NWRI Contribution No. 86-174

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Short Running Title: Phase II Priority Site Selection

Key Words: Toxicity screening, bacterial densities, fecal sterols, Clostridium, coliphage

MANAGEMENT PERSPECTIVE

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

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ABSTRACT

The suitability of a variety of biochemical, microbiological and bioassay tests to become part of a battery of test procedures to identify degraded or degrading sediments and waterbodies are evaluated in this report. Data were obtained from 40 river (Detroit and Niagara) and inshore Lake Erie sampling sites. These data indicate that microbial population, biochemical or bioassay tests performed independently do not provide realistic estimates of priority concern areas and that the battery approach is necessary.

INTRODUCTION

In a previous publication, Dutka et al. (1986) described the results of a study to evaluate the suitability of a variety of microbiological, biochemical and bioassay tests to become part of a "battery of test procedures" which could be used to designate, nationally and internationally, water bodies or sediments that are degraded or are being degraded. This "battery of tests" could also be used to monitor the efficiency of various sewage treatment processes.

In this paper, the second phase of our attempts to develop this battery are described, using the waters and sediments of the lower Detroit River, north shore of Lake Erie and the upper reaches of the Niagara River as testing sites. In the second phase study, the ATP-TOX System, a toxicity screening test (Xu and Dutka, 1987) and the SOS Chromotest with S-9 addition, a new genotoxicity screening test, were added. Also added was a test for Clostridium perfringens spores, as an indicator of long-term and ongoing pollution. The dehydrogenase activity test which was used in the first study was dropped from the second phase.

The final goal of these studies is to develop a "battery of tests" containing two or three toxicant-genotoxic screening tests and two to three microbiological hazard screening tests which can be used internationally to prioritize specific water bodies and sediments for further investigation and or remedial action.

The "battery" approach will make it possible to establish "hot spots", areas of immediate concern, which were not previously suspected due to inappropriate or one-dimensional testing procedures. When the final selection of the "battery of tests" is made, those tests which can be performed on refrigerated or frozen samples, 30-96 hours after collection will be given priority.

Data from phase II sampling sites are presented and the results discussed.

PHASE II. SÉLECTION DE SITES PRIORITAIRES DANS LE CAS DE RÉGIONS DÉGRADÉES, D'APRÈS DES ESSAIS MICROBIENS ET LA RECHERCHE DE SUBSTANCES TOXIQUES

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RÉSUMÉ

Le présent rapport évalue divers essais biochimiques, microbiologiques et biologiques afin de les inclure dans une batterie de tests visant à identifier les sédiments et bassins dégradés ou en cours de dégradation. Les données proviennent de 40 sites d'échantillonnage fluviaux (rivières Détroit et Niagara) et situés près du rivage du lac Erié. Ces données indiquent que l'étude de la population microbienne et les essais biochimiques ou biologiques réalisés de manière indépendante ne fournissent pas des estimations réalistes relatives aux régions les plus durement touchées et qu'il est nécessaire d'utiliser l'approche de la batterie de tests.

PERSPECTIVE-GESTION

Cette série d'études, toujours en cours, vise à identifier les masses d'eau dégradées ou en dégradation à l'aide de divers essais microbiologiques, biochimiques et biologiques. On vise à déterminer si différents tests (coliformes fécaux, streptocoques fécaux, E. coli, Legionella, coliphages, coprostanol, cholestérol, système ATP-TOX, essai de génotoxicité et essai Microtox) peuvent faire partie d'une batterie de tests qui servira à accorder une priorité aux bassins et sédiments ou à certaines zones de bassins, en fonction de la gravité de la dégradation, pour que puissent être prises des mesures correctives ou entreprises d'autres études. L'utilisation d'une batterie de tests devrait permettre de déterminer des régions prioritaires qui jusqu'ici ne semblaient pas causer des problèmes à la lumière des essais unidimensionnels ou inadéquats réalisés. Au moment du choix des tests microbiologiques, biochimiques et biologiques à inclure dans la batterie, seront préférés les tests qui peuvent être réalisés sur des échantillons réfrigérés ou congelés, 24 à 96 heures après le prélèvement. L'essai sur les coliphages, l'un des paramètres présentement étudiés, est particulièrement important puisque ce test permet d'obtenir des renseignements sur la présence potentielle d'organismes indicateurs et de pathogènes bactériens et viraux. Les données sur les coliphages obtenues dans le cadre de ces études seront comparées à des données obtenues dans le cadre d'une étude portant sur trois continents (Sud-est asiatique, Amérique du Aud et Afrique du Nord) et huit pays, réalisée sous la direction de B.J. Dutka grâce au parrainage du Centre de recherche pour le développement international (CRDI), Ottawa, Canada.

METHODS

Sampling Sites

A total of 40 sampling sites were selected for this study during June 1986, 11 within the lower portion of the Detroit River below Windsor, one at Monroe, Michigan, 22 sites along the north shore of Lake Erie and six sites within the upper reaches of the Niagara River (Fig. 1, Table 1).

The sampling sites were selected to reflect river and stream loadings into Lake Erie, industrial and domestic outfalls and for background information some areas thought to be unpolluted.

Sample Collection

All sediments were collected with an Ekman dredge and the whole dredge sample was split between various containers. Where little sediment was available, several casts were made and the samples were split between the various containers and kept on melting ice (Dutka et al., 1986).

