SEDIMENT GENOTOXICITY AND ITS RELATIONSHIP TO THE FREQUENCY OF CHIRONOMID LABIAL PLATE DEFORMITIES

by

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ABSTRACT

Sediment samples were taken from seven locations in the Welland River in December 1986 and April 1987. The DMSO extracts of these sediment samples showed a significant ($p<0.01$) genotoxic effect for one location situated directly below a polyvinyl chloride plant's discharge pipe (station D-1). It was concluded that genotoxic contaminants were associated with the sediments from this location. Analysis of sediment samples from the nearby station D-2 resulted in a significant ($p<0.05$) positive genotoxic response in December but not in April.

Chironomids (midge larvae) taken from each of seven study locations were analyzed for the frequency of chironomid labial plate deformities (over 1000 individual specimens were observed). The samples from station D-1 showed the highest frequency of chironomid labial plate deformities (10.9% ± 3.2%), while samples from the upstream control (station A) displayed the lowest frequency of deformities (3.8% ± 1.3%). All samples were coded to avoid unconscious biases.

The results of the genotoxicity study indicated that station D-1 in the Welland River was contaminated with genotoxic materials. The genotoxic materials may have induced the observed increased frequency in chironomid labial plate deformities.

Samples from stations C and D-1, located in a downstream portion of the river bounded by an industrialized area were slightly toxic according to the alkaline phosphatase inhibition component of the SOS chromotest analyses. The toxicity of these samples was only evident once they had been activated by the S9

(liver extract) mixture.

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INTRODUCTION

Pollution from carcinogenic materials has become a serious problem as large amounts of contaminated municipal and industrial wastes annually enter natural water bodies. For example, over the years, the government of Canada has poured tens of millions of dollars into the Sydney Steel Corporation in Nova Scotia (McMillan, 1988). The technology which was used to produce steel in this case created an enormous lagoon of highly carcinogenic materials that constitutes the largest chemical carcinogenic industrial landfill in eastern Canada. It now costs the Canadian and Nova Scotian tax payers 49 million dollars to excavate and incinerate the Sydney Steel Corporation landfill wastes. This represents the second biggest site clean-up in the history of North America (McMillan, 1988).

Since aquatic communities are sensitive to mutagens, many ecologists have attempted to use invertebrates to quantify the level of carcinogens in aquatic sediments (Warwick, 1985). To the best of the author's knowledge, results reported here comprise the first study of the relationship between the frequency of benthic invertebrate abnormalities and sediment SOS chromotoxicity. The correlation between these two independent tests of genotoxicity constitutes the main focus of my thesis research.

Although many studies have considered or have investigated the genotoxicity of environmental samples and the frequency of deformities in chironomids, this is the first study to use the SOS chromotest in conjunction with the study of the frequency of

chironomid labial plate deformities. The genotoxicity of environmental samples and the frequency of deformities of chironomids were both studied in terms of their relationship to industrial and agricultural discharge sources.

The Welland River was chosen for this study because of its status as a provincially significant wetland area. The Welland River flows east through the City of Welland to the Niagara River. Upstream of the City of Welland, the river is mainly influenced by agricultural runoff. Within and downstream of the City of Welland, the major discharges to the river are from industrial and domestic sources (Steele, 1981; Dickman et al., 1983). The Welland river has been studied since the 1960's (e.g. Johnson, 1964).

Serious shock loading of industrial waste materials in the Welland River has resulted in aquatic plant dead zones below some industrial discharge sites in the Welland River (Dickman et al., 1983, 1986 and 1988). Several industrial discharge effluents contained detectable genotoxic organic compounds, such as chloroform, carbon tetrachloride and dibromomethane (Kaiser and Comba, 1983). These organic compounds have been described as carcinogens (McCann and Ames, 1976).

Discharges of heavy metals were also found at industrial sites, those metals included Cr, Pb, Ni (Dickman et al., 1986) some of which are both toxic and genotoxic (Flessel, 1979).

Previous studies on the Welland River indicated that the frequency of gonadal neoplasms in wild carp-goldfish hybrids was higher in the industrial influenced portion of the Welland River

than in the upstream agricultural portion (Dickman and Steele, 1986). This report prompted the author attempt to determine whether there were any genotoxic materials being discharged into the Welland River.

The seven locations selected for this study were divided into two groups: 1). A control location (site A) which was located in an agricultural area upstream of the city of Welland, and 2). Six "treatment" locations (sites B, C, D-1, D-2, E, and F) which were all located in an industrial area downstream from the city of Welland with the single exception of station B which received insecticides and herbicides from an agricultural area near station A (Fig. 1). It should be noted that similar herbicides and insecticides only reached station A after they had passed through an extensive aquatic plant lined streamcourse. These aquatic plants serve to remove pesticides from the water (Bingham, 1973).

The goals of this study were:

1. To determine the genotoxicity of sediment samples from the Welland River, using the SOS chromotest and coded samples to avoid unconscious biases.
2. To sample benthic invertebrates from the Welland River, at the identical locations that the SOS chromotest sediment mutagenicity test samples had been taken.
3. To observe the frequency of chironomid labial plate deformities at these locations, after first again coding the samples to prevent unconscious bias.

4. To determine the correlation between the level of SOS chromotest genotoxicity and the frequency of chironomid labial plate deformities.
5. To determine the diversity of benthic invertebrates (at the generic level) at each of the seven study sites in the Welland River.

Literature Review

Aquatic toxicological studies of freshwater include two major areas of study:

1.) field studies of species pattern, species diversity, species abundance, etc. (Guafin and Tarzwell, 1952). These field studies have been carried out with bacteria, aquatic plants and invertebrates, etc. (Cairns et al., 1972; Buikema and Herricks, 1978; Dickman et al., 1983; Pettibone and Cooney, 1986).

2.) the determination of pollutants in environmental samples by using some sensitive, inexpensive, convenient and short-term methods to estimate genotoxic pollutant loading (de Serres and Matsushima, 1987).

Both areas have developed rapidly, as genotoxic pollution problems have become more and more evident (Sato et al., 1983; Samoiloff et al., 1983; Xu and Dutka, 1986). In fact, contaminated drinking water is considered as a potential cause of cancer in humans (Velema, 1987).

Genotoxicity of environmental samples

Genotoxins include mutagens, clastogens, carcinogens, and teratogens (Quillardet et al., 1985). Mutagens cause permanent modifications in DNA structure in both prokaryotes and eukaryotes (Starr and Taggart, 1987). Clastogens result in chromosomal changes in eukaryotes (Adler, 1984). Carcinogens cause gene mutations leading to cancer in eukaryotes (Starr and Taggart, 1987). Teratogens cause birth defects (Weis and Weis, 1987). The

differences between these genotoxins are not absolute. For example, 83% of carcinogens tested were also known mutagens according to the Ames test of Maron and Ames (1983). Theoretically, mutagens cause gene mutations, and consequently they may result in chromosomal changes, and finally they may result in the appearance of abnormalities. Because there is little difference between genotoxic groups, especially when one considers eukaryotes and prokaryotes, the grouping of genotoxins is quite confused. Some authors limit mutagens to DNA damaging agents in bacteria (McCann et al., 1975), while others expand their definition of mutagens to include carcinogenic potentiality in higher eukaryotes (Quillardet et al., 1985; Metcalfe et al., 1985). In this thesis the term "genotoxicity" is used in a restricted sense (e.g., DNA damaging agents) as a large range of organisms from bacteria to insect larvae were analyzed in my study.

For the purpose of evaluating the genotoxic potentiality of waste materials in the environment, genotoxicologists have sought a widely available and uncomplicated method of detecting potential genotoxic compounds. Different methods for detecting potential genotoxic materials have been developed (Venitt and Parry, 1984; de Serres and Matsushima, 1987). The yeast *Saccharomyces cerevisiae* has been used as a simple eukaryotic system for detecting genotoxicity of chemicals (Parry and Parry, 1984). Cultured mammalian cells have also been used in estimating genotoxicity of chemicals and environmental samples (Dean and Danford, 1984). However, bacteria are the most widely used test

organisms in genotoxic studies (Hollstein and McCann, 1979).

The Ames test

The Ames test is a well-known mutagenicity test which has been widely used since 1975 to detect drug genotoxicity as well as chemical and environmental genotoxicity (Ames et al., 1975; Walker, 1984; Dutka et al., 1981; Samoiloff et al., 1983; Sato et al., 1983). The Ames test employs Salmonella bacteria which carry a mutation at an easily detected locus, and the mutation could be reversed by mutagens. An increase in the frequency of revertants is related to increasing dosage levels of genotoxic compounds.

Fly ash collected from an oil-burning power plant was demonstrated using the Ames test to contain mutagens (Wei et al., 1984). The acid, base and neutral fractions of samples from petrochemical plant wastes were also found to be mutagenic using the Ames test (Brown and Donnelly, 1984). Metcalfe et al (1985) found that the genotoxic materials associated with the discharge of particulate material in oil refinery effluents were present in the most polar silica gel fraction. Some extensively used pesticides such as organophosphorous insecticides (Usha Ranni et al., 1980) were also demonstrated to be genotoxic using the Ames test.

Many freshwater bodies have been used as dumpsites for waste materials. Metcalfe et al. (1985) observed a positive relationship between the genotoxicity of particulate materials in petroleum refinery effluents and the concentration of oil and grease samples by using the Ames test and the carcinogenicity

test. Samoiloff et al. (1983) reported Tobin Lake (Canada) was contaminated by agricultural runoff, and waste discharge from mining, petrochemical industries, paper mills and municipalities. The neutral compounds extracted from sediments of the lake showed mutagenic activity when tested with the Ames test. Sato et al. (1983) using the Ames test found that some sediments which they removed from the Niagara River and its feeder streams were mutagenic.

The SOS Chromotest

The SOS Chromotest is both more sensitive and more specific than the Ames test (Quillardet et al., 1982), and was therefore adopted in this study as the principal genotoxicity test. The first report on the SOS chromotest was made by Quillardet et al. in 1982.

The SOS chromotest is based on the "SOS" response to DNA-damaging agents in *E. coli* (Walker, 1984; Little and Mont, 1982). When DNA damage or interference with DNA replication occurs, SOS-inducing signal is generated. The SOS signal activates RecA protease activity which cleaves LexA repressor. The LexA represses several SOS genes which response for DNA repair. In *E. coli* K-12 cells, the sfiA (sulA) gene encodes protein that inhabits cell division, possibly, to allow time for DNA repair (Watson et al., 1987). The sfiA gene is repressed by LexA, therefore, sfiA gene has the SOS function. The test strain (*E. coli* K-12 PQ 37) carries a sfiA::lacZ fusion, and has a deletion for the normal lac region so that beta-galactosidase (coded for by lacZ) activity is strictly dependent upon sifA

expression. Thus, the extent of any DNA damage is measured by quantifying the activity of beta-galactosidase in test cells (Quillardet et al., 1982). It has been shown that the results of the SOS chromotest are closely correlated with those of the Ames test (Quillardet et al., 1982; Quillardet and Hofnung, 1985; Dayan et al., 1987). The SOS chromotest was first used to measure genotoxicity of pure chemicals which were mutagenic and/or carcinogenic in other test systems (Quillardet et al., 1982). These studies revealed that the SOS chromotest was reliable in detecting genotoxicity. Genotoxicity studies of 83 compounds were made in order to compare the results of the SOS chromotest and the Ames test with the carcinogenicity test. It was concluded that there were no false positives with the SOS chromotest (e.g. non-carcinogen but SOS inducer) among the 83 tested chemicals. The Ames test on the other hand produced a number of false positives (38%) (Quillardet et al., 1985). Three out of 42 chemicals which displayed mutagenicity in the Ames test did not induce the SOS response in the SOS chromotest (Ohta et al., 1984).

Although the SOS chromotest has been used in examining pure chemicals (Quillardet et al., 1985; Ohta et al., 1984; Olivier and Marzin, 1987), only a few environmental studies using the SOS chromotest have been carried out (Xu and Dutka, 1987). Six samples taken from contaminated areas along Prince Edward Island were tested with the SOS chromotest, five of the six samples displayed weak genotoxicity (Xu and Dutka, 1987).

The SOS chromotest has a major advantage over the Ames test.

In the SOS chromotest, the test strain does not need to survive for as long a time (only two hours) as it does in the Ames test (48 hours). Therefore, the SOS chromotest can be used for detecting toxic mutagens which are undetectable in the Ames test because they kill the bacteria (Quillardet et al., 1982). Since the SOS chromotest has some major advantages over the Ames test (ie. it gives a quantitative response within a few hours and it is more sensitive than the Ames test), it was used as the primary mutagenicity screening tool in this thesis research.

Deformities of benthic invertebrates exposed to mutagenic substances

Because it is well recognized that some pollutants cause mutations, the studies of freshwater pollution were expanded to consider the frequency of benthic invertebrate deformities (Hamilton and Saether, 1971). Genotoxic pollution is a concern because such pollutants frequently accumulate without causing the death of organisms. Instead, long term disabilities occur (Baumann, 1984).

Studies in France revealed that in drinking water, mutagenic substances could be detected by using a micronuclear test (Jaylet et al., 1987). Milbrink (1983) found that the frequency of chaetal deformities in oligochaetes was significantly correlated with mercury pollution in Lake Vanern, Sweden.

Benthic invertebrate chironomid larvae represent some of the most widely distributed genera in freshwater (Roback, 1978). In addition, their larvae represent a sensitive stage in their life

cycle. Research on the frequency of deformities of chironomid larvae in polluted freshwater (Hamilton and Saether, 1971) led to the use of deformities of mouth parts, antennae and chironomid body wall thickness (Hamilton and Saether, 1971; Wiederholm, 1984; Warwick et al., 1987) detecting genotoxic pollution.

The mouthparts of chironomid larvae normally are symmetrical. Deformed specimens are characterized by asymmetrical labial plates and mandibular structures. Unusual thickenings of the chironomid larval body wall were also considered as a deformity by Hamilton and Saether (1971). It was suggested that different types of deformities may be caused by different types of pollutants (Hamilton and Saether, 1971). The susceptability to these malformations seemed to differ among the various chironomid species (Wiederholm, 1984). Some species were far more sensitive to mutagens than others.

In a study of Lake Erie, it was found that deformed chironomid larvae were generally restricted to locations near known sources of industrial pollution (Hamilton and Saether, 1971). Sediment core studies of the Bay of Quinte in Lake Ontario, revealed that the presence of deformed chironomid larvae increased inversely with depth (in most cases, the age of sediment core is determinated by depth, the deeper the older). The increase in deformed chironomid larvae was associated with increased pollution in the bay (Warwick, 1980).

The studies of heavy metal (Hg, Zn, Cd, Ni, Pb, Cu and Cr) polluted lakes in Sweden concluded that in these lakes there were much higher occurrences of deformed chironomids than in

unpolluted lakes (Wiederholm, 1984). Experimental pond studies showed that pollution by a synthetic, coal tar-derived oil could induce a high frequency of deformities in Chironomus decorus (Cushmam, 1984). It was suggested that antennal deformities in chironomid larvae could also be used as a biological screening marker for the detection and evaluation of mutagenic contaminants in aquatic ecosystems (Warwick, 1985). It was found that the number of deformed antennae in Chironomus sp. was higher in samples from Tobin Lake (contaminated with chemical pollutants and toxic residues of agricultural and industrial waste) than Last Mountain Lakes which were comparatively uncontaminated. In the study of Chironomid larvae collected from sediments in the harbour at Port Hope, Ontario, it was found that a direct relationship between the degree of sediment radioactivity and the frequency of deformities in Chironomus spp. existed (Warwick et al., 1987). Hamilton and Saether (1971) reported that aberrant mouth parts, thickened body walls and head capsule malformations might be caused by pollutants from agricultural as well as industrial sources.

Mechanisms of deformity induction by genotoxins

The mechanisms of deformity induction include gene mutations, interference with transcription and translation, disruption of cell division, and metabolic disturbance (Weis and Weis, 1987; Jaylet et al., 1987). Mutagenic or genotoxic compounds cause deformities in animals by disrupting genes (Metcalfe and Sonstegard, 1985). Genotoxic pollutants which

are discharged into water systems as waste materials accumulate in the associated sediments which comprise habitats of many benthic invertebrate taxa (Buikeman and Herricks, 1978; Lafont, 1984; Wetzel, 1983). Although the absolute concentration of genotoxic pollutants may be very low in water and sediment, bioconcentration and accumulation will serve to magnify them in the food chain (Buikema and Herricks, 1978; Tarkpea et al., 1985). For example, PCBs in the water were 8.2 ppt, while in Lake Trout (*Salvelinus namycush*) they were detected at 28.0 ppm (Metcalfe, 1977).

