 0 00 | 0 00 | 0 00 | 0.13 | 2.75 | 0 63 | 0 00 | 0 00 |
| SD | 20.65 | 0 35 | 0.00 | 1 25 | 0 35 | 0 00 | 0 00 | 0 00 | 0.35 | 4 27 | 1 77 | 0 00 | 0 00 |
| Ca | 146 | 12 | 180 | 102 | 30 | 3 | 0 | 0 | 0 | 6 | 0 | 7 | |
| Cb | 156 | 2 | 164 | 88 | 36 | 1 | 0 | 0 | 0 | 0 | 0 | 13 | |
| Cc | 419 | 41 | 181 | 80 | 55 | 14 | 0 | 0 | 0 | 6 | 0 | 5 | |
| Cd | 412 | 5 | 200 | 88 | 30 | 3 | 0 | 0 | 0 | 0 | 0 | 4 | |
| Ce | 146 | 74 | 174 | 70 | 55 | 5 | 0 | 0 | 0 | 1 | 0 | 8 | |
| Cf | 231 | 27 | 91 | 51 | 78 | 2 | 0 | 0 | 0 | 3 | 0 | 8 | |
| Cg | 166 | 0 | 271 | 71 | 25 | 3 | 0 | 0 | 0 | 5 | 0 | 9 | |
| Ch | 344 | 36 | 108 | 101 | 66 | 4 | 0 | 0 | 0 | 8 | 0 | 8 | |
| Ci | 289 | 31 | 178 | 46 | 63 | 2 | 0 | 0 | 0 | 20 | 0 | 28 | |
| Cj | 318 | 0 | 301 | 38 | 38 | 7 | 0 | 0 | 0 | 18 | 0 | 17 | |
| TOTAL | 2629 | 227 | 1796 | 717 | 504 | 44 | 0 | 0 | 0 | 65 | 0 | 107 | |
| MEAN | 262 90 | 22.70 | 178 60 | 71 70 | 50 40 | 4 40 | 0 00 | 0 00 | 0 00 | 6 50 | 0 00 | 10.70 | |
| SD | 108 34 | 23.79 | 66.65 | 21.74 | 23.03 | 3 78 | 0 00 | 0 00 | 0 00 | 7 07 | 0 00 | 7 16 | |

AQUATIC BENTHIC INVERTEBRATE DATA – STANFIELDS – 1990 (cont.)

| STATION | Athesix sp. | Beetle sp. | Ephemerella subvaria | Stenacron interpunctatum | Stenonema vicarium | Paraleptophlebia sp. | Aeschna sp. | Stagnicola elodes | Brachycentrus numerosus | Helicopsyche borealis | Hydropsyche bronta | No. of Taxa | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 1b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| 1c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 1d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 1e | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| 1f | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| 1g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 1h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 1i | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 5 |
| TOTAL | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 | 0.11 | 0 00 | 0 00 | 0.00 | 0 00 | 0.00 | 4 67 | |
| SD | 0 00 | 0 00 | 0.00 | 0.00 | 0 00 | 0 33 | 0 00 | 0 00 | 0.00 | 0 00 | 0 00 | 1 00 | |
| 2a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 2b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 2d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2e | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 2f | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 2h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2i | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2j | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2k | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| TOTAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 0 00 | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 2 36 | |
| SD | 0 00 | 0 00 | 0 00 | 0 00 | 0.00 | 0.00 | 0 00 | 0 00 | 0 00 | 0.00 | 0 00 | 1 50 | |
| 3a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 3c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3e | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 3f | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 3g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3i | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3j | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| TOTAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 1 90 | |
| SD | 0 00 | 0 00 | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 88 | |
| 4a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 4c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| 4e | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4f | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 4h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| TOTAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 2 63 | |
| SD | 0 00 | 0 00 | 0.00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 0 00 | 1 51 | |
| Ca | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 120 | 11 | |
| Cb | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 9 | |
| Cc | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 3 | 55 | 12 | |
| Cd | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 4 | 0 | 0 | 47 | 11 | |
| Ce | 3 | 0 | 0 | 0 | 1 | 6 | 0 | 2 | 0 | 0 | 23 | 13 | |
| Cf | 0 | 0 | 1 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 21 | 12 | |
| Cg | 1 | 1 | 0 | 0 | 1 | 3 | 0 | 0 | 2 | 1 | 21 | 14 | |
| Ch | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 0 | 1 | 24 | 13 | |
| Ci | 3 | 0 | 0 | 0 | 2 | 4 | 0 | 6 | 4 | 0 | 42 | 14 | |
| Cj | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 2 | 0 | 0 | 41 | 10 | |
| TOTAL | 9 | 2 | 2 | 1 | 7 | 26 | 1 | 20 | 6 | 5 | 427 | | |
| MEAN | 0 90 | 0 20 | 0 20 | 0 10 | 0 70 | 2 60 | 0.10 | 2 00 | 0 60 | 0 50 | 42 70 | 11 90 | |
| SD | 1.29 | 0 42 | 0 42 | 0 32 | 1 06 | 2 59 | 0 32 | 1 89 | 1 35 | 0 97 | 29 87 | 1 66 | |

