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

by

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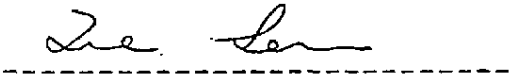
Signature 

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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 waterbodies. 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 is now costing 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 my knowledge, my thesis research is 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 been done on 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 me to 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; Samolloff et al., 1983; Xu and Dutka, 1986). In fact, contaminated drinking water was considered at one time 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). The differences between these genotoxins are not absolute.

For example, 90% of the carcinogens were also known mutagens tested in the Ames test of McCann and Ames (1976). 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). I preferred to use the term "genotoxicity", 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 been looking for 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, 1983; Dutka et al., 1981; Samolloff et al., 1983; Sato et al., 1983). The Ames test employs Salmonella bacteria which carry a mutation at an easily detected locus. 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. 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 (Metcalfe et al., 1985). Some extensively used pesticides such as organphosphorous 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. Samolloff et al. (1984) reported that neutral compounds

extracted from sediments of Tabin Lake (Canada) which was contaminated with agricultural runoff, mining, petrochemical industries, and paper mills and municipalities 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 principle 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). The test strain (E. coli PQ 37) carries a gene fusion of the SOS repair operon (lacZ gene). The test strain 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), I used it as my primary mutagenicity screening tool in my 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).

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, coaltar-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 feild which they could not duplicate in the lab.

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 my 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 genotxins act alos 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-caaled 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 contaminated 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 (Samolloff et al., 1983). Sediment extracts of samples from the Niagara River and its feeder streams appeared to have high levels of toxicity (Sato et al., 1983). Therefore, genotoxic pollutants could exert their effects on the benthic invertebrate community 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 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 pattern in a benthic invertebrate community can be related to the level of toxic materials entering the environment (Hart and Fuller, 1974; Buikema 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 low, 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).

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 patterns 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 portion in an industrialized areas (Fig. 1).

Sampling sites

Site A: the upstream Welland River control site was 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: Welland River entensive agricultural site was located at the confluence of the OWMC Swale and the Welland River, in acore 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 those 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 (Appenix 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), values of the effluents were 8 out of 12 months higher than the MOE requirement, and the discharge contained ammonium ion, vinyl chloride and phosphate. The emergent vegetation at this site was dominated by Potamogeton spp., and Typha spp.

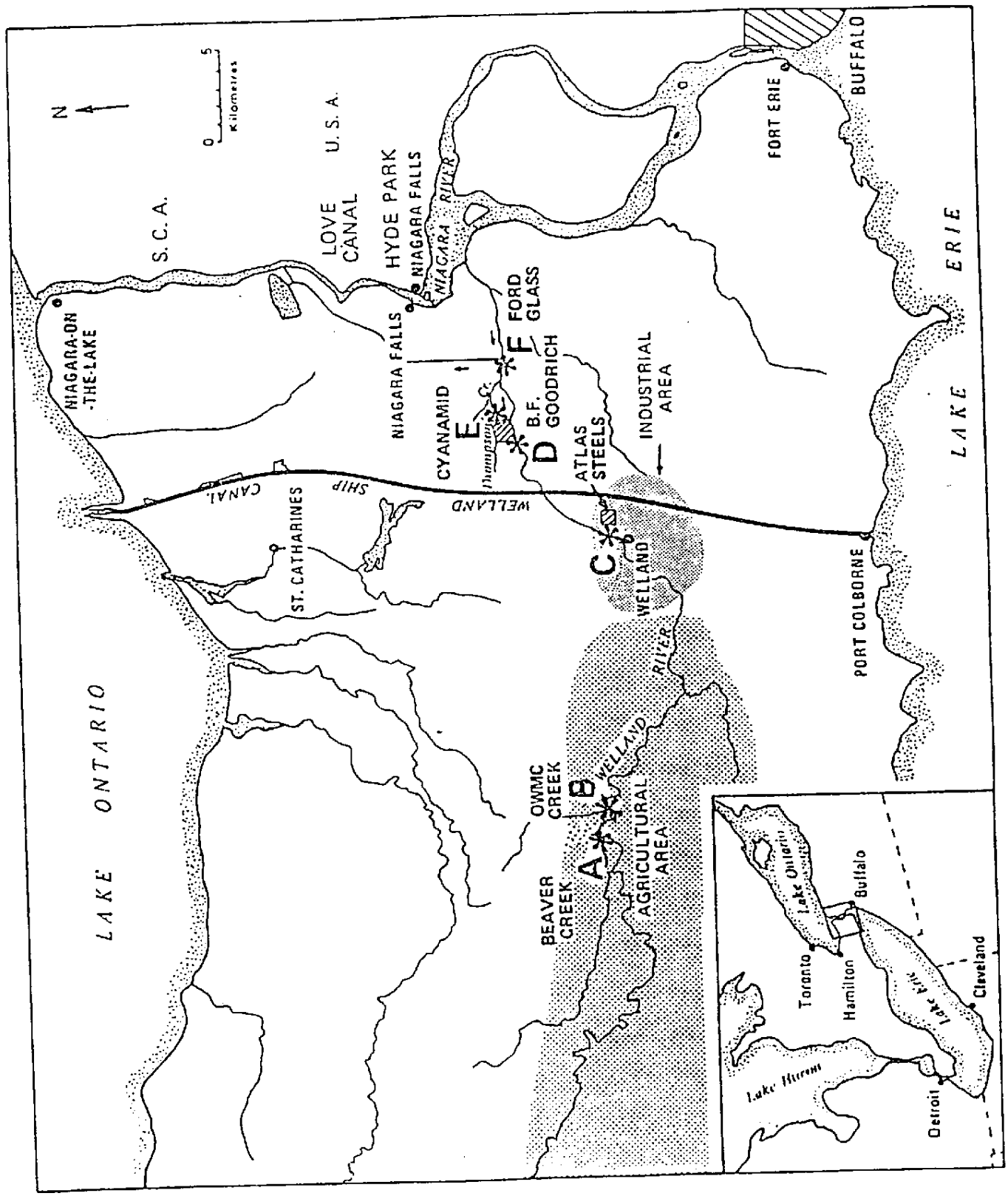
Site D-2 was located at the confluence of the discharge stream of the B.F. Goodrich Co. and the Welland River just downstream of site D-1. The emergent vegetation at this site was dominated by Sagittaria spp., Cladophera spp., and Typha spp.

