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NWRI Contribution No. 99-070

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March 17, 1999

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NWRI Contribution Number: 99-070

MANAGEMENT PERSPECTIVE

The assessment of environmental impacts of combined sewer overflows (CSOs) on receiving waters is rather challenging, because of the intermittent nature of these discharges and great variability in their flow rates and pollutant concentrations. In the current practice, such an assessment is primarily based on chemical characterization of CSOs. With respect to toxic effects of CSOs, this approach has some limitations arising from difficulties with identifying toxic or non-toxic species of contaminants, their bioavailability, and combined effects. Such limitations can be eliminated by toxicity testing, which was applied to CSOs in this study.

In toxicity testing, a battery of eight toxicity tests was applied to CSO samples collected in Toronto and Hamilton. In the earlier phase of this study, a very low frequency of acute toxicity was noted. Consequently, this phase focused more on non-acute CSO toxic effects, by adding genotoxic and low-level chronic toxicity tests. Study results indicate that about 90% of all CSO samples were non-toxic, and would not exert toxic impacts on the receiving waters. The remaining cases of CSO toxicity mostly referred to genotoxicity or chronic toxicity. With the exception of the first flush (i.e. a highly polluted, early part of the overflow) and small receiving waters, CSOs present a low toxic threat to the receiving waters.

This report should be of interest to water managers and researchers dealing with the assessment and control of combined sewer overflows.

SOMMAIRE À L'INTENTION DE LA DIRECTION

L'évaluation des incidences environnementales des déversements d'égouts unitaires (DEU) dans les eaux réceptrices constitue un défi, en raison de la nature intermittente de ces rejets et de la grande variabilité de leurs débits et des concentrations de polluants. Dans la pratique, une telle évaluation est principalement basée sur la caractérisation chimique des DEU. En ce qui a trait aux effets toxiques des DEU, cette approche comporte certaines limites provenant des difficultés à identifier les espèces de contaminants toxiques et non toxiques, à déterminer leur biodisponibilité et leurs effets combinés. Ces limites peuvent être éliminées à l'aide des essais de toxicité, qui ont été appliqués aux DEU au cours de cette étude.

Lors des essais de toxicité, les échantillons de DEU prélevés à Toronto et Hamilton ont été soumis à une batterie de huit essais. Au cours de la première phase de cette étude, on a observé une très faible fréquence de toxicité aiguë. Cette phase a donc plus porté sur les effets toxiques des DEU non aigus, en ajoutant des essais de génotoxicité et de faible toxicité chronique. Les résultats de l'étude révèlent qu'environ 90 % de tous les échantillons de DEU étaient non toxiques et qu'ils n'auraient aucune incidence toxique sur les eaux réceptrices. Les cas restants de toxicité des DEU se rapportaient en majorité à la génotoxicité ou à la toxicité chronique. À l'exception de la première chasse d'eau (c.-à-d. une première partie très polluée du déversement) et des petits plans d'eau récepteurs, les DEU présentent une menace de faible toxicité pour les eaux réceptrices.

Ce rapport devrait intéresser les gestionnaires des eaux et les chercheurs dont les travaux portent sur l'évaluation et le contrôle des déversements d'égouts unitaires.

ABSTRACT

Combined sewer overflow (CSO) discharges have been characterized in the past using chemical parameters. This approach has provided a great deal of information on the input of solids, nutrients, metals, hydrocarbons and trace organic compounds from these discharges to the receiving waters. It does not, however, indicate the bioavailability of these contaminants or their potential impact on biological systems or organisms in the receiving waters. To fill this gap, a battery of acute toxicity, genotoxicity and chronic toxicity tests were applied to a variety of combined sewer overflow discharges. This battery of tests included *Daphnia magna*, Microtox™, Sub-mitochondrial particle bioassays (reverse and conventional electron transport methods), Ames fluctuation test, SOS chromotest, and the fathead minnow and *Ceriodaphnia dubia* chronic toxicity tests. Of these tests, *Daphnia magna* and Microtox™ exposed whole organisms (a freshwater cladoceran and bacteria respectively) to the effluent, demonstrating survival impacts. The sub-mitochondrial particle tests used cellular (beef heart) tissue to determine the impact of the effluent on cell biochemical processes. The Ames fluctuation test and SOS chromotest indicate the effects of the effluent on genetic repair processes (biochemical functions) and hence indicate the degree to which cellular genetic material may be affected. The fathead minnow and *Ceriodaphnia dubia* 7-day tests use whole organisms to assess low level chronic toxicity.

Combined sewer overflow discharges in Toronto and Hamilton (two Great Lakes Areas of Concern) were sampled at sites with various wastewater sources related to such land use as industrial, commercial, institutional, residential and areas with high traffic flow.

The results of this study indicate that most of the CSO discharge appears to be non-toxic, except for the first flush which may be toxic and exert harmful impacts on receiving waters. Remedial measures should therefore focus on the control of the first flush. The most sensitive tests in these investigations appeared to be the Ames fluctuation test, fathead minnow and *Ceriodaphnia dubia*. It is therefore recommended that such tests be included in any future monitoring programs.

RÉSUMÉ

Les rejets de déversements d'égouts unitaires (DEU) ont été caractérisés dans le passé à l'aide de paramètres chimiques. Cette approche a fourni beaucoup de renseignements sur l'apport en solides, nutriments, métaux, hydrocarbures et composés organiques traces de ces rejets dans les eaux réceptrices. Elle n'indique cependant pas la biodisponibilité de ces contaminants, ni leur impact possible sur les systèmes biologiques ou les organismes dans les eaux réceptrices. Afin de remédier à la situation, on a fait subir une batterie d'essais de toxicité, de génotoxicité et de toxicité chronique à une variété de rejets de déversements d'égouts unitaires, notamment l'essai au *Daphnia magna*, Microtox™, les bioessais de particules sub-mitochondriales (méthodes de transfert d'électrons inverse et classique), l'essai de fluctuation de Ames, l'essai SOS Chromotest, et les essais de toxicité chronique avec le tête-de-boule et *Ceriodaphnia dubia*. De tous ces essais, *Daphnia magna* et Microtox™ exposaient des organismes entiers (respectivement un cladocère d'eau douce et une bactérie) à l'effluent, montrant les impacts sur la survie. Les essais de particules sub-mitochondriales utilisaient du tissu cellulaire (coeur de boeuf) pour déterminer l'impact de l'effluent sur les processus biochimiques de la cellule. L'essai de fluctuation de Ames et le SOS Chromotest indiquent les effets de l'effluent sur les processus de réparation de l'ADN (fonctions biochimiques) et, ainsi, le degré auquel le matériel génétique cellulaire peut être affecté. Les essais de 7 jours avec le tête-de-boule et *Ceriodaphnia dubia* utilisent des organismes entiers pour évaluer la faible toxicité chronique.

On a prélevé des échantillons des rejets de déversements d'égouts unitaires à Toronto et Hamilton (deux secteurs préoccupants des Grands Lacs), à des sites où il y avait diverses sources usées provenant de zones industrielles, commerciales, institutionnelles, résidentielles et de circulation intense.

Les résultats de cette étude révèlent que la majeure partie des rejets de DEU ne semblent pas toxiques, à l'exception de la première chasse d'eau, qui peut être toxique et avoir des

effets dommageables sur les eaux réceptrices. Les mesures correctives doivent donc porter sur la première chasse d'eau. Les essais les plus sensibles au cours de cette étude semblent avoir été l'essai de fluctuation de Ames, et les essais avec le tête-de-boule et *Ceriodaphnia dubia*. On recommande donc d'inclure ces essais dans tous les futurs programmes de surveillance.

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1.0 INTRODUCTION

1.1 Background

Combined sewers carry both municipal wastewater and stormwater runoff from urban drainage. In dry weather, the sewer pipes are only partially full. During wet weather (rain/snowmelt), the capacity of the pipe network is rapidly approached. Control structures are installed to divert excess flow from the system before the network becomes hydraulically overloaded. These overflow discharges are called combined sewer overflows (CSOs), and impact adversely on receiving waters. CSOs convey suspended solids and grit, excess nutrients including nitrogen, phosphorus and carbon, toxic ammonia, oxygen demanding wastes, bacteria and pathogenic organisms, heavy metals (mostly copper, lead and zinc), oil and grease and trace organic compounds (including herbicides, pesticides and industrial chemicals). The composition of these effluents varies considerably over the duration of the overflow event and with location.

In the Great Lakes Basin, the impacts of CSOs and stormwater discharges contribute strongly to impairments of beneficial water uses in a number of Areas of Concern (AOCs), (Weatherbe and Sherbin 1994). The Toronto Waterfront and Hamilton Harbour were identified as two AOCs with large impacts caused by wet weather discharges. Combined sewer overflows are an important component of such wet weather discharges in these two areas.

Impacts downstream of CSO outfalls vary based on the frequency of overflows and the strength of the wastewater. Dissolved oxygen depletion may be caused by high biochemical oxygen demand (BOD), chemical oxygen demand (COD) and high ammonia concentrations. Nutrient enrichment, caused by increased concentrations of particulate and soluble phosphorus, nitrogen and carbon, may result in eutrophication. Bacteria and pathogenic organisms directly affect human health through recreational water contact. Toxic impacts may result from discharges of ammonia, chlorides, heavy metals and trace organic contaminants. The treatment of CSO discharges should primarily focus on the reduction of these substances.

Currently, CSO discharge control has been through reduction of overflow volume through flow balancing (e.g., holding tanks), and addition of storage capacity within the system. While these control measures have demonstrated beneficial effects with respect to the frequency, quantity and quality of the overflow effluent (e.g., suspended solids), very little is known with respect to the impact on toxicity. Chemical data do not distinguish between bioavailable forms of chemical contaminants and cannot account for the potential synergistic effects of these chemical cocktails. It is therefore beneficial to use toxicity testing as a measure to determine the ecological impacts of CSOs on the receiving waters.

Large variations in chemical concentrations occur during the course of the CSO discharge. Although acute toxicity was rarely demonstrated for CSO effluent samples (Rochfort et al., 1997), a battery of toxicity tests allowed all types of toxic responses to be registered. In this study, the emphasis for the testing was placed on genotoxic and chronic tests, which were more appropriate for determining less severe toxic impacts. The Ames fluctuation test and SOS chromotest were used as indicators of genotoxicity. Chronic toxicity was assessed using the *Ceriodaphnia dubia* 7-day survival and reproduction test, and the fathead minnow 7-day survival and growth test. Acute toxicity was monitored using the whole organism *Daphnia magna* test, Microtox™ and Sub-mitochondrial particle tests.

The chemical composition of wet weather discharges have been well documented (Ellis et al., 1997). Toxicity testing has proved to be a useful tool in assessing wet weather discharges and identifying highway runoff as one of the most toxic types (Marsalek et al., 1999). This, along with toxicity identification evaluation (TIE) studies, have shown that PAHs and heavy metals are the most common sources of the observed toxicity (Ellis et al., 1997; Sansalone and Buchberger, 1997). Most of these chemicals can be attributed to the operation of motor vehicles, while others (such as chlorides) are associated with seasonal factors, such as highway maintenance practices in cold climates (Novotny et al., 1998). Studies have also shown that wet weather discharges impact receiving waters by changing flow and hydraulic regimes, as well as affecting sediment loading and transport, and these can affect the habitat structure (Ellis and Hvitved-Jacobsen, 1996).

Toxicity testing and benthic monitoring at impacted receiving waters sites was suggested as a suitable compliment to the conventional chemical analysis of grab samples of the CSO discharges (and/or receiving waters), which may be inadequate for impact characterization (Seager and Abrahams, 1990). As many of the chemical constituents remain bound to particulate material, sediment transport may also be an important factor in the impact of the discharges on the receiving waters (Lee et al., 1997).

Combined sewer overflows are somewhat different from stormwater discharges, in that they contain components of urban runoff (mainly metals and PAHs) as well as the high organic, solids, nutrients and ammonia loadings from municipal sewage. Dilution of toxicants in these discharges can result, and as such they do not represent as severe and acute a toxic threat to receiving waters as stormwater discharges. The more subtle effects are not as easily detectable as for stormwater discharges owing to the lower soluble concentrations of pollutants. CSOs may impact significantly on benthic organisms, however, and this was studied in great detail by Seager and Abrahams (1990). They used both a benthic organism (*Gammarus pulex*) and a fish monitor (gill ventilation rates for rainbow trout). Other organisms have also been monitored (either for presence/abundance or for survival), such as *Asselus aquaticus* (Muliss et al., 1993), and *Dreissena polymorpha* (Fabroulet et al., 1993). One of the more sensitive measures of toxic impacts on the aquatic ecosystem, was found to be the assessment of benthic community structure; however, many physical factors (such as periodic scouring) could also adversely affect the populations (Borchardt, 1993).

1.2 Objectives

The objectives of this study were to determine the toxicity of combined sewer overflows in the Toronto and Hamilton areas. For this purpose, samples were collected at a number of sites in the Toronto area, and supplemented by samples from the Hamilton area. First flush samples were also compared with composite samples. A battery of toxicity tests (including *Daphnia magna*, Ames Fluctuation Test, SOS Chromotest, *Ceriodaphnia dubia* and fathead minnow) were used to assess the potential impacts of the effluents on receiving stream ecosystems. Available chemical data were used to correlate toxicity with specific chemical parameters to identify potential causes of the observed toxicity.

2.0 SITE DESCRIPTIONS

2.1 Toronto

2.1.1 Strachan Avenue

The Strachan Avenue sampling site is located on Strachan Avenue in the Western Beaches area of the City of Toronto (Figure 2.1). The CSO outfall is submerged and is not directly accessible. Sampling occurred at the CSO overflow weir prior to discharge. Automated sampling was employed in the 1997 field season. Samples collected in 1996 were collected via manual grab sampling with a stainless steel bucket.

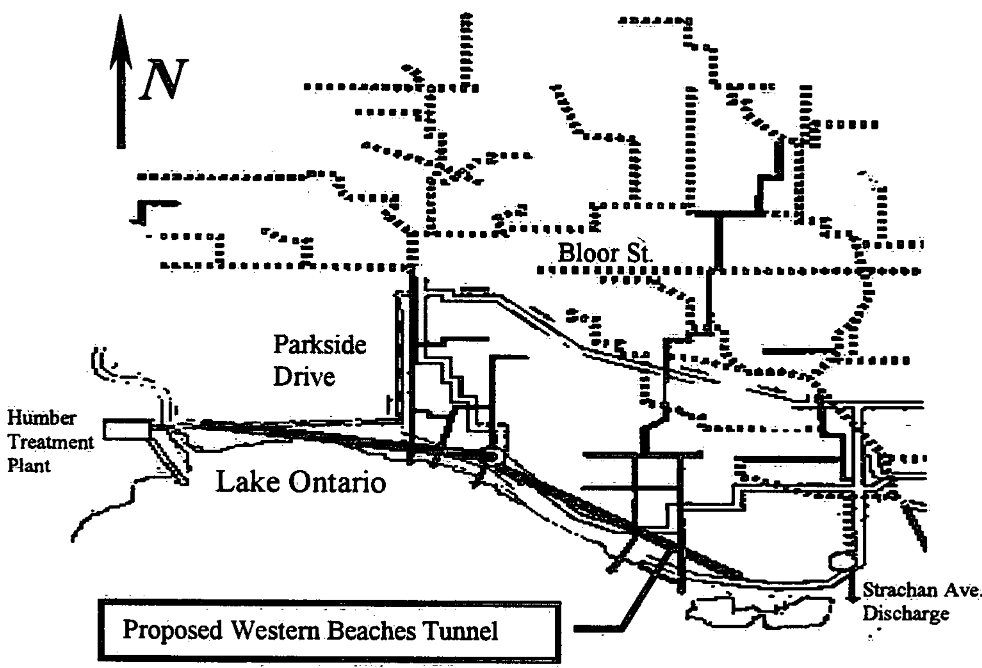
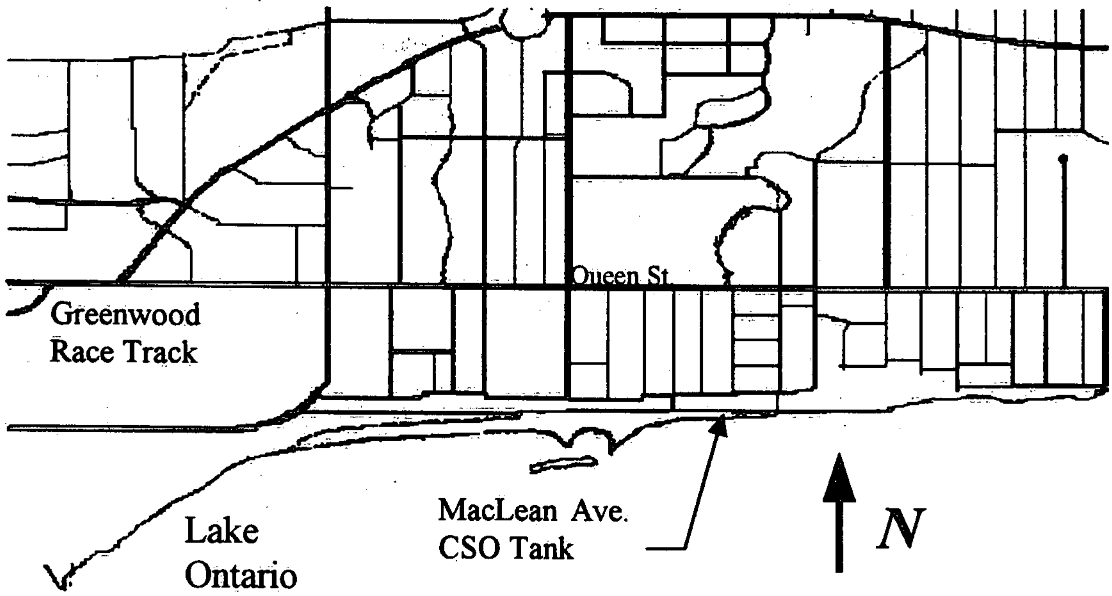


Figure 2. 1: Strachan Avenue CSO Location

2.1.2 MacLean Avenue

The MacLean Avenue site is located at a combined sewer overflow tank, in the Eastern Beaches area of Toronto (Figure 2.2). This site only overflows during very large storm events, due to the high reserve storage capacity in this part of the sewer system. This



site receives street runoff from a largely residential area, with some high traffic flow. Sanitary waste is largely residential, with some commercial and light industrial components.