AQUATIC BENTHIC INVERTEBRATE DATA – STANFIELDS – June 1991

| Station | Stenelmis sp. | Chironomidae | Amicola limosa | Tipulla sp. | Ephemerella bicolor | Physa heterostropha | Gyraulus deflectus | Hydropsyche morosa | Erpobdella punctata | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1a | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1b | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1c | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1d | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1e | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1f | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1g | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1h | 1 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1i | 2 | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1j | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 3 | 71 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MEAN | 0.30 | 7.10 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SD | 0 67 | 2.88 | 0.32 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2a | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| 2b | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2c | 0 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2d | 2 | 8 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 2e | 0 | 2 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 2f | 0 | 5 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 2g | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2h | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2i | 1 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2j | 0 | 4 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 5 | 42 | 8 | 2 | 2 | 1 | 1 | 1 | 1 | 0 |
| MEAN | 0 50 | 4.20 | 0.80 | 0.20 | 0.20 | 0.10 | 0.10 | 0.10 | 0.10 | 0.00 |
| SD | 0.71 | 1.69 | 1.23 | 0.63 | 0.42 | 0.32 | 0 32 | 0.32 | 0.32 | 0.00 |
| 3a | 1 | 108 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3b | 0 | 168 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3c | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3d | 0 | 80 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 3e | 1 | 48 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 3f | 0 | 223 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 3g | 0 | 97 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3h | 0 | 102 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3i | 0 | 108 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3j | 0 | 147 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 2 | 1155 | 1 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| MEAN | 0.20 | 115.50 | 0.10 | 0.00 | 0.40 | 0.00 | 0 00 | 0.00 | 0.00 | 0.00 |
| SD | 0.42 | 50.95 | 0.32 | 0.00 | 0.52 | 0.00 | 0.00 | 0 00 | 0.00 | 0.00 |
| 4a | 0 | 148 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 |
| 4b | 1 | 144 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 4c | 0 | 171 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| 4d | 0 | 153 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4e | 1 | 165 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4f | 0 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4g | 0 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4h | 0 | 168 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4i | 3 | 141 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4j | 2 | 193 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| TOTAL | 7 | 1402 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 0 |
| MEAN | 0.70 | 140.20 | 0.00 | 0.00 | 0.20 | 0 50 | 0.00 | 0.00 | 0.00 | 0.00 |
| SD | 1.06 | 46.04 | 0.00 | 0.00 | 0.63 | 0.71 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ca | 3 | 147 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Cb | 0 | 115 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Cc | 0 | 261 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Cd | 1 | 188 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ce | 0 | 105 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Cf | 0 | 184 | 7 | 0 | 1 | 4 | 0 | 0 | 0 | 0 |
| Cg | 0 | 96 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| Ch | 0 | 125 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Ci | 0 | 242 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cj | 0 | 130 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| TOTAL | 4 | 1573 | 16 | 0 | 4 | 10 | 0 | 0 | 2 | 1 |
| MEAN | 0.40 | 157.30 | 1.60 | 0.00 | 0.40 | 1.00 | 0.00 | 0.00 | 0.20 | 0.10 |
| SD | 0 97 | 56.70 | 2.41 | 0.00 | 0.52 | 1.63 | 0.00 | 0.00 | 0.42 | 0.32 |

AQUATIC BENTHIC INVERTEBRATE DATA – STANFIELDS – JUNE 1991 (cont.)