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.,

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).



Sample preparation for genotoxicity tests

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

The dimethyl sulfoxide (DMSO) sediment extracts were made by adding 25 g (the wet weight of sediment was weighed out and placed in a clean bottle) 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. The distilled water extract procedure was similar to the DMSO extraction procedure except that ultra-pure distilled water replaced the 10% DMSO solution.

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_4 161.1g, $NaH_2PO_4.2H_2o$ 5.5g, 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 Lit.), 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 fraction of 0.1 ml (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 ul 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 condition for these two enzyme assays. In order to screen the pro-genotoxic materials, an S9 mammalian microsomal fraction was used in the SOS chromotest.

Optimal chromotest development time

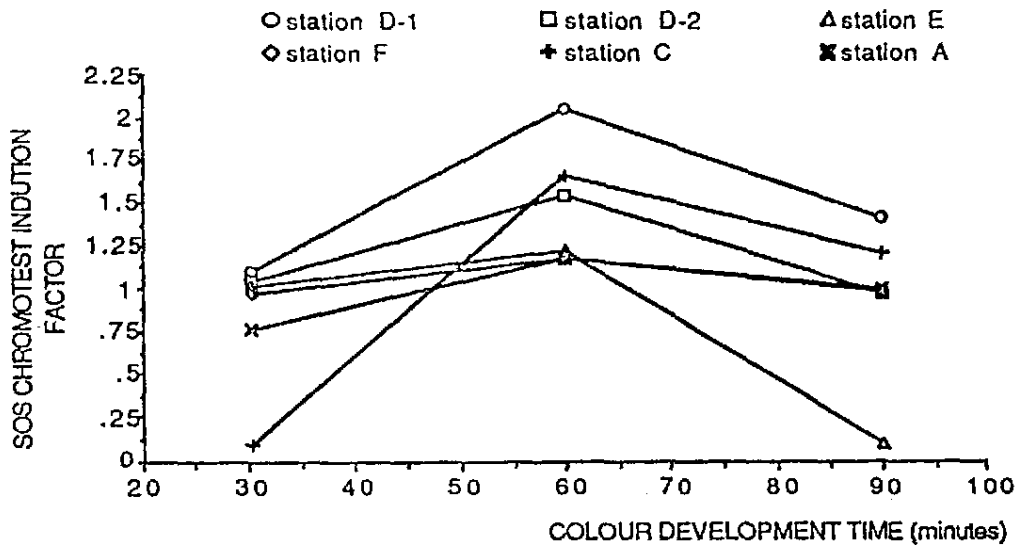
To determine the optimum SOS chromotest time, several tests for induced colour development time were conducted. The results of the optimum SOS chromotest time test showed that induced colour development of 60 minutes was the optimum time (Fig. 2). Therefore, the results of this study were reported as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

Assays for beta-galactosidase

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 been replased 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

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

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

$$I(c) = \frac{R(c)}{R(0)}$$

where R(c) and R(0) represent the ratio (R) at concentrations (C) and (0), 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 made within 12 hours of sample collection. The collected sample, a mixture of mud or clay and water, was poured into 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% glycerine

solution.

The Annelids were carefully picked from the vials, and placed on clean glass slides, mounted in Ammans Loctophenol solution and covered with a glass cover slip. Next, they were each examined under a Leitz compound microscope at 400 x magnification. Dichotomous keys (Pennak, 1953; Pimental, 1967; Brinkhurst and Cook, 1974) 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 means of Leitz compound microscope. Dichotomous keys to the bethic invertebrates were in Pennak (1953).

Evaluation of symmetry patterns and abnormalities

The symmetry of each invertebrate was observed (ie. labial plates of each chironomid specimen was 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.

The Sperman-Kendall rank correlation analysis 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.

Calculation of Species Diversity Indices

Simpson's diversity index:

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

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

Shannon-Wiener's diversity index:

$$H = - \sum_{i=1}^{k} p \log p$$

where p 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). 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. 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 ($P<0.01$).

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.	(April,1987) mean	n	S.D.	10% DMSO -S9 (December,1986) mean	n	S.D.
A	1.173	6	0.123				0.854	4	0.119
B	1.062	4	0.066				0.915	4	0.098
C	1.664	10	0.429				1.072	2	0.007
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. (continuous)

sample sites	Distilled Water Extract+S9 (December,1986) mean	n	S.D.	Distilled Water Extract -S9 (December,1987) mean	n	S.D.
A	1.064	3	0.086	0.873	4	0.033
B	0.993	4	0.019	0.827	4	0.094
C	0.984	4	0.010	0.806	3	0.066
D-1	1.285	4	0.041	1.062	3	0.088
D-2	1.059	4	0.084	1.031	3	0.078
E	1.024	4	0.097	0.827	3	0.031
F	0.964	4	0.026	1.200	6	0.441