Figure 2. 2: MacLean Avenue CSO Sample Location

2.1.3 North Toronto

The North Toronto site is located in East York adjacent to the Don Valley parkway. At this site, a full time secondary treatment facility continuously processes 0.46 m^3/s of wastewater from the trunk sewer (Figure 2.3). The remainder of this wastewater is sent to the Main sewage treatment plant (STP). During wet weather, the combined sewer flow increases, and a control structure diverts excess flow into a CSO treatment system. This system consists of three settling tanks (approximate dimensions 7m wide by 30m long). Effluent from these tanks is collected and mixed with stormwater runoff and the secondary effluent from the treatment plant prior to discharge into the Don River. This location was also used as a demonstration site for the polymer coagulant addition, to improve settling in the clarifiers. Water Technology International (WTI) personnel added the polymer coagulant during selected overflow events. Only one processed effluent sample was collected at this site. All other samples were untreated effluents.

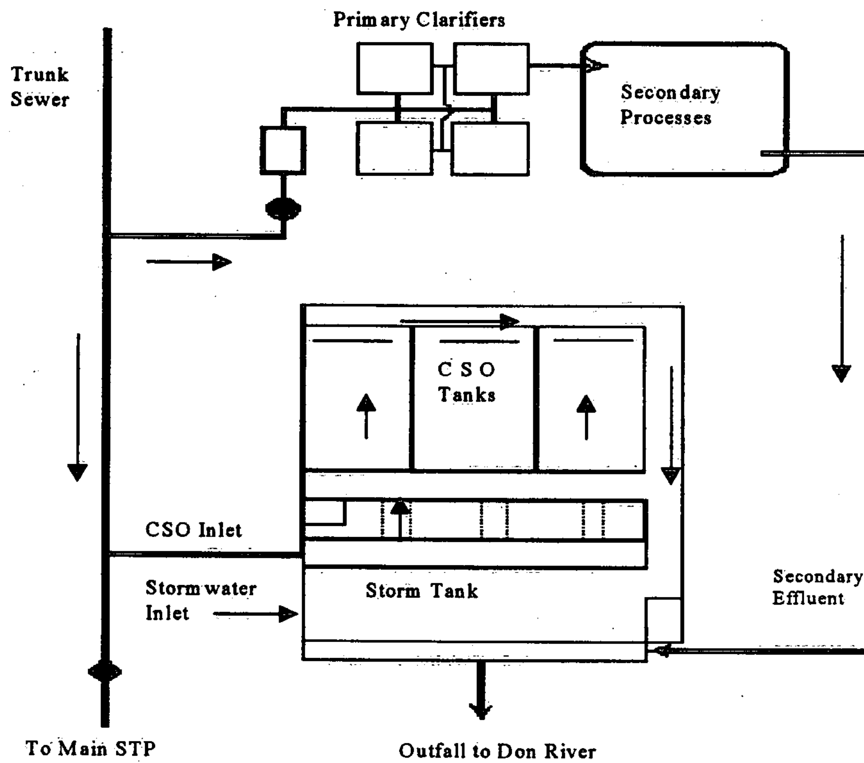


Figure 2. 3: North Toronto CSO Tanks

2.1.4 Massey Creek Site

The Massey Creek site is located in Scarborough. Massey Creek runs through the Metro Park located at Pharmacy Avenue (Figure 2.4). The combined sewer overflow discharges during moderate rainfall events. This location was also used as a demonstration site for the pilot scale polymer coagulant and plate clarifier treatment facility. Figure 2.5 shows the plate clarifier experimental setup. Sewage and stormwater effluent at this site comes mainly from residential sources. Some commercial sources also contribute to this site.

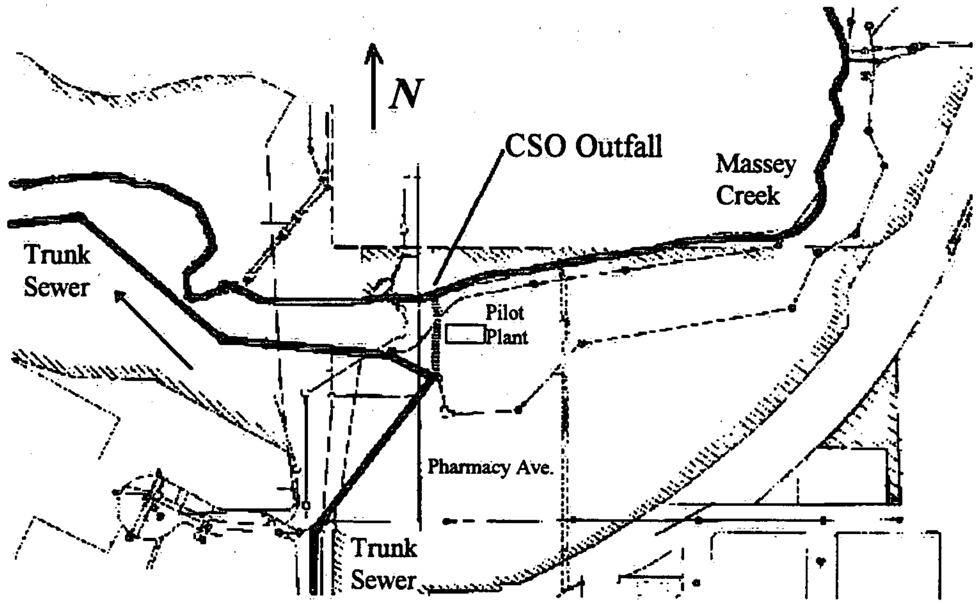


Figure 2. 4: Massey Creek Sampling Site

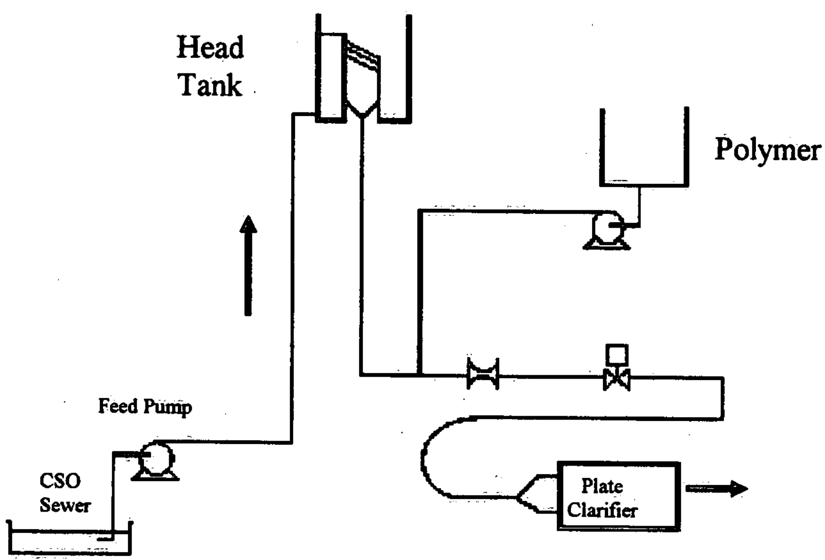


Figure 2. 5: Plate Clarifier Setup at Massey Creek Site

2.1.5 Sixth Street Site

This stormwater only site is located in Etobicoke, near the lakeshore (Figure 2.6). Samples were collected in a catchbasin which is connected via a pipe network to a discharge point on Lake Ontario. These effluents receive no pre-treatment prior to discharge. Samples were collected by an automated sampler which produced an integrated sample over the storm event period.

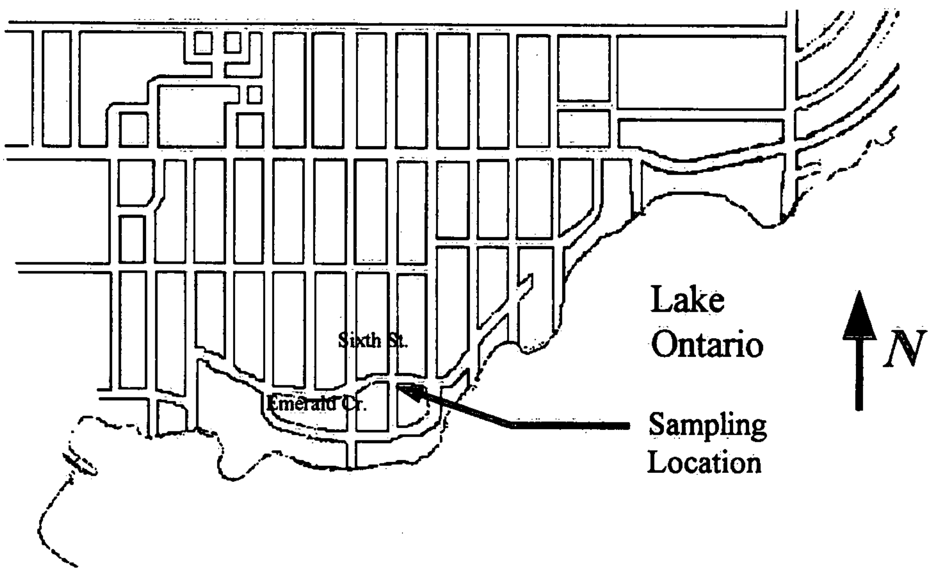


Figure 2. 6: Sixth Street, Etobicoke Sampling Site

2.1.6 Woodcrest Drive Site

This stormwater only site is also located in Etobicoke (Figure 2.7), in a purely residential area. The composite samples were taken by automated sampler during each storm event. The Humber River is the receiving water for this stormwater discharge.

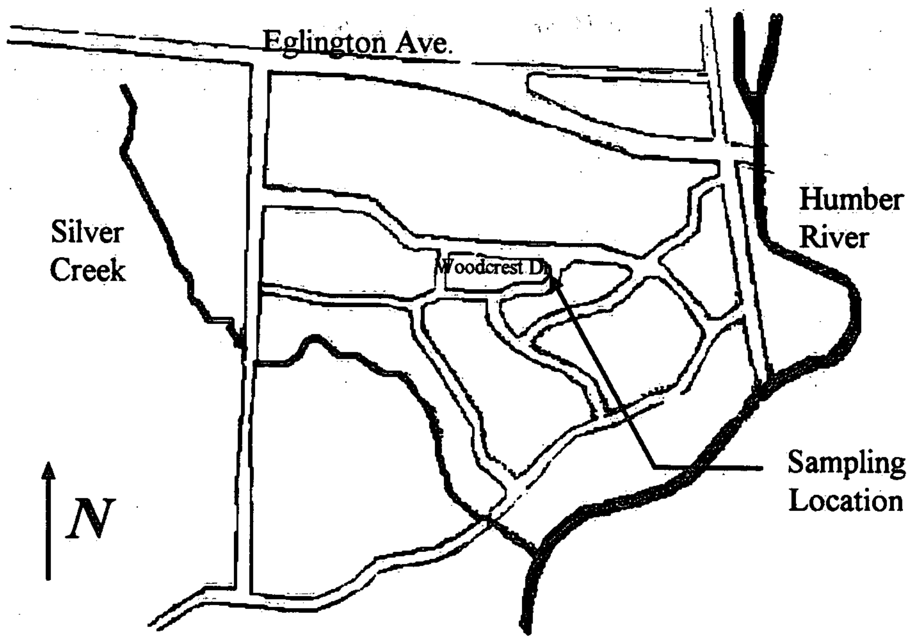


Figure 2. 7 : Woodcrest Drive, Etobicoke Sampling Site

2.1.7 Humber River Site

This sampling site is located close to the Woodcrest Drive site (Figure 2.7). Only one sample was collected from this site, using a grab sampling method. The Humber Valley watershed is drained by the Humber River, and caries surface runoff during rain and snowmelt events. Many stormwater pipes discharge into this river, which eventually empties into Lake Ontario. Considerable dilution results from natural drainage at this site.

2.2 Hamilton

Figure 2.8 indicates the locations of the combined sewer overflow sites in Hamilton. Five CSO sites were chosen to represent all types of land use. All sites were sampled using manual grab sampling techniques, at the surface outfall.

2.2.1 Melvin Avenue

The Melvin Avenue outfall is located at the lower end of the Red Hill Creek. Due to its location near the sewage treatment plant, the site receives well mixed sewage from a

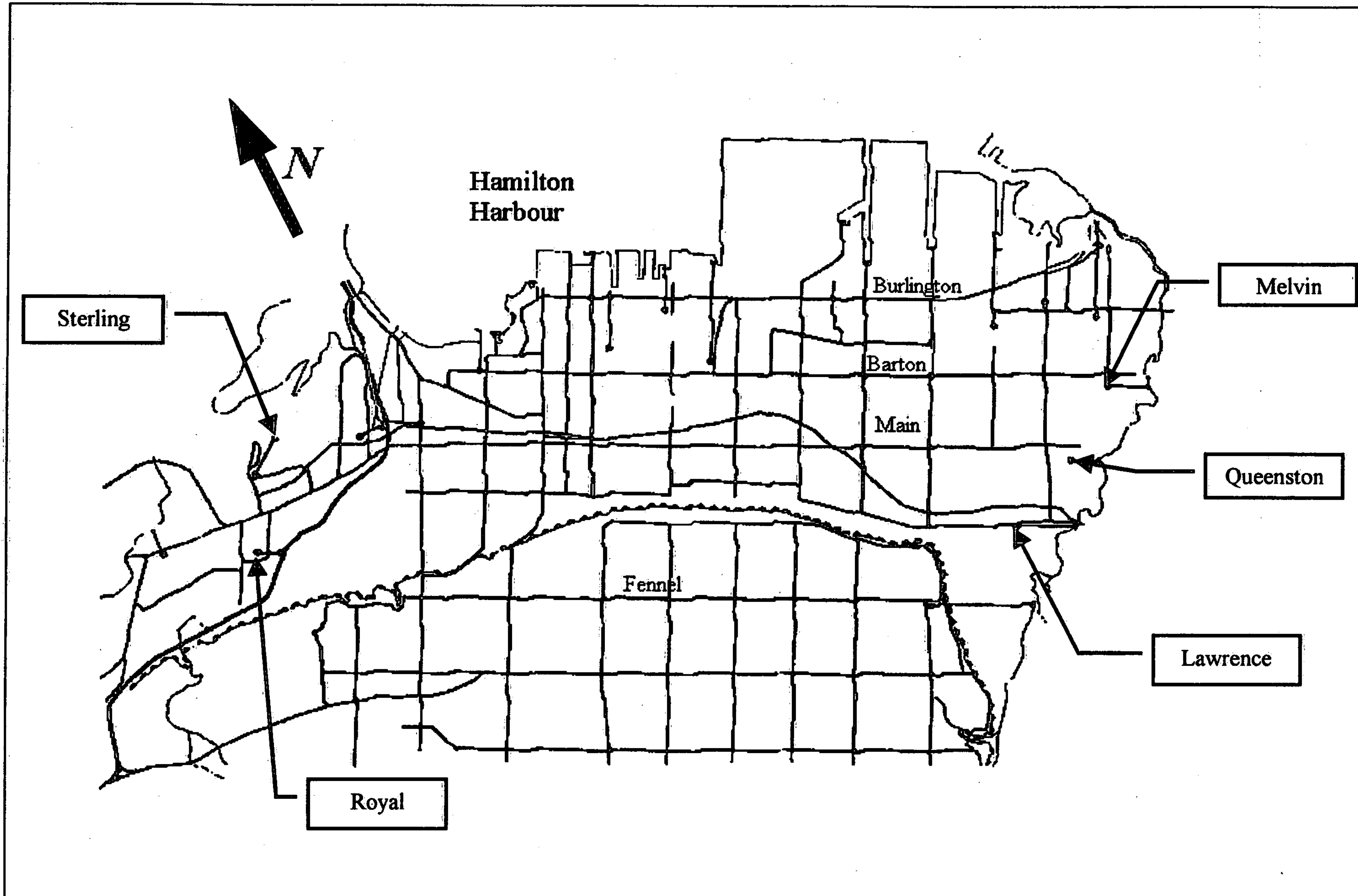


Figure 2.8: Hamilton Combined Sewer Overflow Site Location Map

large number of upstream sources. This site had the greatest commercial stormwater runoff input of the Hamilton sites.

2.2.2 Queenston Road

The Queenston Road outfall is located further upstream on the Red Hill Creek, and the catchment is characterized by less commercial land use, but greater traffic density. Queenston Road is one of the city's major traffic arteries. This site has a partially submerged outfall, and samples were only collected from this site when strong flows were present.

2.2.3 Lawrence Avenue

The Lawrence Avenue outfall is located at the base of the escarpment on the Red Hill Creek watershed. This CSO receives runoff primarily from residential areas, as well as from a major traffic artery.

2.2.4 Sterling Avenue

The Sterling Avenue outfall receives runoff from parking lots and major roads (i.e., Highway 2) near McMaster University, as well as the McMaster hospital and laboratory wastewater. The discharge was located in a small creek, which ultimately feeds into the Coote's Paradise Conservation area.

2.2.5 Royal Avenue

The Royal Avenue outfall is located at the corner of Stroud and Royal Avenues and has a purely residential catchment. This site received both residential runoff and municipal wastewater. Overflows occurred more often at this site, indicating a low reserve capacity in the pipe network.

2.3 Sample Collection

A total of 34 samples were collected from Toronto sites, of which, ten were stormwater runoff. The majority of these combined sewer overflows were untreated (Raw), while in two tests, a polymer coagulant aid was applied. One sample each from Toronto and Hamilton was re-tested after settling at 15°C for 24 hours to simulate storage in the proposed CSO tunnel. A list of all samples collected is presented in Table 2.1. A check mark (✓) indicates that the sample was analyzed for that test. Where a **P** is indicated in the fathead minnow or *Ceriodaphnia* columns, only the 100% pass/fail scan was used (i.e. full strength sample - no dilutions). **E** indicates that the sample was analyzed by an external lab. A cross (×) shows that no sample was submitted for toxicity analysis using that test. Table 2.2 indicates the same parameters for HCSO (Hamilton CSO) samples. Only combined sewer samples were collected from these sites.