Water was collected by bucket from the surface and was partitioned and preserved as described by Dutka et al. (1986).

Microorganism Tests

Legionella, fecal coliforms, fecal streptococci, E. coli and coliphage tests were performed as described by Dutka et al., 1986.

Clostridia and Clostridium perfringens, MPN enumeration techniques as detailed in "Methods for Microbiological Analysis of Waters, Wastewaters and Sediments (Dutka, 1978), were used in the study. Sediment samples were preincubated at 75°C for 15 minutes before inoculating into preheated (70°C) tubes of DRCM broth after which a temperature of 70°C was maintained for 15 min. The tubes were then incubated at 35°C for 48 hr.

All tubes showing signs of H_2S production were subcultured into preheated (50°C and cooled to 35°C) tubes of litmus milk and incubated at 35°C for up to five days. The formation of a "stormy clot" was taken as confirmation of Cl. perfringens presence.

Cl. perfringens is probably the most widespread, pathogenic anaerobic organism on earth and its distribution is considered to be ubiquitous. The natural habitat of this organism and the only place where it can form spores is in the colon of warm-blooded animals. Its occurrence in nature is consequently dependent on the presence of fecal pollution (Bonde, 1963). An excellent review on Cl. perfringens has been prepared by Bonde (1963).

Lactose Fermenting Isolates

A total of 202 isolates were collected and identified from positive A_1 broth tubes (sediments) as well as from typical fecal coliform colonies on MF-MFC plates. Identification procedures included lactose fermentation, oxidase reaction, IMVC tests, motility, H_2S production and inositol and sorbitol fermentation. Isolates were collected to ascertain the sensitivity of the two techniques to select and enumerate E. coli in these waters and sediments. A search was also made for E. coli serotype 0:157, the causal organism of several recent outbreaks of diarrhea in southern Ontario.

BIOCHEMICAL TESTS AND TOXICITY SCREENING TESTS

Fecal sterol and Microtox tests were performed as outlined by Dutka et al. (1986).

Genotoxicity Test

The test consists of colorimetric assays of enzymatic activities after incubating the tester strain, E. coli K12-PQ37, in the presence of various dilutions of sediment extracts and water samples. Sediments were extracted

(20 gm wet wt) with 20 mL APHA Standard Methods Buffer. The mixture was vigorously shaken for 3 minutes, centrifuged for 10 minutes at 4°C at 5000 rpm and the supernatant tested for genotoxicity activity with and without the addition of S-9 mix (Fish et al., 1985). The results were read by a microtitration plate (Elisa) photometer.

Induction factors were calculated as per Quillardet and Hofnung (1985) with values greater than 1.30 being considered significantly different from the negative controls.

ATP-TOX System

ATP-TOX System is a new toxicity screening test (Xu and Dutka, 1987) based on the inhibition of bacterial growth and luciferase activity by toxicants. The organism used in this study was E. coli K12-PQ37 with a five hour incubation. Any single or mixed culture of bacteria can be used in this test. Studies by Xu and Dutka (1986a, 1987) have shown that the ATP-TOX System in pure chemical and sediment extract studies is comparable and complementary to the Microtox test as it also provides indications of low grade toxicant activity which can only be manifested in actively growing cells over several life cycles.

For the ATP-TOX System test on sediments, the sediments were extracted with glass distilled H_2O which was passed through a complete Milli Q system, in the ratio 1 gm wet wt sediment to 1 mL ultra purified water. This mixture was vigorously shaken for 3 minutes, centrifuged at 4°C for 10 min at 5000 rpm and the supernatant tested for toxicant activity. Results were reported as IC_{50}, the percentage of water sample or gm wet wt of sediment required to give a 50% inhibition of light output compared to the negative control.

RESULTS AND DISCUSSION

In Table 1, a brief characterization of the 40 sediments is presented as well as site descriptions and latitudes and longitudes of the sampling sites. The scheme used to award points for specific data values and finally rank the sediments and waters from areas of most concern to least concern is presented in Table 2. This scheme is biased toward toxicant presence and the direct presence of hazardous microorganisms. Samples with the most points are deemed to contain the greatest potential hazard to man and living organisms found in the aquatic ecosystem. High toxicant levels may have reduced microbial levels/activity in some sediment samples, however, cause and effect relationships were not investigated. Sediments composed mainly of sand are suspected of not providing an accurate picture of sediment toxicant levels or bacterial levels due to the larger surface areas compared to clay, silt or black "gooey" sediments.

Table 3 presents the results of four bacterial, one coliphage, two biochemical and three toxicant screening tests on the 40 water samples. Sample collection sites are ranked from most concern (1) to least concern (16), and in this scheme sites with the same number of points are given the same rank, e.g. sites 12, 13, 24, 28, 37 and 40 all have eight points and are all ranked 13th.