| Station | Simulia sp. | Baetis sp. | Ephemerella sp. | Paraleptophlebia sp. | Ophigyomphus carolus | Ferrissia rivularis | Helobdella stagnalis | Micrasema sp. | | No. of Taxa |
|---|---|---|---|---|---|---|---|---|---|---|
| 1a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1e | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1f | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 1i | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 1j | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| TOTAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.30 |
| SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.67 |
| 2a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 2b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 2e | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 2f | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2i | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 2j | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| TOTAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.40 |
| SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.52 |
| 3a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3e | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 3f | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3i | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3j | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| TOTAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.70 |
| SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.67 |
| 4a | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 4b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 4c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4d | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 4e | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4f | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 4g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 4h | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 4i | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4j | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| TOTAL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.90 |
| SD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.88 |
| Ca | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Cb | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 |
| Cc | 0 | 1 | 0 | 0 | 1 | 0 | 5 | 0 | 0 | 6 |
| Cd | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Ce | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| Cf | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 6 |
| Cg | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Ch | 0 | 9 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| Ci | 0 | 2 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Cj | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| TOTAL | 4 | 41 | 18 | 1 | 1 | 1 | 5 | 0 | 1 | |
| MEAN | 0.40 | 4.10 | 1.80 | 0.10 | 0.10 | 0.10 | 0.50 | 0.10 | 0.10 | 4.40 |
| SD | 0.97 | 4.96 | 3.05 | 0.32 | 0.32 | 0.32 | 1.58 | 0.32 | 0.32 | 1.43 |

AQUATIC BENTHIC INVERTEBRATE DATA – TANDEM FABRICS 1990

| Station | Chironomidae | Erpobdella punctata | Amicola limosa | Physa heterostropha | Stenelmis sp. | Tipulia sp. | Coengrionidae | Phasganophora sp. | Stagnicola elodes | Hydropsyche betteni | Helobdella stagnalis | | No. of Taxa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1a | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1b | 78 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 1c | 188 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 1d | 106 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1e | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1f | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1g | 183 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1h | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1i | 63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1j | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1k | 74 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| TOTAL | 904 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 82 18 | 0.09 | 0.09 | 0.09 | 0.00 | 0.00 | 0.00 | 0 00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.27 |
| SD | 55.59 | 0.30 | 0.30 | 0 30 | 0 00 | 0 00 | 0.00 | 0.00 | 0 00 | 0.00 | 0.00 | 0.00 | 0.65 |
| 2a | 296 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2b | 150 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 4 |
| 2c | 142 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2d | 268 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2e | 307 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 |
| 2f | 300 | 0 | 1 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 6 |
| TOTAL | 1463 | 0 | 1 | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | | |
| MEAN | 243.83 | 0.00 | 0 17 | 1.00 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.00 | 2.67 | |
| SD | 76 97 | 0.00 | 0.41 | 1.26 | 0.41 | 0.41 | 0.41 | 0.41 | 0 41 | 0.41 | 0.00 | 1.97 | |
| 3a | 209 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3b | 261 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3c | 225 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3d | 211 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3e | 149 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3f | 186 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3g | 141 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| TOTAL | 1382 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 197 43 | 0.14 | 0.00 | 0.43 | 0 00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.57 | |
| SD | 42 39 | 0 38 | 0.00 | 0.53 | 0 00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 00 | 0.00 | 0 79 | |
| 4a | 19 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4b | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 4c | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 4d | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 4e | 40 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4f | 139 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| TOTAL | 246 | 4 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 41.00 | 0.67 | 0.00 | 0 33 | 0 00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.67 | |
| SD | 50.41 | 0.82 | 0 00 | 0 82 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.82 | |
| Ca | 110 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Cb | 91 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 |
| Cc | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Cd | 40 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Ce | 107 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| Cf | 69 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| TOTAL | 460 | 2 | 0 | 5 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | | |
| MEAN | 76 67 | 0.33 | 0.00 | 0.83 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.17 | 0.17 | 2.17 | |
| SD | 30 90 | 0.52 | 0.00 | 1.17 | 0 41 | 0.00 | 0.00 | 0.00 | 0.00 | 0.41 | 0.41 | 0.75 | |