Table 2.1: Toronto CSO Samples Submitted for Toxicity Testing

TCSO #	Date	Location	*D. magna*	Microtox	SOS chromotest	Ames Fluctuation	SMP	Fathead Minnow	*Ceriodaphnia*
1	August 8 1996	Strachan Ave.	✓	✓	✓	✓	✓	✓E	✓E
2	August 26 1996	Strachan Ave.	✓	✓	✓	✓	✓	✗	✗
3	September 12 1996	Strachan Ave.	✓	✓	✓	✓	✓	✗	✗
4	September 13 1996	Strachan Ave.	✓	✓	✓	✓	✓	✓E	✓E
5	September 24 1996	Strachan Ave.	✓	✓	✓	✓	✓	✓E	✓E
6	September 24 1996	Strachan Ave.	✓	✓	✓	✓	✓	✓E	✓E
7	September 27 1996	Strachan Ave.	✓	✓	✓	✓	✓	✓E	✓E
8	December 17 1996	Strachan Ave.	✓	✓	✓	✓	✓	✓E	✓E
9	July 8 1997	Influent Scarborough	✓	✓	✓	✓	✓	✓E	✓
10	July 8 1997	Effluent Scarborough	✓	✓	✓	✓	✓	✓E	✓
11	July 8 1997	Influent 2 Scarborough	✓	✓	✓	✓	✓	✗	✓
12	July 8 1997	Effluent 2 Scarborough	✓	✓	✓	✓	✓	✗	✓
13	July 8 1997	North Toronto	✓	✓	✓	✓	✓	✓E	✓
14	July 15 1997	Influent Scarborough	✓	✓	✓	✓	✓	✓P	✓

✓ Sample submitted, ✗ No sample submitted, **P** pass/fail scan used, **E** sample was tested externally

Table 2.1 Continued: Toronto CSO Samples Submitted for Toxicity Testing

TCSO #	Date	Location	*D. magna*	Microtox	SOS chromotest	Ames Fluctuation	SMP	Fathead Minnow	*Ceriodaphnia*
15	July 15 1997	North Toronto	✓	✓	✓	✓	✓	✓P	✓
16	August 13 1997	6th St. Etobicoke	✓	✓	✓	✓	✓	✓P	✓
17	August 13 1997	MacLean Ave. Toronto	✓	✓	✓	✓	✓	✓P	✓
18	August 13 1997	Massey Creek – Scarborough	✓	✓	✓	✓	✓	×	✓P
19	August 13 1997	North Toronto	✓	✓	✓	✓	✓	×	✓P
20	August 13 1997	Strachan Ave.	✓	✓	✓	✓	✓	✓P	✓P
21	August 21 1997	6th St. Etobicoke	✓	✓	✓	✓	✓	×	×
22	September 8 1997	6th St. Etobicoke	✓	✓	✓	✓	✓	×	×
23	September 10 1997	6th St. Etobicoke	✓	✓	✓	✓	✓	×	×
24	September 17 1997	Woodcrest Dr. Etobicoke	✓	✓	✓	✓	✓	×	×
25	September 25 1997	6th St. Etobicoke	✓	✓	✓	✓	✓	×	×
26	September 25 1997	Woodcrest Dr. Etobicoke	✓	✓	✓	✓	✓	×	×

✓ Sample submitted, × No sample submitted, **P** pass/fail scan used, **E** sample was tested externally

Table 2.1 Continued: Toronto CSO Samples Submitted for Toxicity Testing

TCSO #	Date	Location	*D. magna*	Microtox	SOS chromotest	Ames Fluctuation	SMP	Fathead Minnow	*Ceriodaphnia*
27	September 29 1997	Woodcrest Dr. Etobicoke	✓	✓	✓	✓	✓	✗	✗
28	October 27 1997	Strachan Ave.	✓	✓	✓	✓	✓	✓	✓
29	November 1 1997	Strachan Ave.	✓	✓	✓	✓	✓	✓	✓
30	November 1 1997	Woodcrest Dr. Etobicoke	✓	✓	✓	✓	✓	✗	✗
31	November 1 1997	Humber Creek, Etobicoke	✓	✓	✓	✓	✓	✗	✗
32	November 21 1997	North Toronto In	✓	✓	✓	✓	✓	✓	✓
33	November 21 1997	North Toronto Out	✓	✓	✓	✓	✓	✓	✓
34	November 21 1997	Strachan Ave.	✓	✓	✓	✓	✓	✗	✓

✓ Sample submitted, ✗ No sample submitted, **P** pass/fail scan used, **E** sample was tested externally

Table 2.2: Hamilton CSO Samples Submitted for Toxicity Testing

HCSO #	Date	Location	*D. magna*	Microtox	SOS chromotest	Ames Fluctuation	SMP	Fathead Minnow	*Ceriodaphnia*
1	December 1 1996	Royal Ave. CSO	✗	✓	✓	✓	✓	✓	✓
2	December 1 1996	Royal Ave. CSO	✗	✓	✓	✓	✓	✓	✓
3-7	December 1 1996	Royal Ave. CSO	✗	✓	✓	✓	✓	✓	✓
8	February 4 1996	Queenston Ave. CSO	✗	✓	✓	✗	✓	✓	✓
9	February 20 1996	Lawrence Ave. CSO	✗	✓	✓	✗	✓	✓	✓
10	May 3 1997	Melvin Ave. CSO	✓	✓	✓	✗	✓	✗	✓P
11	June 16 1997	Melvin Ave. CSO	✓	✓	✓	✗	✓	✓	✓
12	June 16 1997	Sterling Ave. CSO	✓	✓	✓	✗	✓	✓	✓
13	June 20 1997	Lawrence Ave. CSO	✓	✓	✓	✗	✓	✓	✓
14	July 2 1997	Lawrence Ave. CSO	✓	✓	✓	✗	✓	✗	✓
15	July 28 1997	Sterling Ave. CSO	✓	✓	✓	✓	✓	✓	✓

✓ Sample submitted, ✗ No sample submitted, **P** pass/fail scan used, **E** sample was tested externally

3.0 METHODS

The bioassays applied to water samples for this study include *Daphnia magna* 48 hour acute test, Microtox™ 15 minute test, Sub-mitochondrial particle bioassays (reverse and forward electron transport) and the SOS-Chromotest. Dutka (1989) and Dutka (1997) contain detailed descriptions of these toxicological techniques.

Test organisms and tissues are sensitive to different concentrations and mixtures of pollutants. Not all pollutants are bioavailable to all types of organisms and a battery of tests approach helps to reduce the chances that a sample will be identified as non-toxic when it may be toxic using a different test. Table 3.1 lists tests commonly used in aquatic toxicity testing, and the type of effects each can measure.

Table 3.1: Toxicity Tests and Types of Toxicity Detected

Test	**Cytotoxicity**	**Genotoxicity**	**Acute**	**Chronic**
	Causes cellular damage	Causes genetic damage	Short Term	Long Term
Daphnia magna	✓		✓	
Microtox™	✓		✓	
Sub-mitochondrial particle bioassay	✓		✓	
Ames Fluctuation Test		✓	✓	
SOS Chromotest		✓	✓	
Fathead minnow	✓			✓
Ceriodaphnia dubia	✓	✓		✓

3.1 Acute Toxicity and Genotoxicity Tests

3.1.1 Daphnia magna

The cladoceran *Daphnia magna* used in these tests is the largest of the *Daphnia*, often reaching 5 mm in size. The neonates (first-instar young) are approximately 0.9 mm long and are easily observed with the naked eye. Twelve to 24 hour old neonates are most commonly used in acute toxicity tests. In the test, 10 neonates are used for each sample and sample dilution (usually 100, 75, 50, 25 and 10%) to be tested. The neonate organisms are observed after 1 hr, 4 hr, 24 hr and 48 hours incubation at 21±1°C, when the number of dead animals are recorded. A 48 hour LC_{50} or EC_{50} is derived from the pattern of deaths observed (Dutka, 1997). LC_X indicates the concentration at which X% of the organisms die (e.g., LC50 of 25% would indicate that when the test water is diluted to 25% of its original concentration, it would kill 50% of the test organisms). Here, "LC" stands for "lethal concentration". Similarly, the EC_X value shows the concentration at which X% of the organisms are inhibited (generally in growth or reproduction), where "EC" stands for "effective concentration".

3.1.2 Microtox™

Microbics Corporation has developed a photometric technique which uses a marine bioluminescent bacterium's (*Vibrio fischeri* previously known as *Photobacterium phosphoreum*) response to chemical exposure for assessing relative toxicity. In the test, the rehydrated bacteria are incubated (15°C) in the liquid sample and dilutions of the sample for 15-30 minutes. The samples are read in a Microtox™ 500M reader with computer print out. The toxicant concentration (% of sample) at which a fifty percent normalized light loss occurs for a certain exposure time is automatically calculated and reported as the EC_{50} (effective concentration for 50% light loss) of the toxicant (Dutka, 1997).

3.1.3 SMP (Reverse electron transport)

This procedure uses beef heart sub-mitochondrial particles (SMP) to screen for toxicants in liquid samples. The SMP are fragmented portions of the inner membrane of mitochondria (commonly called electron transport particles), which retain the ability to carry out the integrated enzymatic processes of electron transport and oxidative phosphorylation.

This bioassay is based on the ability of ETP to use energy supplied by adenosine triphosphate (ATP) to drive electrons supplied by succinate in a thermodynamically unfavourable direction through mitochondrial respiratory complex II to complex I, reducing NAD to NADH. NAD is nicotinomide adenine dinucleotide, which acts as an electron acceptor in this biochemical reaction. NADH is the reduced form of the NAD complex (containing one additional hydrogen atom).

To perform the test, thawed and reconstituted electron transport particles are added to a cuvette containing test reagent and the toxicant or environmental sample. ATP is added to drive the electron transport process and the reaction rate is monitored using a spectrophotometer. Toxicity is determined by comparing the rate of electron transport in the cuvettes containing the test samples to the rate observed in control cuvettes (Dutka, 1997).

3.1.4 SMP (Forward electron transport)

This procedure also uses beef heart sub-mitochondrial particles. The Forward (or Conventional) Electron Transport assay (FET or CET) is based on the forward movement of electrons from NADH through mitochondrial respiratory enzyme complexes I, III and IV. This is the direction of normal flow of electrons through these enzymes during cellular respiration. The conversion of NADH to NAD is monitored spectrophotometrically at 340 nm.

To perform the test, thawed and reconstituted electron transport particles are added to a cuvette containing test reagent and the toxicant or environmental sample. NADH is added as an electron donor and the rate of NADH oxidation is monitored using a spectrophotometer. The toxicity of the sample is determined by comparing the rate of NADH depletion in the sample cuvettes to the rate observed in control cuvettes (Dutka, 1997).

3.1.5 Ames Fluctuation Test

The fluctuation assay is a modification of the Ames' *Salmonella* mutagenicity test (Dutka, 1997). The fluctuation test is used in preference to the Ames plate incorporation test in samples where levels of mutagenic chemicals if present are expected to be below the detection limit of the Ames test.

Unlike the Ames test, the induction and expression of mutated *Salmonella* cells in the fluctuation assay occurs in a liquid suspension medium in a micro-well plate. The sample and dilutions are mixed with a basic growth medium, then these suspensions are distributed in 0.2 mL aliquots into the wells of a 96 micro-well plate and incubated for five days. Over this period, only mutated cells will grow if there is no toxicity. Growth is detected by an acidic change in pH in the medium from purple to yellow.

This test is a bacterial reverse-mutation test, using a mutant strain (or strains) of *Salmonella typhimurium*, carrying mutations in the operon coding for histidine synthesis. The *Salmonella typhimurium* strain TA100 was used for these experiments. When bacteria are exposed to mutagenic compounds under certain conditions, reverse mutation from amino acid (histidine) auxotropy to prototrophy occurs.

In this test, mutagenic activity is assumed when a sample induces a dose related increase in the number of wells containing growth of mutated cells. The number of positive wells induced by one or more doses of the sample must significantly exceed those of a control plate containing no sample.

This procedure is available in kit format from Environmental Biodetection Products Inc. (EBPI), in Brampton, Ontario, Canada.

3.1.6 SOS Chromotest

This test for the presence of bioavailable genotoxicants is based on a colorimetric assay of microbial enzymatic activities. Sample plates are read after incubating the genetically engineered tester strain (*E. coli* K12-PQ37) with a suspected liquid sample. The *E. coli* K12-PQ37 has been altered so that the ß-galactosidase gene (lacZ) is fused to the sulA gene. The sulA gene is part of the error-prone SOS repair system.

In the test, an exponential growth phase culture of the *E.coli* is introduced into the wells of a microtitration plate containing samples and controls. After a two hour incubation at 35°C, ß-galactosidase activity (SOS response activity) is measured by changes in the optical density of the sample at 615 nm in a microtitration plate reader. This measures the level of ß-galactosidase via its effect on the indicator compound 5-bromo-4-chloro-3-indolyl-ß-D-galactoside. Thus the greater the amount of ß-galactosidase produced, the greater the SOS response pathway has been induced, and thus the greater the genotoxicant concentration in the sample. This kit test can run with or without S-9 (Arochlor induced liver homogenate), and can be read visually or by a spectrophotometer (Dutka, 1997).

3.2 Chronic Toxicity Testing

Two types of 7-day chronic toxicity tests, using the Cladoceran *Ceriodaphnia dubia* and fathead minnow *Pimephales promelas* were performed. Chronic toxicity was assessed by determining the effect on *C. dubia* survival and reproduction and fathead minnow survival and growth.

3.2.1 Fathead Minnow

Ten fathead minnow larvae less than 24 hours old are placed in each test beaker. A total of four, 500 mL replicates for each concentration tested are used, and compared against a set of four replicate controls. The control water and dilution water used here is municipal tap water de-chlorinated by continuous aeration for at least 4 days. A series of dilutions (100, 50, 25, 12.5 and 6.25%) of the whole test solution are made for several tests at each site. Daily renewals of the test solutions are performed over the 7-day period of the test. At the end of the test, the larvae are removed from the test solution and allowed to depurate in control water for 1 to 2 hours. The larvae are then counted, dried at 100°C for < 24 hours and weighed.

The Environment Canada protocol EPS-1/RM/22 (1992) was followed regarding fathead minnow culturing, feeding and test conditions. Measurements for pH, conductivity, temperature, dissolved oxygen, ammonia and hardness were taken throughout the test duration to help provide insight as to changes in water quality over time.

3.2.2 Ceriodaphnia dubia

A *Ceriodaphnia dubia* neonate, less than 24 hours old is placed in each test cup. A total of ten 20 mL replicates for each concentration tested are compared with a set of 10 replicate controls. The control water and dilution water is tap water, de-chlorinated by continuous aeration for at least 4 days. A series of dilutions (100%, 50%, 25%, 12.5% and 6.25%) of the whole test solution is made for several tests at each site. Daily renewals of the test solutions are performed over the 7-day period of the test, at which time the neonates have matured and produced 3 broods of young.

The Environment Canada protocol EPS-1/RM/21 (1992) was followed regarding *Ceriodaphnia* culturing, feeding and test conditions.

3.3 Chemical Testing

The Ontario Ministry of Environment and Energy (MOEE) and Water Technology International (WTI) laboratories provided results of water quality analyses, including biochemical oxygen demand (C-BOD_5), solids (suspended, total and dissolved), nitrogen (nitrite, nitrate + nitrite, ammonia + ammonium, Total Kjeldahl nitrogen), phosphorus (phosphate, total phosphorus), carbon (dissolved organic, dissolved inorganic), silicon and total metals (Al, Ba, Be, Cd, Co, Cr, Cu, Fe, Pb, Mg, Mn, Mo, Ni, Ag, Sr, Ti, Va, Zn, Ca). All samples were analysed according to Standard Methods (APHA, 1989).

4.0 RESULTS

A summary of all data collected during the study of urban wet weather discharges in the Toronto and Hamilton Areas of Concern is presented in this section. Table 4.1 identifies the date, time, location, land use, rainfall, antecedent dry period, method and type of sample collected in the Toronto area. Table 4.2 identifies the same parameters for samples collected in Hamilton.

A comprehensive chemical characterization was not possible for all samples due to limited sample volume or inadequate collection and preservation techniques (applied by others). All water chemistry results have been combined and are summarized in Appendix A.

4.1 Acute Toxicity and Genotoxicity Test Results

Table 4.3 shows a suggested interpretation of the acute and genotoxic test results, using a four point toxicity scale (Rochfort et al., 1998). This scale brackets the responses of a variety of toxicity tests so that they can be compared more easily, and is based on a concept found in Dutka (1988). The four categories consist of "no toxicity", "potential toxicity", "confirmed (moderate) toxicity" and "severe toxicity". Table 4.4 shows the toxicity point value (TPV) results for four types of acute and two types of genotoxic test results for samples collected in Toronto. Table 4.5 shows the same test results obtained for samples collected in Hamilton. A summary of the raw acute toxicity and genotoxicity test data collected during this study can be found in Appendix B.