In all uses of indicator organisms, we are dealing with a concept, a concept that usually works and is protective (and possibly over protective) of users of natural and potable waters. We believe that due to increasing stresses on water supplies, and rising analytical costs, we must develop cheaper, simpler, more stable and quicker indicator systems which will reflect both bacterial and viral contamination from sewage and farmland runoff. Coliphage appears to be one of the most obvious candidates. Although a review of literature on the coliphage test indicates that coliphage may be an ideal test for approximation of health hazard due to fecal pollution, there appears to be a reluctance to accept research implications to local water quality estimation, even though the procedure has now been documented by North America's two major method standardization organizations, APHA and ASTM. To overcome this reluctance, it may be necessary for each area or jurisdiction considering the use of coliphage, to establish coliphage relationships to fecal coliforms, E. coli, fecal streptococci and other traditional indicators or pathogens. These veting studies for coliphage could be considered inappropriate, as there are no direct numerical relationships between coliforms, fecal coliforms, E. coli and the degree of hazard related to the incidence and infectivity rate of waterborne Salmonella, Shigella, Cholera viruses and also to coprostanol, the absolute indicator of fecal contamination (Dutka and El-Shaarawi, 1975).

In these studies, coliphage data are being collected and evaluated against traditional indicator system data. In this study as in earlier studies (Dutka et al., 1986; Dutka et al., 1986a), statistical analyses of the data indicates that fecal coliforms and coliphages are positively correlated and that the coliphage values can be indicated or predicted by using fecal coliforms, fecal streptococci and E. coli data.

In Table 4 the ratios between fecal coliforms, fecal streptococci, E. coli and coliphage are summarized. This table was developed by listing all the ratios between the parameters and then establishing the mean and median values from this list. One interesting observation noted in Table 4 is the relative stability of the fecal streptococci/coliphage ratios, especially as their only common factor is their fecal origin and they are not part of each others reproductive cycles. The mean fecal coliform/coliphage ratios are very similar to those found in raw source drinking water by the Atlantic Corporation (1979) and are supportive of the concept that coliphages are indicators of microbial health hazards in natural fresh waters.

From Table 3 it can be seen that site #38, Niagara River at Tonawanda, N.Y. had the highest fecal coliform, E. coli, fecal streptococci and coliphage counts. Unfortunately due to laboratory limitations, coprostanol levels (biochemical indicators of fecal pollution) were not tested for on this sample, an ideal site to have confirmed fresh fecal contamination with coprastanol/indicator bacteria levels. Sample #15, Sturgeon Creek on Lake Erie, with its elevated fecal streptococci counts, presents a typical picture of farmland runoff.

Of the 112 isolates collected from fecal coliform MF plates, 77.7% were found to be E. coli, none of which proved to be serotype 0:157. However, samples 7, 9, 33 and 35 indicated only a 15% E. coli confirmation rate. Excluding these isolates from the total, the confirmation rate as E. coli was 91.3%. These studies confirm the basic reliability of the MFC medium to enumerate fecal coliform and E. coli and the lessened requirement for specific media to enumerate E. coli (MTEC agar).

Only one isolation of Legionella pneumophila occurred during this study, at sample site #5, Detroit River, mouth of Canard River (Table 3). These results combined with earlier studies on the presence of Legionella organisms in Canadian rivers and lakes are supportive of our belief that Legionella are present in Canadian fresh waters but at very low levels, and are not a major or consistent part of the natural aquatic microflora (Dutka et al., 1986; Dutka and Ewan, 1986; Dutka et al., 1983; Dutka and Ewan, 1983). Thus, as reported by Dutka et al, 1986, the recovery of Legionella organisms from mesotrophic and eutrophic waters is a rarity. Observations made during this and the previous study in this series suggest that Legionella organism enumeration provides insufficient information to be part of any future battery of tests to discern areas of concern in natural water systems.

Coprostanol and cholesterol concentration estimates were only performed on 50% of the water samples due to laboratory limitations. The presence of cholesterol in all samples tested, as noted in an earlier study (Dutka et al., 1986) is difficult to explain. It has been suggested (personal communication) that this cholesterol may reflect local bird populations as birds are sources of cholesterol and little if any of coprostanol. Unfortunately, bird populations densities at the sampling sites are unknown. The only two water samples positive for coprostanol were #8 Little River and #9 Rouge River mouths taken in the Detroit River. The coprostanol finding at

Site #8 is supportive of concerns expressed in a local newspaper about the pumping of raw sewage into Little River (Windsor Star, 1986). The ubiquitous presence of cholesterol continues to present a problem in understanding the implications of the fecal sterol data.

Two toxicity screening tests were used in this study, the Microtox test and the ATP-TOX System (Xu and Dutka, 1987). As in Phase I studies (Dutka et al., 1986), water samples tested neat and concentrated 10x all proved to be negative for toxicants via the Microtox Test. However, with the ATP-TOX System only five samples #10, 14, 15, 18 and 38 indicated the presence of no toxic activity. The increased sensitivity of the ATP-TOX System over the Microtox procedure can partially be explained by the knowledge that when rapidly growing bacterials cells are exposed to low concentrations of toxicants, growth inhibition usually occurs. Nevertheless, several life cycles will proceed and the toxic effect can be estimated by comparing sample cell growth to the control via ATP content (Xu and Dutka, 1987). Port Burwell (#22) and Port Rowan (#23) surface water samples proved to be the most toxic water samples of this study based on ATP-TOX System.