indicate statistical significance

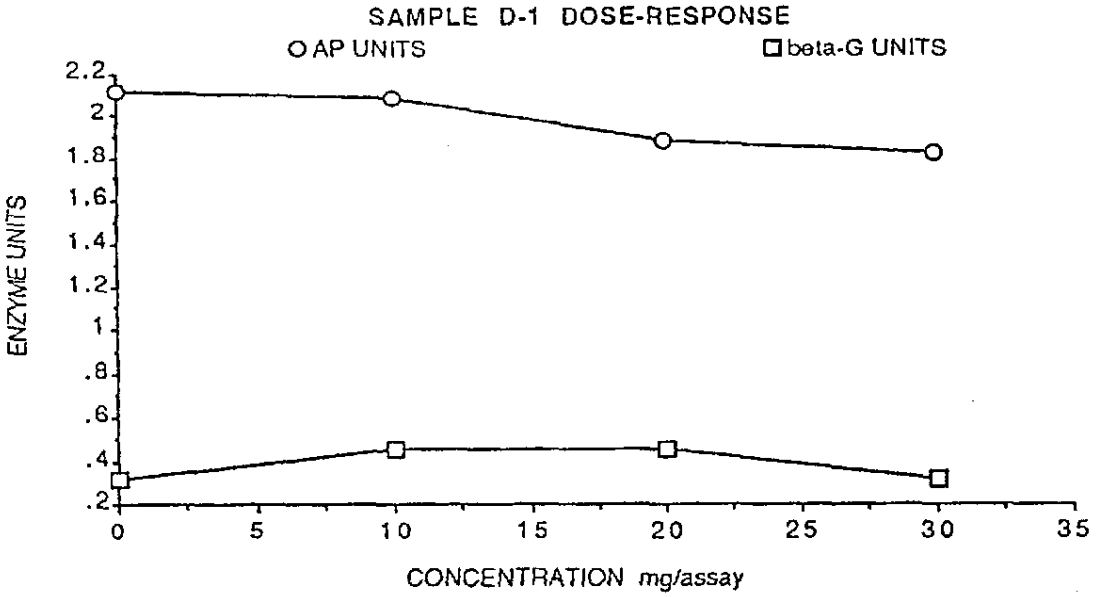
Of the samples assayed, only samples from station D-1 were pro-genotoxic in DMSO extract. To construct a dose-dependent response pattern for the samples which gave a positive response to the SOS chromotest, the enzyme activities of beta-galactosidase and alkaline phosphatase were assayed. The dose-response 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 inhibition 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.5). DMSO