Trends can be identified by combining the results of the acute and genotoxic tests on the 34 Hamilton and Toronto samples. Twenty out of 192 tests were positive (Figure 4.1). Ninety percent of the acute toxicity tests produced negative results. Very few samples were toxic overall (having a TPV of 2 or 3), and most were completely non-toxic. However, 35% of samples (12 of 34) were positive for at least one of the six acute and genotoxic tests applied (Figure 4.2). Only 9% of samples

Table 4.1: Sample Collection Information for Toronto

Date (d-m-y)	Time (h:m)	Location	TCSO #	Land Use	Rainfall (mm)	Antecedent Dry Period (d)	Sample Type	Collection Method	Treatment
08-Aug-96	14:00	Strachan	1	I,C,R,T	7.8	5	R	M	N
26-Aug-96	-	Strachan	2	I,C,R,T	3.4	2	R	M	N
12-Sep-96	13:00	Strachan	3	I,C,R,T	8.4	0	R	M	N
13-Sep-96	13:00	Strachan	4	I,C,R,T	31	0	R	M	N
24-Sep-96	14:00	Strachan	5	I,C,R,T	11.8	1	R	M	N
24-Sep-96	+24 h[1]	Strachan	6	I,C,R,T	11.8	1	R	M	Y
27-Sep-96	14:00	Strachan	7	I,C,R,T	7.8	0	R	M	N
17-Dec-96	16:00	Strachan	8	I,C,R,T	23	0	R	M	N
08-Jul-97	16:45	PI scarb	9	C,R,T	9.8	1	R	M	N
08-Jul-97	16:55	PE scarb	10	C,R,T	9.8	1	R	M	Y
08-Jul-97	17:15	PI scarb2	11	C,R,T	9.8	1	R	M	N
08-Jul-97	17:16	PE scarb2	12	C,R,T	9.8	1	R	M	Y
08-Jul-97	17:45	N Tor	13	I,C,R,T	9.8	1	R	M	N
15-Jul-97	15:20	PI Scarb	14	C,R,T	6.2	6	R	M	N
15-Jul-97	15:45	N Tor	15	I,C,R,T	6.2	6	R	M	N
12-Aug-97	22:00	6th Et	16	R	17.8	0	C	A	N
12-Aug-97	22:00	MacLean	17	I,C,R,T	17.8	0	C	A	N
13-Aug-97	2:00	PI Scarb	18	C,R,T	17.8	0	R	M	N
13-Aug-97	2:00	N Tor	19	I,C,R,T	17.8	0	R	M	N
13-Aug-97	2:00	Strachan	20	I,C,R,T	17.8	0	C	A	N
21-Aug-97	0:01	6th Et	21	R	2.2	0	C	A	N
08-Sep-97	-	6th Et	22	R	0.1	0	C	A	N
10-Sep-97	9:30	6th Et	23	R	11.8	0	C	A	N
17-Sep-97	13:00	Wcrest Et	24	R	3	4	C	A	N
25-Sep-97	15:00	6th Et	25	R	8	2	C	A	N
25-Sep-97	15:00	Wcrest Et	26	R	2.8	0	C	A	N
29-Sep-97	10:00	Wcrest Et	27	R	27.8	4	C	A	N
27-Oct-97	10:00	Strachan	28	I,C,R,T	25.4	0	C	A	N
01-Nov-97	15:00	Strachan	29	I,C,R,T	25.4	0	C	A	N
01-Nov-97	10:30	Wcrest Et	30	R	25.4	0	C	A	N
01-Nov-97	11:00	Humber Cr	31	I,C,R,T	25.4	0	R	M	N
21-Nov-97	14:00	N Tor	32	I,C,R,T	2.4	0	R	A	N
21-Nov-97	14:00	N Tor effluent	33	I,C,R,T	2.4	0	R	A	Y
21-Nov-97	14:00	Strachan	34	I,C,R,T	2.4	0	C	A	N

Key to Location Abbreviations: PI - plate clarifier influent, PE plate clarifier effluent, Wcrest Et - Woodcrest Dive City of Etobicoke, 6th Et - 6th Street City of Etobicoke
Key to Land Use Abbreviations: I - Industrial, C - Commercial, R - Residential, T - High traffic area
Key to Sample Type: F - First flush, R - Random, C - Composite
Key to Collection Method: A - Automatic, M - Manual

NOTE: 1 - sample TCSO-5 settled for 24 h at 15C

Table 4.2: Sample Collection Information for Hamilton

Date (d-m-y)	Time (h:m)	Location	HCSO #	Land Use	Rainfall (mm)	Antecedent Dry Period (d)	Sample Type	Collection Method	Treatment
01-Dec-96	15:30	Royal	1	R	9.1	0	R	M	N
01-Dec-96	+24h[1]	Royal	2	R	9.1	0	R	M	Y
01-Dec-96		75[2]	3	R,T	9.1	0	R	M	N
01-Dec-96		66	4	R,T	9.1	0	R	M	N
01-Dec-96		50	5	R,T	9.1	0	R	M	N
01-Dec-96		33	6	R,T	9.1	0	R	M	N
01-Dec-96		25	7	R,T	9.1	0	R	M	N
04-Feb-97	20:00	Queenston	8	C,R,T	11.9	3	R	M	N
20-Feb-97	22:00	Lawrence	9	C,R,T	8.8	0	R	M	N
03-May-97	9:30	Melvin	10	C,R,T	18.6	0	R	M	N
16-Jun-97	18:00	Melvin	11	C,R,T	16.2	3	F	M	N
16-Jun-97	18:30	Sterling	12	I,C,R,T	16.2	3	F	M	N
20-Jun-97	15:00	Lawrence	13	C,R,T	4.4	0	F	M	N
02-Jul-97	21:20	Lawrence	14	C,R,T	8.5	0	F	M	N
28-Jul-97	19:30	Sterling	15	I,C,R,T	17.5	0	F	M	N

Key to Location Abbreviations: I - Industrial, C - Commercial, R - Residential, T - High traffic area
Key to Sample Type: F - First flush, R - Random
Key to Collection Method: A - Automatic, M - Manual

NOTE: 1 - sample TCSO-5 settled for 24 h at 15C
NOTE: 2 - samples were mixed with Skyway Bridge Runoff (%cso shown)

Table 4.3: Toxicity Point Values Corresponding to Raw Toxicological Data

Effect Level	**Toxicity Point Value**	***Daphnia magna* EC**	**Microtox EC50**	**Sub-Mitochondrial Particle (RET and CET)**	**SOS Chromotest**	**Ames Fluctuation Test**
		Percent	*Percent*	*Percent Inhibition*	*Genotoxicity Induction Factor*	*Positive/ Negative*
No Toxicity Present	**0**	EC10 at 100%	> 100	0 - 9	< 1.00	Negative
Indication of Potential Toxicity	**1**	EC20 - EC40 at 100%	> 40	10 - 50	1.0 - 1.29	Positive at P = 0.1
Confirmed Toxicity	**2**	EC50 at 100%	40.0 - 10.0	51 - 90	1.30 - 2.00	Positive at P = 0.05
Severe Level of Toxicity	**3**	EC50 at 75% and below	9.0 and below	91 - 100	2.01 and above	Positive at P = 0.01 or greater

EC - Effective concentration required to inhibit some percentage of the organism tested. (An EC20 at 100% indicates that 20% of the organisms were affected by the 100% solution)
RET - Reverse Electron Transfer
CET - Conventional (Forward) Electron Transfer

Table 4.4: Toxicity Point Values for Acute and Genotoxic Tests in Toronto

Date	Location	TCSO #	Acute Toxicity Tests				Genotoxicity Tests	
			D.magna	Microtox	Submitochondrial particles RET	Submitochondrial particles CET	Fluctuation Test	SOS Chromotest
08-Aug-96	Strachan	1	0	0	1	0	0	1
26-Aug-96	Strachan	2	0	0	1	0	0	1
12-Sep-96	Strachan	3	0	0	1	0	0	1
13-Sep-96	Strachan	4	0	0	1	1	0	1
24-Sep-96	Strachan	5	0	0	1	1	0	1
24-Sep-96	Strachan [1]	6	0	0	1	1	0	1
27-Sep-96	Strachan	7	0	0	1	0	0	1
17-Dec-96	Strachan	8	0	0	0	1	0	1
08-Jul-97	PI Scarb	9	0	0	0	0	2	1
08-Jul-97	PE Scarb	10	0	0	1	0	0	1
08-Jul-97	PI Scarb2	11	0	0	1	0	2	1
08-Jul-97	PE Scarb2	12	0	0	1	0	0	0
08-Jul-97	N Tor	13	0	0	0	0	2	1
15-Jul-97	PI Scarb	14	0	0	1	0	3	1
15-Jul-97	N Tor	15	0	0	0	0	2	1
12-Aug-97	MacLean	17	1	0	0	0	2	1
13-Aug-97	PI Scarb	18	0	0	0	1	2	1
13-Aug-97	N Tor	19	0	0	0	0	0	1
13-Aug-97	Strachan	20	0	0	0	0	0	0
27-Oct-97	Strachan	28	0	0	1	1	0	2
01-Nov-97	Strachan	29	0	0	1	0	2	1
21-Nov-97	N Tor	32	0	2	2	2	3	2
21-Nov-97	N Tor effluent	33	0	0	2	2	3	2
21-Nov-97	Strachan	34	0	0	0	0	3	1

Key to Place Abbreviations: PI - plate clarifier influent, PE plate clarifier effluent, Scarb - Scarborough
N-Tor - North Toronto N Tor Effluent - Treated Effluent from North Toronto
NOTE 1: sample was settled for 24 h at 15C

Table 4.5: Toxicity Point Values for Acute and Genotoxic Tests in Hamilton

Date	Location	HCSO #	Acute Toxicity Tests				Genotoxicity Tests	
			D.magna	Microtox	Submitochondrial particles RET	Submitochondrial particles CET	Fluctuation Test	SOS Chromotest
01-Dec-96	Royal	1	n/a	0	0	1	1	1
01-Dec-96	Royal [1]	2	n/a	0	0	1	2	1
04-Feb-97	Queenston	8	n/a	0	1	1	n/a	1
20-Feb-97	Lawrence	9	n/a	0	1	0	n/a	1
03-May-97	Melvin	10	0	0	1	1	n/a	1
16-Jun-97	Melvin	11	0	0	1	1	n/a	1
16-Jun-97	Sterling	12	0	0	1	0	n/a	1
20-Jun-97	Lawrence	13	0	0	1	0	n/a	1
02-Jul-97	Lawrence	14	0	0	1	0	n/a	0
28-Jul-97	Sterling	15	n/a	0	1	0	0	1

n/a - sample was not tested
NOTE 1: Sample was settled 24 h at 15C

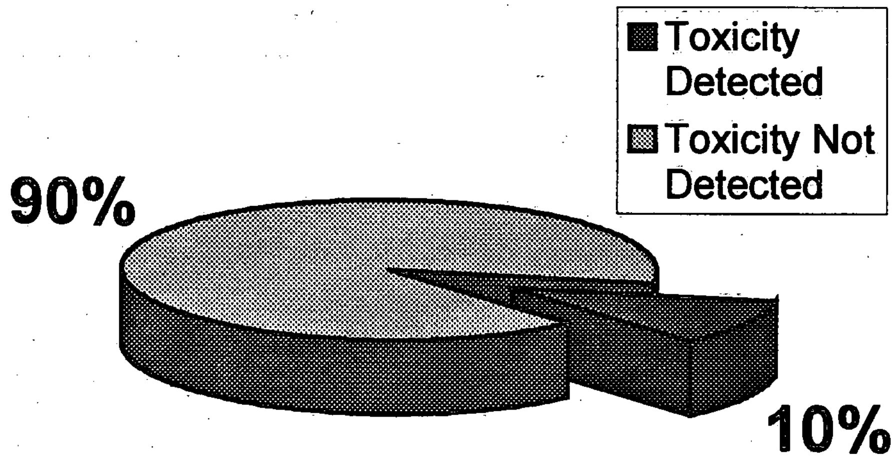


Figure 4. 1: Number of Acute Positive Tests

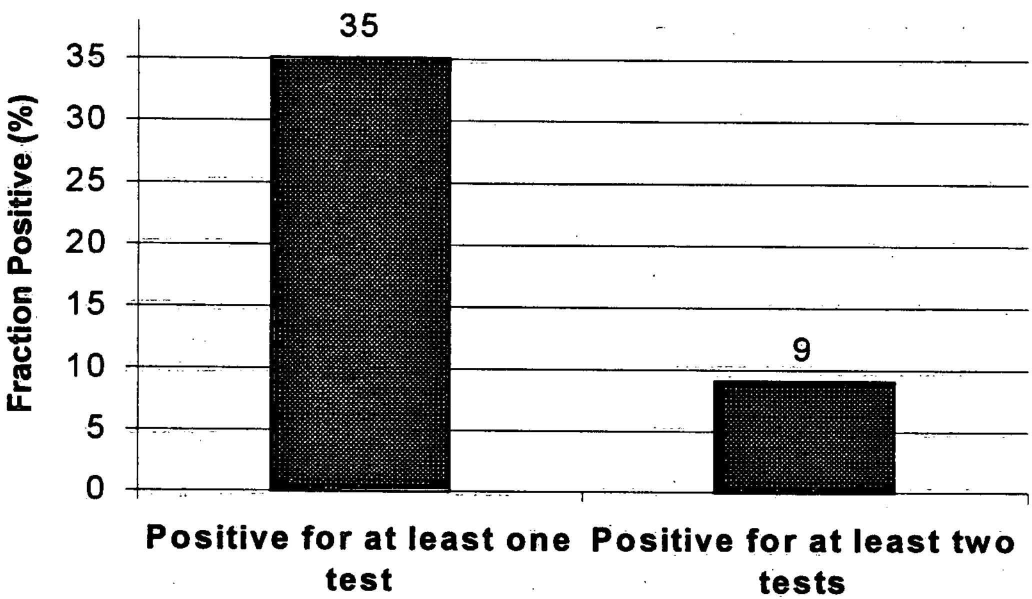


Figure 4. 2: Fraction of Acute Samples Positive for One or Two Tests

(3 of 34) were positive for two tests, and only two samples were positive for four or more tests (both were samples from North Toronto - TCSO 32 and 33).

Of the CSO samples which were positive for acute toxicity or genotoxicity, the Ames fluctuation test resulted in the highest number of positive responses (12 of 27 = 44%), (Tables 4.4 and 4.5), with some results displaying extreme toxicity point values of 3. The next most sensitive test was the SOS chromotest, with 3 positive responses in 34 samples. The acute sub-mitochondrial particle bioassays scored only 2 positive results in 34 samples, and Microtox™ yielded only one positive result in 34 samples. The mean TPV response of each test to the CSO samples (Figure 4.3) indicates that the Ames fluctuation test shows the greatest test response to the toxicants found in CSOs (1.07), and was therefore the most sensitive of the tests applied. Four CSO samples from Toronto registered extreme toxicity (TPV = 3) to the Ames test. Notably, the *Daphnia magna* whole organism toxicity tests did not generate strong results with any of the CSO effluents tested. The two most sensitive tests (Ames fluctuation test and SOS chromotest) were genotoxic tests, indicating that the primary concern associated with CSO discharges was genotoxicity rather than acute toxicity.

4.2 Chronic Toxicity Test Results

A pass/fail summary of the fathead minnow (FHM) survival and growth and the *Ceriodaphnia dubia* (*Ceriodaphnia*) survival and reproduction chronic toxicity test results for Toronto samples is presented in Table 4.6. Samples that were non-toxic are designated by "blank" rectangles, while toxic samples are indicated by darkly shaded areas. Where no sample was available for testing, "n/a" appears. Table 4.7 presents the same results for samples collected in Hamilton. Table 4.8 shows the experimental values for the Toronto chronic toxicity tests, including the "No Observed Effect Concentration" (NOEC), the concentration at which 25% inhibition of growth (fathead minnow) and reproduction (*Ceriodaphnia*) occurs (IC25), the pass/fail status of the test, and the associated significance (P-Value) of the pass/fail judgement. Table 4.9 shows the same values for Hamilton samples. The raw data for the fathead minnow tests are summarized in Appendix C. The raw data for the *Ceriodaphnia* tests are summarized in Appendix D.

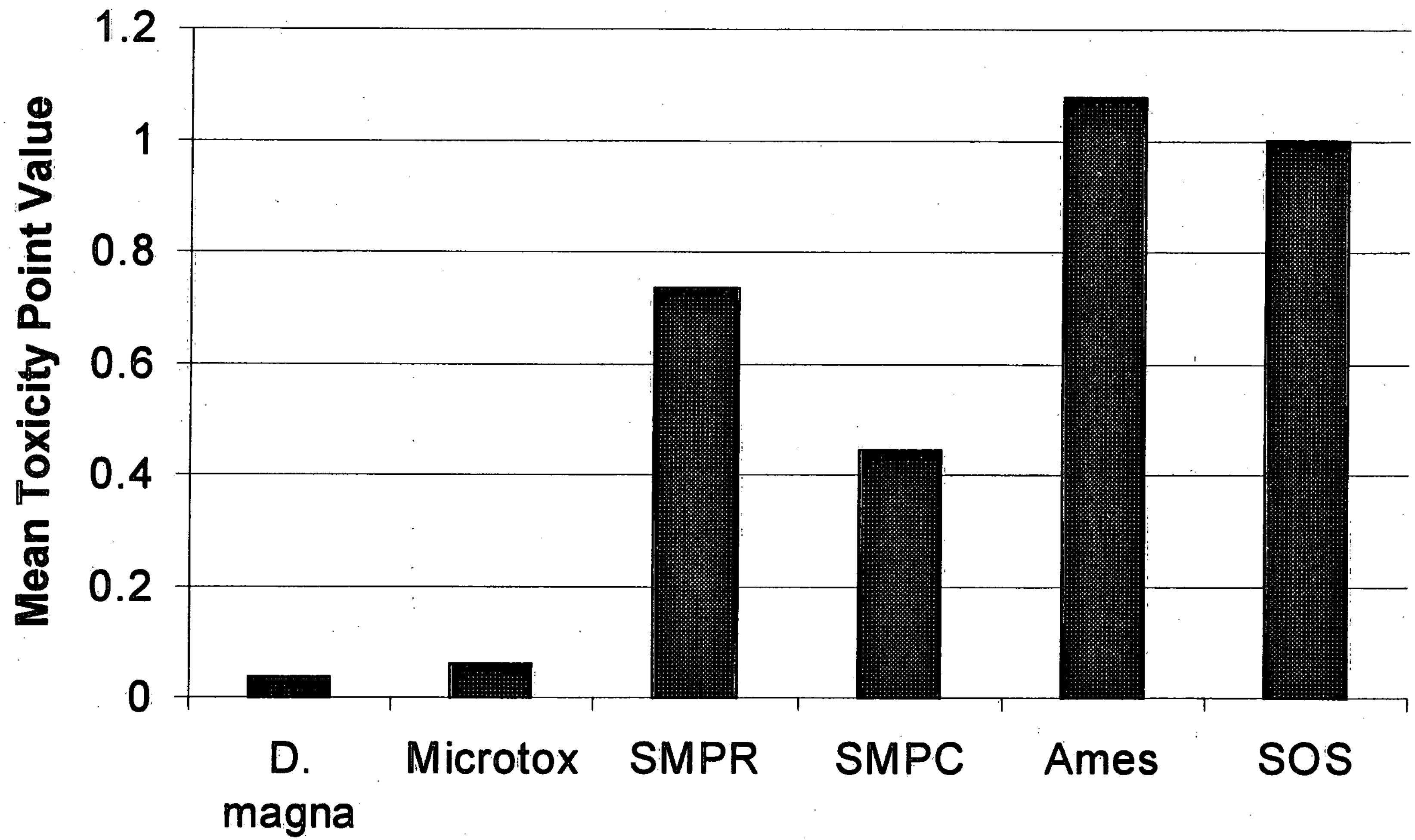


Figure 4.3: Average TPV Response for Each Test

Table 4.6: Fathead Minnow Survival and Growth and *Ceriodaphnia* Survival and Reproduction - Toronto CSOs

Sample	FHM Survival	FHM Growth	CD Survival	CD Reproduction
1	n/a	n/a		
4		(shaded)		
5		(shaded)		
7				
8				
9		(shaded)	(shaded)	(shaded)
10		(shaded)		
11	n/a	n/a	(shaded)	(shaded)
12	n/a	n/a		
13		(shaded)		(shaded)
14	(shaded)	(shaded)		
15	(shaded)	(shaded)	(shaded)	(shaded)
17		(shaded)		
18				
19				
20		(shaded)		
28	(shaded)	(shaded)		
29				
32	(shaded)	(shaded)		(shaded)
33	(shaded)	(shaded)		(shaded)
34	n/a	n/a		