In the genotoxicity screening tests using a modified SOS Chromotest procedure (Xu and Dutka, 1986), both water and sediment extract samples were tested without heating or sterilizing. With each microplate 4NQO (4-nitroquinoline 1-oxide) was used as a positive control without S-9 and 2AA (2-aminoanthracene) was used as a positive control with S-9. Distilled water was the negative control for the water samples and buffer water was the negative control for the sediment extracts. In the water samples tested for genotoxicity, two samples #1B, Monroe Michigan and #31 offshore Port Maitland had Induction Factors greater than 1.30. This level is considered significantly different from the negative control (IF=1.00) and these two samples could be considered to contain genotoxic material. However, there were several samples which had increased Induction Factors, e.g. #8, #9, #18, #28, #32, #35, #37, #38, #39 and #40 which could be considered, in unconcentrated water samples to show genotoxic activity below the threshold of significance. Interestingly, sample #31 with the highest Induction Factor for genotoxicity also had a fairly high ATP-TOX System value, 33%.

Based on the point scheme developed in Table 2, the ten water quality sampling sites of greatest potential concern are:

1. Detroit River at Rouge River mouth, #9 sample;
2. Detroit River at Little R. mouth, #8 sample;
3. Niagara River at Tonawanda, N.Y., #38 sample;
4. Wheatley Harbour, #17 sample;
5. Port Stanley, #21 sample;
6. Port Burwell, #22 sample;
7. Port Maitland offshore, #31 sample;
8. Detroit River at Canard River mouth, #5 sample;
9. Detroit River at Turkey Creek, #7 sample;
10. Detroit R. at Grosse Ile WTP, #11 sample (Table 3).

Fecal coliform/E. coli population estimates via Al Broth MPN procedure in the sediment samples are shown in Table 5. Previously, studies have shown that the Al Broth procedure had a high specificity for E. coli enumeration (Dutka et al., 1986). In this study, only 73.8% of the positive Al Broth tubes tested were confirmed to have E. coli and none were serotype 0:157. Interestingly isolates collected from samples 7, 9, 33 and 35 showed a 86.6% E. coli content while isolates collected from mFC agar plates from the water samples at the same stations had only a 15% confirmation of E. coli. The point well made here is that anytime a new area is being sampled, isolate confirmation should be undertaken to establish which population is being enumerated.

Several of the sediments contained fecal coliform/E. coli populations of 160,000 or greater/10 g wet weight of sediment, notably #1B Monroe Michigan; #8 and #9 Detroit River at Little River and Rouge River mouths; #26 Port Dover on the Lynn River; and #29 Selkirk at mouth of Sandusk Creek.

There is little information on the distribution of Cl. perfringens spores in Canadian waters and sediments. A 1977 study by Dutka, reported Cl. perfringens levels varying from 17 to >2400 per 10 g wet weight of sediment in the six Qu'Appelle lakes in Saskatchewan. Bonde (1963) reports investigations showing 10-100 per mL Cl. perfringens spores in river water polluted with sewage, raw sewage with 10-1000/mL, effluent from percolating filters with 1-100/mL, spring waters with 0-7800/mL and river and stream waters 3-568 per mL. In this study the maximum number of spores recovered was 34 per 10 gm wet wt sediment. Two locations, Site #8 and Site #9, where very large concentrations of Cl. perfringens were expected, had fecal coliform densities >160,000 and coprostanol values of 35 and 29 ppm, only had densities of 10 and <2 per 10 g wet wt Cl. perfringens.

Many sediment samples with very low and also very high fecal coliform counts were found to have <2 Cl. perfringens spores/10 g wet wt. Based on previous data (Dutka, 1977) and literature reports (Bonde, 1963), the Cl. perfringens spore counts found during this study were atypical. It is difficult to select the best hypothesis from the variety of explanations possible for these low level results. The data from this study must be accepted as showing areas where long-term fecal pollution has occurred, i.e. results >2 per 10 g wet weight. Further investigations using this parameter will be carried out to establish the validity of Cl. perfringens data as part of a "battery of tests" to establish priority areas of concern.

Cholesterol concentrations continue to be a conundrum (Dutka et al., 1986). In all samples of water and sediment tested with two exceptions, #12 and #19 sediments (Table 5), cholesterol levels were found, while only 11 of 27 sediments appeared to contain coprostanol, the absolute indicator of fecal material presence. Data from some of the samples positive for coprostanol are difficult to understand, e.g. #21 with fecal coliform MPN 12/10 g and Cl. perfringens <2/10 g and #1 with fecal coliform MPN 540/10 g and Cl. perfringens 14/10 g, while negative samples such as #7 have fecal coliform densities of 11,000/10 g and 6 Cl. perfringens/10 g or #39 with fecal coliform counts of 350/10 g and Cl. perfringens of 4/10 g. Continued evaluation of the fecal sterol parameters is required. The philosophy of using fecal sterols to indicate areas polluted by feces is sound, the data obtained from single point studies are often difficult to understand and interpret.

Six of the sediment water extracts were found to have toxic activity as measured by the Microtox test. The sample with the highest concentration of toxicants was site #8, Detroit River at Little River mouth, which required 0.39 g of wet weight sediment to provide an EC_{50} effect. All the other positive sites required between 0.44-0.49 g wet weight of sediment to produce a toxic effect. Thus, the Microtox test data show that the concentration of water soluble toxicants in the positive sediments is quite low, just above threshold levels.

The sediment extracts were tested with and without the addition of S-9 by the SOS Chromotest procedure for genotoxic activity (Xu and Dutka, 1986a). The samples were tested at two sediment concentration levels 0.5 g/mL and 1.0 g/mL. All samples tested without S-9 addition showed a dose response effect. Samples #1, #1B, 2, 3, 7, 10, 11, 21, 22 and 23 all showed strong inducing potency for genotoxic activity. With the exception of sample #33 all the other sediment extracts (1.0 g/mL) showed increased induction factors (negative control IF=1.00) and could be considered to be indicative of genotoxic activity below the threshold of significance.