extracts without S9 in the SOS chromotest did not appear to inhibit protein biosynthesis (Fig. 6), 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. 7). 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 activated by metabolic activation.

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


SAMPLE D-1 DOSE-RESPONSE
O 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

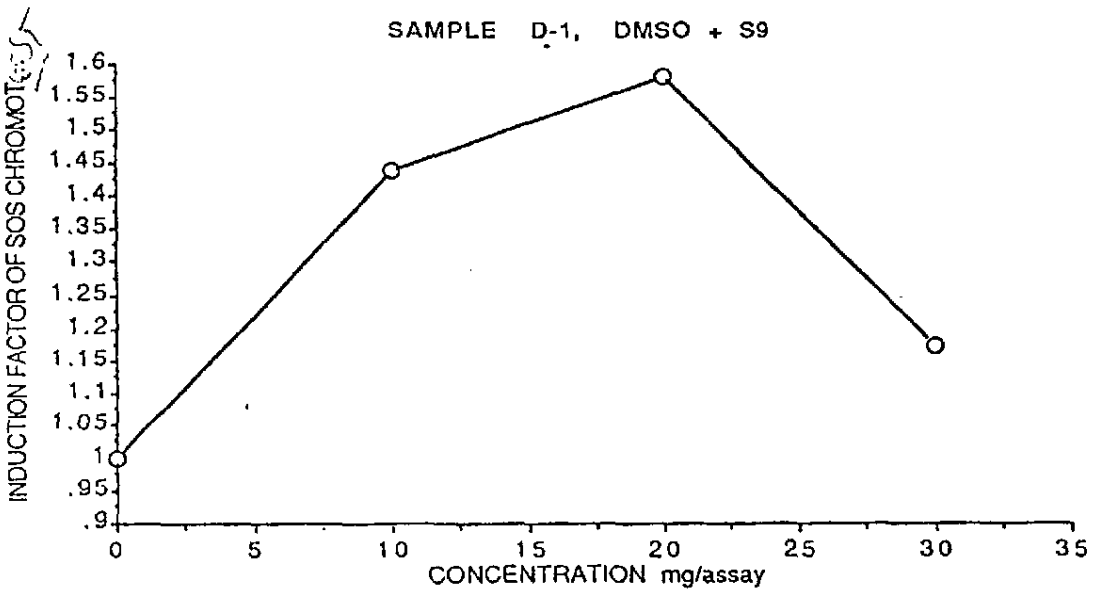
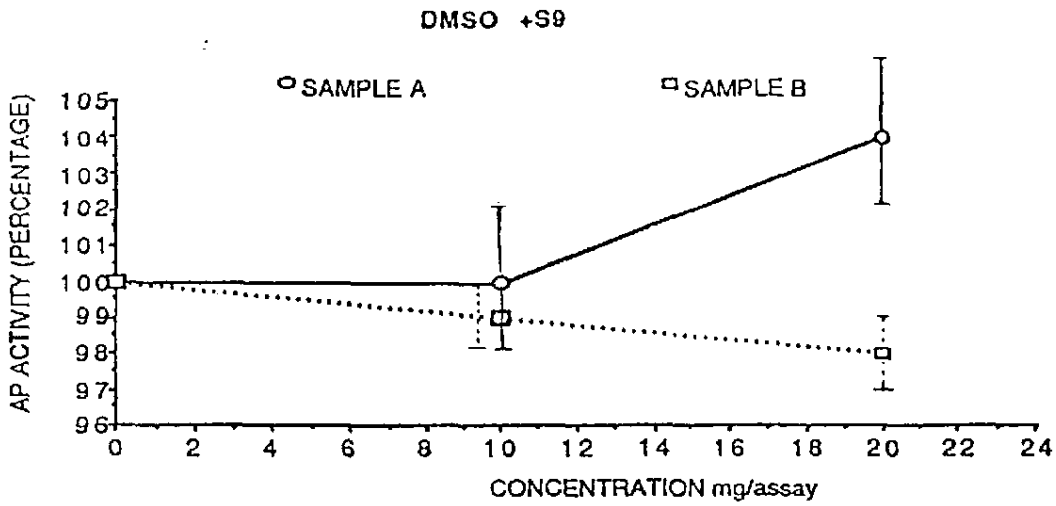
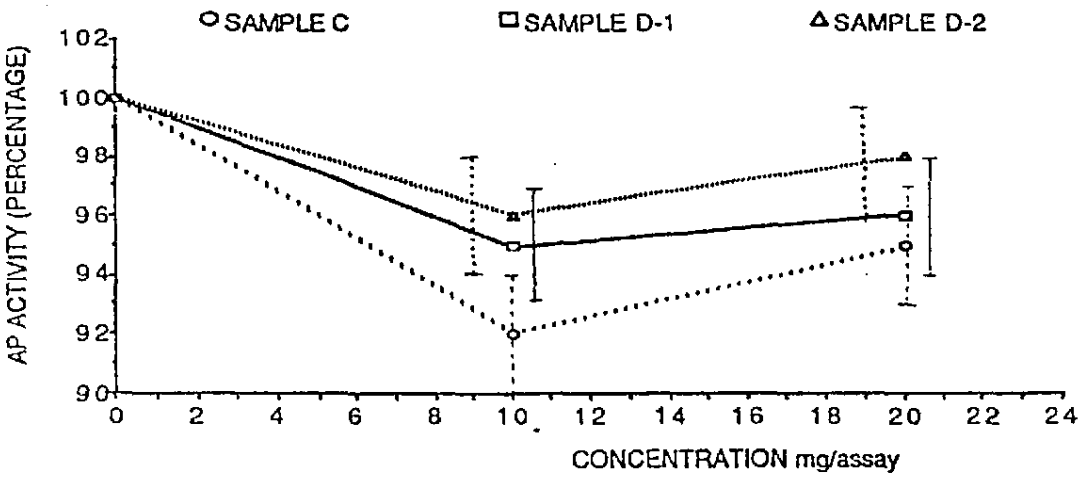