Table 4.7: Fathead Minnow Survival and Growth *Ceriodaphnia* Survival and Reproduction Hamilton CSOs

Sample	FHM Survival	FHM Growth	CD Survival	CD Reproduction
1				
2				
3-7	(shaded)	(shaded)	(shaded)	(shaded)
8				
9				
10	n/a	n/a		
11	(shaded)	(shaded)		
12	(shaded)	(shaded)		(shaded)
13	(shaded)	(shaded)		(shaded)
14	n/a	n/a		
15	(shaded)	(shaded)	(shaded)	(shaded)

Pass		**Fail**	(shaded)	**No Sample**	n/a

Table 4.8: Raw Data for Toronto CSO Chronic Toxicity Tests

Date	Location	TCSO #	Fathead Minnow NOEC (%)	Fathead Minnow IC25 (%)	Fathead Minnow Pass Fail	Fathead Minnow Sign.level p value	*Ceriodaphnia* NOEC (%)	*Ceriodaphnia* IC25 (%)	*Ceriodaphnia* Pass Fail	*Ceriodaphnia* Sign.level p value
08-Aug-96	Strachan	1	-	-			100	> 100	Pass	
13-Sep-96	Strachan	4	100	88	Fail	0.05	100	> 100	Pass	
24-Sep-96	Strachan	5	100	88	Fail	0.05	100	> 100	Pass	
27-Sep-96	Strachan	7	100	> 100	Pass		100	> 100	Pass	
17-Dec-96	Strachan	8	100	> 100	Pass		100	> 100	Pass	
08-Jul-97	PI scarb	9	25	37.9	Fail	0.05	12.5	70	Fail	0.001
08-Jul-97	PE scarb	10	50	70.3	Fail	0.05	50	95	Pass	
08-Jul-97	PI scarb2	11	-	-			50	n/a 1	Fail	0.001
08-Jul-97	PE scarb2	12	-	-			25	100	Pass	
08-Jul-97	N Tor	13	50	60.7	Fail	0.05	50	65	Fail	0.001
15-Jul-97	PI Scarb	14	-	-	Fail	0.001	100	> 100	Pass	
15-Jul-97	N Tor	15	-	-	Fail	0.001	50	90	Fail	0.001
12-Aug-97	MacLean	17	-	-	Fail	0.001	100	> 100	Pass	
13-Aug-97	PI Scarb	18	-	-			-	-	Pass	
13-Aug-97	N Tor	19	-	-			-	-	Pass	
13-Aug-97	Strachan	20	-		Fail	0.001	-	-	Pass	
27-Oct-97	Strachan	28	> 50	50	Fail	0.001	> 100	100	Pass	
01-Nov-97	Strachan	29	-	-			> 100	50	Pass	
21-Nov-97	N Tor	32	> 6.25	6.25	Fail	0.001	42	25	Fail	0.001
21-Nov-97	N Tor effluent	33	> 6.25	6.25	Fail	0.001	35	12.5	Fail	0.001
21-Nov-97	Strachan	34	-	-			> 100	100	Pass	

Key to Locations: PI - plate clarifier influent, PE plate clarifier effluent,
Wcrest Et Woodcrest Dive City of Etobicoke, 6th Et 6th Street City of Etobicoke
n/a 1 - LC50 of 75 % (LC50 - concentration causing 50% lethality)
Pass/Fail - indicates test was performed using full strength effluent only, "-" test was not applied to these samples,
NOEC - No observed effect concentration - concentration at which no negative effects were noted
IC25 - Inhibiting concentration causing a 25% decrease in growth or reproduction

Table 4.9: Raw Data for Hamilton Chronic Toxicity Tests

Date	Location	HCSO #	Fathead Minnow NOEC (%)	Fathead Minnow IC25 (%)	Fathead Minnow Pass Fail	Fathead Minnow Sign.level p value	*Ceriodaphnia* NOEC (%)	*Ceriodaphnia* IC25 (%)	*Ceriodaphnia* Pass Fail	*Ceriodaphnia* Sign.level p value
01-Dec-96	Royal	1	100	> 100	Pass		100	> 100	Pass	
01-Dec-96	Royal	2	100	> 100	Pass		100	> 100	Pass	
01-Dec-96	Royal 1	3		28 1	Fail	0.001		36.5 1	Fail	0.001
04-Feb-97	Queenston	8	100	> 100	Pass		100	> 100	Pass	
20-Feb-97	Lawrence	9	100	> 100	Pass		100	> 100	Pass	
03-May-97	Melvin	10	-	-			-	-	Pass	
16-Jun-97	Melvin	11	50	75	Fail	0.001	100	> 100	Pass	
16-Jun-97	Sterling	12	25	50	Fail	0.001	12.5	25	Fail	0.001
20-Jun-97	Lawrence	13	12.5	3	Fail	0.001	25	45	Fail	0.001
02-Jul-97	Lawrence	14	-	-			100	> 100	Pass	
28-Jul-97	Sterling	15	12.5	12.5	Fail	0.05	50	n/a 2	Fail	0.001

1 HCSO samples 3 – 7 were mixtures of CSO and Highway runoff. IC25 results are presented as % CSO
2 - LC50 of 65%, (LC50 - concentration causing 50% lethality)
" - " test was not applied to these samples
NOEC - No observed effect concentration - concentration at which no negative effects were noted
IC25 - Inhibiting concentration causing a 25% decrease in growth or reproduction

The overall results of the chronic toxicity tests for Toronto and Hamilton (Figure 4.4) were 42 failures (35% positive for toxicity), and 78 passes. When the fathead minnow survival and growth are viewed separately, more than 60% of samples passed the survival tests, while less than 40% passed the more sensitive growth test (Figure 4.5). While nearly 85% of samples passed the *Ceriodaphnia* (CD) survival test, less than 70% passed the more sensitive reproductive test (Figure 4.5). These results are indicative of low level chronic toxicity.

While the majority of these samples were non-toxic, those samples that showed positive toxicity did not usually exhibit "acute" toxicity to the test organism. However, the "first flush" samples collected from sites in Hamilton did show more severe toxicity than composite samples collected elsewhere.

4.3 Chemical Contribution to Toxicity Responses

In an attempt to use the scant chemistry data provided by others, the chemistry data were related to the toxicity results, using a suggested chemical point value system. The chemical concentrations were then converted into chemical point values (CPV) for ease of comparison. This index (Table 4.10) uses a four point scale, and is largely based on the Canadian Water Quality Guidelines. These guidelines are designed to protect receiving water quality and therefore objective limits for the parameters are generally conservative. A CPV of zero indicates concentrations below the lowest guideline levels. CPVs of 2 and 3 suggest concentrations above those found for the guidelines. These CPVs can be used to identify the chemical parameters that result in toxicity. Only dissolved oxygen (DO), conductivity, biochemical oxygen demand (BOD), ammonia (NH_3), Al, Cu, Fe and Pb were used in this index. The effect of Cd could not be considered as all sample concentrations were below the detection limit of 10 μg/L. Table 4.11 shows the converted chemistry data for Toronto CSO samples, and Table 4.12 shows the same data for Hamilton sites. The sum TPV (derived from the five acute and genotoxic tests) is also listed for these samples. While all of the parameters listed above are likely to contribute to the toxicity of the sample, some factors have a greater effect than others. Additive and synergistic effects may also occur.

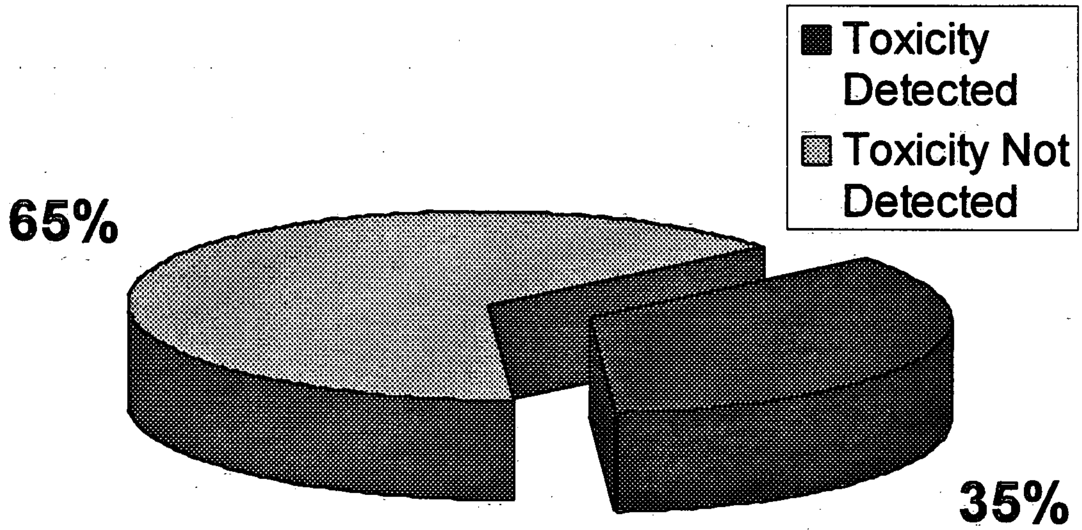


Figure 4.4: Chronic Toxicity Test Results Toronto and Hamilton

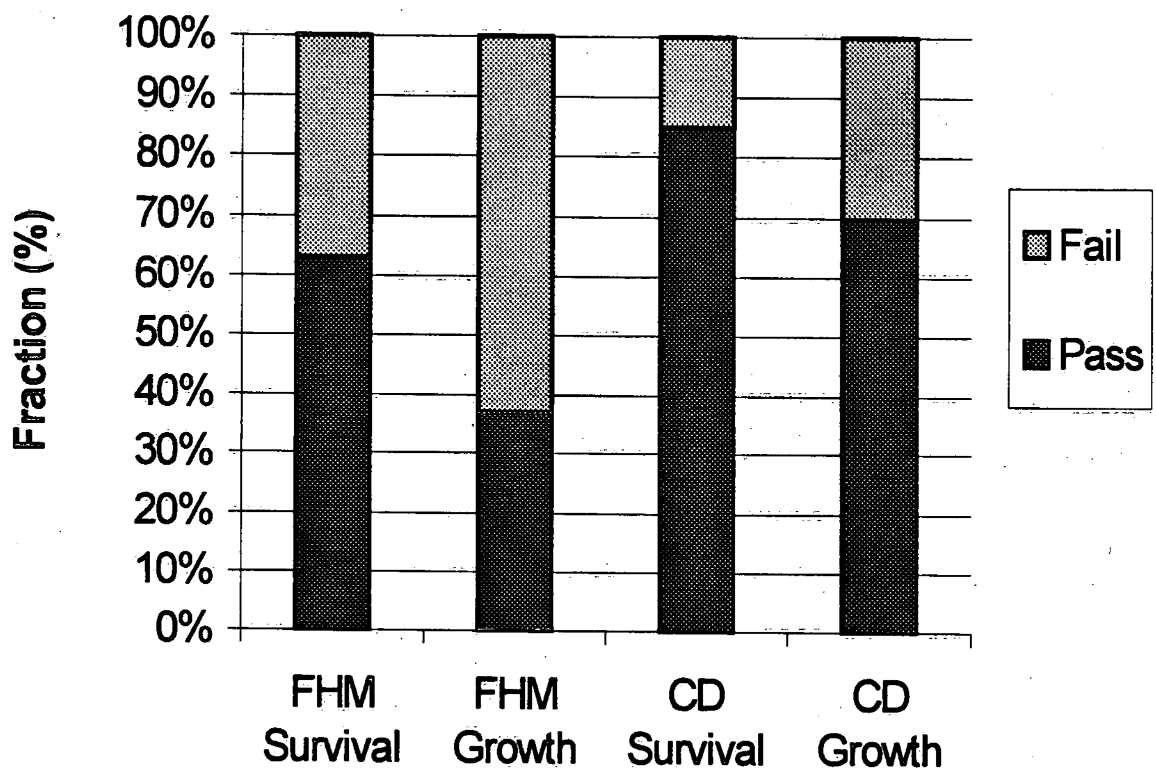


Figure 4.5: Breakdown of Pass Fail Results for Chronic Toxicity Tests in Toronto and Hamilton

Table 4.10: Chemistry Point Value Index

Parameter	Units	CPV Level 0	CPV Level 1	CPV Level 2	CPV Level 3
Dissolved Oxygen[1]	mg/L	> 5.00	4.00 - 5.00	3.00 - 4.00	< 3.00
Conductivity[2]	μS/cm	< 150	150 - 500	500 - 1000	> 1000
BOD[2]	mg/L	< 15	15 - 50	50 – 150	> 150
COD[2]	mg/L	< 100	100 – 250	250 – 400	> 400
Ammonia[1]	mg/L	< 1.00	1.00 – 2.00	3.00 – 5.00	> 5.00
Aluminum	μg/L	< 5	5 - 100	100 – 5000	> 5000
Copper	μg/L	< 200	200 - 500	500 – 5000	> 5000
Iron	μg/L	< 100	100 - 1000	1000 - 5000	> 5000
Lead	μg/L	< 7	7 - 100	100 – 200	> 200

[1] Index based on fathead minnow protocol (Environment Canada, 1992)
[2] Index based on observed toxicity related to highway runoff
For all other parameters, index was based on Canadian Water Quality Guidelines (1995).

Table 4.11: Toronto CSO Chemistry Point Value Data

Sum TPV	Date	Location	TCSO #	DO	Cond	CBOD-5	NH3(N)	Al	Cu	Fe	Pb
3	13-Sep-96	Strachan	4	0	1						
3	24-Sep-96	Strachan	5	0	2						
2	27-Sep-96	Strachan	7	0	3						
2	17-Dec-96	Strachan	8	0	1						
3	08-Jul-97	PI scarb	9	2	1	2	2		2	2	0
2	08-Jul-97	PE scarb	10	0	1	1	2		0	1	0
4	08-Jul-97	PI scarb2	11			2	2		1	2	0
1	08-Jul-97	PE scarb2	12			1	2		0	0	0
3	08-Jul-97	N Tor	13	0	1	1	2		1	2	0
5	15-Jul-97	PI Scarb	14	2	1	2	1		2	2	1
3	15-Jul-97	N Tor	15	3	1	2	3		2	2	1
4	12-Aug-97	MacLean	17	1	1	2	1		1	2	1
4	13-Aug-97	PI Scarb	18			3	1		2	2	1
1	13-Aug-97	N Tor	19			2	2		2	2	1
0	13-Aug-97	Strachan	20	2	1	3	3		3	3	3
4	27-Oct-97	Strachan	28	1	1						
4	01-Nov-97	Strachan	29				0		0	2	1
11	21-Nov-97	N Tor	32	3	2	3	3		2	2	1
9	21-Nov-97	N Tor effluent	33	3	2	3	3		0	2	1

Table 4.12: Hamilton CSO Chemistry Point Value Data

Sum TPV	Date	Location	HCSO #	DO	Cond	CBOD-5	NH3(N)	Al	Cu	Fe	Pb
3	01-Dec-96	Royal	1	0	3			2	0	2	1
4	01-Dec-96	Royal	2	0	3			2	0	1	0
3	04-Feb-97	Queenston	8	0	3						
2	20-Feb-97	Lawrence	9	0	3						
3	03-May-97	Melvin	10	0	1						
3	16-Jun-97	Melvin	11	1	2			2	1	1	1
2	16-Jun-97	Sterling	12	3	1		2	2	1	2	1
2	20-Jun-97	Lawrence	13	2	1		1	2	1	2	1
1	02-Jul-97	Lawrence	14			0	1	2	0	1	0
2	28-Jul-97	Sterling	15	3	1	2	1	2	3	3	1

This CPV interpretation is used to simplify comparison and highlight some of these factors. These chemistry data were compared to the acute toxicity and genotoxicity of CSO samples on a per-test basis using the sum of the toxicity point values as an indication of overall toxic response. Chronic toxicity data could not be characterized in the same way as the acute data, and therefore were used to support acute comparisons. Incomplete data sets meant that associations were more difficult to establish and consequently, no conclusive trends could be identified.

It was found that low levels of initial dissolved oxygen characterized most of the samples registering acute toxicity and genotoxicity. Most of these samples were also likely to be toxic to fathead minnow growth (if not also survival), and possibly *Ceriodaphnia* reproduction. For fathead minnow, all IC25 values were below 50% when DO was low. At DO levels above 5.00 mg/L (CPV of 0), this toxicity was reduced. Dissolved oxygen plays a critical part in toxicity tests where longer exposures are required, as oxygen is essential for organism survival.

Although high conductivity (an indicator of dissolved solids - including the chloride ion) did not provide a reasonable estimate of acute toxicity, it was found to have some correlation to chronic toxicity. While this may hold for most cases, some samples with high conductivity readings did not show any chronic toxicity. Conductivity was therefore not a reliable predictor of sample toxicity, despite the fact that some correlation between conductivity and acute toxicity had been observed for highway runoff (Rochfort et al., 1997).

Samples with very high BOD values (CPV of 3) tended to show strong toxic responses in the chronic toxicity tests. Samples with a CPV level 2 for BOD were also quite toxic in chronic tests. The same was found to be true for some of the acute toxicity tests. The presence of oxygen demanding substances in the wastewater can often exert a toxic effect on organisms in the receiving stream. This association was not always true, however, as one sample with very high BOD (TCSO-20) was determined to be non-toxic by all tests, except fathead minnow growth. Overall, it would appear that BOD is a good chemical indicator of sewage strength and toxicity, however, it may not provide adequate information to estimate sample toxicity in all cases.

Ammonia is a contaminant commonly associated with combined sewer overflow discharges and can often contribute to oxygen depletion and toxicity (particularly in fish). As such, chronic toxicity appeared to be well correlated to ammonia concentrations. Samples with high ammonia concentrations showed strong toxic responses in fathead minnow and *Ceriodaphnia* tests. Notably, CPV level 3 ammonia samples also had level 3 initial dissolved oxygen levels. As the CPV levels declined, the IC25 values generally increased, suggesting that sample toxicity may be strongly influenced by ammonia concentrations. A combination of high BOD/COD and ammonia concentrations are likely to have resulted in the low dissolved oxygen and contributed to the ultimate failure of the fathead minnow experiments.