To locate the sources which cause the highest occurrence of deformities in chironomids, Hamilton and Saether (1971) tested several pesticides (including some known genotoxins) on lab cultures of *Chironomus* spp. The authors failed to observe the same high frequencies of deformed chironomid larvae as they obtained in their field study. They concluded that synergistic effects occur in the field which they could not duplicate in the lab. Warwick (1985) resorted the speciemns of *Chironomus* sp. which had been studied by Hamilton and Saether (1971), and he found that the antannae of those *Chironomus* sp. were deformed. Thus, the organs which are selected to be detected are important. It was concluded that antannae were more sensitive to low levels of genotoxins, while labial plates (mentum) and mandibles were sensitive to higher levels (Warwick, 1985).

The studies in experimental ponds revealed that a coal oil derived product did increase the frequency of deformities in *Chironomus decorus* labial plates (Cushman, 1984), while in other

studies, it was found that petroleum refinery effluents (Metcalfe et al., 1985) and some crude oils (Vandermeulen et al. 1985) were genotoxic. It was suggested that genotoxic sediments in water bodies cause a high frequency of deformities in benthic invertebrates (Warwick et al., 1987).

To the best of the author's knowledge, there are no published studies of sediment genotoxicity using the SOS chromotest and the frequency of benthic invertebrate deformities for the same sample locations.

Toxicity vs genotoxicity of environmental samples

Some genotoxins act also as general toxins, e.g. $K_2Cr_2O_7$ (Olivier and Marzin, 1987), and neocarcinostatin (Quillardet et al., 1982). It was noted that some environmental samples might contain these so-called toxic mutagens. Vandermeuleu et al. (1985) found that crude oils and oil products were toxic to the Ames test bacteria. Chemical fractions of sediments from comtaminated Tobin Lake demonstrated in biological assay systems that major toxic constituents of the sediments were neutral compounds eluted from the Florisil columns by 1:1 hexane-dichloromethane, and those fractions were the major mutagenic constituents of the sediments (Samoiloff et al., 1983). Sediment extracts of samples from the Niagara River and its feeder streams appeared to have high levels of toxicty (Sato et al., 1983). Therefore, genotoxic pollutions could exert their effects on the benthic invertebrate commmunity in two ways: 1.) DNA-damaging activity. 2.) direct toxicity to the organisms.

Underestimation of the genotoxicity of environmental samples can be due to the toxic effects of the contaminated samples (Vandermeulen et al. 1985; Metcalfe et al. 1985). The SOS chromotest has another advantage over the Ames test; the SOS chromotest can also detect the toxicity of samples by their suppression of alkaline phosphatase activity (Quillardet et al. 1982). In the SOS test strain, E. coli K-12 PQ 37, alkaline phosphatase is noninducible by genotoxins. It is constantly synthesized by living cells. Monitoring the alkaline phosphatase activity can screen for the survival of the bacteria. If a tested sample causes a decrease of alkaline phosphatase activity, it can be considered as a protein biosynthesis inhibitor and therefore, the sample is toxic to the bacteria (Quillardet et al., 1982). In my study, the toxicity of samples was estimated since their toxicity could have serious impacts on the aquatic benthic invertebrate community.

Measures of generic richness

Changes in species composition and density in a benthic invertebrate commmunity can be related to the level of toxic materials entering the environmnet (Hart and Fuller, 1974; Buikman and Herricks, 1978; Wiederholm, 1984). Earlier studies of freshwater toxins in the environment were mainly field studies.

Species diversity and species richness are two ways to characterize community structure. In a normal aquatic system, the number of individuals of each species is comparatively lwo, and the number of species is high. As the pollution levels increase,

a reduction in the complexity of the community is observed as a reduction in the the number of species. At the same time the abundance of a few species generally increases (Cairns et al., 1972; Hart, 1974).

Cushman (1984) reported that a coal-derived oil product reduced the species diversity of benthic insects in his experimental study ponds. In Lytle Creek, U.S.A., an aquatic invertebrate community survey was conducted, and it was found that upstream of a sewage outfall, higher species richness occurred than at the discharge site, and with distance downstream the species richness recovered (Gaufin et al., 1952). Thus, species richness generally decreases when toxic pollutants enter an aquatic system (Cairns et al., 1972; Cushman, 1984). In the Welland River, aquatic macrophytes and attached microbiotic species richness decreased downstream of a number of industrial discharge sites, and then recovered with distance (Dickman et al. 1983 and 1988; ; Albanese et al., 1988). Fish populations declined in species richness where waste materials from agricultural and industrial effluents in the Welland River were high (Steele, 1981).

By comparing the species pattern of benthic invertebrates in normal (control areas) with that of polluted areas, it has been found that not only do species composition and density change, but a shift in the dominant species also occurs (Buikema and Herricks, 1978). Therefore, it was concluded that those dominant species surviving in polluted environments were tolerant of the sort of pollutants to which they were exposed. It was

found that a high relative abundance of tolerant species in a particular location was related to the specific pollutants in each location (Hart and Fuller, 1974).

MATERIALS AND METHODS

The Welland River

The Welland River is a slow flowing, shallow river and it is a provicially significant class 1 wetland in Niagara. The river begins in Ancaster, flows east to the Niagara River which brings water into Lake Ontario. Upstream of the City of Welland the river passes through agricultural areas while downstream of Welland the river passes an industrialized area (Fig. 1).

Sampling sites

Site A was the upstream Welland River control site located at the confluence of Beaver Creek and the Welland River, in the agricultural area, upstream of the City of Welland. The emergent aquatic vegetation at this site was dominated by *Potamogeton* spp., *Nuphar* spp., *Nymphaea* spp., *Typha* spp., *Phragmites* spp., *Sagittaria* spp., *Pontederia* spp., *Polygonum* sp., *Vallisnaria* spp. and *Cladophora* spp.

Site B was the Welland River intensive agricultural site located at the confluence of the OWMC Swale and the Welland River in a corn and wheat agricultural area, downstream of site A, and upstream of the City of Welland. The aquatic emergent vegetation at this site was similar to that at site A.

Site C was located at the confluence of the discharge pipe of the Atlas Specialty Steels Co. and the Welland River, downstream of the City of Welland. The mean discharge from the Atlas-Mansfield storm sewer from which their waste stream entered the Welland River was 2458.9 m^3/day (Ontario Ministry of the

Environemt (MOE), 1987). Their effluent contained high levels of cadmium, nickel, chromium, lead, zinc, copper, cobalt and iron (Appendix 1). The aquatic emergent vegetation at this site was dominated by Sagittaria spp., and Phragmites spp.

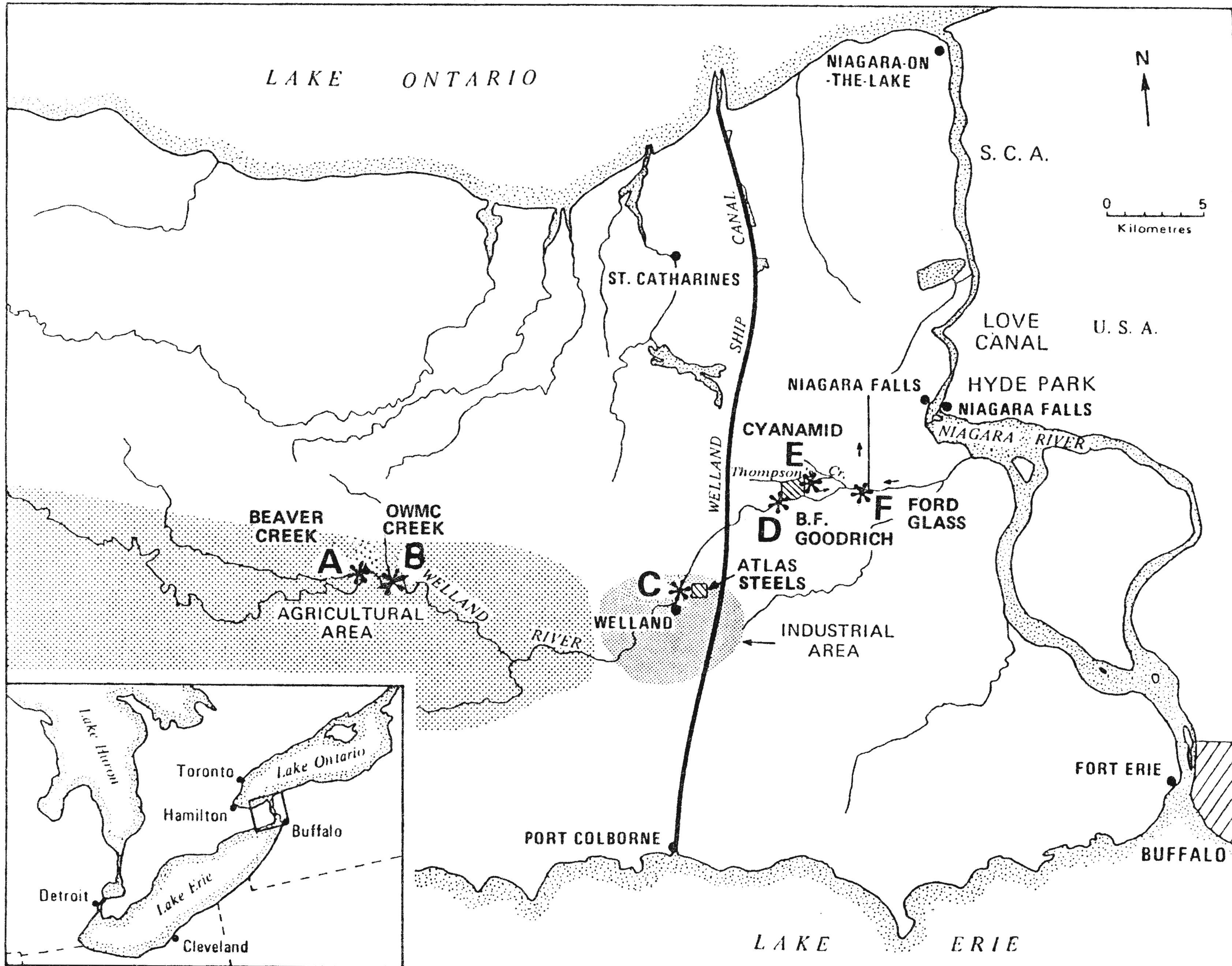
Site D-1 was located below the discharge pipe of the B.F. Goodrich Co. which produces polyvinyl chlorides. The mean discharge from the sewer was 2213.58 m^3/day (MOE, 1987). Biological Oxygen Demand (BOD), a measure of the quantity of oxidizable material present in a water sample, had values of the effluents during 8 out of 12 months higher than the MOE acceptability level, and the discharge contained ammonium ion, vinyl chloride and phosphate. The emergent vegetation at this site was dominated by Potamogeton spp., and Typha spp.

Site D-2 was located at the confluence of the discharge stream of the B.F. Goodrich Co. and the Welland River just downstream of site D-1. The emergent vegetation at this site was dominated by Sagittaria spp., Cladophora spp., and Typha spp.

Site E was located in Thompson Creek which was used as the main discharge stream of the Cyanamid Chemical Co. The company produces organic and inorganic nitrogen and phosphorus products. The mean discharge to this creek from Cyanamid was 27342 m^3/day (MOE, 1987). The effluents contained detectable concentrations of cyanide, ammonia, urea, nitrate, phosphorus, chromium, nickel, and zinc. There was no aquatic vegetation at this sample site due to the high toxicity of their ammonia waste discharges during frequent shock loading events (Dickman et al., 1983 and 1988).

Site F was located at the confluence of the discharge channel of the Ford Glass Co. with the Welland River, downstream of site D-2. The discharge from their sewer was 8,554 m^3/day (MOE, 1987). The effluents contained iron, ammonium ion and phosphate. The dominant emergent aquatic plant species at this site were *Typha* spp., *Sagittaria* spp., and *Pontederia* spp.

Figure 1. Map of the study area (modified from Dickman et al., 1983). Sites A, B, C, D (D-1 and D-2), E, and F were the locations where samples were taken for both the SOS chromotest and benthic invertebrate analyses.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WELLAND
INDUSTRIAL AREA
Lake Huron
Toronto
Lake Ontario
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Cleveland
FORT ERIE
PORT COLBORNE
BUFFALO
LAKE ERIE

Sample preparation for genotoxicity tests

Seven sets of sediment samples were collected from the Welland River at the same sites as previously described in December of 1986 and April of 1987 (Fig.1) The sediments for genotoxicity testing were placed into sterilized plastic bags and within 2 hours were returned to the laboratory and stored at 4°c in a refrigerator. Extraction of samples was done within 24 hours of sample collection.

The dimethyl sulfoxide (DMSO) sediment extracts were done by adding 25 g wet weight of sediment to 25 ml of 10% DMSO in distilled water. The bottle was then stoppered and vortexed for 2 minutes to make the contents homogeneous. The mixture was then poured into a sterilized centrifuge tube and centrifuged for 20 minutes at 2000 rpm at 4°C (IEC CENTRA-7R refrigerated centrifuge). The suspension was next filtered through a 0.22um filter. This was done for purposes of sterilization to permit its use in the SOS chromotest which requires sterilized constitients. The distilled water extract procedure was similar to the DMSO extraction procedure except that ultra-pure distilled water replaced the 10% DMSO solution.

SOS chromotest bacterium

The E.coli K-12 PQ 37 strain was kindly provided by Dr. B.J. Dutka of the Canada Centre for Inland Waters. The genetic markers of this strain are thr, leu, his, pyr D, thi, gal E, gal K, Iac U169, sr1300::Tn10, rpo B, rps L, sfiA::Mud (Ap Lac) cst, rfa,

uvrA, trp::Muc' and Pho' (Quillardet et al., 1982).

Media and Buffers for the SOS chromotest

L medium: 1% Bacto tryptone, 0.5% Bacto yeast extract, 1% NaCl, pH 7.2. Bacteria were cultured in L medium supplemented with 20 ug/ml ampicillin.

B buffer: Na_2HPO_4 16.10g, NaH_2PO_4 .$2H_2o$ 5.5g, KCl 0.75g, $MgSo_4$· H_2o. 0.25g, sodium dodecyl sulfate(SDS) 1g, beta-mercaptoethanol 2.7ml per 1 double distilled water, adjusted to pH 7.0 with HCl or NaOH.

P buffer: tris(hydroxymethyl)aminomethane 121g, SDS 1g per 1 double distilled water, adjusted to pH 8.8 with HCl.

ONPG solution (4 mg/ml): 400mg O-nitrophenyl-b D-galacto-pyranoside (ONPG) per 100 ml of 0.1 M phosphate buffer pH 7.0.

PNPP solution (4 mg/ml): 400 mg p-nitrophenyl phosphate disodium (PNPP) per 100 ml of P buffer.

Activation mixture (S9 mixture): per 10 ml of rat liver S9 (purchased from Litron Laboratories Ltd), salt solution (1.65 M KCl + 0.4 M $MgCl.6H_2o$) 0.2 ml; G6P(1 M) 0.05 ml; NADP(0.1 M) 0.15 ml; P buffer 2.5 ml; L medium 6.1 ml; S9 1 ml.

The SOS Chromotest

The SOS chromotest test procedure was based on the published methods of Quillardet et al. (1985). A 0.1 ml fraction of the overnight culture was reconstituted in 5 ml of fresh L medium at 37°C for 2 hours,

One ml of reconstituted culture (OD 600 = 0.15-0.2) was then diluted in 9 ml of fresh L medium (without metabolic activation)

or 9 ml of fresh S9 mixture (with metabolic activation). Fractions of 0.3 ml were placed into a series of sterilized test tubes (ice bathed) containing 20 µl of sample extract to be tested. The mixture was incubated by shaking for 2 hours at 37°C. After incubation, the fractions were used for beta-galactosidase and alkaline phosphatase assays. Two parallel series of test tubes were set up at the same time and under the same conditions for these two enzyme assays. In order to screen the pro-genotoxic materials, an S9 mammalian microsomal fraction was used in the SOS chromotest.