SAMPLE D-1, DMSO + S9
INDUCTION FACTOR OF SOS CHROMOT
1.6
1.55
1.5
1.45
1.4
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
.95
.9
0
5
10
15
20
25
30
35
CONCENTRATION mg/assay

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


DMSO +S9
SAMPLE A
SAMPLE B
AP ACTIVITY (PERCENTAGE)
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)
102
100
98
96
94
92
90
0 2 4 6 8 10 12 14 16 18 20 22 24
CONCENTRATION mg/assay

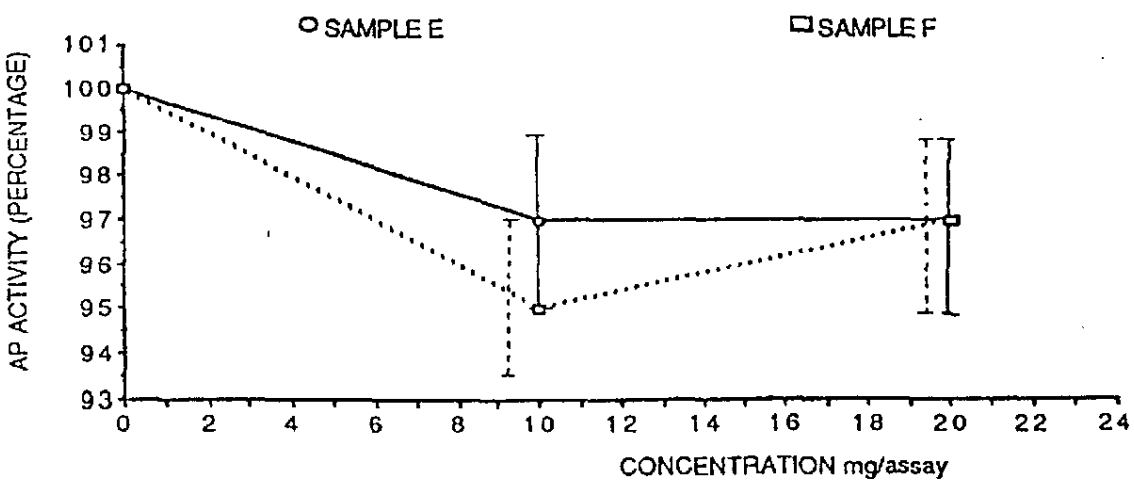
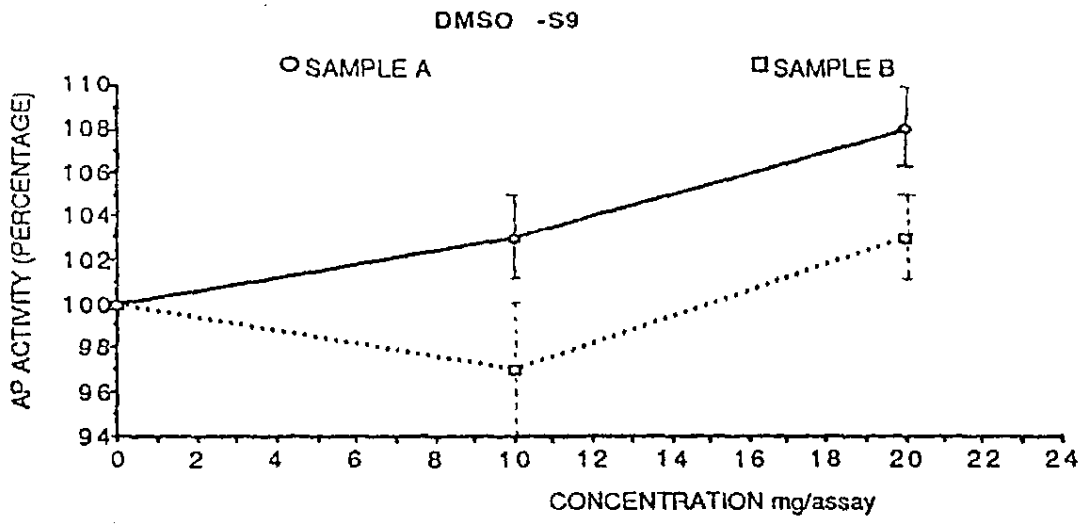
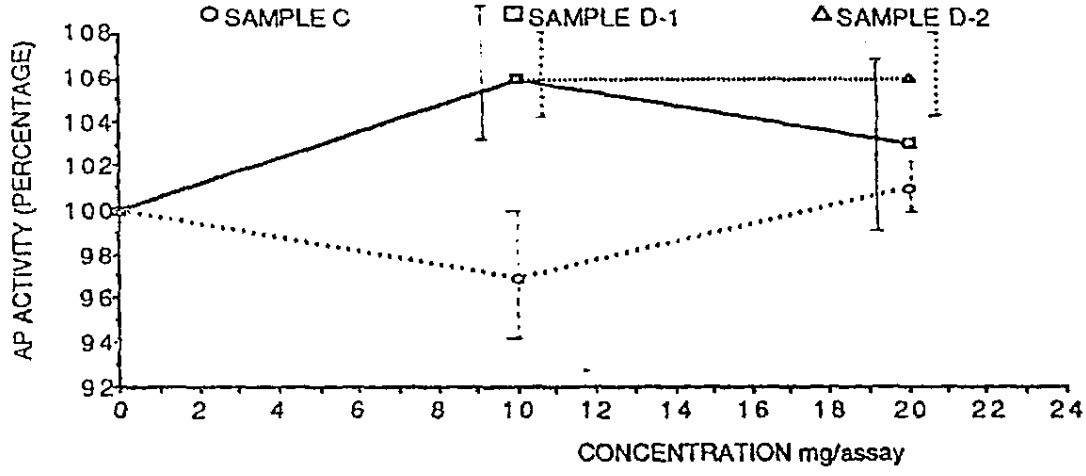