The addition of S-9 to the SOS Chromotest procedure resulted in 14 of the 40 samples showing a decreasing effect with increased substrate level and only one sample, #22 (Port Burwell) indicated the presence of a strong positive genotoxic effect with an Inducing Factor of 1.45. This sample when tested without S-9 addition produced the highest indication of genotoxic activity with an Inducing Factor of 1.67.

Fourteen of the samples tested with S-9 (compared to 39 without S-9) showed an increased Induction Factor over the negative control (IF=1.00) and could be considered to show genotoxic activity below the threshold of significance. Possibly with better extraction procedures (XAD resins, liquid/liquid) and/or more concentrated water extracted samples, most of the samples would show strong genotoxic capability. Interestingly, sample #33 which did not show any genotoxic activity potential without S-9 addition showed a slight degree of genotoxic activity potential with the addition of S-9 to the SOS Chromotest procedure. Nonetheless, the data clearly indicate that S-9 addition tends to dampen genotoxic activity expression as measured by the SOS Chromotest in water extracted sediments.

Based on these and earlier studies (Xu, Dutka and Kwan, 1986; and Xu and Dutka, 1986a), the modified SOS Chromotest appears to have some advantages over the Ames test in testing environmental samples for mutagenic or genotoxic activity. The use of the microplate with its 96 wells makes the test easy to perform, especially when a large number of samples are to be screened for genotoxicity. In performing the test, two end points can be reached, the SOSIP (expressed as Inducing Factor) and toxicity (as shown by alkaline phosphatase activity. In addition, the procedure requires the use of only one bacterial strain which reduces the number of tests required. Moreover, the results can be easily visualized by two simple colorimetric enzyme assays.

When tested for toxicant activity, by the ATP-TOX System, 18 of the 40 sediment extracts indicated the presence of toxicant activity compared to 13 by the Microtox test. Only sediment extract from sample #39 was positive for toxicant activity by both the Microtox and ATP-TOX System. Clearly, the Microtox and ATP-TOX System are sensitive to different toxicants or classes of toxicants as well as having overlapping sensitivities. The ATP-TOX System results showed a closer correlation with the modified SOS Chromotest, in that of the 10 SOS Chromotest positive samples with Inducing Factors greater than 1.30, seven were also positive for toxicant activity as measured by the ATO-TOX System while only two were positive by the Microtox test. One very notable correlation was in sample #22 Port Burwell, where both the ATP-TOX System and SOS Chromotest indicated that this sediment extract was the most toxic and most genotoxic of the 40 sediment extracts tested. Contrarily the second most genotoxic sediment extract, Sample #11 (Detroit River-Grosse Ile WTP) also showed a slight positive toxic activity as measured by the Microtox test but not by the ATP-TOX System.

From these data and other ATP-TOX System data (Xu and Dutka, 1987), it would appear that the ATP-TOX System is a sensitive and easily performed screening test. It is at least as sensitive as the Microtox test for toxicant activity and adds an additional dimension, life cycle effects, to rapid toxicity screening test batteries. Clearly, the ATP-TOX System is complementary to the Microtox test toxicity screening procedure.

Based on the point ranking scheme developed in Table 2, the top ten sediments for priority concern (Table 5) would be: 1, sample #8, Detroit R. at Little R.; 2, Sample #2, Detroit R., temporary dump site; 3, Detroit R., old dump site; 4, Sample #22, Port Burwell; 5, Sample #9, Detroit R. and Rouge R.; 6, Sample #7, Detroit R. at Turkey Creek; 7, Sample #11, Detroit R. at Grosse Ile WTP; 8, Sample #23, Port Rowan; 9, Sample #38, Niagara R. at Tonawanda; 10, Sample #26, Port Dover-Lynn R. This priority ranking scheme is based on the inclusion of the fecal sterol parameters with the missing data points.

Comparing the top ten areas of concern from Table 3, water samples and Table 5, sediment samples, there are six common stations, which are listed below:

Table 3 Water Sample Rank	Table 5 Sediment Sample Rank	Sample Site
1	5	#9 Detroit R. - mouth of Rouge R.
2	1	#8 Detroit R. - mouth of Little River downstream of WTP
3	9	#38 Niagara R. - mouth of Two Mile Creek at Tonawanda, USA
6	4	#22 Port Burwell - offshore
9	6	#7 Detroit R. - mouth of Turkey Creek
10	7	#11 Detroit R. at Grosse Ile WTP

Thus based on this study the areas of highest priority concern would be sample sites #8, #9, #22, #38, #7 and #11.

Again as previously noted (Dutka et al., 1986), and from the data presented in Tables 3 and 5, it is obvious that microbial populations, biochemical tests or toxicant/mutagen screening tests performed independently are not sufficient to provide realistic estimates of priority concern areas and that the battery approach is required.

Refining of the proposed battery of short-term tests will continue to be a priority with emphasis on parameters which can be preserved for 24-96 hours before testing must be initiated.