<u>Optimal chromotest development time</u>

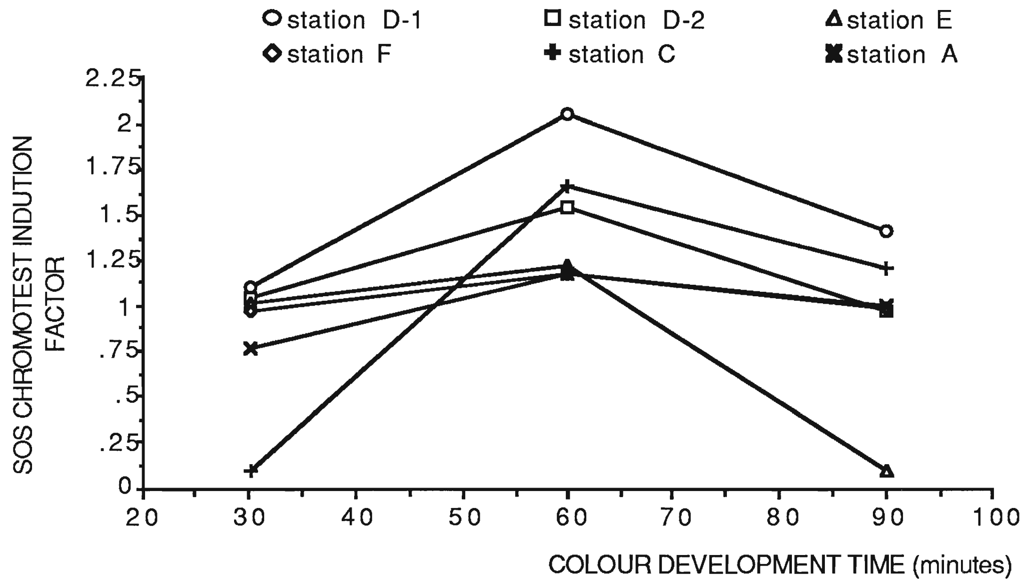
To determine the optimum SOS chromotest time, several tests for induced colour development time were conducted. The results of the optimum SOS chromotest time test showed that induced colour development of 60 minutes was the optimum time (Fig. 2). Therefore, the results of this study were reported as 60 minutes colour development times except where otherwise noted.

<u>Assays for beta-galactosidase</u>

To each test tube 2.7 ml of B buffer was added to the beta-galactosidase series and incubated at 37°C for 15 minutes. The enzyme assay was then initiated by the addition of 0.6 ml of ONPG solution per tube. The assay of color development was made at 30 minutes, 60 minutes, and 90 minutes after addition of the substrate.

The absorbance at 420 nm was read against a colorimeter blank consisting of an assay in which the bacterial culture had

Figure 2. The SOS chromotest induction factor and colour development time (test concentration = 20 mg wet weight /assay).



SOS CHROMOTEST INDUCTION FACTOR AND THE COLOUR DEVELOPMENT TIME

been replaced by either L medium in experiments performed without metabolic activation or activation mixture (S9 mix) in experiments performed with metabolic activation.

Assays of alkaline phosphatase

The alkaline phosphatase assay was similar to that of the beta-galactosidase assay except that instead of B buffer, P buffer was used and instead of ONPG solution, 4mg/ml PNPP solution was used.

Controls for the SOS chromotest

Positive controls in each set of assays were included. 4NQO (4-nitroquinoline-N-oxide) was used in the experiments without metabolic activation and 2AA (2-aminoanthrancene) was used in experiments with metabolic activation. The results obtained from the positive controls were compared with previously published values (Quillardet et al., 1985; Xu and Dutka, 1987).

Negative controls in each set of assays were also included. 10% DMSO solution and double distilled sterilized water were used in all experiments using DMSO extract and ultra-pure distilled water extracts.

The induction factor for each sample was compared with the negative control to determine the statistical significance of the test.

Calibration of the SOS chromotest

The units of enzyme activity were calculated according to Quillardet and Hofnung's (1985):

$$\text{Enzyme units} = \frac{1000 \times A420}{t}$$

In this formula, A420 is the optical density at 420 nm read from the incubation mixture, and t is the length of time for incubation in the presence of the substrate (ONPG or PNPP) in minutes.

The ratio of beta-galactosidase units to alkaline phosphatase units reflects the induction of the sfiA gene (Quillardet et al., 1982):

$$R = \frac{A420\ B \times tP}{A420\ P \times tB}$$

where A420 B and A420 P represent the optical density at 420 nm read from the incubated mixture. Where tB and tP represent the reaction time from beta-galactosidase and alkaline phosphatase. The induction factor I(c) is

$$I(c) = \frac{R(c)}{R(o)}$$

where R(c) and R(o) represent the ratio (R) at concentrations (c) and (o), respectively. The induction factor of each sample was compared with the negative control in order to evaluate the statistical significance of the test.

Ekman dredge samples

Invertebrate samples were collected from the bottom of the Welland River (10 to 40 cm depth) at seven locations during August and September of 1987. An Ekman Dredge (15.3 x 15.3 x 23.0 cm) was used to collect the sediment samples. The dredge was set up and placed on the surface of the sediment and carefully pressed into the mud to a depth of approximately 4 cm. Before I lifted the dredge out of the sediment, I checked to see that the jaws were tightly closed. At least three replicate dredges were randomly collected at each location and then mixed together to form a single composite sample which was then placed into a plastic bag and returned to the laboratory. Three of these composite samples were taken at each location (ie. 9 Ekman samples) and these were returned to the laboratory for sorting.

Sample preservation and mounting techniques

K.A.A. solution (Pimentel,1967):

kerosene	10 ml
acetic acid	20 ml
95% ethanol	100 ml

5% formalin solution:

10% formalin	50 ml
distilled water	50 ml

Ammans Lactophenol (Pimentel, 1967):

kerosene	20 ml
lactic acid	20 ml
glycerol	40 ml

distilled water 20 ml

20% Glycerin solution (Pennak, 1953):

glycerin 20 ml

70% ethanol 80 ml

70% ethanol:

95% ethanol 70 ml

distilled water 25 ml

Separation of the invertebrates

Separation of invertebrates from the sediment sample was done within 12 hours of sample collection. The collected sample, a mixture of mud or clay and water, was poured onto a large white enamel plate and gently mixed with clean water so that the movement of invertebrates could be easily observed. The invertebrates were carefully separated from the sample with forceps and an eye dropper. The separated invertebrates were preserved in K.A.A. solution (Pimentel, 1967). After one day, I replaced the K.A.A. solution with 5% formalin, and stored the invertebrates in vials at room temperature. All vials were coded by Dr.M. Dickman (my thesis supervisor) to avoid unconscious bias before classifying and counting the specimens at each station.

Classification of the invertebrates

Two days before the identification of the specimens, I replaced the 5% formalin solution with a 70% ethanol solution. For Annelida, one day before identification, I placed the specimens in Ammans Lactophenol solution. For Diptera, one day before observation, I placed the specimens into 20% glycerin

solution.

The annelids were carefully picked from the vials, and placed on clean glass slides, mounted in Ammans Lactophenol solution and covered with a glass cover slide. Next, they were each examined under a Leitz compound microscope at 400 x magnification. Dichotomous keys (Pennak, 1953; Pimental, 1967; Brinkhurst 1986) were used for identifying the collected annelid specimens.

The specimens of Diptera were mounted in pure glycerin solution, observed under a Leitz microscope at 400 x magnification. A dichotomous key (Pennak, 1953) was used for classifying the specimens (Wiederholm, 1983).

Large specimens of other classes were observed under the dissecting microscope. Smaller ones mounted in 70% ethanol solution were identified by using a Leitz compound microscope. Dichotomous keys to the benthic invertebrates in Pennak (1953) were used for identification at generic level.

Evaluation of symmetry patterns and abnormalities

The symmetry of each invertebrate was observed (ie., labial plates of each chironomid specimen were checked for symmetry). If a deformed structure was found, it was recorded (see results section). The number of chironomids and the number of malformations observed were listed and were later transcribed into tables (see results section).

Statistical Analyses

The Student's "t" test was used to determine the difference between controls and tested samples (Sokal and Rohlf, 1981).

The Spearman-Kendall rank correlation analysis (Sokal and Rohlf, 1981) was used for non-parametric tests and the relationship between the frequency of chironomid larvae labial plate deformities and the SOS chromotest induction factor level

Correlation coefficients were used for parametric tests (Sokal and Rohlf, 1981).

Calculation of Species Diversity Indices

Simpson's diversity index (Simpson, 1949):

$$D = \frac{N (N - 1)}{\Sigma n (n - 1)}$$

where N was the total number of specimens, n was the individual number of different species.

Shannon-Wiener's diversity index (Shannon, 1948):

$$H = - \sum_{i=1}^{k} p_i \log_2 p_i$$

where p_i was the proportion of individual species, and k was the number of species.

RESULTS

Genotoxicity of sediment samples

Distilled water extracted sediment samples were tested using the SOS chromotest. All samples gave a negative response to the SOS chromotest with or without metabolic activation (Table 1).

The 10% DMSO extracts of sediment samples yielded a higher induction factor value with metabolic activation than did the sample without metabolic activation (Table 1). With metabolic activation, the samples from site D-1, collected under the discharge pipe of the B.F.Goodrich Co. showed the highest induction factor (I.F = 2.053 ± 0.361), and the samples from station B, located in the agriculturally influenced portion of the Welland River, showed the lowest induction factor (I.F = 1.062 ± 0.066).

Four samples were collected in December, 1986, at stations D-1, D-2, E and F. The DMSO extract with metabolic activation for station D-1 gave a significant positive response ($P<0.01$), stations D-2 and E gave a weaker but still significant positive response ($p<0.05$). The genotoxicity of sediment extracts was higher in the industrial-influenced portion than it was in the agriculturally influenced portion of the river (Table 1). Without metabolic activation, all DMSO extracts of samples gave negative responses to the SOS chromotest (Table 1).

To confirm these results, another set of samples was taken at the same locations in the river four months after the first set of samples was taken, and other three samples from stations

Table 1. The SOS chromotest induction factor of sediment extracts from seven locations in the Welland River (test concentration = 20 mg wet weight/ assay).

Table 1. Induction Factor of seven samples

sample sites	10% DMSO extract +S9 (December,1986) mean	n	S.D.	10% DMSO extract +S9 (April,1987) mean	n	S.D.	10% DMSO -S9 (December,1986) mean	n	S.D.
A				1.173	6	0.123			
B				1.062	4	0.066			
C				1.664	10	0.429			
D-1	1.638*	4	0.135	2.053*	13	0.361	1.231	3	0.140
D-2	1.272	4	0.070	1.537	9	0.427	1.200	3	0.240
E	1.450*	4	0.168	1.209	10	0.126	0.995	3	0.140
F	1.049	4	0.032	1.179	11	0.209	0.978	6	0.058

Table 1. (continued)

sample sites	10% DMSO -S9 (April. 1987) mean	n	s.d.	Distilled Water Extract +S9 (December,1986) mean	n	S.D.	Distilled Water Extract +S9 (april, 1987) mean	n	S.D.
A	0.854	4	0.119				1.064	3	0.086
B	0.915	4	0.098				0.993	4	0.094
C	1.072	2	0.007				0.984	4	0.010
D-1				1.285	4	0.041			
D-2				1.059	4	0.084			
E				1.024	4	0.097			
F				0.964	4	0.026			

Table 1. (continued)

sample site	Distilled Water Extract -S9 (December, 1986) mean	n	s.d.	Distilled Water Extract -S9 (April, 1987) mean	n	s.d.
A				0.873	4	0.033
B				0.827	4	0.094
C				0.806	3	0.066
D-1	1.062	3	0.088			
D-2	1.031	3	0.078			
E	0.827	3	0.031			
F	1.200	6	0.441			

* positive response according to Student's "t" Test.

A, B and C were collected. At this time, only the DMSO extract of the sediment samples from station D-1 gave a significant positive SOS chromotest response with metabolic activation ($P<0.01$).

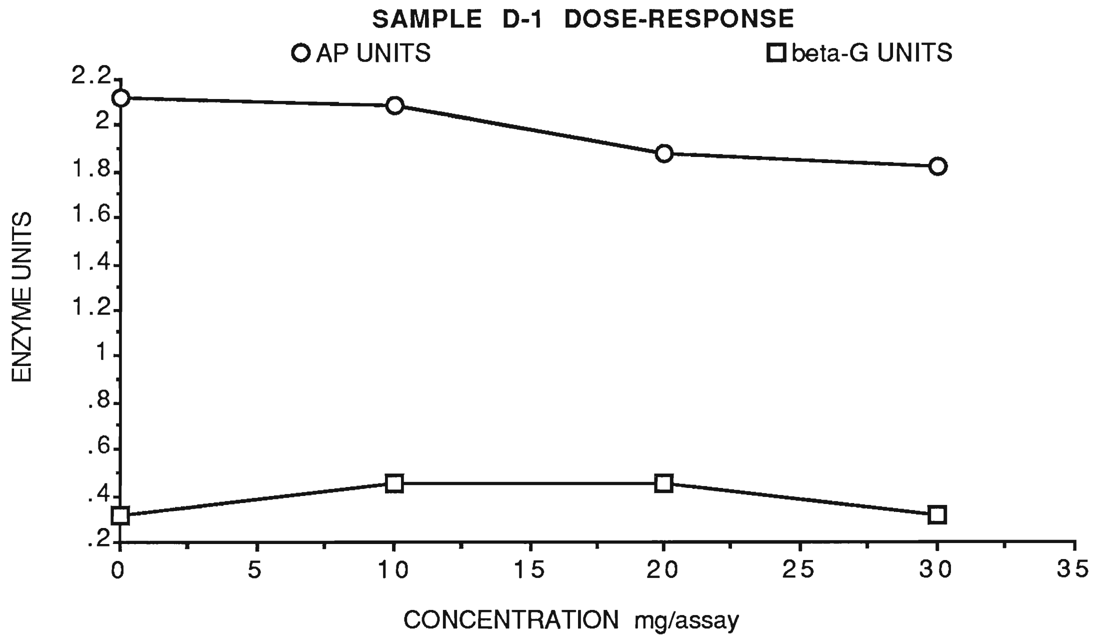
Of the samples assayed, only samples from station D-1 were pro-genotoxic in DMSO extract. To construct a dose-dependent response pattern for the samples which gave a positive response to the SOS chromotest, the enzyme activities of beta-galactosidase and alkaline phosphatase were assayed. The dose-response curve for samples from station D-1 showed that the induction factor rose to a maximum at a concentration of 20mg/assay, and then decreased (Fig. 3).

The results of the alkaline phosphatase assay for DMSO extracts with S9 for samples from station D-1 indicated that the enzyme units decreased with increasing sample concentration (Fig. 4). In DMSO extracts with S9 added, almost all samples except for those from station A had slight inhibitory effects on general protein biosynthesis when doses were increased (Fig. 5). According to the alkaline phosphatase activity levels (as percentage of initial level), DMSO extracts from stations C and D-1 showed a significant ($p<0.05$) inhibition effect on protein biosynthesis (Fig.4). DMSO extracts without S9 in the SOS chromotest did not appear to inhibit protein biosynthesis (Fig. 5), though station F did display a slight inhibition effect ($0.05< p <0.10$).

Among the distilled water extracts, only samples from stations C and D-1 showed a significant ($p<0.05$) inhibition of protein biosynthesis when tested with S9 (Fig. 6). Distilled

water extracts of samples from stations F without S9 slightly inhibited protein biosynthesis ($0.05<p<0.10$) (Fig. 7). These results implied that some water soluble contaminants in samples from stations C and D-1 were toxic when activated by metabolic activation.

Figure 3. The dose-response relationship for the SOS chromotest.


SAMPLE D-1 DOSE-RESPONSE
AP UNITS
beta-G UNITS
ENZYME UNITS
2.2
2
1.8
1.6
1.4
1.2
1
.8
.6
.4
.2
0
5
10
15
20
25
30
35
CONCENTRATION mg/assay

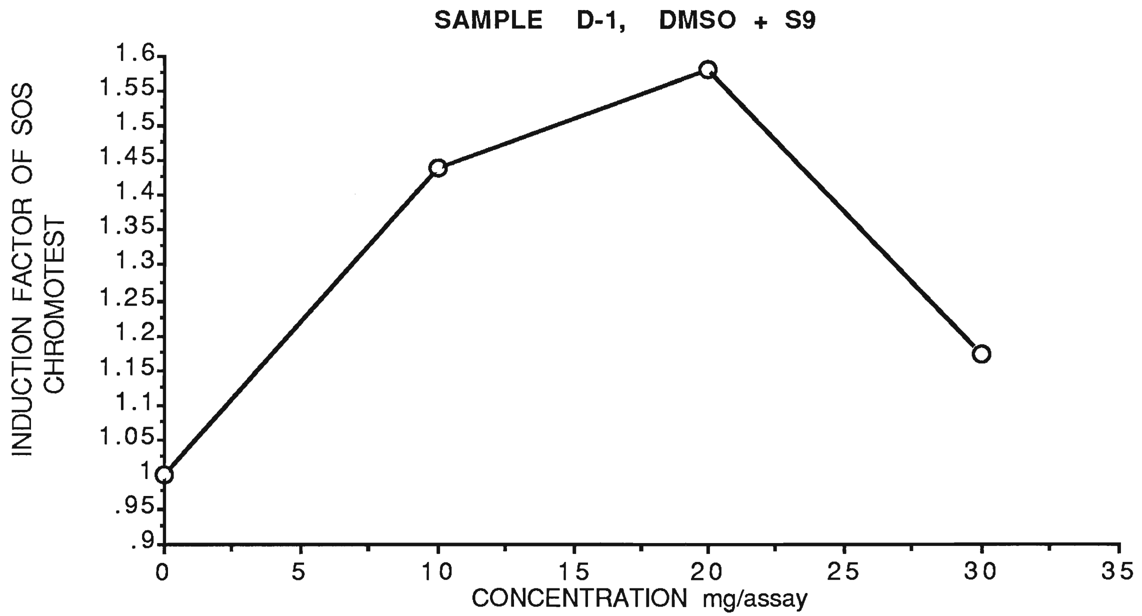

SAMPLE D-1, DMSO + S9
INDUCTION FACTOR OF SOS CHROMOTEST
1.6
1.55
1.5
1.45
1.4
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
.95
.9
0
5
10
15
20
25
30
35
CONCENTRATION mg/assay

Figure 4. The percent alkaline phosphatase activity in the SOS chromotest of 10% DMSO extracts with S9 (vertical bars represent one standard deviation); n = 3 or more.