SAMPLE E
SAMPLE F
AP ACTIVITY (PERCENTAGE)
101
100
99
98
97
96
95
94
93
0 2 4 6 8 10 12 14 16 18 20 22 24
CONCENTRATION mg/assay

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


DMSO -S9
SAMPLE A
SAMPLE B
AP ACTIVITY (PERCENTAGE)
110
108
106
104
102
100
98
96
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)
108
106
104
102
100
98
96
94
92
0 2 4 6 8 10 12 14 16 18 20 22 24
CONCENTRATION mg/assay

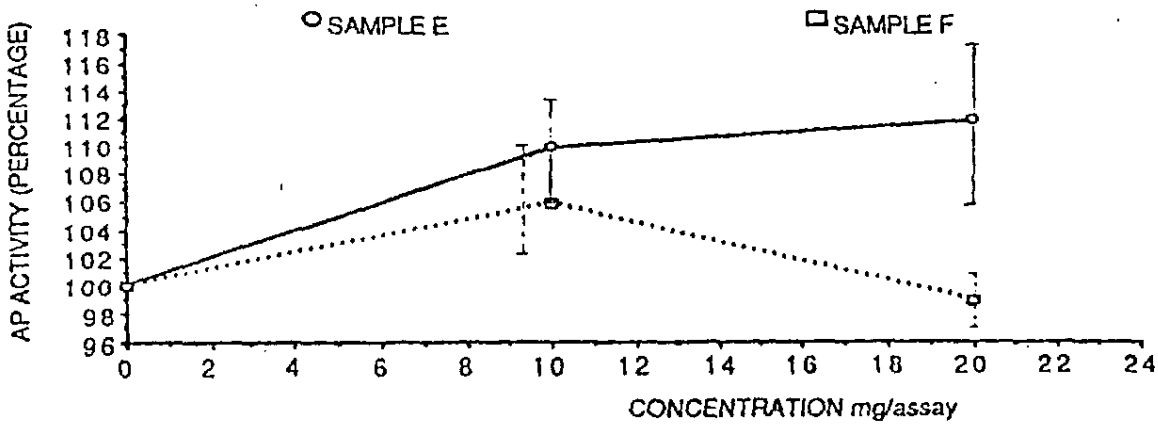
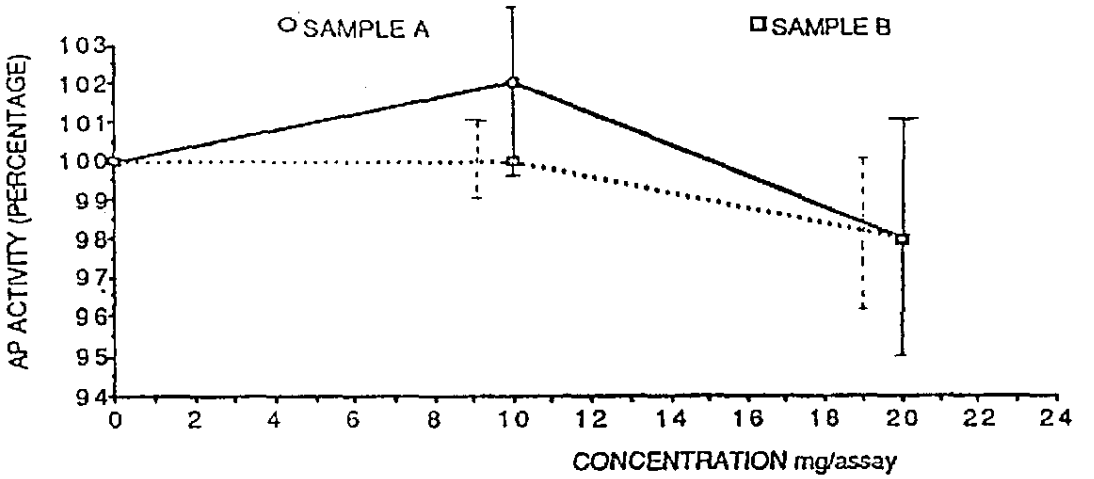
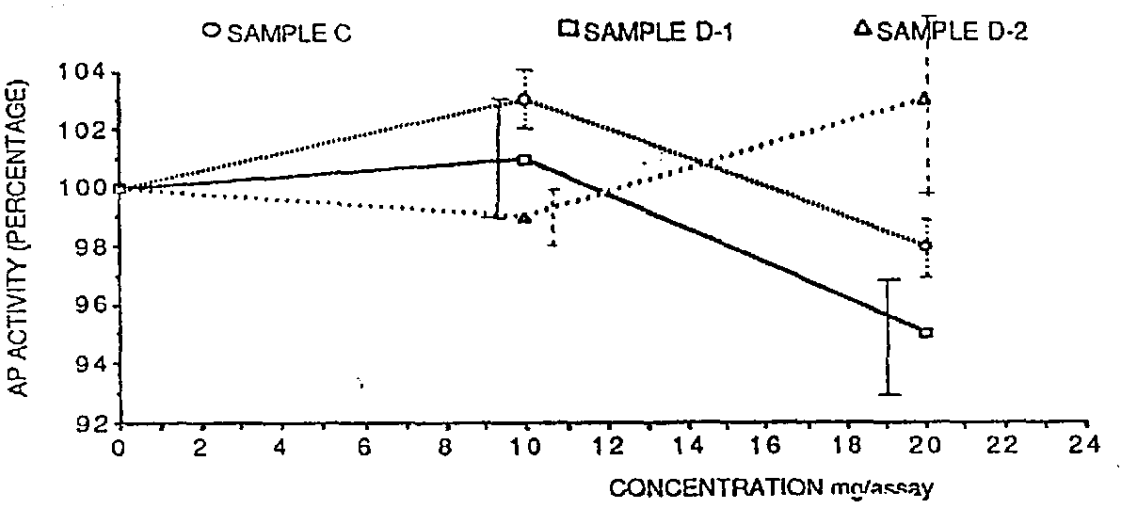