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Table 1 Sampling Site Location and Sediment Description

Site #	Location	Latitude N	Longitude W	Sediment Description
1	Detroit River mouth-old dump site	41°57'38"	83°08'05"	silty mud-brown with black & grey streaks
1B	Monroe, Michigan, USA-mouth of River Raisin	41°54'05"	83°20'42"	brown silt, black streaks, oil film on surface
2	Detroit River mouth-temporary dump site	42°00'13"	83°08'58"	silty mud, brown with black streaks
3	Detroit River-Amherstburg near Bois Blanc Island	42°06'12"	83°06'54"	grey and brown sand, gravel
4	Detroit River-Amherstburg near Allied Chemical	42°07'10"	83°06'48"	gravel and rocks, some sand
5	Detroit River-near mouth of Canard River	42°08'51"	83°07'03"	fine sand, some organic matter
6	Detroit River-Fighting Island offshore of waste bed	42°11'22"	83°07'07"	black organic, sandy with clay
7	Detroit River-Mouth of Turkey Creek	42°14'44"	83°06'24"	black, sandy, weeds
8	Detroit River-mouth of Little River-downstream of WTP	42°20'26"	82°55'50"	black, gooey, smells of H_2S
9	Detroit River-mouth of Rouge River	42°16'30"	83°06'40"	black gooey
10	Detroit River-Wyandotte near Pt. Hennepin	42°12'05"	83°08'47"	grey clay, sandy-seaweeds
11	Detroit River-Grosse Ile WTP	42°07'30"	83°10'24"	black sand
12	Mouth of Big Creek	42°02'19"	83°03'14"	medium coarse brown sand
13	Mouth of Cedar Creek	42°00'22"	82°46'28"	medium coarse brown sand, black particles
14	Leamington-beach area	42°01'36"	82°36'10"	medium coarse brown sand
15	Mouth of Sturgeon Creek	42°01'04"	82°34'41"	coarse brown sand
16	Point Pelee-offshore	41°54'53"	82°30'38"	fine brown sand
17	Wheatley Harbour-fish processing plant	42°04'32"	82°27'02"	brown mud, sand and gravel
18	Rondeau Harbour-outside Marina	42°15'58"	81°54'43"	sand
19	Port Glasgow-offshore	42°30'53"	81°36'52"	sand
20	Patrick Point-offshore	42°35'44"	81°28'05"	sand
21	Port Stanley-harbour mouth	42°39'30"	81°12'52"	sandy silt
22	Port Burwell-offshore	42°38'43"	80°48'40"	silt
23	Port Rowan-offshore	42°36'56"	80°26'28"	silt
24	Turkey Point-offshore beach area	42°41'13"	80°18'12"	sandy silt
25	Port Ryerse-offshore	42°45'49"	80°15'24"	sandy silt
26	Port Dover-Lynn River 1 km upstream	42°47'28"	80°11'56"	black mud
27	Port Dover-offshore	42°46'48"	80°11'08"	silt
28	Nanticoke-offshore	42°47'58"	80°02'29"	sand and black particles
29	Selkirk-mouth of Sanduck Creek	42°48'32"	80°02'29"	sandy silt
30	Port Maitland-harbour	42°51'33"	79°34'36"	sandy silt
31	Port Maitland-offshore	42°50'38"	79°35'31"	sandy silt
32	Port Colborne-harbour	42°52'53"	79°15'23"	sandy silt
33	Crystal Beach-offshore	42°52'23"	79°04'09"	sand
35	Niagara River-Fort Erie-Canada Customs	42°55'49"	78°54'49"	sandy silt
36	Lackawana-offshore	42°48'07"	78°51'46"	sand
37	Niagara River-Peace Bridge	42°54'48"	78°54'32"	black sand
38	Niagara River-Tonawanda USA-mouth of Two Mile Creek	43°00'38"	78°54'26"	black humus
39	Niagara River-Grand Island USA-opposite N. Tonawanda	43°01'55"	78°53'42"	sandy silt
40	Niagara River-mouth of Black Creek	42°58'52"	79°01'24"	sandy silt

Table 2 Point Awarding Scheme Used to Rank Samples, Based on Suspected Contained Hazards

Fecal Streptococci Fecal Coliform/*E. coli* Sediment 10g/100 mL MPN Water/100 mL	Pts	Coliphage /100 mL	Pts	*Legionella* /L	Pts	Coprostanol Sediment ppm Water ppb	Pts	Cholesterol Sediment ppm Water ppb	Pts	*Clostridium perfringens* /10 gm sediment	Pts
<100	1	5 - 24	1	1 - 33	2	<1.0	1	<2.0	1	2 - 10	2
100 - 500	2	25 - 100	2	34 - 100	4	1 - 3	3	2.1 - 4	2	11 - 25	4
501 - 2,500	3	101 - 250	3	101 - 500	6	3.1 - 5	5	4.1 - 6	3	26 - 50	6
2,500 - 16,000	4	251 - 1000	4	501 - 1000	8	5.1 - 7	7	6.1 - 8	4	51 - 100	8
16,000-	7		7	1000+	10	7+	10	8+	5	100+	10

ATP TOX System: IC_{50} % Water Sample	ATP TOX System: IC_{50} % wet wt	Pts	Microtox EC_{50}/g wet wt or /mL	Pts	Genotoxicity Induction Factor per .5 mL Water Sample or per g wet wt of Sediment with and without S-9	Pts
50	.41 - .5	2	<0.1	10	1.0 - 1.29	1
35 - 44	.31 - .40	4	.11 - .2	8	1.30 - 1.50	3
20 - 34	.21 - .30	6	.21 - .3	6	1.51 - 2.0	5
10 - 19	.10 - .20	8	.31 - .4	4	2.1 - 3.0	7
<10	<.1	10	.4+	2	3.1+	10