Only total heavy metals data were available for these samples. It would have been preferable to use dissolved metal concentrations that are more indicative of bioavailable forms. All sample Al levels were moderately high for this small data set, however, the associated toxicity point values of those samples ranged from almost non-toxic to moderately toxic. Al, therefore was not a suitable parameter to correlate to toxicity. Even though Cu CPVs were very high for some samples, these samples were not acutely toxic. A greater degree of acute toxicity was found for samples with only moderate levels of Cu. Cu was therefore not a good indicator of potential acute toxicity. *Ceriodaphnia* showed a somewhat stronger toxicity response for high levels of Cu and Fe. The samples with the highest concentration of Fe (CPV of 3) should have demonstrated a significant acute toxic effect, however they were only slightly toxic. Pb also showed the same trends. It is possible that the Fe and Pb levels measured may not have been bioavailable, or that there were combined effects, which lowered the toxicity of Fe or Pb. These data indicate that it is important to use toxicity testing to help determine potential ecosystem effects, as contaminant monitoring does not provide enough information to adequately characterize the effluents.

Some samples were of specific interest because of their highly toxic (or non-toxic) nature. The two most toxic samples (TCSO-32 and 33) were collected from North Toronto (one before and one after treatment with polymer coagulant). Strong responses were noted in Microtox™, Sub-mitochondrial particles, Ames fluctuation test and SOS chromotest, as well as fathead minnow survival and growth, and the *Ceriodaphnia* reproduction. Notably, *Daphnia magna* and *Ceriodaphnia* survival were not affected by these toxicants. These samples also had low initial dissolved oxygen, high conductivity readings, high BOD and ammonia as well as some high metal concentrations.

A sample from Strachan Avenue (TCSO-20) was found to be non-toxic to all acute, genotoxic and chronic tests (with the only exception being fathead minnow growth), despite the fact that it had very high metals concentrations, as well as being low in dissolved oxygen, high in BOD and ammonia.

With such a diverse array of toxicity responses, it becomes apparent that chemical characterization alone is not enough to positively identify samples which will result in the greatest impact on the receiving waters.

4.4 Urban Stormwater Runoff Toxicity Test Results

Stormwater samples collected in Etobicoke were examined separately from the combined sewer overflow samples because of their different nature. Stormwater sites contribute to the receiving waters similar pollutants as those in CSOs, but they do not contain the additional sanitary waste found in CSO discharges. In these separate stormwater systems, small (low intensity or short duration) rainfall events produce discharges more frequently than CSOs. Only ten samples were collected from three different sites in Etobicoke, all of which were located in residential areas.

The toxicity point value results of the acute and genotoxic tests are presented in Table 4.13. Very little acute toxicity was noted for these samples, which was expected in the residential locations selected. Genotoxic responses were somewhat higher, including several confirmed toxicity (TPV level 2) responses in both Ames fluctuation test and SOS chromotest. The Ames fluctuation test registered 50% (5 of 10) of the samples as having positive toxicity, and the SOS chromotest showed 30% (3 of 10) samples positive. Notably, Microtox™ showed all samples as non-toxic, and there were no positive detects for toxicity by any other test.

A summary of the chemical point values (CPV) is presented in Table 4.14. The limited amount of chemical data made comparisons difficult, and therefore it is impossible to

Table 4.13: Toxicity Point Values for Etobicoke Stormwater Samples

Date	Location	TCSO #	Acute Toxicity Tests				Genotoxicity Tests	
			D.magna	Microtox	Submitochondrial particles RET	Submitochondrial particles CET	Fluctuation Test	SOS Chromotest
12-Aug-97	6th Et	16	1	0	0	0	2	1
21-Aug-97	6th Et	21	1	0	0	0	0	1
08-Sep-97	6th Et	22	1	0	0	0	0	1
10-Sep-97	6th Et	23	0	0	1	0	2	1
17-Sep-97	Wcrest Et	24	0	0	1	0	2	2
25-Sep-97	6th Et	25	0	0	1	1	0	2
25-Sep-97	Wcrest Et	26	1	0	0	0	0	2
29-Sep-97	Wcrest Et	27	1	0	0	1	2	1
01-Nov-97	Wcrest Et	30	0	0	1	1	0	1
01-Nov-97	Humber Cr	31	0	0	1	0	2	1

Key to Place Abbreviations:
Wcrest Et - Woodcrest Dive City of Etobicoke, 6th Et - 6th Street City of Etobicoke

Table 4.14: Etobicoke Stormwater Sample Chemistry Point Value Data

Date	Location	TCSO #	Sum TPV	DO	Cond	CBOD-5	NH3(N)	Al	Cu	Fe	Pb
12-Aug-97	6th Et	16	**4**	0	3	1	0		0	2	0
21-Aug-97	6th Et	21	**2**				0		2	1	3
10-Sep-97	6th Et	23	**4**				0		2	1	3
17-Sep-97	Wcrest Et	24	**5**				0	2	0	1	0
25-Sep-97	Wcrest Et	26	**3**				0	0	0	1	0
29-Sep-97	Wcrest Et	27	**5**			0	0	0	0	0	0

Key to Place Abbreviations:
Wcrest Et - Woodcrest Dive City of Etobicoke, 6th Et - 6th Street City of Etobicoke

draw conclusions on the significance of chemical effects on toxicity. It is significant to note that the most toxic sample from this location (TCSO-27) had very low levels of metals, ammonia and BOD. This sample produced moderate toxicity responses in the Ames fluctuation test and the SOS chromotest. This type of response was not expected from an urban runoff sample. Previous research had demonstrated that areas impacted by higher traffic flow produced strong acute toxicity responses (Marsalek et al., 1998). The raw toxicity data from these experiments are summarized in Appendix B.

Only one set of chronic toxicity experiments was performed on these samples due to limited sample volumes collected by the automated samplers. The chronic tests were performed on the August 13, 1997 sample from 6th Street in Etobicoke (TCSO 16). No chronic toxicity was detected in this sample for either the fathead minnow or *Ceriodaphnia* tests.

5.0 DISCUSSION

Analysis of complex systems is often best approached using multivariate analysis, however, identification of primary contributors of toxicity by this method was not possible due to insufficient and missing data. Correlation of specific parameters with toxicity may not be accurate due to limited sample sizes, and as such, only general trends could be identified.

5.1 Acute and Genotoxic Test Responses

The majority of the TPV of acute toxicity test responses (Tables 4.4 and 4.5) were below 3 (the lowest level for confirmed presence of toxicity). Genotoxicity test results were notably higher than the acute test results because of the bioavailability of the genotoxicants present. These tests were therefore more suitable for detecting the toxic impacts of low level contamination found in the CSO samples. It would therefore be beneficial to include at least one genotoxic test in future combined sewer overflow investigations.

The *Daphnia magna* acute test showed very little toxic response overall. This whole organism test is highly sensitive to chemical imbalances. The lack of responses from either first flush or composite samples indicated that the levels of bioavailable toxins were very low and that this organism was not sensitive enough to detect the level of toxicity exhibited by these samples. It may still be useful to include this test in a toxicity-screening program. The Microtox™ test has been well utilized in the testing of industrial discharges. It is primarily sensitive to metal toxicity, often found in highway runoff. Only one positive response was detected using this test, including first flush samples. This particular sample (TCSO 32), was also found to be very toxic by other tests as well. As only one response was detected for all of these experiments, it would not be recommended for inclusion in toxicity testing of combined sewer overflows. The sub-mitochondrial particle bioassays (forward and reverse electron transport) did show a range of responses for these samples although none indicated the presence of severe toxicity. Despite the fact that only a low level of toxicity was registered, these types of tests may merit some consideration with respect to screening potential in combined sewer overflow monitoring, as these tests are easy and inexpensive to perform.

The Ames Fluctuation test registered the strongest genotoxic response to the CSO effluents. The Ames test indicates the presence of genotoxicants in the effluents and can also register acute toxicity. It is therefore the preferred test to be used with these types of discharges. The long period of incubation (4 days) does mean that the test results are not immediately available, but the test is most suited as a monitoring tool. The genotoxic type tests are more readily applicable to the lower level of toxicity found in these samples. The SOS Chromotest tended to confirm the results of the Ames Fluctuation tests. The SOS Chromotest also responds to the genotoxic effect of the pollutants in the effluents tested. It is an easy and inexpensive test to perform in the laboratory, and may be the most suitable test to be used as a screening tool on CSO samples (Dutka, 1997).

The toxicity reduction performance of the CSO treatment measures (clarification and coagulant addition) was very difficult to quantify using the acute toxicity and genotoxicity tests. There were not enough samples to determine that toxicity reduction occurred on a regular basis. The samples tested did show indications that some toxicity reduction could occur, although some samples appeared to increase in toxicity. Further testing would be required to establish a useful performance database on which to base future designs. First flush samples were generally more toxic than composite samples, although toxicity levels varied considerably between sites and for different events. Such strong variations make absolute comparisons more difficult.

5.2 Chronic Toxicity Test Results

The chronic toxicity tests were far better at detecting toxicity from CSOs, and provided a full range of responses, from non-toxic to acutely toxic. Samples that were toxic for acute tests were also likely to be toxic when tested for chronic toxicity. The results for the fathead minnow and *Ceriodaphnia* chronic toxicity tests are summarized in Tables 4.7 and 4.8. The fathead minnow growth and survival tests failed more often than the

Ceriodaphnia survival and reproduction tests, indicating that it was potentially the most sensitive to the substances causing toxicity in these samples.

First flush samples (Hamilton samples HCSO 8-15, Table 4.8), consistently showed the greatest toxic responses in fathead minnow. These first flush samples were characterized by lower initial dissolved oxygen levels, but were otherwise similar to the composite samples collected elsewhere. The fathead minnow larvae were adversely affected by low dissolved oxygen in the samples (along with higher ammonia levels).

Ceriodaphnia tests usually failed in reproduction rather than survival. Only severely toxic samples resulted in organism death. First flush samples, and those taken under winter conditions (which may contain high contaminant levels, including chloride from road salt), were generally the most toxic.

Both the fathead minnow and *Ceriodaphnia* tests were highly sensitive to variations in CSO effluent toxicity. As such, they would be recommended for use in a CSO monitoring program. Drawbacks to these tests are the amount of time required (7 days), and the volume of sample that must be collected (40L for both tests).

5.3 Relationship Between Toxicity and Chemical Parameters

Classification of selected water chemistry results using the chemical point value (CPV) index facilitated comparison with the toxicity test results. The parameters used (dissolved oxygen, conductivity, BOD, ammonia, Al, Cu, Fe and Pb) represent a suite of the most commonly observed parameters in toxicity testing. The acute and genotoxic test results showed that dissolved oxygen levels, BOD and ammonia were consistently high when samples were toxic. Both fathead minnow and *Ceriodaphnia* were strongly influenced by dissolved oxygen, BOD and ammonia. The lack of complete water chemistry data for each sample prevented the identification of parameter specific associations using multivariate analysis. While metal concentrations did not appear to influence toxicity, the synergistic effects of these complex chemical systems are best addressed with toxicity testing. Measuring individual parameters (e.g. metals concentrations) to monitor such discharges may not provide enough information for a comprehensive assessment.

5.4 Urban Stormwater Runoff

Most of the stormwater runoff samples collected during wet weather discharges in Etobicoke (Table 4.13) were non-toxic. The composite samples were collected from catchbasins in urban residential areas, where low traffic density and lack of commercial activity was a contributing factor in the low toxic responses. Some samples did show low level genotoxic effects, but the severe acute effects were absent. The toxicity observed in the genotoxic tests is likely to be related to some high metal ion concentrations noted for these samples. Although only one chronic toxicity test was performed on these effluents, there was no indication of toxicity to *Ceriodaphnia*. Unfortunately chemistry data for these stormwater sites were very limited, and no comprehensive comparisons could be performed.

5.5 Combined Sewer Overflow Testing

The lack of complete chemical data for samples tested demonstrated the benefits of using toxicity testing to identify potentially damaging discharges. The testing of CSO effluents using a battery of toxicity tests served as an index, which could be used to determine relative differences between the sample effluents. The test conditions were not indicative of the effects of dilution in the receiving waters, and although initial impacts from a CSO discharge may be rather severe, a rapid recovery can be expected in most cases due to mixing and dilution in receiving waters. In some experiments with fathead minnow and *Ceriodaphnia*, positive increases in growth and reproduction occurred as a result of the excess nutrients available in the effluents (a hormesis effect). In toxic samples, the hormesis effect was generally noted after dilution below the toxic threshold, but on non-toxic samples, even the full strength effluent could exhibit this effect. While the first flush appears to contain higher amounts of toxic substances, the majority of the discharge volume appears to be non-toxic. If the first flush could be controlled, ecosystem damage may be reduced.

6.0 CONCLUSIONS

First flush CSO effluent showed a higher degree of toxicity than samples collected later in the overflow event or as a composite, particularly with respect to genotoxicity and chronic toxicity. Very few of the samples tested exhibited acute toxicity. The majority of the overflow volume appears to be non-toxic, therefore remedial measures aimed at reducing the toxic impacts of these discharges should target the first flush.

Genotoxicity and chronic toxicity tests appear to be the most suitable tests to use when attempting to characterize CSO toxicity because of their sensitivity in detection of lower levels of toxicity which are associated with these discharges. These tests should be used only to identify relative differences between sites and to compare discharges from different events. They cannot be used to accurately predict impacts on receiving waters. The wide variation in effluent quality noted during these investigations in Toronto and Hamilton indicates that these discharges are difficult to characterize. The toxicity of the effluents is directly related to the conditions at the time of discharge. These factors include quality and type of wastewater, intensity and duration of rainfall, antecedent dry period, degree of pollutant buildup and capacity of the sewer network.

The impact of CSO discharges on the receiving waters also depends on a number of factors. These factors include total volume of discharge, the size of the receiving water body, degree of circulation/flushing, existing condition of the receiving water body at the time of overflow, impacts of other discharges and spills and the frequency of overflow events.

The recovery of an aquatic ecosystem impacted by combined sewer overflow discharges may be rapid after an overflow event, depending on conditions in the receiving waters. In some cases the addition of nutrients by CSO discharges may temporarily enrich parts of the receiving water and encourage aquatic growth.

ACKNOWLEDGEMENT

The authors gratefully acknowledge financial support of this study by Environment Canada's Great Lakes 2000 Cleanup Fund and collection and chemical analysis of combined sewer overflow samples by Conor Pacific Environmental Technologies Inc. and the Ontario Ministry of Environment and Energy.

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APPENDIX

A

Chemistry Data

Toronto CSO Chemistry Data – Water Chemistry

Toronto CSO Chemistry Data – Total Metals

Hamilton CSO Chemistry Data – Water Chemistry

Hamilton CSO Chemistry Data – Total Metals

Toronto CSO Chemistry Data *Water Chemistry*

Date	Location	TCSO #	DO (mg/L)	pH	Cond (uS/cm)	Hardness (mgCaCo3/L)	TSS (mg/L)	TDS (mg/L)	TOTAL SOLIDS (mg/L)	CBOD-5 (mg/L)	COD (mg/L)	Nitrite (N) (mg/L)	Nitrate + Nitrite (N) (mg/L)	NH3(N) (mg/L)	TKN(N) (mg/L)	PO4(P) (mg/L)	TP(P) (mg/L)	DOC (mg/L)	Dissolved IC (mg/L)	Si (SiO2) (mg/L)
08-Aug-96	Strachan	1																		
26-Aug-96	Strachan	2																		
12-Sep-96	Strachan	3																		
13-Sep-96	Strachan	4	7.30	7.50	302	72														
24-Sep-96	Strachan	5	7.90	7.80	502	120														
24-Sep-96	Strachan*	6																		
27-Sep-96	Strachan	7	8.20	8.30	1043	238														
17-Dec-96	Strachan	8	8.30	7.90	288	82														
08-Jul-97	PI scarb	9	3.90	7.30	384	90				108	352			4.80	13.70		2.84			
08-Jul-97	PE scarb	10	7.00	7.30	343	76				27	62			4.21	7.30		0.92			
08-Jul-97	PI scarb2	11								84	172			4.21	10.40		1.95			
08-Jul-97	PE scarb2	12								28	77			3.81	6.34		0.73			
08-Jul-97	N Tor	13	5.70	7.20	342	80				49	184			4.21	6.34		0.73			
15-Jul-97	PI Scarb	14	3.99	7.06	295					66	240			2.34	10.30		1.78			
15-Jul-97	N Tor	15	2.92	6.97	371					137	370			5.24	15.30		3.13			
12-Aug-97	6th Et	16	5.52	7.47	1633	420				18	69			0.92	2.66		0.88			
12-Aug-97	MacLean	17	4.75	7.15	195	60				125	206			1.15	7.02		2.06			
13-Aug-97	PI Scarb	18								238	264			2.88	8.45		2.33			
13-Aug-97	N Tor	19								103	240			4.11	10.80		2.65			
13-Aug-97	Strachan	20	3.17	7.22	452	120				151	521			2.03	10.60		2.72			
21-Aug-97	6th Et	21												0.29	1.40		0.21			
08-Sep-97	6th Et	22																		
10-Sep-97	6th Et	23												0.83	2.56		0.14			
17-Sep-97	Wcrest Et	24										0.015	0.675	0.21	1.04	0.0365	0.16			
25-Sep-97	6th Et	25																		
25-Sep-97	Wcrest Et	26										0.037	0.455	0.26	1.16	0.079	0.16			
29-Sep-97	Wcrest Et	27		6.94			7	38	46	2.2	5	0.034	1.65	0.15	0.68	0.039	0.08			
27-Oct-97	Strachan	28	4.97	7.07	354	100														
01-Nov-97	Strachan	29									91			0.92	3.47		1.28			
01-Nov-97	Wcrest Et	30																		
01-Nov-97	Humber Cr	31																		
21-Nov-97	N Tor	32	1.62	7.49	803	160				157	324			14.00	22.00		3.70			
21-Nov-97	N Tor effluent	33	1.16	7.43	839	140				151	309			18.00	25.00		3.40			
21-Nov-97	Strachan	34																		