SAMPLE E
SAMPLE F
AP ACTIVITY (PERCENTAGE)
118
116
114
112
110
108
106
104
102
100
98
96
0 2 4 6 8 10 12 14 16 18 20 22 24
CONCENTRATION mg/assay

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

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
0 2 4 6 8 10 12 14 16 18 20 22 24
CONCENTRATION mg/assay

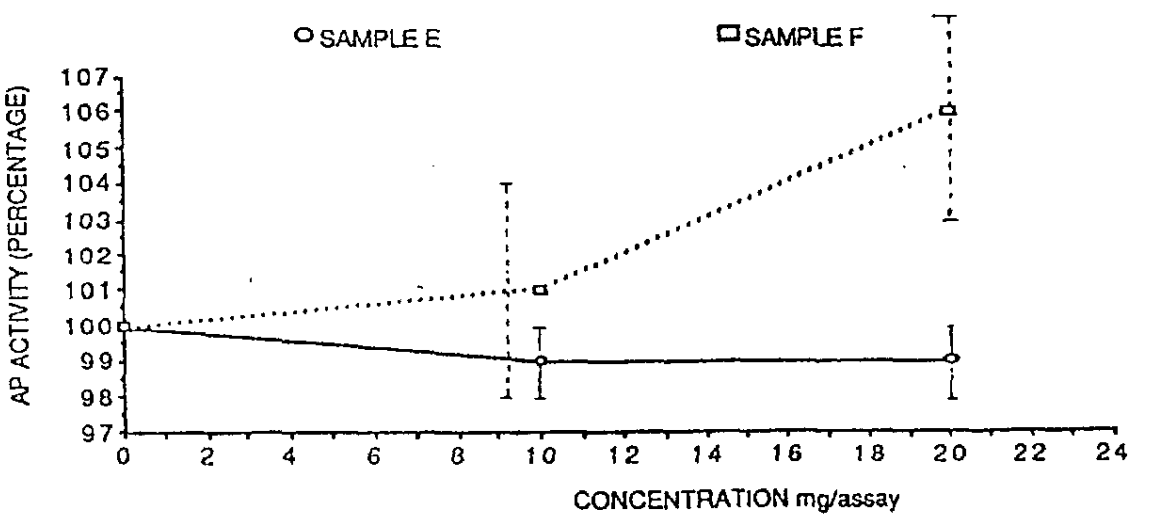
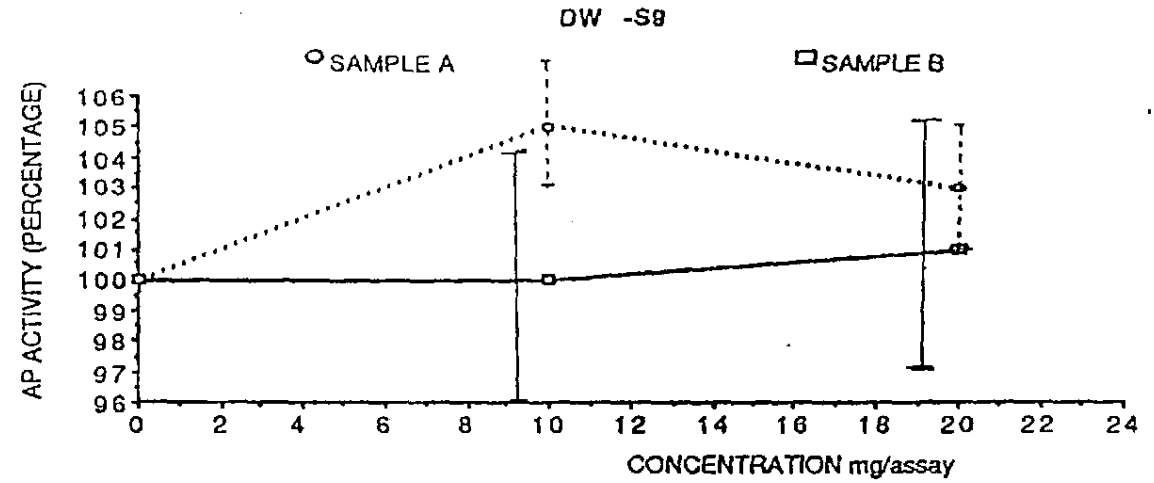
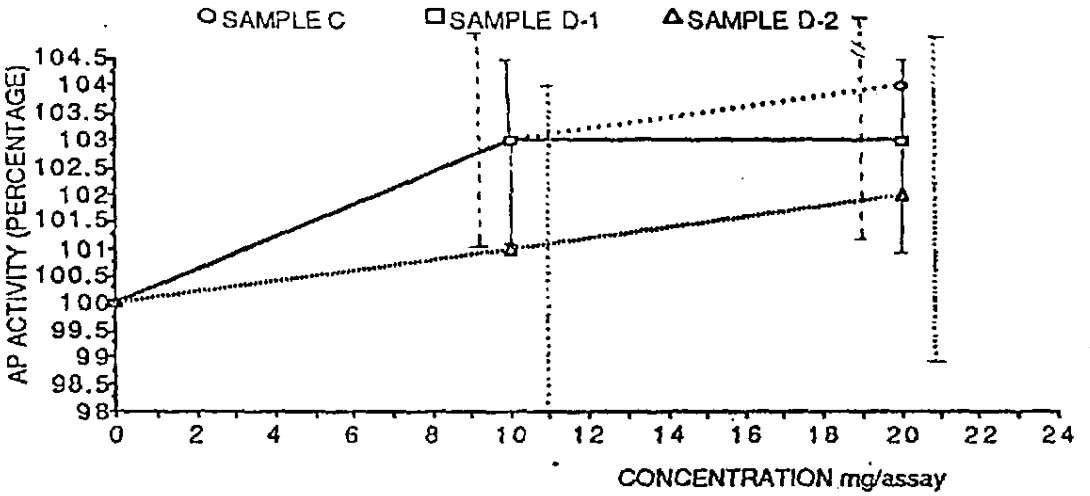
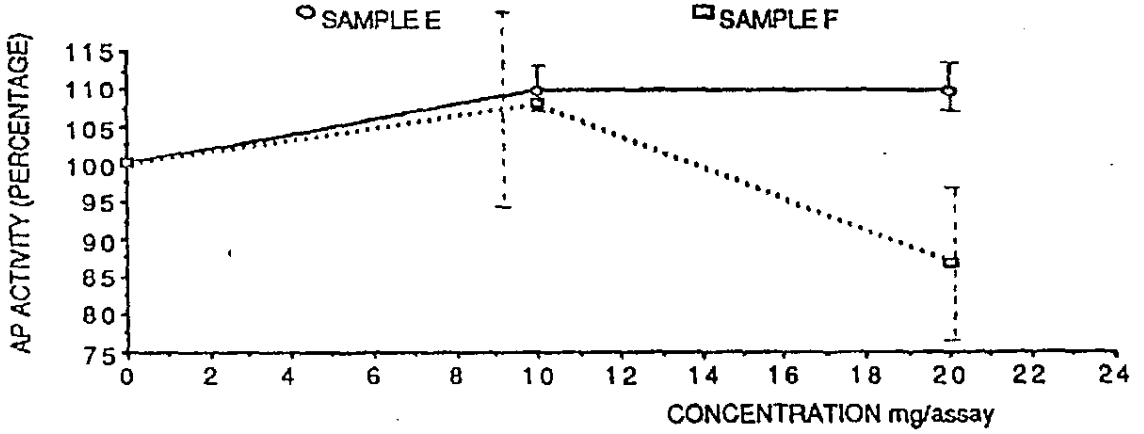

SAMPLE E
SAMPLE F
AP ACTIVITY (PERCENTAGE)
107
106
105
104
103
102
101
100
99
98
97
0 2 4 6 8 10 12 14 16 18 20 22 24
CONCENTRATION mg/assay

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


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 was 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, 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 agricultural 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 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 carring 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

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.

Figure 8. "Normal" structure of chironomid labial plate (modified from Wiederholm, 1983).