Table 3 Results of Water Sample Analyses

Sample No. and Site	Fecal Coliform MF-mFC /100 mL	E.coli MF-mTEC /100 mL	Coliphage /100 mL	Legionella /Litre	Fecal Streptococci MF-KF /100 mL	Fecal Sterol Coprostanol ppb	Fecal Sterol Cholesterol ppb	Microtox EC_{50}/mL 10x conc.	SOS Chromotest Induction Factor/.5 mL with S-9	ATP-TOX System IC_{50} % of Water Sample	Points	Rank
1 Detroit R. mouth-old dump	3	<1	<5	0	<1	<0.05	2.55	neg	.78	50	5	16
1B Monroe, Michigan	10	<1	10	NT[1]	<1	-	-	neg	1.44	48	9	12
2 Detroit R.-temp. dump	8	4	<5	NT	2	-	-	neg	.78	49	7	14
3 Detroit R.-Amherstburg near Bois Blanc Island	110	88	20		22	-	-	neg	0.89	43	9	12
4 Detroit R.-Amherstburg	158	100	20	NT	26	-	-	neg	0.67	42	10	11
5 Detroit R.-Canard R.	1490	930	5	30	12	-	-	neg	0.78	42	14	7
6 Detroit R.-Fighting Is.	86	28	<5	0	10			neg	1.00	50	6	15
7 Detroit R.-Turkey Cr.	460	190	15	0	63	<0.05	2.54	neg	1.00	46	13	8
8 Detroit R.-Little River	1380	1500	245	0	310	3.24	5.86	neg	1.22	48	23	2
9 Detroit R.-Rouge River	7800	2700	120	0	270	1.95	5.86	neg	1.11	46	24	1
10 Detroit R.-Wyandotte	52	42	15	0	2	-	-	neg	1.00	neg	5	16
11 Detroit R.-Grosse Ile WTP	240	53	25	NT	30	<0.05	2.39	neg	0.78	46	12	9
12 Big Creek-mouth	44	17	5	0	44			neg	0.89	48	8	13
13 Cedar Creek-mouth	24	70	<5	0	10			neg	1.00	42	8	13
14 Leamington	25	17	<5	0	75	<0.05	3.14	neg	0.89	neg	5	16
15 Sturgeon Creek	1110	980	<5	0	1870			neg	1.00	neg	11	10
16 Point Pelee	<1	<1	<5	NT	5	<0.05	2.04	neg	.89	47	7	14
17 Wheatley Harbour	1160	1120	20	0	770	<0.05	4.71	neg	1.00	43	18	4
18 Rondeau Harbour	32	14	<5	0	30	<0.05	2.41	neg	1.11	neg	6	15
19 Port Glasgow	73	58	10	0	67	<0.05	1.67	neg	1.00	47	10	11
20 Patrick Point	2	1	5	0	<1	<0.05	1.64	neg	1.00	47	6	15
21 Port Stanley	530	750	35	0	54	<0.05	2.37	neg	.44	39	16	5
22 Port Burwell	250	85	10	0	35	<0.05	2.29	neg	1.00	21	15	6
23 Port Rowan	10	2	<5	0	10	<0.05	2.63	neg	.89	20	11	10
24 Turkey Point	<1	<1	5	0	<1	<0.05	1.76	neg	.78	31	8	13
25 Port Ryerse	<1	<1	<5	0	<1	-	-	neg	.89	30	6	15
26 Port Dover-Lynn River	230	91	15	neg	73	<0.05	2.01	neg	.89	30	13	8
27 Port Dover	17	17	15	NT	5	-	-	neg	1.00	27	11	10
28 Nanticoke	<1	<1	<5	neg	3	-	-	neg	1.11	33	8	13
29 Selkirk-mouth of Sandusk Cr.	410	400	85	neg	106	-	-	neg	1.00	36	12	9
30 Port Maitland-harbour	81	85	5	neg	17	-		neg	1.11	32	10	11
31 Port Maitland-offshore	8	6	5	NT	14	-		neg	1.56	33	15	6
32 Port Colborne-harbour	21	32	80	neg	11	<.05	2.24	neg	1.22	42	11	10
33 Crystal Beach	10	2	<5	neg	2			neg	0.78	41	7	14
35 Niagara River-Lake Erie	48	18	<5	neg	5	<.05	2.45	neg	1.11	47	10	11
36 Niagara R.-Lackawana	<1	3	<5	NT	1			neg	1.00	48	7	14
37 Niagara R.-Peace Bridge	5	1	<5	NT	2			neg	1.11	40	8	13
38 Niagara R.-Tonawanda	>8000	>8000	1445	neg	4500			neg	1.11	neg	20	3
39 Niagara R.-Grand Is.	1	2	<5	neg	1	<.05	2.44	neg	1.22	50	8	13
40 Niagara R.-Black Cr.	13	16	<5	neg	11	<.05	2.08	neg	1.22	49	8	13