Toronto CSO Chemistry Data ***Total Metals***

Date	Location	TCSO #	Al (ug/L)	Ba (ug/L)	Be (ug/L)	Cd (ug/L)	Co (ug/L)	Cr (ug/L)	Cu (ug/L)	Fe (ug/L)	Li (ug/L)	Mg (ug/L)	Mn (ug/L)	Mo (ug/L)	Ni (ug/L)	Pb (ug/L)	Sr (ug/L)	V (ug/L)	Zn (ug/L)
08-Aug-96	Strachan	1																	
26-Aug-96	Strachan	2																	
12-Sep-96	Strachan	3																	
13-Sep-96	Strachan	4																	
24-Sep-96	Strachan	5																	
24-Sep-96	Strachan*	6																	
27-Sep-96	Strachan	7																	
17-Dec-96	Strachan	8																	
08-Jul-97	PI scarb	9				< 10		25	105	3300						< 44			
08-Jul-97	PE scarb	10				< 10		< 24	< 18	176						< 44			
08-Jul-97	PI scarb2	11				< 10		34	91	2660						< 44			
08-Jul-97	PE scarb2	12				< 10		< 24	< 18	74						< 44			
08-Jul-97	N Tor	13				< 10		< 24	73	2970						< 44			
15-Jul-97	PI Scarb	14				< 10		29	129	4430						53			
15-Jul-97	N Tor	15				< 10		25	190	4090						49			
12-Aug-97	6th Et	16				< 10		13	109	2010						< 44			
12-Aug-97	MacLean	17				< 10		< 24	78	2120						26			
13-Aug-97	PI Scarb	18				< 10		< 24	131	3620						22			
13-Aug-97	N Tor	19				< 10		20	141	2530						34			
13-Aug-97	Strachan	20				< 10		38	214	12700						228			
21-Aug-97	6th Et	21				45.1		487	2090	578						3680			
08-Sep-97	6th Et	22																	
10-Sep-97	6th Et	23				8		1320	2620	187						2150			
17-Sep-97	Wcrest Et	24	1280	24.4	< 0.03	< 0.6	< 1.5	< 1	21.5	528		9.26	36.2	< 0.8	< 1.5	< 11	176	1.96	36.3
25-Sep-97	6th Et	25																	
25-Sep-97	Wcrest Et	26	166	5.64	< 0.03	< 0.6	< 1.5	< 1	7.01	252		1.07	24.1	< 0.8	< 1.5	< 11	24.5	3.36	36.2
29-Sep-97	Wcrest Et	27	75.1	4.38	< 0.03	< 0.6	< 1.5	< 1	7.12	97.8		0.878	14.6	< 0.8	< 1.5	< 11	24.5	1.34	35.2
27-Oct-97	Strachan	28																	
01-Nov-97	Strachan	29				< 10		18	32	2480						30			
01-Nov-97	Wcrest Et	30																	
01-Nov-97	Humber Cr	31																	
21-Nov-97	N Tor	32				< 10		7	136	1091						11			
21-Nov-97	N Tor effluent	33				< 10		4	12	1095						14			
21-Nov-97	Strachan	34																	

Hamilton CSO Chemistry Data *Water Chemistry*

Date	Location (or %CSO)	HCSO #	DO (mg/L)	pH	Cond (uS/cm)	Hardness (mgCaCo3/L)	TSS (mg/L)	TDS (mg/L)	TOTAL SOLIDS (mg/L)	CBOD-5 (mg/L)	COD (mg/L)	Nitrite (N) (mg/L)	Nitrate + Nitrite (N) (mg/L)	NH3(N) (mg/L)	TKN(N) (mg/L)	PO4(P) (mg/L)	TP(P) (mg/L)	DOC (mg/L)	Dissolved IC (mg/L)	Si (SiO2) (mg/L)
01-Dec-96	Royal	1	6.39	7.93	1127		68													
01-Dec-96	Royal	2	6.41	7.93	1099															
01-Dec-96	75	3					326													
01-Dec-96	66	4					262													
01-Dec-96	50	5					516													
01-Dec-96	33	6					833													
01-Dec-96	25	7					774													
04-Feb-97	Queenston	8	6.50	7.40	1152	96														
20-Feb-97	Lawrence	9	5.52	7.76	2330															
03-May-97	Melvin	10	5.73	7.35	355	160														
16-Jun-97	Melvin	11	4.26	7.16	681	200	64	544	490			n/a	n/a	n/a	n/a	6.25	0.52	35	25	1.7
16-Jun-97	Sterling	12	2.96	7.16	332	80	171	370	200			0.01	0.20	3.35	0.54	9.20	1.88	17	36	2.2
20-Jun-97	Lawrence	13	3.15	6.89	274	60	175	332	158			0.03	0.25	1.20	0.30	10.10	2.16	8	23	2.0
02-Jul-97	Lawrence	14					37	1510	1470	11		0.01	0.20	1.85	0.68	5.40	1.26	8	103	8.6
28-Jul-97	Sterling	15	2.75	6.73	160	60	320	436	116	67		0.08	1.05	1.20	0.12	2.30	0.48	11	14	1.2

Hamilton CSO Chemistry Data ***Total Metals***

Date	Location	TCSO #	Al (ug/L)	Ba (ug/L)	Be (ug/L)	Cd (ug/L)	Co (ug/L)	Cr (ug/L)	Cu (ug/L)	Fe (ug/L)	Li (ug/L)	Mg (ug/L)	Mn (ug/L)	Mo (ug/L)	Ni (ug/L)	Pb (ug/L)	Sr (ug/L)	V (ug/L)	Zn (ug/L)
01-Dec-96	Royal	1	1400	51	< 500	< 1	1	4	14	2230	7		114	1	2	12	893	3	59
01-Dec-96	Royal	2	566	44	< 500	< 1	< 1	2	15	846	6		49	1	2	5	900	2	34
01-Dec-96	75	3	n/a	142	< 500	< 1	4	34	156	n/a	13		828	5	19	100	777	32	n/a
01-Dec-96	66	4	3440	149	< 500	3	4	32	131	11500	16		826	6	16	102	766	33	611
01-Dec-96	50	5	4290	189	< 500	4	4	44	176	15400	18		1130	8	22	133	696	46	811
01-Dec-96	33	6	7550	391	710	8	9	92	405	29800	27		2240	15	53	253	694	96	1800
01-Dec-96	25	7	6460	297	610	7	8	78	291	26100	28		1940	12	37	230	614	77	1380
04-Feb-97	Queenston	8																	
20-Feb-97	Lawrence	9																	
03-May-97	Melvin	10																	
16-Jun-97	Melvin	11	450			< 1	7	4	71	998			218	15	< 2	15			225
16-Jun-97	Sterling	12	1200			< 1	< 1	10	98	1640			125	13	< 2	15			175
20-Jun-97	Lawrence	13	3160			< 1	< 1	14	50	4930			316	2	< 2	30			199
02-Jul-97	Lawrence	14	270			< 1	2	3	7	658			2000	2	< 2	< 5			15
28-Jul-97	Sterling	15	3400			< 1	2	29	218	6400			331	11	10	70			383

APPENDIX

B

Raw Data for Acute Toxicity and Genotoxicity Tests

Toronto CSO Acute and Genotoxic Data

Hamilton CSO Acute and Genotoxic Data

Toronto CSO Acute and Genotoic Toxicity Test Results

Date	Location	TCSO #	*Acute Toxicity Tests*					*Genotoxicity Tests*		
			D.Magna	Microtox 1X		Submitochondria particles		Fluctuation test		SOS
			48 hrs. EC100	EC50 %	EC10 %	RET % response	CET % activity	+ or -	Sign.level	Chromotest Induction Factor
08-Aug-96	Strachan	1	0	>100	----	86	95	-		1.06
26-Aug-96	Strachan	2	0	>100	----	76	100	-		1.03
12-Sep-96	Strachan	3	0	>100	----	83	100	-		1.05
13-Sep-96	Strachan	4	0	>100	----	81	75	-		1.04
24-Sep-96	Strachan	5	0	>100	----	77	61	-		1.10
24-Sep-96	Strachan*	6	0	>100	----	76	80	-		1.12
27-Sep-96	Strachan	7	0	>100	----	84	100	-		1.07
17-Dec-96	Strachan	8	0	>100	----	99	72	-		1.05
08-Jul-97	PI scarb	9	0	>100	----	100	100	+	0.05	1.11
08-Jul-97	PE scarb	10	10	>100	----	81	100	-		1.09
08-Jul-97	PI scarb2	11	0	>100	----	79	100	+	0.05	1.12
08-Jul-97	PE scarb2	12	10	>100	----	87	100	-		0.98
08-Jul-97	N Tor	13	0	>100	----	92	100	+	0.1	1.02
15-Jul-97	PI Scarb	14	0	>100	----	88	100	+	0.001	1.12
15-Jul-97	N Tor	15	0	>100	----	93	100	+	0.05	1.07
12-Aug-97	6th Et	16	30	>100	----	99	100	+	0.1	1.07
12-Aug-97	MacLean	17	40	>100	----	93	100	+	0.05	1.05
13-Aug-97	PI Scarb	18	10	>100	----	90	86	+	0.1	1.13
13-Aug-97	N Tor	19	0	>100	----	96	100	-		1.06
13-Aug-97	Strachan	20	0	>100	----	100	100	-		0.95
21-Aug-97	6th Et	21	30	>100	----	100	96	-		1.03
08-Sep-97	6th Et	22	30	>100	----	100	100	-		1.01
10-Sep-97	6th Et	23	0	>100	----	77	100	+	0.05	1.16
17-Sep-97	Wcrest Et	24	0	>100	----	87	100	+	0.05	1.29
25-Sep-97	6th Et	25	0	>100	----	87	75	-		1.34
25-Sep-97	Wcrest Et	26	40	>100	----	91	100	-		1.30
29-Sep-97	Wcrest Et	27	20	>100	----	96	77	+	0.1	1.27
27-Oct-97	Strachan	28	0	>100	----	81	72	-		1.36
01-Nov-97	Strachan	29	0	>100	----	70	100	+	0.1	1.12
01-Nov-97	Wcrest Et	30	0	>100	----	79	68	-		1.07
01-Nov-97	Humber Cr	31	0	>100	----	83	100	+	0.1	1.11
21-Nov-97	N Tor	32	0	12.04	3.62	44	44	+	0.001	1.33
21-Nov-97	N Tor effluent	33	0	>100	----	39	58	+	0.001	1.32
21-Nov-97	Strachan	34	0	>100	----	96	100	+	0.001	1.26

Key to Place Abbreviations: PI - plate clarifier influent, PE plate clarifier effluent,
Wcrest Et Woodcrest Dive City of Etobicoke, 6th Et 6th Street City of Etobicoke

Hamilton CSO Acute and Genotoxic Toxicity Test Results

Date	Location	HCSO #	*Acute Toxicity Tests*					*Genotoxicity Tests*		
			D.Magna 48 hrs. EC100	Microtox 1X		Submitochondria particles		Fluctuation test		SOS Chromotest
				EC50 %	EC10 %	RET % response	CET % activity	+ or -	Sign.level	Induction Factor
01-Dec-96	Royal	1	n/a	>100	----	95	67	-*		1.10
01-Dec-96	Royal	2	n/a	>100	----	100	95	+	0.05	1.11
01-Dec-96	75	3	n/a	>100	----	91	71	+	0.05	1.08
01-Dec-96	66	4	n/a	>100	----	0	100	-*		1.11
01-Dec-96	50	5	n/a	>100	----	71	100	-		1.14
01-Dec-96	33	6	n/a	>100	----	64	100	+	0.01	1.11
01-Dec-96	25	7	n/a	>100	----	65	100	+	0.05	1.05
04-Feb-97	Queenston	8	n/a	>100	----	72	76	n/a		1.06
20-Feb-97	Lawrence	9	n/a	>100	----	86	96	n/a		1.07
03-May-97	Melvin	10	10	>100	----	76	86	n/a		1.00
16-Jun-97	Melvin	11	0	>100	----	51	72	n/a		1.06
16-Jun-97	Sterling	12	0	>100	----	80	100	n/a		1.11
20-Jun-97	Lawrence	13	0	>100	----	88	100	n/a		1.02
02-Jul-97	Lawrence	14	10	>100	----	82	100	n/a		0.99
28-Jul-97	Sterling	15	n/a	>100	----	80	100	-		1.03

n/a - test was not applied in this case, * - samples demonstrated a dose response and if concentrated may be positive

APPENDIX

C

Raw Data for Fathead Minnow Chronic Toxicity Tests

Test No: TCSO-4

Pollutech 1996b

Survival

	Dilutions					
	C	6.25	12.5	25	50	100
Rep. 1	10	10	6	10	9	7
Rep. 2	7	9	10	10	7	9
Rep. 3	10	10	8	9	8	9
Mean	9.00	9.67	8.00	9.67	8.00	8.33
(S.D.)	1.73	0.58	2.00	0.58	1.00	1.15

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.49	0.44	0.52	0.52	0.50	0.40
Rep. 2	0.62	0.40	0.53	0.58	0.54	0.39
Rep. 3	0.28	0.45	0.47	0.55	0.56	0.35
Mean	0.46	0.43	0.51	0.55	0.54	0.38
(S.D.)	0.17	0.03	0.03	0.03	0.03	0.03

Test No: TCSO-5

Pollutech 1996b

Survival

	Dilutions					
	C	6.25	12.5	25	50	100
Rep. 1	10	10	10	10	8	9
Rep. 2	10	10	10	9	10	9
Rep. 3	7	8	10	10	8	10
Mean	9.00	9.33	10.00	9.67	8.67	9.33
(S.D.)	1.73	1.15	0.00	0.58	1.15	0.58

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.57	0.67	0.66	0.62	0.62	0.49
Rep. 2	0.66	0.67	0.79	0.72	0.53	0.53
Rep. 3	0.65	0.68	0.74	0.58	0.57	0.59
Mean	0.63	0.67	0.73	0.64	0.57	0.54
(S.D.)	0.05	0.01	0.06	0.08	0.04	0.05

Test No: TCSO-7

Pollutech 1996b

Survival

	Dilutions					
	C	6.25	12.5	25	50	100
Rep. 1	8	10	8	10	10	8
Rep. 2	10	10	9	9	8	10
Rep. 3	10	9	8	10	8	10
Mean	9.33	9.67	8.33	9.67	8.67	9.33
(S.D.)	1.15	0.58	0.58	0.58	1.15	1.15

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.87	0.87	0.81	1.00	0.77	0.78
Rep. 2	0.69	0.92	0.73	0.61	0.80	0.93
Rep. 3	0.76	0.86	0.69	0.86	0.80	0.71
Mean	0.77	0.88	0.74	0.82	0.79	0.81
(S.D.)	0.09	0.03	0.06	0.20	0.02	0.11

Test No: TCSO-8

Pollutech 1996c

Survival

	Dilutions					
	C	6.25	12.5	25	50	100
Rep. 1	10	10	10	10	7	6
Rep. 2	10	10	8	10	8	10
Rep. 3	10	10	9	8	8	10
Mean	10.00	10.00	9.00	9.33	7.67	8.67
(S.D.)	0.00	0.00	1.00	1.15	0.58	2.31

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.85	0.84	0.70	0.76	0.76	0.83
Rep. 2	0.88	0.80	0.69	0.91	0.76	0.76
Rep. 3	0.90	0.97	0.81	0.87	0.87	0.73
Mean	0.88	0.87	0.73	0.84	0.80	0.77
(S.D.)	0.03	0.09	0.07	0.08	0.07	0.05

Test No: TCSO-9

Pollutech 1997a

Survival

	Dilutions					
	C	6.25	12.5	25	50	100
Rep. 1	10	10	9	10	9	0
Rep. 2	9	10	10	10	9	0
Rep. 3	10	10	10	10	7	0
Mean	9.67	10.00	9.67	10.00	8.33	0.00
(S.D.)	0.58	0.00	0.58	0.00	1.15	0.00

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.73	0.64	0.63	0.62	0.42	
Rep. 2	0.63	0.68	0.74	0.66	0.40	
Rep. 3	0.72	0.72	0.63	0.64	0.43	
Mean	0.69	0.68	0.67	0.64	0.42	
(S.D.)	0.05	0.04	0.06	0.02	0.02	

Test No: TCSO-10

Pollutech 1997a

Survival

	Dilutions					
	C	6.25	12.5	25	50	100
Rep. 1	10	10	10	9	10	5
Rep. 2	10	10	10	10	10	9
Rep. 3	10	10	10	10	10	9
Mean	10.00	10.00	10.00	9.67	10.00	7.67
(S.D.)	0.00	0.00	0.00	0.58	0.00	2.31

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.59	0.72	0.67	0.63	0.64	0.22
Rep. 2	0.68	0.57	0.73	0.60	0.68	0.43
Rep. 3	0.80	0.75	0.62	0.63	0.61	0.44
Mean	0.69	0.68	0.68	0.62	0.64	0.36
(S.D.)	0.10	0.10	0.05	0.01	0.03	0.12

Test No: TCSO-13

Pollutech 1997a

Survival

	Dilutions					
	C	6.25	12.5	25	50	100
Rep. 1	10	10	10	10	10	6
Rep. 2	10	10	10	10	10	8
Rep. 3	10	10	10	10	10	5
Mean	10.00	10.00	10.00	10.00	10.00	6.33
(S.D.)	0.00	0.00	0.00	0.00	0.00	1.53

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.57	0.68	0.68	0.65	0.55	0.26
Rep. 2	0.61	0.65	0.65	0.64	0.52	0.31
Rep. 3	0.54	0.61	0.67	0.60	0.51	0.23
Mean	0.57	0.65	0.67	0.63	0.53	0.27
(S.D.)	0.03	0.03	0.02	0.02	0.02	0.04

Test No: TCSO-14

Survival

Dilutions	C	6.25	12.5	25	50	100
Rep. 1	10	10	9	10	10	2
Rep. 2	10	10	10	9	10	4
Rep. 3	10	10	10	10	9	6
Rep. 4	10	10	10	10	9	6
Mean	10.00	10.00	9.75	9.75	9.50	4.50
(S.D.)	0.00	0.00	0.50	0.50	0.58	1.91

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.24	0.22	0.24	0.31	0.29	0.15
Rep. 2	0.21	0.27	0.25	0.27	0.26	0.13
Rep. 3	0.23	0.23	0.28	0.27	0.25	0.18
Rep. 4	0.24	0.24	0.25	0.28	0.30	0.15
Mean	0.23	0.24	0.26	0.28	0.27	0.15
(S.D.)	0.01	0.02	0.02	0.02	0.02	0.02

Test No: TCSO-15

Survival

Dilutions	C	6.25	12.5	25	50	100
Rep. 1	10					7
Rep. 2	10					8
Rep. 3	10					4
Rep. 4	10					4
Mean	10.00					5.75
(S.D.)	0.00					2.06

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.24					0.16
Rep. 2	0.21					0.11
Rep. 3	0.23					0.15
Rep. 4	0.24					0.15
Mean	0.23					0.14
(S.D.)	0.01					0.02

Test No: TCSO-16

Survival

Dilutions	C	6.25	12.5	25	50	100
Rep. 1	10					10
Rep. 2	9					9
Rep. 3	4					10
Rep. 4	3					9
Mean	6.50					9.50
(S.D.)	3.51					0.58