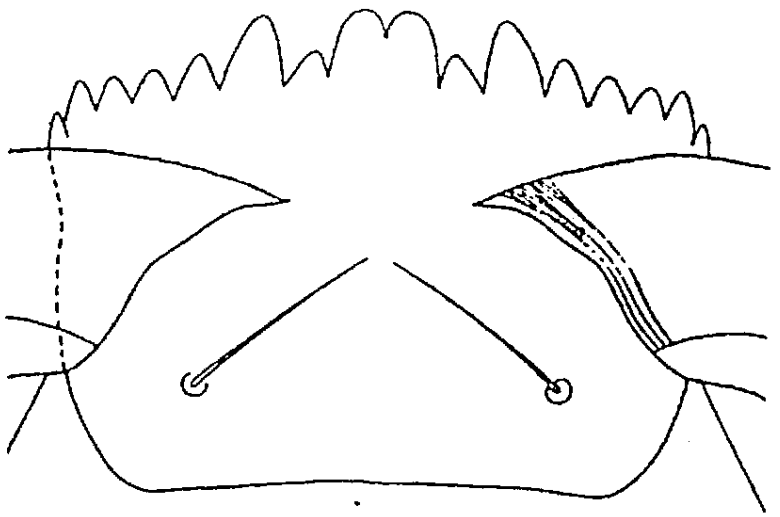
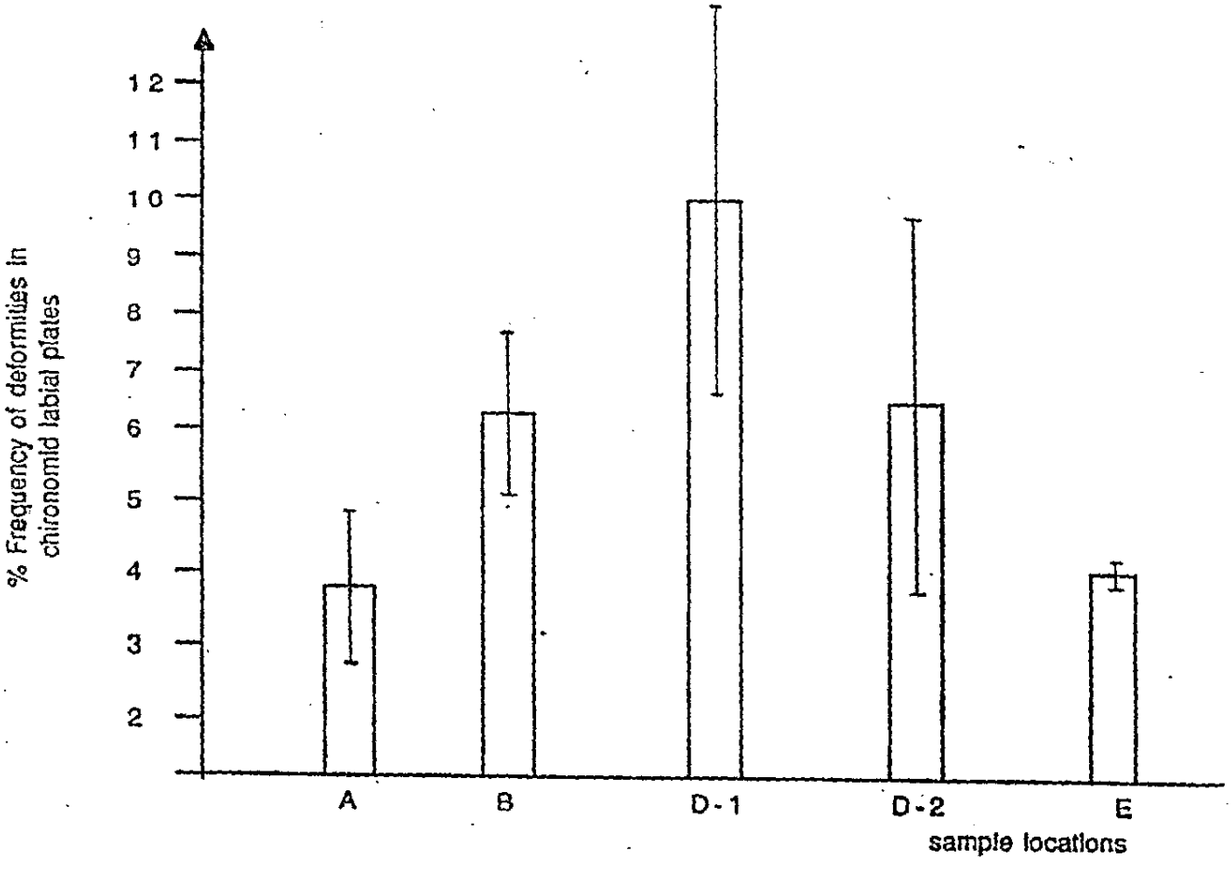


Figure 9. Deformed chironomid labial plate.

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).



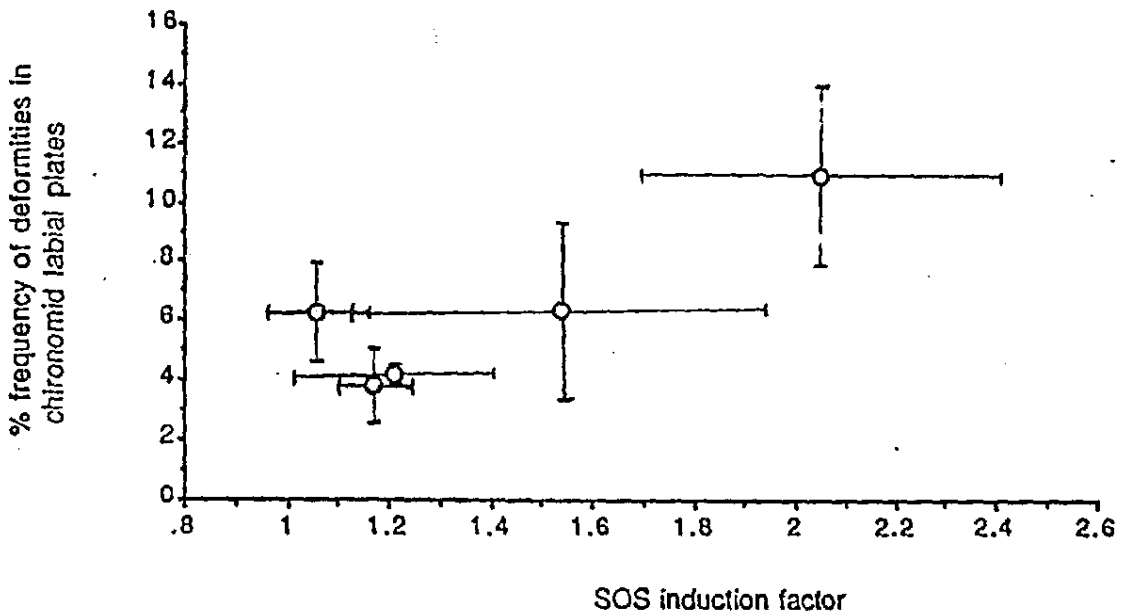
The correlation between the genotoxicity of sediments and the frequency of deformities in chironomid labial plates

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 (Sperman-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