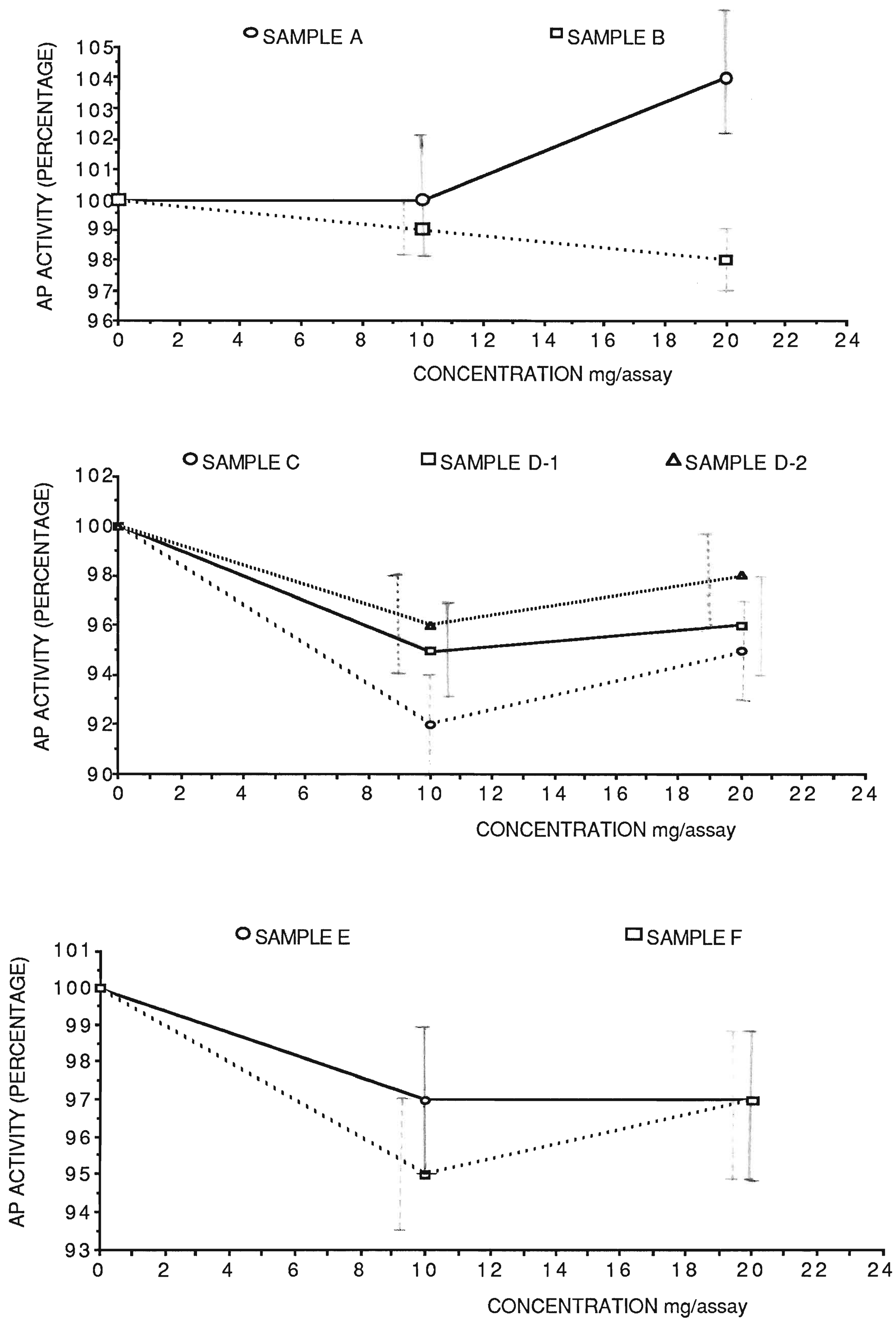
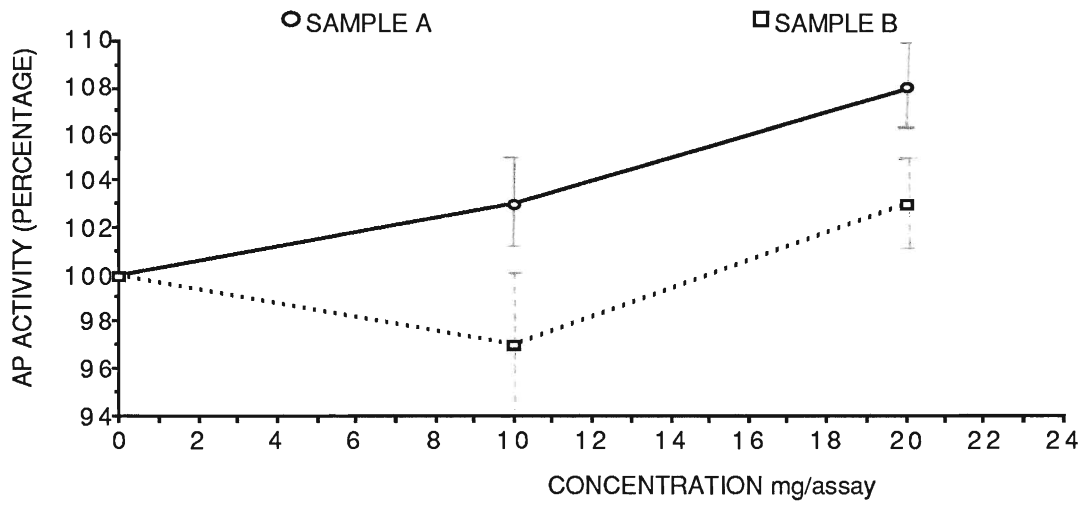
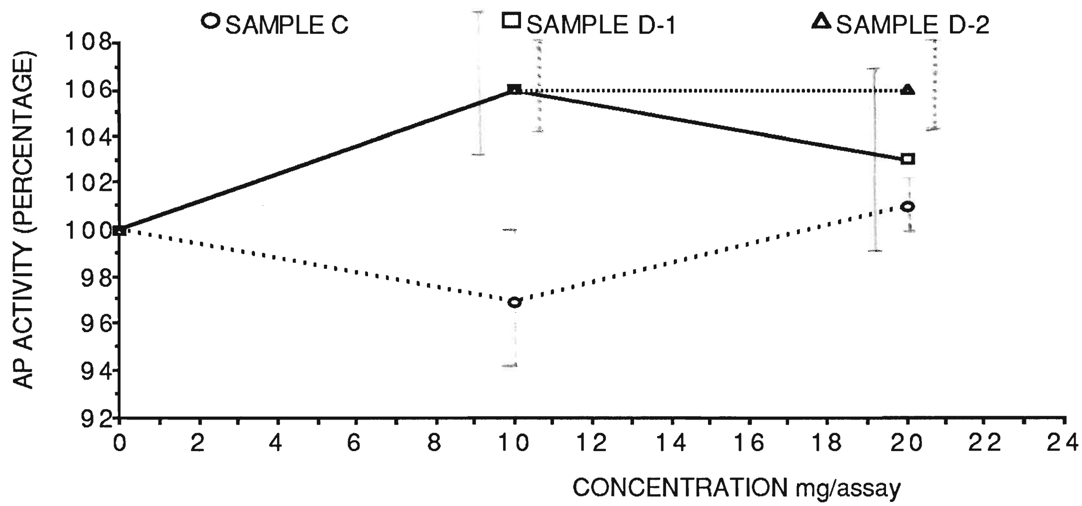

DMSO +S9
SAMPLE A
SAMPLE B
AP ACTIVITY (PERCENTAGE)
CONCENTRATION mg/assay
SAMPLE C
SAMPLE D-1
SAMPLE D-2
AP ACTIVITY (PERCENTAGE)
CONCENTRATION mg/assay
SAMPLE E
SAMPLE F
AP ACTIVITY (PERCENTAGE)
CONCENTRATION mg/assay

Figure 5. The percent alkaline phosphatase activity in the SOS chromotest of 10% DMSO extracts without S9 (vertical bars represent one standard deviation); n = 3 or more.


DMSO -S9
SAMPLE A
SAMPLE B
AP ACTIVITY (PERCENTAGE)
94
96
98
100
102
104
106
108
110
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCENTRATION mg/assay


SAMPLE C
SAMPLE D-1
SAMPLE D-2
AP ACTIVITY (PERCENTAGE)
92
94
96
98
100
102
104
106
108
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCENTRATION mg/assay

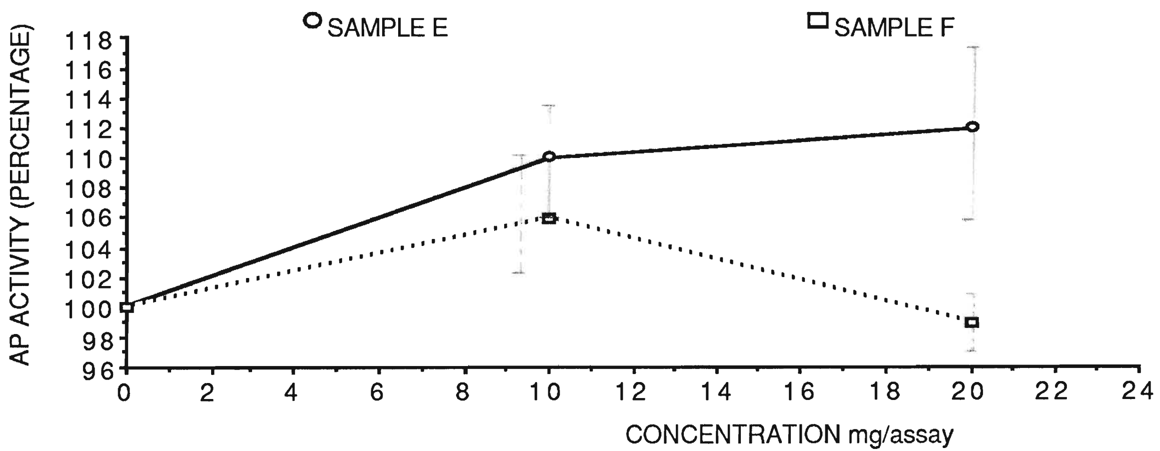
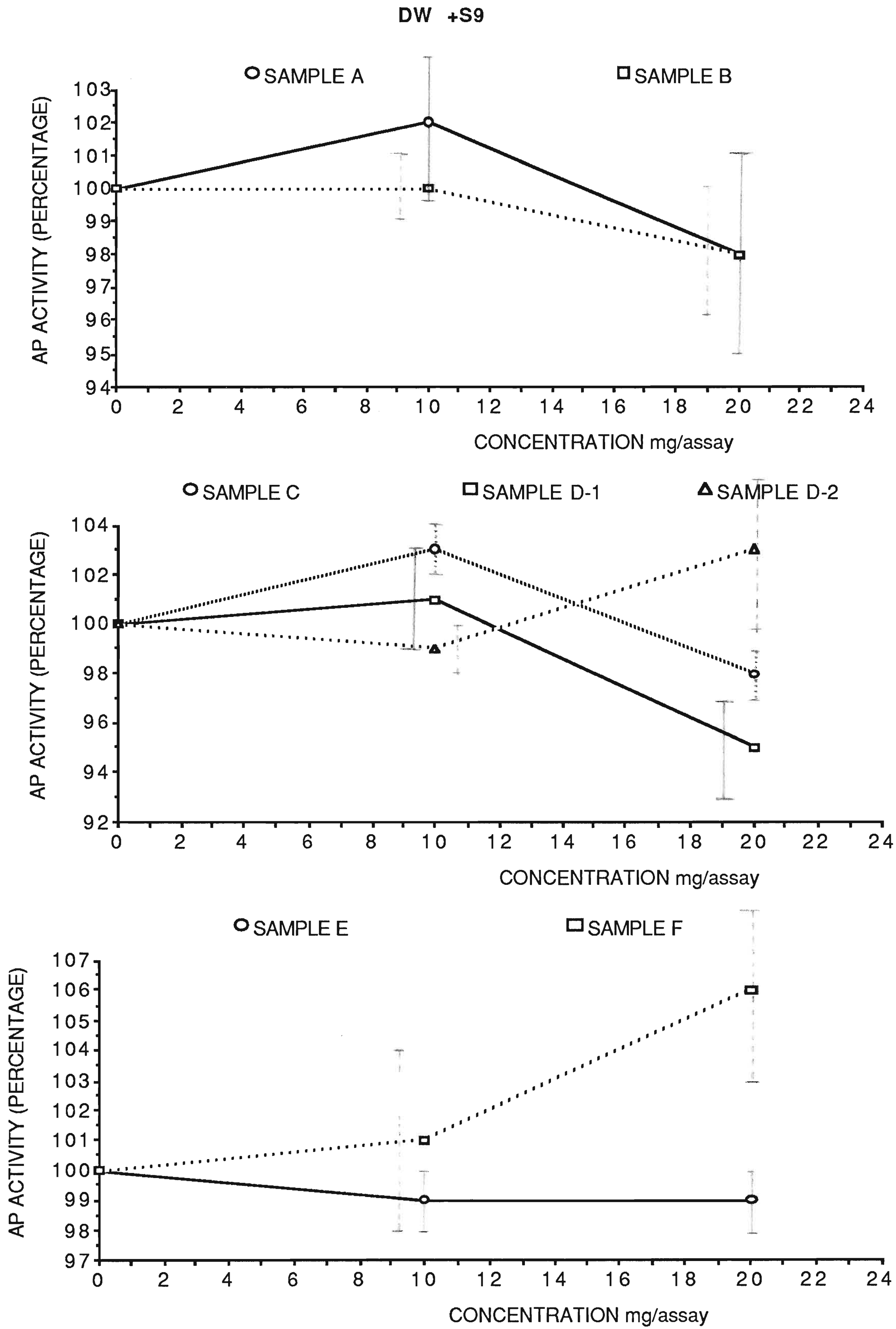
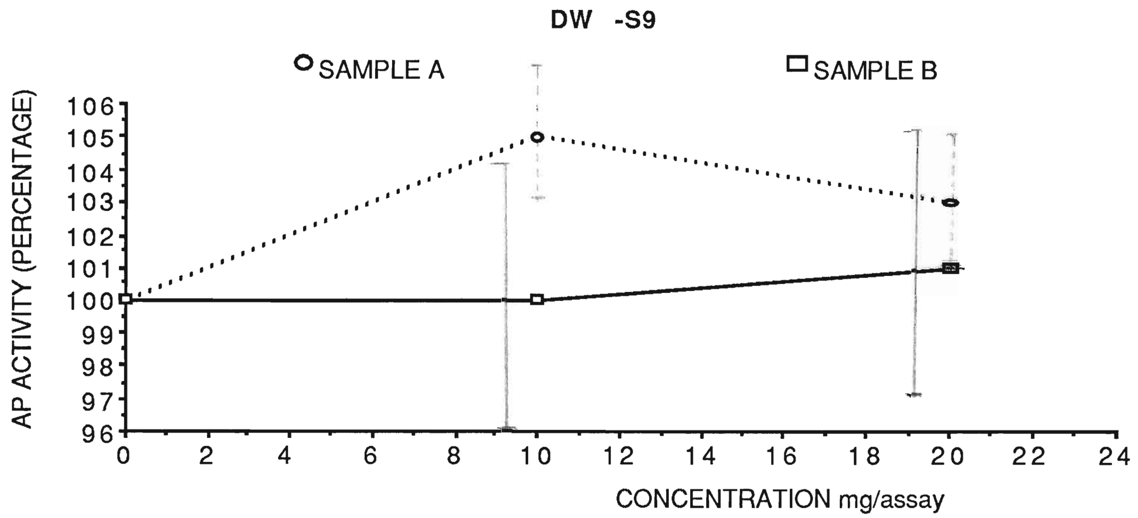