NT[1] = not tested

Table 4 Coliphage Ratios to Fecal Coliforms, Fecal Streptococci and *E. coli*

Sampling Area	Fecal coliform		*E. coli*		Fecal streptococci	
	coliphage		coliphage		coliphage	
	mean	median	mean	median	mean	median
Detroit R. Samples	44	7.8	23	4.7	2.2	1.3
Northshore Lake Erie Samples	62	9.4	55	8.1	94	3.4
Niagara River Samples	12	4.2	7.2	3.2	3.8	2.5
Total Survey Area	49	7.8	38	4.2	53	2.3

Table 5 Results of Sediment Analyses

Sample No. and Site	Fecal Coliform E. coli A1 Broth 10 g/100 mL MPN	Cl. perfringens MPN	Fecal Sterol: Coprostanol ppb	Fecal Sterol: Cholesterol ppb	Microtox EC_{50}/mL 10x conc.	SOS Chromotest Induction Factor/ g wet wt: With S-9	SOS Chromotest Induction Factor/ g wet wt: Without S-9	ATP-TOX System IC_{50}/ g wet wt	Points	Rank
1 Detroit R. mouth-old dump site	540	14	0.20	0.22	neg	1.06	1.53	0.25	22	3
1B Monroe, Michigan, USA	>160,000	17	-	-	neg	1.16	1.46	neg	15	9
2 Detroit R.-temp. dump site	160,000	12	-	-	neg	1.03	1.55	0.25	23	2
3 Detroit R.-Amherstburg near Bois Blanc Island	220	4	-	-	neg	0.74	1.47	0.38	11	14
4 Detroit R.-Amherstburg	280	<2	-	-	neg	0.77	1.24	neg	3	22
5 Detroit R.-Canard R.	920	2	<0.05	0.17	neg	0.87	1.21	neg	6	19
6 Detroit R.-Fighting Is.	2400	<2	-	-	neg	0.84	1.26	0.19	12	13
7 Detroit R.-Turkey Cr.	11,000	6	<0.05	0.19	neg	0.87	1.42	0.19	19	5
8 Detroit R.-Little River	>160,000	10	35.2	23.1	0.39	0.61	1.29	neg	26	1
9 Detroit R.-Rouge River	>160,000	<2	29.1	23.1	0.48	0.77	1.26	neg	20	4
10 Detroit R.-Wyandotte	35,000	8	-	-	neg	0.90	1.32	neg	8	15
11 Detroit R.-Grosse Ile WTP	28,000	13	0.12	0.17	neg	1.23	1.61	neg	18	6
12 Big Creek-mouth	12	2	<0.05	<0.05	neg	0.97	1.21	neg	4	21
13 Cedar Creek-mouth	49	<2	<0.05	0.10	neg	0.87	1.37	0.40	13	11
14 Leamington	920	2	<0.05	0.13	neg	1.00	1.18	0.33	11	14
15 Sturgeon Creek	79	<2	-	-	neg	0.94	1.16	neg	2	23
16 Point Pelee	2	<2	<0.05	0.12	neg	0.94	1.13	neg	3	22
17 Wheatley Harbour	16,000	<2	<0.05	0.25	neg	0.87	1.21	neg	9	16
18 Rondeau Harbour	>16,000	<2	<0.05	0.08	neg	0.77	1.05	neg	8	17
19 Port Glasgow	>16,000	2	<0.05	<0.05	neg	0.74	1.08	0.38	14	10
20 Patrick Point	2200	<2	0.07	0.10	neg	0.84	1.08	0.12	14	10
21 Port Stanley	12	2	0.09	0.22	neg	1.10	1.39	0.42	8	17
22 Port Burwell	>1600	2	0.21	0.57	neg	1.45	1.67	0.02	22	3
23 Port Rowan	9	4	<0.05	0.24	neg	1.13	1.37	0.05	17	7
24 Turkey Point	22	9	<0.05	0.14	neg	0.87	1.07	neg	5	20
25 Port Ryerse	2800	5	-	-	neg	0.97	1.20	neg	7	18
26 Port Dover-Lynn River	160,000	<2	<0.05	0.47	neg	1.00	1.00	0.25	15	9
27 Port Dover-offshore	170	4	-	-	neg	0.90	1.09	0.42	7	18
28 Nanticoke	2	<2	-	-	neg	0.90	1.02	neg	2	23
29 Selkirk-mouth of Sandusk Cr.	>160,000	<2	<0.05	0.25	neg	1.00	1.28	neg	9	16
30 Port Maitland-harbour	>16,000	<2	0.11	0.34	neg	1.06	1.17	0.38	14	10
31 Port Maitland-offshore	17	34	-	-	neg	1.10	1.28	0.46	10	15
32 Port Colborne-harbour	12	12	<0.05	0.26	neg	1.16	1.22	neg	5	20
33 Crystal Beach	22	<2	<0.05	0.13	neg	1.13	0.96	neg	3	22
35 Niagara River-Lake Erie	920	7	0.16	0.99	neg	0.97	1.07	neg	8	17
36 Niagara R.-Lackawana	920	6	-	-	neg	0.94	1.00	neg	6	19
37 Niagara R.-Peace Bridge	46	7	-	-	0.46	1.00	1.04	neg	6	19
38 Niagara R.-Tonawanda	>160,000	2	2.01	1.80	0.46	1.03	1.20	neg	16	8
39 Niagara R.-Grand Is.	350	4	<0.05	0.91	0.49	1.10	1.24	0.50	10	15
40 Niagara R.-Black Cr.	1600	<2	0.22	5.68	neg	1.13	1.20	0.40	12	13