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.42					0.36
Rep. 2	0.41					0.36
Rep. 3	0.38					0.37
Rep. 4	0.33					0.38
Mean	0.38					0.37
(S.D.)	0.04					0.01

Test No: TCSO-28

Survival

	C	6.25	12.5	25	50	100
Rep. 1	10	10	10	9	10	
Rep. 2	10	9	9	9	9	
Rep. 3	10	10	9	9	8	
Rep. 4	9	9	10	9	9	
Mean	9.75	9.50	9.50	9.00	9.00	
(S.D.)	0.50	0.58	0.58	0.00	0.82	

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.37	0.59	0.61	0.68	0.43	
Rep. 2	0.50	0.59	0.60	0.61	0.48	
Rep. 3	0.47	0.60	0.62	0.60	0.48	
Rep. 4	0.43	0.59	0.62	0.58	0.47	
Mean	0.44	0.59	0.61	0.62	0.46	
(S.D.)	0.06	0.01	0.01	0.04	0.02	

Test No: TCSO-32

Survival

	C1	1.06	3.13	C2	6.25	12.5
Rep. 1	10	10	8	8	8	
Rep. 2	7	9	8	9	8	
Rep. 3	9	9	8	8	9	
Rep. 4	10	10	8	9	5	
Mean	9.00	9.50	8.00	8.50	7.50	
(S.D.)	1.41	0.58	0.00	0.58	1.73	

Growth

	C1	1.06	3.13	C2	6.25	12.5
Rep. 1	0.59	0.48	0.59	0.43	0.55	
Rep. 2	0.69	0.57	0.64	0.53	0.71	
Rep. 3	0.62	0.64	0.55	0.53	0.58	
Rep. 4	0.54	0.60	0.63	0.59	0.66	
Mean	0.61	0.57	0.60	0.52	0.63	
(S.D.)	0.06	0.07	0.04	0.07	0.07	

Test No: TCSO-33

Survival

	C1	1.06	3.13	C2	6.25	12.5
Rep. 1	10	9	8	8	6	
Rep. 2	7	9	9	9	10	
Rep. 3	9	7	8	8	9	
Rep. 4	10	10	9	9	9	
Mean	9.00	8.75	8.50	8.50	8.50	
(S.D.)	1.41	1.26	0.58	0.58	1.73	

Growth

	C1	1.06	3.13	C2	6.25	12.5
Rep. 1	0.59	0.72	0.63	0.43	0.70	
Rep. 2	0.69	0.66	0.61	0.53	0.60	
Rep. 3	0.62	0.74	0.64	0.53	0.63	
Rep. 4	0.54	0.71	0.62	0.59	0.70	
Mean	0.61	0.71	0.62	0.52	0.66	
(S.D.)	0.06	0.04	0.01	0.07	0.05	

Hamilton CSO Fathead Minnow Raw Data

Test No: HCSO-9

Survival

	Dilutions C	6.25	12.5	25	50	100
Rep. 1	10	9	8	10	10	10
Rep. 2	9	10	10	10	10	9
Rep. 3	9	8	8	9	7	10
Rep. 4	9	10	10	10	10	7
Mean	9.25	9.25	9.00	9.75	9.25	9.00
(S.D.)	0.50	0.96	1.15	0.50	1.50	1.41

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.29	0.28	0.33	0.31	0.35	0.30
Rep. 2	0.26	0.37	0.33	0.40	0.33	0.28
Rep. 3	0.29	0.25	0.34	0.30	0.31	0.26
Rep. 4	0.26	0.28	0.31	0.36	0.29	0.31
Mean	0.27	0.29	0.33	0.34	0.32	0.29
(S.D.)	0.02	0.05	0.01	0.05	0.03	0.02

Test No: HCSO-10

Survival

	Dilutions C	6.25	12.5	25	50	100
Rep. 1	9					10
Rep. 2	9					8
Rep. 3	10					10
Rep. 4	10					9
Mean	9.50					9.25
(S.D.)	0.58					0.96

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.49					0.33
Rep. 2	0.40					0.30
Rep. 3	0.38					0.31
Rep. 4	0.48					0.36
Mean	0.44					0.32
(S.D.)	0.06					0.02

Test No: HCSO-11

Survival

	Dilutions C	6.25	12.5	25	50	100
Rep. 1	9	10	10	10	10	
Rep. 2	8	10	10	10	8	
Rep. 3	10	10	10	10	8	
Rep. 4	10	10	10	10	9	
Mean	9.25	10.00	10.00	10.00	8.75	
(S.D.)	0.96	0.00	0.00	0.00	0.96	

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.42	0.50	0.52	0.45	0.45	
Rep. 2	0.49	0.49	0.42	0.47	0.43	
Rep. 3	0.44	0.47	0.47	0.47	0.49	
Rep. 4	0.47	0.47	0.49	0.45	0.43	
Mean	0.45	0.48	0.48	0.46	0.45	
(S.D.)	0.03	0.02	0.04	0.01	0.03	

Test No: HCSO-12

Survival

	C1	1.0625	3.125	C2	6.25	12.5	25
Rep. 1	9	9	10	10	9	9	9
Rep. 2	8	10	9	10	10	10	10
Rep. 3	10	10	10	10	10	9	8
Rep. 4	10	9	10	10	10	10	9
Mean	9.25	9.50	9.75	10.00	9.75	9.50	9.00
(S.D.)	0.96	0.58	0.50	0.00	0.50	0.58	0.82

Growth

	C1	1.0625	3.125	C2	6.25	12.5	25
Rep. 1	0.42	0.50	0.54	0.44	0.50	0.41	0.37
Rep. 2	0.49	0.49	0.40	0.50	0.43	0.44	0.35
Rep. 3	0.44	0.45	0.50	0.53	0.44	0.42	0.34
Rep. 4	0.47	0.48	0.52	0.53	0.50	0.42	0.32
Mean	0.45	0.48	0.49	0.50	0.47	0.42	0.34
(S.D.)	0.03	0.02	0.06	0.04	0.04	0.01	0.02

Test No: HCSO-13

Survival

	C	6.25	12.5	25	50	100
Rep. 1	10	9	10			
Rep. 2	8	9	8			
Rep. 3	10	9	10			
Rep. 4	9	9	10			
Mean	9.25	9.00	9.50			
(S.D.)	0.96	0.00	1.00			

Growth

	C	6.25	12.5	25	6.25	12.5
Rep. 1	0.49	0.50	0.42	0.43		
Rep. 2	0.49	0.44	0.48	0.53		
Rep. 3	0.47	0.48	0.42	0.53		
Rep. 4	0.43	0.39	0.46	0.59		
Mean	0.47	0.45	0.44	0.52		
(S.D.)	0.03	0.05	0.03	0.07		

Test No: HCSO-15

Survival

	C	6.25	12.5	25	50	100
Rep. 1	10	10	8	8		
Rep. 2	10	10	9	6		
Rep. 3	10	9	9	9		
Rep. 4	10	8	9	7		
Mean	10.00	9.25	8.75	7.50		
(S.D.)	0.00	0.96	0.50	1.29		

Growth

	C	6.25	12.5	25	50	100
Rep. 1	0.28	0.36	0.41	0.36		
Rep. 2	0.32	0.31	0.38	0.43		
Rep. 3	0.30	0.33	0.33	0.31		
Rep. 4	0.33	0.39	0.36	0.40		
Mean	0.31	0.35	0.37	0.38		
(S.D.)	0.02	0.03	0.03	0.05		

APPENDIX

D

Raw Data for *Ceriodaphnia dubia* Chronic Toxicity Tests

TCSO-1 Pollutech 1996a
Aug 8 1996 Strachan Ave.

	C	100	50	25	12.5	6.25
	25	32	25	28	26	26
	23	30	35	32	24	25
	25	7	38	29	25	26
	24	31	34	35	27	25
	30	26	31	28	23	22
	27	30	33	27	25	23
	25	30	30	32	8	22
	25	32	33	28	24	22
	23	31	33	32	26	22
Mean	**25.2**	**27.7**	**32.4**	**30.1**	**23.1**	**23.7**

TCSO-4 Pollutech 1996b
Sep 13 1996 Strachan Ave.

	C	100	50	25	12.5	6.25
	18	26	28	24	20	20
	20	24	25	31	26	23
	0	24	22	22	0	17
	23	26	26	28	23	21
	20	21	28	25	26	19
	20	31	26	29	20	26
	20	21	24	27	26	19
	18	28	29	28	25	21
	20	27	25	25	29	22
	18	27	25	27	20	19
Mean	**17.7**	**25.5**	**25.8**	**26.6**	**21.5**	**20.7**

TCSO-5 Pollutech 1996b
Sep 24 1996 Strachan Ave.

	C	100	50	25	12.5	6.25
	18	29	25	23	23	22
	20	25	22	22	23	23
	16	24	26	27	0	16
	18	24	26	28	23	23
	17	22	23	23	25	23
	10	25	23	20	21	21
	18	25	24	25	22	18
	18	23	22	23	26	17
	17	27	20	26	22	22
	22	3	23	25	23	24
Mean	**17.4**	**22.7**	**23.4**	**24.2**	**20.8**	**20.9**

TCSO-7 Pollutech 1996b
Sep 27 1996 Strachan Ave.

	C	100	50	25	12.5	6.25
	13	27	27	13	15	11
	7	27	13	11	13	12
	19	31	31	24	23	24
	23	28	26	29	24	27
	18	29	26	31	26	25
	21	17	30	25	27	25
	27	23	30	28	29	23
	29	23	25	30	25	26
Mean	**19.6**	**25.6**	**26.0**	**23.9**	**22.8**	**21.6**

TCSO-8 Pollutech 1997a
Dec 17 1996 Strachan Ave.

	C	100	50	25	12.5	6.25
	26	27	24	24	23	23
	20	26	25	21	23	20
	21	23	26	25	25	24
	23	27	26	20	25	26
	25	24	23	25	25	19
	21	27	24	22	24	24
	24	23	22	22	22	23
	23	22	23	20	19	23
	19	26	22	23	24	22
	23	23	24	23	26	24
Mean	**22.5**	**24.8**	**23.9**	**22.5**	**23.6**	**22.8**

TCSO-9
July 8 1997 Scarborough Plate Influent

	C	100	50	25	12.5	6.25
	26	10	25	28	32	31
	25	4	24	38	30	33
	27	11	22	32	39	37
	30	7	6	17	36	33
	16	0	27	38	40	31
	18	12	13		29	31
	28		23		29	31
	17		19		30	30
	22		31			25
	8					
Mean	**21.7**	**7.3**	**21.1**	**30.6**	**33.1**	**31.3**

TCSO-10
July 8 1997 Scarborough Plate Effluent

	C	100	50	25	12.5	6.25
	26	23	23	30	29	27
	25	16	22	30	29	33
	27	9	24	27	31	26
	30	23	18	12	26	28
	16	14	30	29	28	32
	18	17	25	28	28	37
	28	5	22	27		
	17		14	29		
	22		22			
	8					
Mean	**21.7**	**15.3**	**22.2**	**26.5**	**28.5**	**30.5**

TCSO-11
July 8 1997 Scarborough Plate Influent

	C	100	50	25	12.5	6.25
	22		38	30	33	29
	17		20	25	23	37
	16		38	33	36	31
	12		19	48	34	29
	26		24	38	31	28
	25		33	42	36	18
	15		30	32	34	39
	29		35	34	35	19
	19		29	34		27
	24		40	31		
Mean	**20.5**		**30.6**	**34.7**	**32.8**	**28.6**

TCSO-12
July 8 1997 — Scarborough Plate Effluent

	C	100	50	25	12.5	6.25
	22	13	18	21	18	12
	17	25	18	18	22	10
	16	18	19	20	23	16
	12	19	21	20	22	11
	26	24	22	29	26	9
	25	4	14	24	21	19
	15	16	19	22	14	20
	29	5	14	21	20	21
	19	13	22	25	15	13
	24	19	17		19	12
Mean	**20.5**	**16.6**	**18.4**	**22.2**	**20.0**	**14.3**

TCSO-13
July 8 1997 — North Toronto Influent

	C	100	50	25	12.5	6.25
	31	11	21	34	25	26
	22	4	25	33	40	38
	28	4	27	27	33	33
	29	6	28	29	35	34
	25	3	24	31	33	33
	17	13	31	36	37	31
	26	4	32	37	33	32
	21		16	30	28	16
	19		32	32	34	27
					33	26
Mean	**24.2**	**6.4**	**26.2**	**32.1**	**33.1**	**29.6**

TCSO-14
July 15 1997 — Scarborough Influent

	C	100	50	25	12.5	6.25
	22	20	21	32	30	17
	23	21	31	32	30	19
	17	18	32	16	18	33
	23	20	38	23	31	30
	26	27	30	37	37	33
	21	24	33	27	40	29
	14	23	34	27	18	31
	19	29		23		28
	19	35				23
		29				26
Mean	**20.4**	**24.6**	**31.3**	**27.1**	**29.1**	**26.9**

TCSO-15
July 15 1997 — North Toronto Influent

	C	100	50	25	12.5	6.25
	25	14	32	30	32	25
	25	13	26	33	34	25
	16	15	33	28	31	16
	31	13	28	32	34	31
	30	4	38	30	39	30
	24		26	21	10	24
	31		32	27	10	31
	29		29	34	39	29
	28		19	32	33	28
	25			26	34	25
Mean	**26.4**	**11.8**	**29.2**	**29.3**	**29.6**	**26.4**

TCSO-16
Aug 12 - 97 — 6th St. Etobicoke

	C	100	50	25	12.5	6.25
	19	22	27	33	38	32
	16	30	32	29	39	30
	23	36	36	27	38	35
	18	24	37	32	29	30
	15	36	34	39	34	29
	25	31	35	35	38	32
	17	26	43	36	27	19
	16	27	35	31	34	32
		31	34	27	25	31
				40	33	27
Mean	**18.6**	**29.2**	**34.8**	**32.9**	**33.5**	**29.7**

TCSO-17
Aug 12 - 97 — MacLean Ave. Toronto

	C	100	50	25	12.5	6.25
	19	27	42	41	32	39
	16	36	43	38	35	34
	23	31	42	39	24	31
	18	11	19	43	41	37
	15	34	28	37	38	39
	25	31	38	32	38	34
	17	18	17	18	37	35
	16		18	42	35	35
			42	35	38	32
				36	37	
Mean	**18.6**	**26.9**	**32.1**	**36.1**	**35.5**	**35.1**

TCSO-18
Aug 12 - 97 — Massey Creek - Scarborough

	C	100
	27	27
	24	26
	28	20
	20	18
	23	23
	27	19
	24	34
	22	16
	25	
	18	
Mean	**23.8**	**22.9**

TCSO-19
Aug 12 - 97 — North Toronto

	C	100
	27	17
	24	30
	28	27
	20	19
	23	18
	27	25
	24	27
	22	
	25	
	18	
Mean	**23.8**	**23.3**

TCSO-20
Aug 13 - 97 — Strachan Ave - Western Beaches

	C	100
	19	32
	16	39
	23	27
	18	33
	15	35
	25	28
	17	15
	16	24
		28
Mean	**18.6**	**29.0**

TCSO-22
Sept 8 - 97 — 6th St. Etobicoke

	C	100	50	25	12.5	6.25
	16	29	40	35	35	33
	23	29	36	43	34	30
	28	29	32	35	41	32
	25	23	39	37	38	33
	26	30	46	37	36	34
	15	22	39	40	35	30
	24	28	24	29	39	29
	7	23	32	39	37	38
	23		35	34	34	33
	22			38	19	
Mean	**20.9**	**26.6**	**35.9**	**36.7**	**34.8**	**32.4**

TCSO-23
Sept 10 - 97 — 6th St. Etobicoke

	C	100	50	25	12.5	6.25
	13	19	22	19	19	20
	4	23	22	20	17	18
	9	19	44	19	23	23
	5	21	25	21	21	14
	12	18	19	24	28	14
	16	19	14	20	21	19
	19	16	16	18	18	18
	14	43	22	21	33	19
	12	35	16	44	10	19
	11		24	15	16	12
Mean	**11.5**	**23.7**	**22.4**	**22.1**	**20.6**	**17.6**

TCSO 28
Oct 27 - 97 — Cerio/FHM — Strrachan Ave. W. Beaches, Toronto

	C	100	50	25	12.5	6.25
	19	19	26	34	28	28
	12	23	38	35	31	23
	14	18	35	35	37	32
	13	29	38	33	31	32
	11	22	37	31	37	10
	12	26	35	29	43	28
	20	19	32	32	33	31
	13	29	42	44	29	24
	10	30	34	35	32	16
			34	33		
Mean	**13.8**	**23.9**	**35.1**	**34.1**	**33.4**	**24.9**

TCSO 29
Nov 1 - 97 — Strachan Ave. W. Beaches, Toronto

	C	100	50	25	12.5
	14	17	17	42	20
	16	16	34	36	38
	13	12	37	38	41
	19	11	19	39	38
	22	18	44	43	20
	18	19	21	16	31
	13	21	49	39	13
	21		47	33	32
	25		41	40	41
	19			36	22
Mean	**18.0**	**16.3**	**34.3**	**36.2**	**29.6**

TCSO 32
Nov 21 - 97 — Cerio/FHM — Influent North Toronto

	C	100	50	25	12.5	6.25
	19	2	14	18	24	21
	12	3	11	18	29	31
	18	6	7	21	32	26
	22	3	11	24	29	30
	23	4	14	28	28	30
	24	3	10	22	24	31
	23	0	13	24	29	29
	21	0	9	21	29	38
	13	0		16	28	32
	19				30	28
Mean	**19.4**	**2.3**	**11.1**	**21.3**	**28.2**	**29.6**

TCSO 33
Nov 21 - 97 — Effluent from Clarifier North Toronto

	C	100	50	25	12.5	6.25
	19	0	12	27	35	14
	12	0	12	11	38	19
	18	0	8	29	34	35
	22		10	14	33	30
	23		6	11	21	20
	24		14	11	26	25
	23		8	25	22	24
	21		10	24	38	40
	13		4	10	23	30
	19				25	
Mean	**19.4**	**0.0**	**9.3**	**18.0**	**29.5**	**26.3**

TCSO 34
Nov 21 - 97 — Strrachan Ave. W. Beaches, Toronto

	C	100	50	25	12.5
	15	21	27	26	22
	16	2	28	31	32
	12	24	27	31	17
	11	27	27	32	15
	12	27	29	19	16
	19	14	10	15	18
	15	14	13	18	20
	20	15	32	32	32
	22	18	12	25	30
	23		20	31	26
Mean	**16.5**	**18.0**	**22.5**	**26.0**	**22.8**

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