SAMPLE E
SAMPLE F
AP ACTIVITY (PERCENTAGE)
96
98
100
102
104
106
108
110
112
114
116
118
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCENTRATION mg/assay

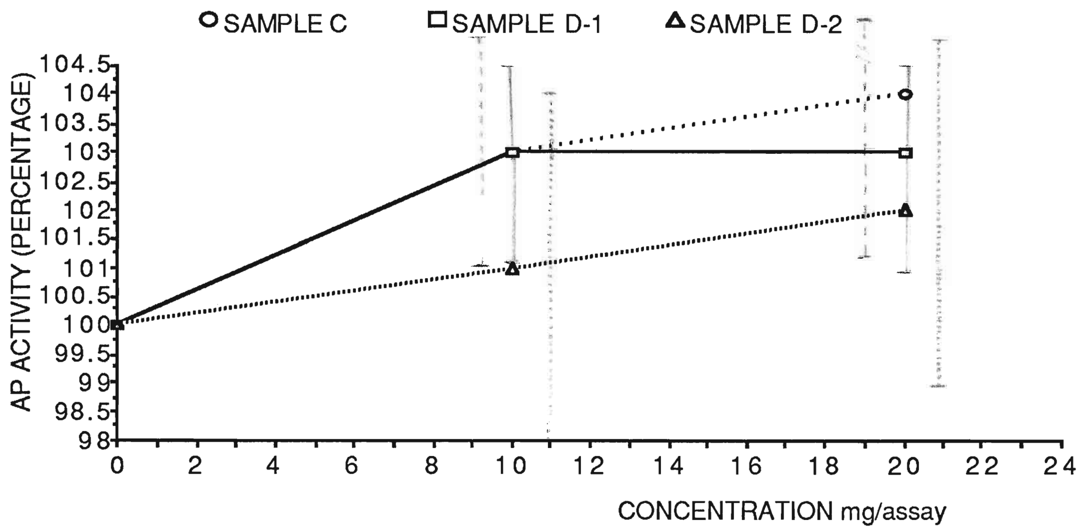
Figure 6. The percent alkaline phosphatase activity in the SOS chromotest of distilled water extracts with S9 (vertical bars represent one standard deviation); n = 3 or more.

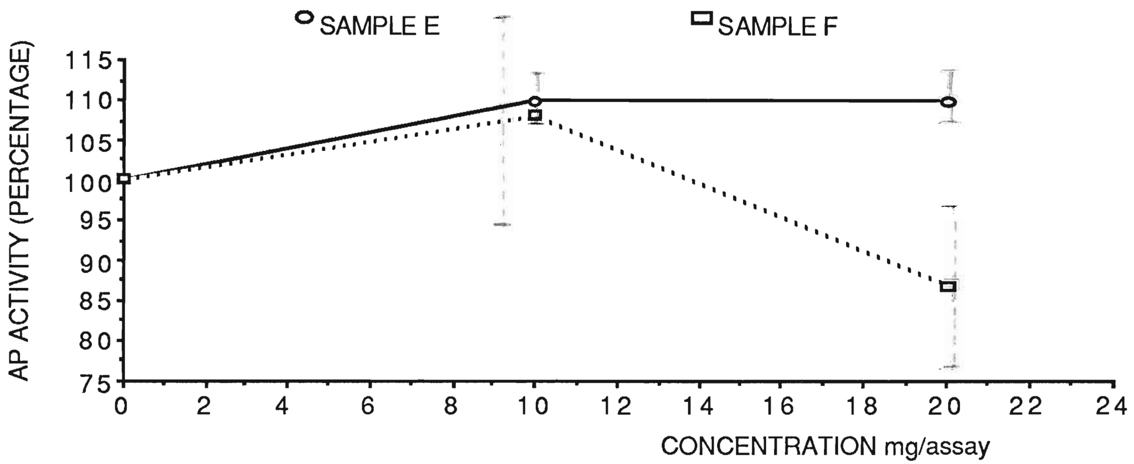


DW +S9
SAMPLE A
SAMPLE B
AP ACTIVITY (PERCENTAGE)
103
102
101
100
99
98
97
96
95
94
0
2
4
6
8
10
12
14
16
18
20
22
24
CONCENTRATION mg/assay
SAMPLE C
SAMPLE D-1
SAMPLE D-2
AP ACTIVITY (PERCENTAGE)
104
102
100
98
96
94
92
CONCENTRATION mg/assay
SAMPLE E
SAMPLE F
AP ACTIVITY (PERCENTAGE)
107
106
105
104
103
102
101
100
99
98
97
CONCENTRATION mg/assay

Figure 7. The percent alkaline phosphatase activity in the SOS chromotest of distilled water extracts without S9 (vertical bars represent one standard deviation); n = 3 or more.


DW -S9
SAMPLE A
SAMPLE B
AP ACTIVITY (PERCENTAGE)
106
105
104
103
102
101
100
99
98
97
96
0 2 4 6 8 10 12 14 16 18 20 22 24
CONCENTRATION mg/assay


SAMPLE C
SAMPLE D-1
SAMPLE D-2
AP ACTIVITY (PERCENTAGE)
104.5
104
103.5
103
102.5
102
101.5
101
100.5
100
99.5
99
98.5
98
0 2 4 6 8 10 12 14 16 18 20 22 24
CONCENTRATION mg/assay


SAMPLE E
SAMPLE F
AP ACTIVITY (PERCENTAGE)
115
110
105
100
95
90
85
80
75
0 2 4 6 8 10 12 14 16 18 20 22 24
CONCENTRATION mg/assay

The frequency of labial plate deformities in Chironomids

The appearance of normal mouth parts in the chironomid labial plate is shown in Fig. 8. The structure is symmetrical and well defined. The deformed chironomid labial plates included various types of asymmetry, such as mild asymmetry in the numbers of lateral teeth (Fig. 9), fused processes on the median teeth, and gaps in the labial plate. Deformities of mandibles and antennae were not analyzed, although some deformed mandibles were occasionally noted.

Six out of 15 chironomid genera which were found in the agriculturally influenced portion of the Welland River appeared to have deformed individuals, while 6 out of 10 genera in the industrial influenced portion of the river had deformed specimens (Table 3). Labial plate deformities of chironomid larvae showed that the highest frequency of deformed labial plates was associated with the industrial influenced portion of the Welland River (station D-1, 10.90% ± 3.23%, Fig. 11). The control site A displayed the lowest frequency of deformities (3.84% ± 1.32%) (Fig. 10). The frequency of deformed chironomid labial plates (6.42% ± 3.30%) was higher at station D-2 than at the agricultural site A. Site E displayed a slightly higher frequency of deformities (4.20% ± 0.28%) than site A. Site B (6.29% ± 1.71%), located at the confluence of the Welland river and an old discharge pipe carrying farm field runoff yielded a higher frequency of deformed Chironomid labial plates than the nearby upstream site A (Fig. 10), though the difference was not significant ($p > 0.05$). Station F displayed a very low abundance

Figure 8. "Normal" structure of chironomid labial plate (modified from Wiederholm, 1983). Where "LP" indicated labial plate (mentum), "VP" indicated ventromental plate.

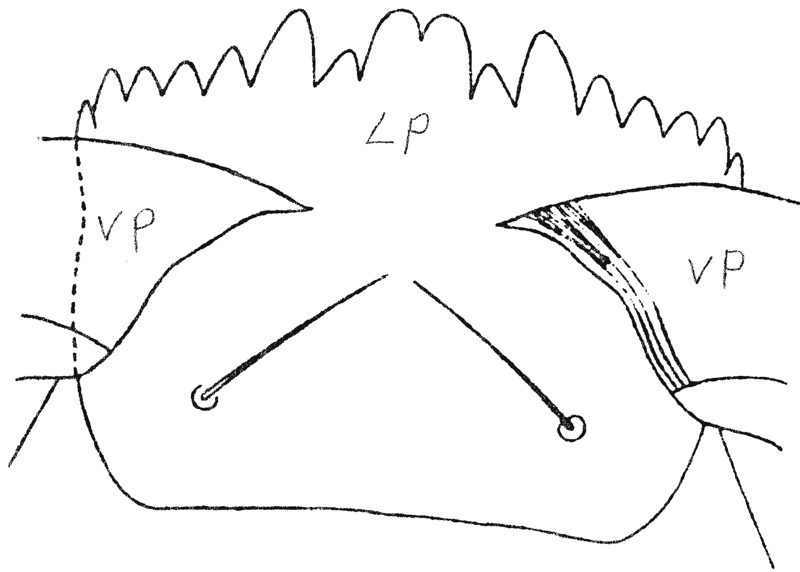
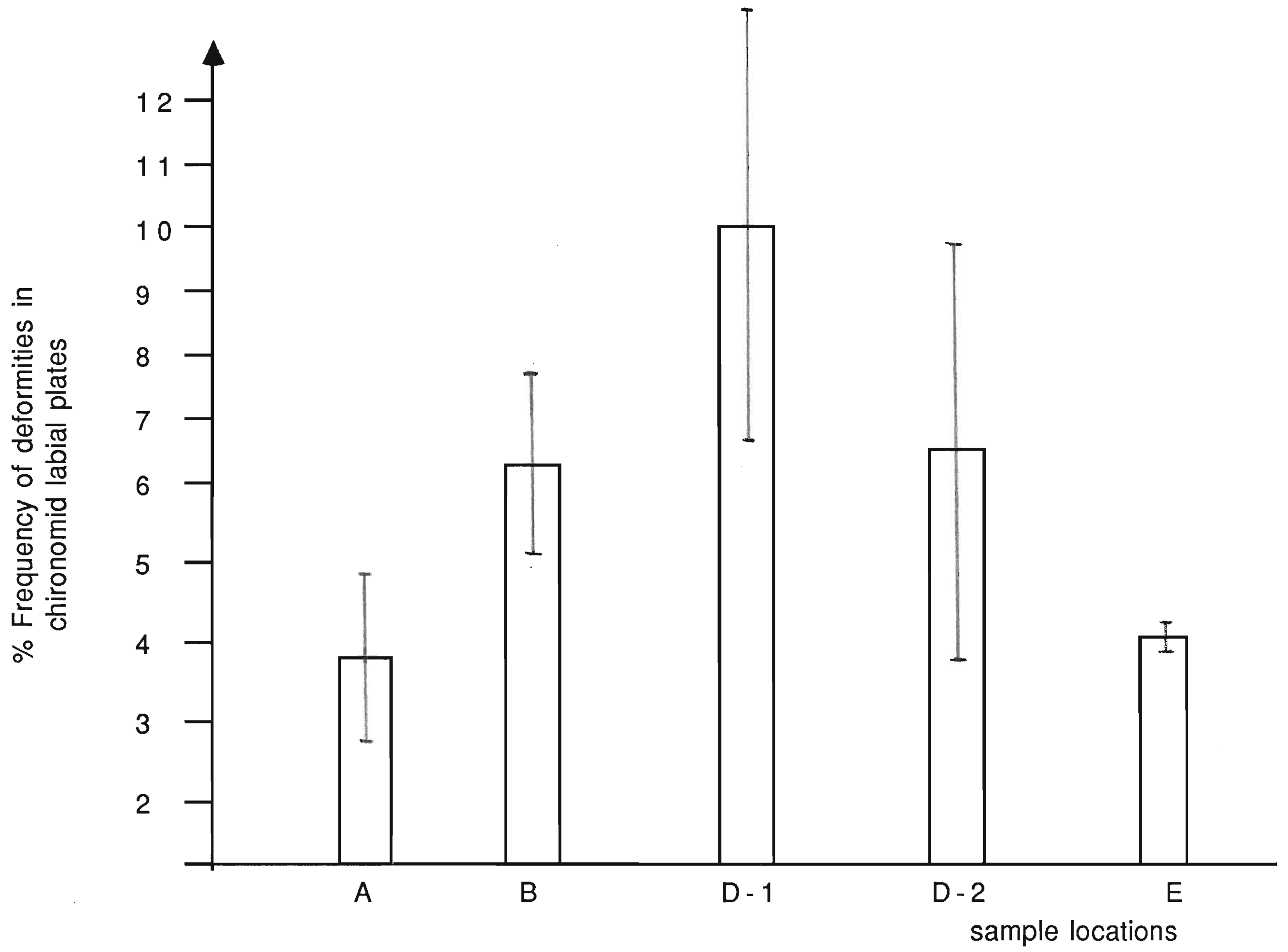

LP
VP
VP

Figure 9. Deformed chironomid labial plate.

Figure 10. The frequency (percentage) of deformities in chironomid labial plates at 5 sample sites in the Welland River (vertical lines represent one standard deviation); n = 3 ro more.


% Frequency of deformities in chironomid labial plates
12
11
10
9
8
7
6
5
4
3
2
A
B
D-1
D-2
E
sample locations

of chironomids as only 3 specimens were found in three composite samples. Therefore, site F was not included in the chironomid deformity analyses.

The difference in the frequency of deformities in Chironomid labial plates was statistically significant between site A and D-1, though there were no significant differences between other stations.

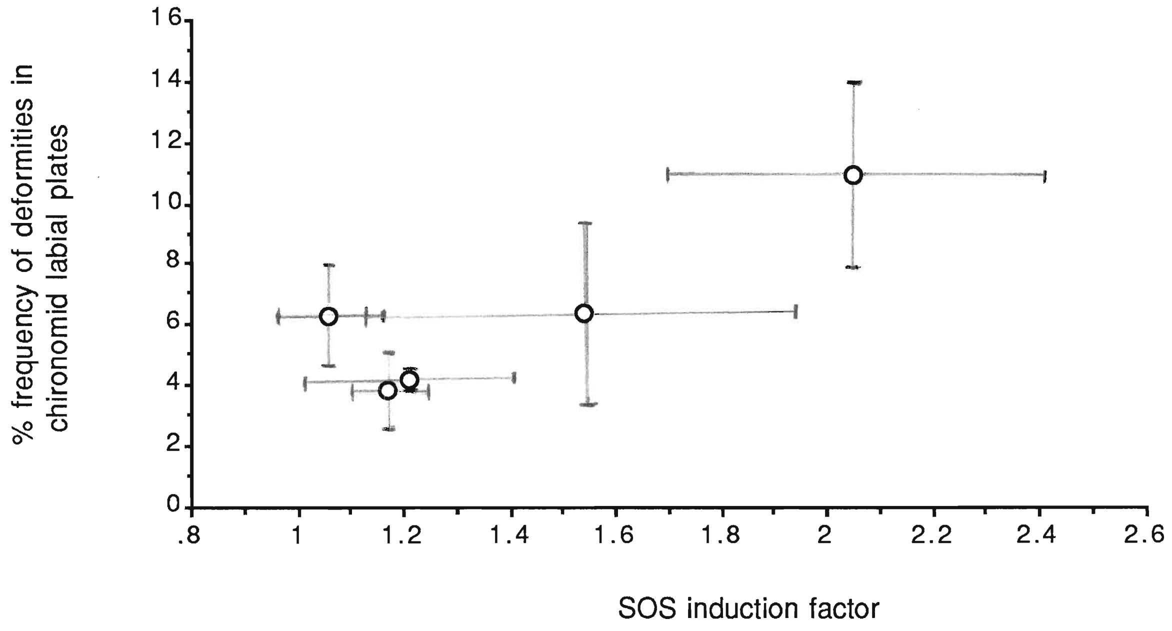
The correlation between the genotoxicity of sediments and the frequency of deformities in chironomid labial plates

The highest genotoxicity of sediments and the highest frequency of deformities in chironomid labial plates occurred at industrial site D-1 which was at the end of the discharge pipe of a company which produces polyvinyl chlorides (Fig. 1). The correlation between the genotoxicity of the sediment samples and the frequency of deformities of labial plates of chironomid larvae showed a significant positive correlation ($r = 0.88$, $p < 0.05$). However, when tested using a non-parametric test (Spearman-Kendall Rank Test) the correlation was not significant at the $p < 0.05$ level because a high correlation coefficient of 0.9 is required by the non-parametric test for five or less degrees of freedom when using the less powerful non-parametric test.