AQUATIC BENTHIC INVERTEBRATE DATA – BRITEX – 1990

| Station | Chironomidae | Gammaris tigrinus | Gammaris oceanicus | Stagnicola elodes | Physa heterostropha | Hydropsyche morosa | Stenelmis sp. | Amicola lumosa | No. of Taxa |
|---|---|---|---|---|---|---|---|---|---|
| 1a | 12 | 238 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 1b | 3 | 302 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 1c | 15 | 325 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 1d | 10 | 814 | 21 | 0 | 0 | 0 | 0 | 0 | 3 |
| 1e | 2 | 315 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 1f | 4 | 432 | 8 | 0 | 0 | 0 | 0 | 0 | 3 |
| 1g | 6 | 279 | 4 | 0 | 0 | 0 | 0 | 0 | 3 |
| 1h | 0 | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 1i | 0 | 366 | 16 | 0 | 0 | 0 | 0 | 0 | 2 |
| TOTAL | 52 | 3146 | 49 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 5.78 | 349.56 | 5.44 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.22 |
| SD | 5.40 | 199.89 | 7.99 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.67 |
| 2a | 1 | 230 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2b | 0 | 369 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2c | 2 | 298 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2d | 0 | 633 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2e | 8 | 426 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2f | 2 | 175 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2g | 0 | 153 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2h | 11 | 311 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| TOTAL | 24 | 2595 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 3.00 | 324.38 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.63 |
| SD | 4.17 | 155.39 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.52 |
| 3a | 1 | 643 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3b | 0 | 507 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3c | 0 | 507 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3d | 8 | 198 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3e | 0 | 301 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3f | 0 | 891 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 3h | 21 | 182 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 3i | 0 | 309 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| TOTAL | 30 | 3538 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 3 75 | 442.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.38 |
| SD | 7.50 | 243.53 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.52 |
| 4a | 1 | 794 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4b | 3 | 418 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4c | 1 | 368 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4d | 11 | 333 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4e | 21 | 480 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4f | 9 | 305 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4g | 2 | 279 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4h | 3 | 613 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 4i | 10 | 510 | 0 | 0 | 0 | 0 | 0 | 0 | |
| TOTAL | 61 | 4100 | 0 | 0 | 0 | 0 | 0 | 0 | |
| MEAN | 6.78 | 455.56 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.00 |
| SD | 6.65 | 166.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ca | 0 | 731 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Cb | 14 | 597 | 0 | 1 | 0 | 0 | 0 | 0 | 3 |
| Cc | 5 | 269 | 0 | 0 | 1 | 0 | 0 | 0 | 3 |
| Cd | 8 | 294 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Ce | 4 | 312 | 0 | 1 | 0 | 1 | 0 | 0 | 4 |
| Cf | 7 | 421 | 0 | 2 | 0 | 0 | 0 | 0 | 3 |
| Cg | 10 | 567 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Ch | 3 | 622 | 0 | 1 | 0 | 0 | 1 | 0 | 4 |
| Ci | 7 | 891 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Cj | 3 | 420 | 0 | 0 | 0 | 0 | 1 | 0 | 3 |
| Ck | 7 | 328 | 0 | 0 | 0 | 0 | 0 | 1 | 3 |
| TOTAL | 68 | 5452 | 0 | 5 | 1 | 1 | 2 | 1 | |
| MEAN | 6.18 | 495.64 | 0.00 | 0.45 | 0.09 | 0.09 | 0.18 | 0.09 | 2.73 |
| SD | 3.82 | 202.03 | 0.00 | 0.69 | 0.30 | 0.30 | 0.40 | 0.30 | 0.90 |

# Appendix K

## Estimated Receiving Water-to-Effluent Dilution Ratios Required to Prevent Reproductive Impairment of *C. dubia*

Estimated Receiving Water-To-Effluent Dilution Ratios Required To Prevent Reproductive Impairment of C. dubia

| Sample and Date | NOEC | Ch.V. | IC50 |
|---|---|---|---|
| Tandem Fabrics (Sept. 11/12) | 19:1 | 14:1 | 14:1 |
| Britex (Sept. 17/18) | 15:1 | 11:1 | 12:1 |
| Tandem Fabrics (Sept. 24/25) | 157:1 | 112:1 | 56:1 |
| Britex (Oct. 1/2) | 31:1 | 22:1 | 18:1 |
| Stanfields (Oct. 9/10) | 15:1 | 11:1 | 11:1 |
| Stanfields (Oct. 29/30) | 31:1 | 22:1 | 16:1 |

NOEC - highest concentration which showed no significant observable effect.

Ch.V. - chronic value: geometric mean between NOEC (defined above) and LOEC (lowest concentration resulting in a significant effect): "effect" was measured in terms of reproductive impairment (number of young) relative to controls.

IC50 - concentration which causes a 50% reduction in the reproduction of exposed organisms.

34002681