It is interesting that station B showed a relatively high frequency of deformities in chironomid labial plates, but the sediment samples had the lowest SOS chromotest induction factor (negative responses). Reasons for this are offered in the discussion section.

The standard error for the frequency of deformities was very high (Fig.11). As a result, the differences between most sites were not significant. However, the values of sites A and D-1 were significantly different ($p<0.05$).

Figure 11. Correlation between the genotoxicity and the frequency of deformities in chironomid labial plates (vertical bars represent one standard deviation of % deformity frequency estimates, and horizontal bars represent one standard deviation in SOS induction factor estimates)


% frequency of deformities in chironomid labial plates
16
14
12
10
8
6
4
2
0
.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4
2.6
SOS induction factor

Benthic invertebrates

Invertebrates were sampled at the same seven locations as described for the genotoxicity tests (Fig 1). During a one month period of sampling, it was found that the generic richness was higher at the control station and lowest in the industrial-influenced portions of the Welland River (Fig. 12). The most abundant benthic invertebrates in the agricultural control sections were oligochaetes, crustaceans and chironomids (Table 2). In the industrial sections, the most abundant species were oligochaetes and chironomids; crustaceans were not found. It is noteworthy that site C which was polluted by heavy metals from a steel company's effluents was essentially bereft of all benthic invertebrates (Table 3). Because crustaceans and ephemeropterans were essentially absent in the industrial-influenced portion of the Welland river during the entire sampling period, It was concluded that they were pollution sensitive taxa (Table. 3).

There were 13 Crustacean genera in my samples from the agricultural area, only 1 genus was found in the industrial area, a single specimen of a cyclopoid copepod. Mayfly nymphs (abundant upstream) were essentially absent in the industrial area during the sampling period.

The highest density of benthic invertebrates was at the B.F. Goodrich site D-2, (11,899 $\pm$ 2,721 /m^2 surface area of sediment), while the lowest density of benthic invertebrates was at the Atlas Steels site C, (114 $\pm$ 85 /m^2, Table 2). The agricultural (control) site had higher benthic invertebrate density than the industrial sites except for sites D-1 and D-2 where substantial

Figure 12. Generic richness of benthic invertebrates at seven locations in the Welland River (number of genera per 700 cm^2) (vertical lines represent one standard deviation); n = 3.

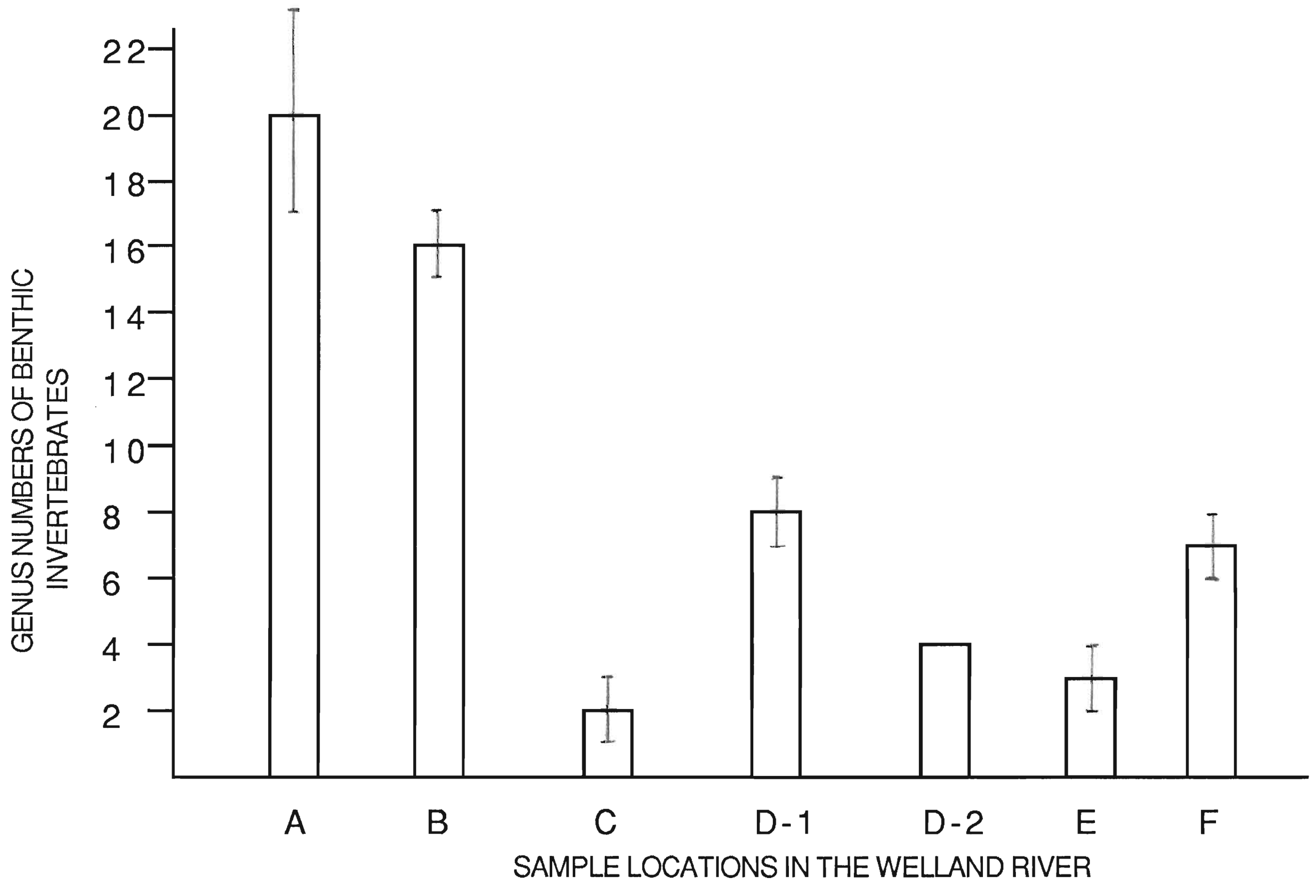

GENUS NUMBERS OF BENTHIC INVERTEBRATES
22
20
18
16
14
12
10
8
6
4
2
A
B
C
D-1
D-2
E
F
SAMPLE LOCATIONS IN THE WELLAND RIVER

Table 2. Density of benthic invertebrates at seven locations in the Welland River (number of specimens /m^2) (mean $\pm$ standard deviation); n = 3.

Table 2. Benthic Invertebrate Density (number of specimens $/m^2$).

sample site	Total	oligochaetes	chironomids
A	4654 ± 219	138 ± 42	551 ± 241
B	5610 ± 1352	306 ± 80	4932 ± 1257
C	114 ± 85	109 ± 81	0.0
D-1	8329 ± 1842	7944 ± 1990	347 ± 126
D-2	11899 ± 2721	11510 ± 2806	375 ± 153
E	1325 ± 584	7 ± 7	1318 ± 591
F	3860 ± 1377	3794 ± 1390	14 ± 8

Table 2 (continued)

sample site	oligochaetes	chironomids		
	Limnodrilus sp.	*Tanypus* sp.	*Ablabesmyia* sp.	*Chironomus* sp
A	117 ± 42	0.0	223 ± 107	0.0
B	299 ± 81	634 ± 634	14 ± 6	14 ± 8
C	33 ± 10	0.0	0.0	0.0
D-1	2051 ± 1068	237 ± 163	19 ± 5	24 ± 24
D-2	11486 ± 2806	223 ± 163	0.0	142 ± 58
E	0.0	64 ± 64	0.0	0.0
F	3272 ± 1251	5 ± 5	0.0	0.0

Table 2. (continued)

sample site	chironomids		isopods	amphipods
	Pseudochironomus sp.	*Tanytarsus* sp.		
A	152 ± 98	104 ± 50	332 ± 104	124 ± 33
B	36 ± 27	2119 ± 950	50 ± 32	11 ± 11
C	0.0	0.0	0.0	0.0
D-1	5 ± 5	0.0	0.0	0.0
D-2	0.0	0.0	0.0	0.0
E	0.0	0.0	0.0	0.0
F	0.0	0.0	0.0	0.0

Table 3. Benthic invertebrate taxa at seven locations in the Welland River (number of specimens per 700 cm^2), n = 3. Double values with slash (/) represent the number of deformed specimens/total number of counted.

SPECIES	A1	A2	A3	B1	B2	B3	B4	C1	C2	C3	D1,1	D1,2	D1,3	D2,1	D2,2	D2,3	E1	E2	F1	F2	F3
TURBELLARIA																					
RHABDOCOELIDA																					
Hydrolimax sp.	5	6	3	2	16	10	15					1									9
NEMATODA																					
ENOPLIDA																					
DORYLAIMIDAE																					
Actinolaimus sp.						1															
ANNELIDA																					
OLIGOCHEATA																					
PLESIOPORA																					
NAIDIDAE																					
Aulodrilus sp.																			1		
Aulophorus sp.											4	398	645	2							
Dero sp.																1					
Nais sp.	2										5	92	85						4	1	
Stylaria sp.	1																				
PLESIOPORA																					
TUBIFICIDAE																					
Limnodrilus sp.	7	14	4	34	11	12	27	3	3	1	262	166	5	304	1088	427			175	114	402
Peloscolex sp.								16												4	1
Tubifex sp.	1					1					11			2			1		29	14	45
HIRUDINEA																					
RHYNCHOBDELLIDA																					
ERPOBDELLIDA																					
Mooreobdella sp.																			1	2	
Batracobdella sp.							1														
CRUSTACEA																					
CLADOCERA																					
CALYPTOMERA																					
DAPHNIDAE																					
Daphnia sp.	26		71																		
Scapholeberis			4																		
CHYDORIDAE																					
CHYDORIDAE																					
Graptoleberis	2																				
EURYCERCINAE																					
Eurycercus sp.			2																		
COPEPODA																					
EUCOPEPODA																					
CYCLOPIDAE																					
Cyclops sp.	41	9	41	3	2	3	5				1										
OSTRACODA																					
CYPRIDAE																					
Candona sp.				1																	
Cypridopsis sp.		4	3	7																	
ISOPODA																					
ASELLOTA																					
ASELLIDAE																					
Asellus sp.	21	37	12	1	3		10														
AMPHIPODA																					
GAMMARIDAE																					
Gammarus sp.	13	8	5		1																
HYDRACHRINA																					

SPECIES	A1	A2	A3	B1	B2	B3	B4	C1	C2	C3	D1,1	D1,2	D1,3	D2,1	D2,2	D2,3	E1	E2	[illegible]	F2	F3
ARRENURIDAE																					
Arrenurus sp.	4	2	8			1															
LIMNESIIDAE																					
Limnesia sp.	4																				
UNIONICOLIDAE																					
Koenikea sp.		1	2																		
Neumania sp.	1						1														
INSECTA																					
COLEOPTERA																					
HALIPLIDAE																					
Peltodytes sp.																			1		1
ELMIDAE																					
Dubiraphia sp.	1			1		3	1														
DIPTERA																					
ORTHORRHAPHA																					
CERATOPOGONIDAE																					
Bezzia sp.			1								5										
Palpomyia sp.						1	1								3					1	
STRATIOMYIIDAE																					
Oxycera sp.	1																				
TENDIPEDIDAE																					
CHIRONOMINAE																					
Chironomous sp.						2	2					1/5		7	. 16	1/5					
Cryptochironomous sp.				4	3	9	5														
Cryptotendipes sp.	1			2/2																	
Dicrotendipes sp.	1				1																
Endochironomous sp.	7					16											·/1				
Glyptotendipes sp.		1																			
Polypedilum sp.				15/92	6/75	4/52	5/217									1					
Paedochironomous sp.	7	1	24	2		8							1/1								
Tanytarsus sp.	2/13	1	2/8		5/126	5/145	14/324														
Tribelos sp.					1																
ORTHOCLADIINAE																					
Cricotopus sp.																	1/50				
Psectrocladius sp.													1					6.120			
TANYPODINAE																					
Ablabesmyia sp.	1/20	1	26	1	1		2				1	2	1								
Coelotanypus sp.			1		1	1	2														
Larsia sp.																					
Natarsia sp.											.2										
Pentaneurini sp.							1														
Procladius sp.	3	1		13/50		23	47								1				2		
Tanypus sp.				14/178							/28	14	8		1/9	38		9		1	
EPHEMEROPTERA																					
BAETIDAE																					
Caenis sp.	54	22	395	6	7	7	4														
BAETIDAE																					
Baetis sp.			1																		
ODONATA																					
COENAGRIONIDAE																					
Coenagrion sp.	2	3	11			3		1													2
LIBELLULIDAE																					
Ladona sp.	2	4																		4	
Libellula sp.			1																		

quantities of organic matter were discharged (MOE, 1987). Site D-2 showed the highest oligochaete density, (11,510 ± 2,806 /m^2 ,Table 2). Sites D-1, D-2 and F had higher oligochaete densities than that of the agricultural control section, while sites C and E were lower than the control in oligochaete densities (Table 2). The chironomid density was higher in the agricultural section, especially site B. chironomid density at site B (OWMC Swale) was over 10 times higher than at any other sites (Table 2). At the Atlas industrial site C, all chironomid species were absent.

Although the number of oligochaete genera in the agricultural and industrial areas was 5 and 6, respectively, Aulophorus spp. (Dero spp.) and Audrilus spp. were only found in the industrial-influenced portions of the river. The most abundant species of oligochaetes in both agricultural and industrial influenced sections was Limnodrilus spp., but the relative density of Limnodrilus spp. was much higher in the industrially influenced sections of the Welland River (Tables 2 and 4). At sites A and B, the relative densityn of Limnodrilus spp. ranged from 5.24% ± 3.53% to 5.43% ± 1.18% of the total benthic invertebrates, while in the industrial sections it was 34.92% ± 23.39% to 95.50% ± 2.56% except at station E.

In the agricultural area, 15 chironomid genera were found, while in the industrial influenced portions of the Welland River only 10 chironomid genera were observed. Tanytarsus spp., Cryptochironomus spp. and Coelotanypus spp. were not found in the industrial influenced portions of the river. Cricotopus spp., Psectrocladius spp. and Natarsia spp. were not found in the

Table 4. Relative density of benthic invertebrate taxa at seven locations in the Welland River (percentage abundance) (mean ± standard deviation); n = 3.

Table 4. Relative density of benthic invertebrates (percentage abundance)

sample iste	oligochaetes	chironomids	isopods	amphipods
A	5.80 ± 3.38	11.82 ± 5.14	14.28 ± 9.15	4.39 ± 1.60
B	5.56 ± 1.13	87.25 ± 1.03	0.75 ± 0.37	0.11 ± 0.11
C	98.33 ± 1.67	0.0	0.0	0.0
D-1	93.67 ± 4.07	5.67 ± 3.43	0.0	0.0
D-2	95.71 ± 2.58	4.20 ± 2.61	0.0	0.0
E	0.96 ± 0.96	99.04 ± 0.96	0.0	0.0
F	97.58 ± 1.04	0.55 ± 0.28	0.0	0.0

Table 4. (continued)

sample site	oligochaetes	chironomids		
	Limnodrilus sp.	*Tanypus* sp.	*Ablabesmyia* sp.	*Chironomus* sp
A	5.24 ± 3.53	0.0	4.45 ± 2.16	0.0
B	5.43 ± 1.18	15.61 ±15.61	0.25 ± 0.09	0.25 ± 0.16
C	71.67 ± 28.33	0.0	0.0	0.0
D-1	34.92 ± 23.39	3.88 ± 2.33	0.24 ± 0.06	0.25 ± 0.25
D-2	95.50 ± 2.56	2.95 ± 2.56	0.0	1.15 ± 0.25
E	0.0	3.36 ± 3.36	0.0	0.0
F	83.28 ± 2.07	0.24 ± 0.24	0.0	0.0

Table 4. (continued)

sample site	chironomids	
	Pseudochironomus sp.	*Tanytarsus* sp.
A	2.54 ± 0.88	2.52 ± 1.45
B	37.81 ± 12.63	0.82 ± 0.66
C	0.0	0.0
D-1	0.04 ± 0.04	0.0
D-2	0.0	0.0
E	0.0	0.0
F	0.0	0.0

agricultural areas. The most abundant species of Chironomids in the agricultural sections were *Tanytarsus* spp. and *Ablabesmyia* spp., while in the industrial sections, *Tanypus* spp., *Pseudochironoms* spp. and *Chironomus* spp. dominated (Tables 2 and 4). The relative density of chironomids was higher in the agricultural areas, at stations A and B (11.82% ± 5.14% and 87.25% ± 1.03%, respectively). In the industrial areas, the relative density of chironomids was low (0.55% ± 0.28% to 5.67% ± 3.45%) except at station E (99.04% ± 0.96%).

It is interesting that stations A and B displayed substantial differences in their species composition and density. Station B had slightly lower species richness and diversity than station A (Fig. 8 and Table 5), while site B displayed a slightly higher density of some of the more pollution tolerant benthic invertebrates (Table 2). There was a great difference in the relative density of chironomid and crustacean species between stations A and B. The relative density of chironomids was greater at station B (87.25% ± 1.03%) in comparison with station A (11.82% ± 5.14%). The most abundant chironomid species shifted from *Ablabesmyia* spp. at station A to *Tanypus* spp. and *Pseudochironomus* spp. at station B (Table 5). At the same time, the relative density of isopods and amphipods was lower at station B (0.75% ± 0.37% for isopods and 0.11% ± 0.11% for amphipods) as compared with station A (14.28% ± 9.15% for isopods and 4.39% ± 1.60% for amphipods).

Comparing the relative density of oligochaetes and Chironomids along the river, it was found that a significant

negative correlation (Spearman-Kendall Rank test) ($r = -1$, $p < 0.01$) was observed. In other words, as the relative density of oligochaetes increased the relative density of chironomids decreased (Table 4).

Species diversity and species richness of bethic invertebrates

The species diversity at the generic level was higher in the agriculturally influenced portions of the Welland River than in the industrialized areas. The difference in generic diversity between the agricultural and industrialized areas was statistically significant ($p < 0.05$). The lowest generic diversity was recorded at site C where Simpson's index was 0.85 ± 0.45 and Shannon-Wiener's index was 0.09 ± 0.09 (Table 5). The highest generic diversity was observed at the agricultural control site A (Simpson's index was 6.15 ± 2.20, Shannon-Wiener's index was 0.86 ± 0.13). The differences in generic diversity were consistent with the generic richness data set. Locations displaying high generic richness also displayed high generic diversities. The agricultural control site A displayed the highest generic richness (20 ± 3 per 700 cm^2 surface area of sediment) while the industrial sample station C had the lowest generic richness (2 ± 1 per 700 cm^2) (Fig. 12). The difference in generic richness between the agricultural and industrial influenced portions of the Welland River was statistically significant ($p < 0.05$).

At site D-1, there was a relatively higher generic richness (8 ± 1 per 700 cm^2) and diversity (Simpson's index 1.75 ± 0.32) than at any other industrial sites (Fig. 12). This was due to the 2 additional oligochaete species found at D-1, *Auldrilus* spp. and *Aulophorus* spp. (Table 3). The decrease in generic richness and diversity in the industrial influenced portion of the Welland River was mainly the result of absent Crustacean species and Mayfly nymphs (Table 3). At station F, generic richness and

diversity increased significantly compared with the upstream stations D-2 and E (Fig. 12 and Table 5). This was due to the increase in the number of oliogochaete species and dragonfly nymph species at station F (Table 3).

Table 5. Generic diversity of benthic invertebrates at seven locations in the Welland River (mean ± standard deviation); n = 3.

Table 5 Species diversity of benthic invertebrates at generic level.

sample site	Simpson's index	Shannon-Wiener's index
A	6.15 ± 2.20	0.86 ± 1.23
B	3.06 ± 0.18	0.66 ± 0.04
C	0.85 ± 0.45	0.09 ± 0.09
D-1	1.75 ± 0.32	0.35 ± 0.08
D-2	1.10 ± 0.06	0.10 ± 0.04
E	1.11 ± 0.03	0.10 ± 0.01
F	1.42 ± 0.07	0.28 ± 0.04

DISCUSSION

Sediment genotoxicity

Each extract of the sediment samples was tested at least three times using the SOS chromotest. Absolute values for the induction factors varied slightly between experiments. This was a function of the initial number of bacteria and the degree of activity of the S9 mixture which was used (Quillardet et al., 1985). The DMSO extract of samples from station D-1 gave a significant positive response ($p < 0.01$) in both the December and April samples. I inferred from this that there were some potential genotoxic materials at station D-1 under the discharge pipe of the B.F. Goodrich Co. The presence of vinyl chloride, a known genotoxin, in their discharge water (MOE, 1987) was the most probable causative agent.

Although sample D-2 and sample E gave weak positive responses in the SOS chromotest in the first collection of samples ($p < 0.05$, December, 1986), they were not positive in the second collections (April, 1987). I therefore considered the genotoxicity at sample sites D-2 and E to be possibly seasonal and less strong than D-1.

DMSO is widely used as an organic solvent in genotoxicity tests (Ames et al., 1975; Quillardet et al., 1982). DMSO sample extracts were the only fraction that gave a positive response to the genotoxicity test. Therefore, it was concluded that some organic materials were genotoxic in the sediments of sample D-1. The industrial MOE (1987) reported that the B.F. Goodrich

effluent at station D-1 contained detectable concentrations of vinyl chloride (0.09 kg per day or 0.041 ppm). Vinyl chloride has been demonstrated to be a potential genotoxin when activated by the S9 mixture (liver extract) (McCann et al., 1975). Therefore, vinyl chloride in the sediments at station D-1 may have induced the observed SOS response at that site. Although DMSO extracts of sample D-1 yielded a positive response in the SOS chromotest, the induction factor was not very high (2.053 ± 0.361 at 20 mg wet weight/assay) compared to the positive control 2AA (29.11 ± 5.01 at 10 ug/assay). The genotoxicity of the B.F. Goodrich sediments was therefore not strong. This may be due to the low concentration of potential genotoxic compounds in the sediments at this site.

Low levels of mutagenic contaminants in sediments from Prince Edward Island were also reported by Xu and Dutka (1986). Their genotoxicity studies of sediment samples from Prince Edward Island revealed that the induction factor (positive response to the SOS chromotest) was also quite low (the highest induction factor for their samples was 1.65) (Xu, and Dutka, 1986).

Short-term assays of genotoxicity for environmental samples have been developed in a number of studies. The SOS chromotest was recommended as a sensitive genotoxicity test (Quillardet et al., 1982 and 1985). In the present study, it was tried to use the Ames test to detect samples which showed positive responses in the SOS chromotest, all the results of the Ames test were negative (it did not list in the result section). it was found that the SOS chromotest was more sensitive than the Ames test.

There are three possible explanations why the Ames test yielded negative results at station D-1 while the SOS test was positive. First, the SOS chromotest is more sensitive than the Ames test (Quillardet et al., 1982). Aquatic ecosystems in proximity to human activity will contain literally hundreds of contaminants and their by-products. The concentration and dissolution rates of each of these chemicals vary in different aquatic systems (Samoilloff et al., 1983; Dutka et al., 1981). Generally, the concentrations of organic lipophilic compounds are lower in the water phase than in surface sediments (Wetzel, 1983). A previous study conducted in our lab (World Wildlife Report, 1987) showed that the liquid samples which were taken at the same locations as the sediment samples gave negative responses in both the SOS chromotest and the Ames test (Veltri, personal comm.). It appeared that negative genotoxicity responses from water samples and positive responses from sediment samples at the same test stations were the result of the higher concentrations of DMSO soluble materials in the sediments. Therefore, it may be speculated that the concentrations of the potential genotoxic compounds were very low in the water of the Welland River and were lower than the detection level of the Ames test and the SOS chromotest.

Second, the inducible SOS repair in *E. coli* K-12 PQ 37 responds to mutagens and indirect genotoxic agents (Quillardet et al., 1985). The SOS system is inducible when a cell's DNA is damaged or its DNA replication is inhibited (Walker, 1984). In the Ames test, the reversions have to be caused by DNA

base-mutagen actions. For example, the TA98 strain in the Ames test detects mutagens that cause base-pair substitutions, while the TA100 strain detects various frameshift mutagens (Maron and Ames, 1983). The different mechanisms in the SOS chromotest and the Ames test may result in different responses to the same genotoxin. For example, hydroxyurea, has been shown to have teratogenic and carcinogenic effects on mammals, and is a strong inhibitor of DNA synthesis (Timson, 1975). Hydroxyurea gave a positive response to the SOS chromotest and a negative response to the Ames test (Quillardet et al., 1985). The results of my research indicated that the potential genotoxic compounds in the samples from the Welland River were indirectly genotoxic and would be expected to be mutagenic only when metabolically activated.

The third factor is that the Ames test needs low toxicity levels if the test strain is to survive. Therefore, the Ames test cannot be used to detect mutagenic compounds if they are so toxic that they kill the Ames test bacteria (Ames et al., 1975; Quillardet et al., 1985). The alkaline phosphatase activity results of my study indicated that the extracts from station D-1 were toxic (Fig. 3), This toxicity (Appendix 1.) could be the result of B.F. Goodrich ammonia discharges (Hellawell, 1988). The toxicity of the samples might therefore have caused in the negative responses observed in the Ames tests which K.Veltri and I carried out (World Wildlife Report, 1987).

It is also worthy of note that the positive response of the organic compounds extracted by DMSO in the SOS chromotest were

obtained only in the presence of the S9 metabolic activitor. The S9 mixture was prepared from rat livers and contained enzymes that metabolize pro-mutagenic substances converting them to mutagens (Ames et al., 1975; Venitt and Parry, 1984). Thus, the data obtained in this study implies that some organic compounds in the samples tested may be pro-genotoxic.

The present study suggested that the sediments at one site in the industrially influenced portions in the Welland River was contaminated with potential genotoxic waste materials. Further work on impacts of genotoxic pollution on the aquatic ecosystem in the Welland River is warranted.

Deformities of chironomid labial plates

Deformities in chironomid larvae occur naturally. In Parry Sound, Georgian Bay, 0.0-1.3% of the Chironomus cucini collected were deformed (Hare and Carter, 1976). Wiederholm (1984) reported that in the unpolluted (control) areas the frequency of deformities in chironomid larvae ranged from 0.7-0.8%. In my study, it was found that the frequency of natural occurrence of deformities in chironomid labial plates was 3.84% $\pm$ 1.32% (station A, Fig. 10). Thus, in the Welland River, the frequency (%) of naturally deformed chironomid labial plates was slightly higher when compared with other studies.

Because these deformities are very easily detected, and chironomids are ubiquious in freshwater and relatively resistant to organic pollution, they have commonly been used as pollution indicators (Hamilton et al., 1971; Wiederholm, 1984; Warwick et al., 1987).

Fifteen Chironomid genera were found in the agriculturally influenced portions of the Welland River and 10 in the industrially influenced portions. Six out of 15 genera in the control sections appeared to have deformed individuals, six out of 10 genera in the industrial sections had deformed specimens (Table 3). Therefore, it was concluded that in the industrialized influenced portions of the Welland River chironomids had higher proportions of deformed taxa than in the agricultural areas. This may indicate that industrial pollution in the Welland River was more mutagenic than the pesticides and related agricultural chemicals released into the river in the agricultural areas.

In most cases, deformed chironomid labial plate teeth were shorter than normal ones. In some cases, there were extra teeth. Deformed labial plates were invariably asymmetrical. The highest percentage of deformities in chironomid labial plates was found at station D-1 (10.90% ± 3.23%). This was significantly higher ($p<0.05$) than at station A (3.84% ± 1.32%). Although chironomids at station D-2 had higher frequencies of deformed labial plates than chironomids at station A, the difference was not significant at the $p<0.05$ level (Fig. 10). Station D-1 was affected by B.F. Goodrich Co. effluent which according to the MOE (1987) and my SOS chromotest analyses contained some pro-genotoxic materials.

The frequency of deformities in chironomid labial plates in the agricultural areas was not very high when compared to the industrial sections. Wiederholm (1984) found that heavily polluted lakes had higher frequencies of deformities in chironomids than did less polluted lakes (4.1%-10.7% in polluted

lakes, 1.4% to 1.6% in slightly polluted lakes and 0.7% to 0.8% in control lakes). In heavily polluted lakes the frequency of labial plate deformities ranged from 3.8% to 25.0%. At Port Hope, Ontario, Chironomus sp. within the heavily polluted inner harbour area showed a very high frequency of deformities (83%) compared with the outer harbour (14%) where levels of radioactivity were much lower. From the results of the frequency of deformities analyses, the high percentages of deformed chironmid labial plates at station D-1 may be associated with the genotoxic pollution at this site.

Station B was located in the agricultural influenced portion of the river. At this station, it was found that a higher frequency of deformities in chironomid labial plates (6.29% ± 1.71%) occurred than at the upstream station A (3.84% ± 1.32%). Both stations were assumed to be affected by agricultural effluents (Dickman et al., 1983). Station A was located in an aquatic plant-lined stream course while station B was located at the end of a pipe draining agricultural runoff from a storm sewer near the proposed dumpsite of the Ontario Waste Management Corporation. The observed difference in the frequency of chironomid labial plate deformities between these two locations may be because aquatic plants play an important role in removing hazardous waste materials from the water (Bingham, 1973). Few aquatic plants occurred at station B while many occurred at station A.

Chironomids from station E had a slightly higher frequency of deformities in labial plates (4.20% ± 0.28%) Than the

chironomids from upstream control station A, but the difference was not statistically significant ($p>0.05$).

Correlations between genotoxicity and the frequency of deformities in chironomid labial plates.

The cause-effect relationship between high chironomid deformity frequencies and certain effluents of industrial discharges was discussed by Warwick et al. (1987). Theoretically, deformities are caused by interruption of a cell's physiological activities by some agents such as DNA-damaging substances (Warwick et al., 1987). Warwick (1980) found that in the sediment cores taken from the Bay of Quinte the frequency of deformed chironomid larvae increased from 0.09% in the pre-European sediments to 1.06% in the 1950's sediments and to 1.99% in the present day (1972) chironomid population of the bay. It was concluded that the increase in the frequency of chironomid deformities was associated with the increased level of pollution in the bay. Warwick et al. (1987) suggested that genotoxins induce an elevated frequency of deformities in chironomids. Wiederholm (1984) related the high frequencies of deformed chironomids to the presence of heavy metals including Ni, Pb, Cr etc. which were demonstated to be genotoxic (Fleesel, 1979). However, no studies have been published on sediment genotoxicity using the SOS chromotest and the frequency of deformities in chironomid labial plates.

The present study revealed that the highest sediment genotoxicity and the highest frequency of deformities in

chironomid labial plates both occurred at the same site (station D-1) (Table 2 and Fig. 10). The correlation between sediment genotoxicity and the frequency of deformities in chironomid labial plates was tested statistically. In the parametric test, the correlation coefficient was 0.88, which was a statistically significantly positive correlation ($p<0.05$). However, this correlation was not significant when the non-parametric test was employed ($p<0.05$). The reason for adopting the non-parametric test was that the sample size (number of replicates and stations, Appendix 3) was small. It would not be safe to use a parametric test with so few samples (Ward, 1978).

Other studies have concluded that there were detectable carcinogenic chemicals in the industrially influenced portion of the Welland River (Kaiser and Comba, 1983). At station D, water samples contained chloroform (75 ng/l), carbon tetrachloride (15 ng/l) and dibromomethane (10 ng/l). Those compounds were reported to be carcinogens (McCann et al., 1975; Quillardet et al., 1985). There were detectable levels of vinyl chloride (0.09 kg/day or 0.041 ppm. MOE, 1987) in the water samples taken at station D. Vinyl chloride was demonstrated to be genotoxic (McCann et al., 1975). At station E, water samples contained even higher concentrations of chloroform (1800-3750 ng/l, in 1980; 150- 300 ng/l, in 1981), carbon tetrahloride (120 ng/l, in 1980; 15-60 ng/l, in 1981) and dibromonethane (15-20 ng/l, 1980; 5 ng/l, 1981) (Kairser and Comba, 1983).

The DMSO extracts of sediment from station D-1 were pro-genotoxic, while DMSO extracts of sediments from stations D-2

and E were possibly pro-genotoxic. It was therefore inferred that genotoxic pollutants from industrial sources in the sediments of the Welland River may have caused the high frequency of deformities in chironomids observed.

Sediment from station B had the lowest SOS chromotest induction factor (negative response), but site B displayed a relatively high frequency of deformities in chironomids (Table 1 and Fig. 8). This may be due to the agricultural effluent which contained genotoxic compounds. Some genotoxins are undetectable in bacterial tests. The best example of this is PCB (Arochlor 1254) which is a carcinogen but does not give a positive response in either the SOS chromotest or the Ames test (Quillardet et al., 1985). The possibility that agricultural chemicals were responsible for the high frequency of deformities in chironomid labial plates at station B cannot be ruled out. Ecologically, the main difference between stations A and B is that station A was located at a bigger creek whose water passed through an extensive aquatic plant lined stream course, while station B was located at the end of a small sewer and its water flowed directly into the Welland River through this pipe.

Aquatic plants play an important role in removing pollutants from the water (Bingham, 1973). Station A was a more natural site in terms of its generic richness (Fig. 12) and the relative abundance of individual benthic invetebrate genera (Table 2). Therefore, station B may be slightly polluted by agricultural effluents.

Toxicity vs genotoxicity of the sediment samples

Sample extracts from stations C and D-1 were found to be toxic according to the alkaline phosphatase test which is part of the SOS chromotest. In the SOS chromotest, alkaline phosphatase was used to check protein biosynthesis activity. Alkaline phosphatase was noninducible by DNA damaging agents. During the test period, alkaline phosphatase activity should be maintained at its initial level or increased if the test samples are not toxic (Quillardet et al., 1982). A decrease in the enyzme activity of alkaline phosphatase is considered to be an inhibititory response to a toxin and protein biosynthesis of the E. coli test strain is reduced (Quillardet et al., 1982). Comparing the alkaline phosphatase activity in DMSO extracts with and without the S9 mixture, major differences were noted (Figs. 4 and 5). When tested with S9, samples from stations C and D-1 showed a significant decrease in alkaline phosphatase activity ($p < 0.05$). In contrast, when tested without S9, none of the samples except those from station F ($0.05 < p < 0.10$) inhibited protein biosynthesis. Although it was reported that the S9 mixture displayed a slight inhibitory effect on protein biosynthesis (Quillardet et al., 1985), the results of the inhibition of protein biosynthesis in this study were obviously not because of the S9 mixture itself as indicated by the controls (Appendix 2). Thus the S9 (liver extract) converted some materials into toxins. It was concluded, therefore, that potential toxic contaminants occurred in the sediments of the Welland River, especially near the discharge pipe of the B.F.

Goodrich Co. and the Atlas-Mansfield storm sewer. Those potential toxic organic compounds might be associated with agricultural and industrial effluents. As the effluents contained many compounds which were not identified, it was impossible to determine which compounds caused the toxicity responses. I concluded, however, that in many cases, chemical toxicity prevented positive Ames test mutagenicity analyses from being detected with the presence of S9 mixture.

The distilled water extracts of the Welland River sediments in the presence of the S9 (liver extracts) caused alkaline phosphatase activity to decrease significantly ($p< 0.05$) in samples taken from stations C and D-1, while without S9, only sample F showed a slight ($p>0.05$, $p <0.10$) inhibition of protein biosynthesis. The results of the distilled water extracts of samples from stations C and D-1 implied that the toxicity of some DMSO and water soluble compounds was activated by the S9 mixture. For station F, the addition of the S9 (liver extract) mixture seemed to detoxify the sediment DMSO extracts (Figs. 5 and 7). Several other studies were found in which the addition of S9 increased the toxicity of sample materials , and decreased toxicity of other compounds (Vanderneulen et al., 1985; Sato et al., 1983).

Studies of the Welland River showed that the sediments from site C (near the discharge location of the Atlas Steels Co.) contained very high concentrations of heavy metals, such as Pb, Zn, Ni, and Cr etc. (Dickman et al., 1988). Cr, Ni, and Pb have been reported to be both toxic and genotoxic (Fleesel, 1984;

Olivier et al., 1987). Site E was contaminated with ammonia and nitrates at toxic levels (Dickman et al., 1988).

Benthic invertebrate community structure

In my research, only live benthic invertebrates (ie., those which moved during sorting) were preserved. Although the classification of these benthic invertebrates was at the generic level, the data from the agricultural and industrially influenced portions of the Welland River watershore are comparable.

The generic richness and diversity was significantly lower in the industrial influenced portion of the Welland River especially at station C, where only a few oligochaete and dragonfly nymph specimens were found. In the agriculturally influenced portion of the Welland River, there were 13 genera of crustaceans. At station A, the relative abundance of isopods and amphipods were 14.28% $\pm$ 9.15% and 4.39% $\pm$ 1.60%, respectively. At station B, the relative abundance of isopods was 0.75% $\pm$ 0.37%, and 0.11% $\pm$ 0.11% for amphipods. In the industrialized area, the crustacean species decreased dramatically. Crustacean species are very sensitive to many types of pollution (Gaufin and Tarzwell, 1952; Buikeman et al., 1978). The results of my study were in accord with Gaufin and Tarzwell's observations. Gaufin and Tarzwell (1952) reported that in the domestic sewage polluted zone of Lytle Creek there were no crustacean species. Crustaceans had lower Lc50 values for various pollutants including pesticides and heavy metals than any other invertebrate species (Buikeman et al., 1978). For example, the Lc50 for PCB was 10-200 ug/l for

Gammarus spp., while PCB toxicity for dragonflies was 800-1000 ug/l (Buikeman and Herricks, 1978). A concentration of 0.025 ppm of Baytex (fenthion) was lethal to chironomid larvae, while 0.01 ppm of Baytex exceeded the toxic limits for the isopod (*Palomonetes*) and the amphipod (*Hyalella*) (Muirhead-Thomson, 1971).

Sediment type also affects the distribution of benthic invertebrates (Wetzel, 1983). In the Welland River, the structure of bottom sediment from upstream to downstream was fairly uniform. A clay base was found at all stations in both the industrial and the agricultural influenced sites in the river watershore except a few sites. Therefore, differences of invertebrate community at the industrial discharge sites were attributed to the impacts of industrial effluents and not to substrate differences.

At station C, the precipitation of discharged materials resulted in a "reef" structure in front of the confluence of the Atlas Steels Co. discharge stream and the Welland River. The observed changes in invertebrate species composition at this site was probably caused by industrial discharges of heavy metals at this site.

The seasonal changes of crustacean species pattern in the Welland River were not studied. Although during the sampling period few crustaceans were found in the industrially influenced portions of the river, it was not possible to conclude that crustaceans were absent from the industrial influenced section of the river because benthic invertebrate samples were only taken in

August and September.

In the agricultural influenced portions the Welland River, stations A and B displayed slight differences in genera. Generic richness and diversity decreased slightly ($0.05< p <0.10$) at station B (16 ± 1.1 per 700 cm^2). The remarkable difference was in the relative density of individual species (genera). At station A, the relative density of chironomids was 11.82% ± 5.14%, while at station B it increased to 87.25% ± 1.03% (Table 4). Isopod and amphipod relative densities at station A were 14.28% ± 9.15% and 4.39% ± 1.60%, respectively, while at station B it was 0.75% ± 0.37% for isopods and 0.11% ± 0.11% for amphipods. Therefore, it implies that station A had a more "normal" aquatic assemblage than did station B. It may be because station A was located below a dense aquatic plant stand which may have played a role in removing hazardous agricultural chemicals from the water (Bingham, 1973).

At station C, there were no chironomid larvae found. Sediment heavy metal concentrations at station C were high (3927 ± 600 ppm Nickel, 3712 ± 130 ppm Chromium, 800 ± 200 ppm Lead, 1088 ± 190 ppm Zinc, 753 ± 77 ppm Copper, and 237 ± 43 ppm Cobalt) (Dickman et al., 1986). The studies on avoidance response of Chironomus sp. to sediment containing heavy metals revealed that the threshold avoidance was 213-422 ppm Cadmium, 4385-8330 ppm Zinc, and 799-1513 ppm chromium (Wentsel et al., 1977). Therefore, the absence of chironomids at station C is likely due to the high concentrations of sediment heavy metals at this station, especially Nickel and Chromium.

At station D-1, the generic richness and diversity were significantly higher than at any other industrial site, though they were significantly lower than the agricultural control site. The high species richness at station D-1 was apparently due to the increase in oligochaete species at this station. The discharge effluent of the B.F. Goorich Co. contained high levels of organic materials (BOD was 43.37 kg/day. MOE, 1987). The discharge effluent of the B.F. Goodrich Co. contained high levels of organic materials (BOD was 43.37 kg/day. MOE, 1987). the oligochaete species, Limnodrilus spp. and Dero spp. are tolerant of high BOD and high organic pollution (Hart et al., 1972; Lafont, 1984). Therefore, more tolerant oligochaete taxa appeared at station D-1 due to the organic pollution there. Another possibility to explain the higher species richness at station D-1 is that site D-1 was located in a branch stream. Station D-1 was not affected by the main current of the river which carries industrial effluents from upstream industrial areas. This may be more obvious when comparing site D-1 with D-2 which was located at the confluence of the Welland River and the discharge stream of the B.F. Goodrich Co. At site D-2, species richness and diversity were significantly lower than at site D-1, while site D-2 was affected by both the B.F. Goodrich effluent and the upstream materials carried in the current of the river. Additionally, sediment structure was different at site D-1 and D-2. Site D-1 was more rocky than other parts of the Welland River. The difference in substrate may also account for the observed species differences.

Oligochaete density was significantly higher at most industrial discharge sites in the Welland River (Table 4). At stations A and B, the relative density of oligochaetes was 5.80% ± 3.38% and 5.56% ± 1.13%, respectively, while in the industrial sites it exceeded 90% except at station E where ammonia concentrations were so high that they were likely inhibitory.

My research results showed that in the industrially influenced portions of the river, generic richness and diversity were lower than at the upstream control site (Fig. 12) and at the same time the relative density of oligochaetes was greater (Table 5). This is consistent with the findings of other researchers. Brinkhurst and Cook et al. (1974) concluded that increased amount of organic effluents tend to increase the individual number of oligochaetes but the number of other benthic species often declines. It was proposed that a bottom invertebrate community containing 80% or more tubificids indicated a high degree of organic enrichment or organic pollution (Aston, 1973). Tubificid relative density at stations C, D-2, and F were in excess of 95%. *Limnodrilus* spp. were suggested to be pollution indicators (Brinkhurst and Cook, 1974). The relative abundance of *Limnodrilus* spp. increased significantly in the industrially influenced portion of the Welland River. At the agricultural stations, the relative density of *Limnodrilus* spp. was 5.24% ± 3.53% and 5.43% ± 1.18% for station A and B, respectively. In the industrial stations. it ranged from 34.92% ± 23.39% to 95.50% ± 2.56% except at station E (Table 5).

The lower benthic invertebrate generic richness and

diversity in the industrially influenced portion of the Welland River may be due to toxic pollution. As discussed in the previous section, the river may be contaminated with potential toxic organic pollutants, especially at stations C and D-1. Stations C and E had very low densityies of benthic invertebrates (114 ± 85 m^{-2} and 1325 ± 534 m^{-2}, respectively) compared with the agricultural area stations A and B (4654 ± 219 m^{-2} and 5610 ± 1352 m^{-2}). The toxicity of samples from station C was evident from both DMSO and distilled water extracts (Figs. 4 and 6). The high concentration of toxic heavy metals in sediments at station C was also noted. At station C, Nickel was about 90 times higher in its concentration than at upstream sites (3929 ± 600 ppm), Chromium about 120 times higher (3712 ± 130 ppm), Lead about 11 times higher (800 ± 200 ppm), Zinc about 8 times higher (1088 ± 190 ppm), and Copper about 27 times higher (753 ± 77 ppm) (Dickman et al., 1986).

The high content of cyanide (2.3 kg/day or 0.092 ppm. Niagara River Toxics Committee, 1984) and ammonia at site E may be one of the reasons for the observed low species richness and low densities of benthic invertebrates at station E. Another reason was that at stations C and E serious shock loading was found (Dickman et al., 1983 and 1988). Shock loads are defined as short term sporadic discharges. At station C, it was recorded that the specific conductivity increased about 8 times when sporadic discharges occurred and at station E the specific conductivity increased about 4 times during shock loading events (Dickman et al., 1986). Shock loads damaged the aquatic plants in

the Welland River, and it was concluded that shock loads were a more serious pollution problem than heretofore believed (Dickman et al., 1988). Invertebrate community structure at stations C and E may be in part a response to these shock loads which altered their habitat by eliminating many of the natural aquatic plants at these sites.

The relative density of oligochaetes and chironomids along the Welland River benthic zone showed a significant negative correlation (Table 4.). This is suggestive of competitive exclusion, an hypothesis which was not tested in this study.

CONCLUSIONS

1. The SOS chromotest revealed that organic soluble (DMSO soluble) contaminants in the sediments from station D-1 (an industrial discharge site in the Welland River near a polyvinyl chloride plant) resulted in significantly higher genotoxicity at this site.
2. Sediment genotoxicity was higher in the industrial influenced portions of the Welland River than in the agricultural influenced portions.
3. The highest frequency of deformities in chironomid labial plates was found at station D-1. The lowest frequency of deformities in chironomids was found at the control site (station A).
4. The correlation between genotoxicity of sediments and the frequency of deformities in chironomid labial plates was statistically significant ($P<0.05$) using a parametric test. The non-parametric correlation test (Spearman-Kendall Rank Test) was not significant ($p>0.05$).
5. The generic richness and diversity of benthic invertebrates was lower in the industrial influenced portions of the Welland river than in the agricultural areas.
6. The sediment toxicity of samples extracted in the DMSO was high for samples from stations C and D-1. Toxicity was also found in the distilled water extracts of sample sediments from stations C and D.
7. The most abundant benthic invertebrate genera in the agricultural section of the Welland River were chironomids,

oligochaetes, and crustaceans, while in the industrialized section of the river, oligochaetes and chironomids dominated and the crustaceans were extremely rare.

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Appendix 1. Industrial discharges in the Welland River.

(MOE, 1987) (kg/day).

Appendix 1. Industrial discharges in the Welland River (MOE, 1987) (kg/day).

discharges	sample sites			
	C	D	E	F
Cadmium	0.085	----	----	----
Chromium	15.6	----	2.28	----
Iron	47.7	----	----	0.703
Phosphate	----	0.89	20.14	3.2
Ammonium ion	----	4.95	----	3.28
Nitrogen	----	----	2106.59	----
Kjeldahl organic.	----	----	10.79	11.9
Oil and grease	31.0	----	----	24.1
Vinyl chloride	----	0.09	----	----
Suspending solids	1148.3	24.76	6.67	145.10
BOD	----	43.37	16.63	47.6
flow (m^3/day)	24584.9	2213.6	27342.0	8554.0

Appendix 2. The percent alkaline phosphatase activity in the SOS chromotest of seven sediment samples taken from the Welland River.

ALKALINE PHOSPHATASE ACTIVITY (PERCENTAGE)

SAMPLE SITES	CONCENTRATION (mg /assay)	DMSO + S9			DMSO - S9			DW + S9			DW - S9		
		MEAN	S.D.	n	MEAN	S.D.	n	MEAN	S.D.	n	MEAN	S.D.	n
A	0.0	100		12	100		4	100		4	100		4
	10.0	100	2	9	103	2	4	102	3	4	105	2	4
	20.0	104	2	10	108	2	4	98	3	3	103	2	4
B	0.0	100		4	100		4	100		4	100		4
	10.0	99	1	4	97	3	3	100	1	4	100	4	4
	20.0	98	1	4	103	2	4	98	2	4	101	4	4
C	0.0	100		12	100		2	100		4	100		4
	10.0	92	2	12	97	3	2	103	1	4	103	2	4
	20.0	95	2	12	101	1	2	98	1	4	104	3	4
D - 1	0.0	100		12	100		4	100		4	100		4
	10.0	95	2	12	106	3	3	101	2	4	103	2	3
	20.0	96	2	12	103	4	3	95	2	4	103	2	3
D - 2	0.0	100		12	100		4	100		4	100		4
	10.0	96	2	12	106	2	3	99	1	4	101	3	3
	20.0	98	2	12	106	2	3	103	3	4	102	3	3
E	0.0	100		11	100		4	100		4	100		4
	10.0	97	2	12	110	4	3	99	1	4	110	3	3
	20.0	97	2	12	112	6	3	99	1	4	110	3	3
F	0.0	100		12	100		5	100		3	100		5
	10.0	95	2	12	106	4	5	101	3	3	108	12	5
	20.0	97	2	12	99	2	5	106	3	3	87	9	5

Appendix 3. The % frequency of deformities in chironomid labial plates at five stations in the Welland River.

Appendix 3. The % frequency of deformities in chironomid labial plates

replicates	sample sites				
	A	B	D-1	D-2	E
1	5.66%(53)	13.37%(329)	9.76%(41)	2.27%(44)	3.92%(51)
2	0.0%(5)	4.26%(258)	4.76%(21)	0.0%(7)	4.40%(134)
3	3.39%(59)	1.95%(256)	9.09%(11)	7.14%(28)	---------
4	7.69%(39)	3.17%(600)	20.0%(5)	7.69%(13)	---------
5	2.44%(41)	8.43%(83)	--------	21.43%(14)	---------
6	---------	6.54%(107)	--------	0.0%(5)	---------
mean	3.84%	6.29%	10.90%	6.42%	4.20%
S.D.	1.32	1.71	3.23	3.30	0.28