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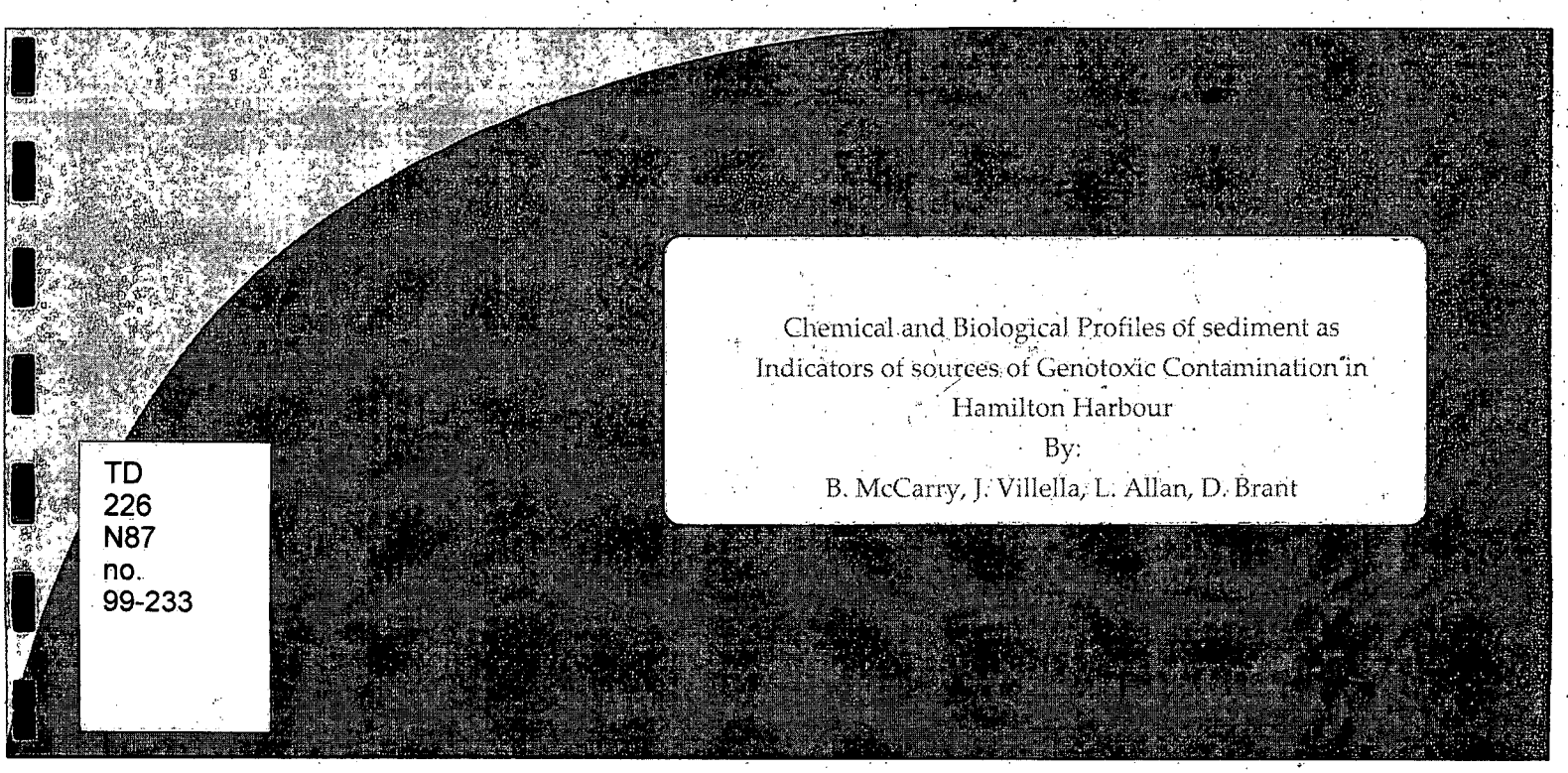

Chemical and Biological Profiles of sediment as Indicators of sources of Genotoxic Contamination in Hamilton Harbour

By:

B. McCarry, J. Villella, L. Allan, D. Brant

99-233

MANAGEMENT PERSPECTIVE

- **Title:** Chemical and Biological Profiles of Sediment as Indicators of Sources of Genotoxic Contamination in Hamilton Harbour, ~~Part I: Analysis of Polycyclic Aromatic Hydrocarbons and Thia-Arene Compounds, and:~~ Part II: Bioassay-Directed Fractionation Employing the Ames *Salmonella*/Microsome Assay.

- **Authors: C.H. Marvin**, AERB, NWRI; B.E. McCarry, J. Villella, L.M. Allan and D.W. Bryant, Departments of Chemistry and Biochemistry, McMaster University.

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- **Citation:**

- EC Priority/Issue: Great Lakes 2000 Program, Hamilton Harbour RAP. This work supports the EC priority of implementing ecosystem initiatives focused on ecosystems of national priority including AOCs in the Great Lakes and Atlantic Coastal Areas. Business Line = Nature, Outcome = Conservation of biodiversity in healthy ecosystems, Result = Priority ecosystems are conserved and restored, Sub-result = Great Lakes AOCs restored under GL2000.

- Current Status: This work is an investigation of chemical and bioassay response profiles as tracers of sources of contamination in Hamilton Harbour and is presented as a series of two papers. The findings in both phases of the study are complementary in that the presumed presence of two primary sources of organic contaminants to the harbour are substantiated; contamination originating from areas of historically contaminated sediments, and contamination originating in the watershed and entering the harbour through a tributary. This conclusion is based on samples from Redhill Creek and Windermere Arm exhibiting chemical and bioassay profiles characteristic of mobile combustion emissions while samples from the open harbour exhibited profiles suggesting the influence of both mobile and industrial emissions.

- Next Steps: Publish in a scientific journal and communicate to RAP processes. Any continuation of this work is contingent upon funding.

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CHEMOSPHERE

Chemical and biological profiles of sediments as indicators of sources of contamination in Hamilton Harbour. Part II: Bioassay-directed fractionation using the Ames *Salmonella*/microsome assay

Christopher H. Marvin [a,b,*], Brian E. McCarry [a], John Villella [a], Laurie M. Allan [a], Douglas W. Bryant [c]

[a] *Department of Chemistry, McMaster University, Hamilton, Ont., Canada L8S 4M1*

[b] *Aquatic Ecosystem Restoration Branch, National Water Research Institute, Environment Canada, P.O. Box 5050, 867 Lakeshore Road, Burlington, Ont., Canada L7R 4A6*

[c] *Department of Biochemistry, McMaster University, Hamilton, Ont., Canada L8S 3Z5*

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Abstract

Bottom sediment and suspended sediment samples from Hamilton Harbour (western Lake Ontario) and from a major tributary were profiled using a bioassay-directed fractionation approach. Sample extracts were fractionated using an alumina/Sephadex gel clean-up procedure to afford non-polar aromatic fractions which were characterized using chemical analyses and the Ames/microsome bacterial assay in *Salmonella typhimurium* strains YG1025 with the addition of oxidative metabolism (S9), and YG1024 without S9. Non-polar aromatic fractions of selected samples were separated by normal phase HPLC into 1-min fractions which were subjected to bioassay analyses. The bioassays using strain YG1025+S9, a TA100-type strain, were performed to assess genotoxicity arising from the presence of polycyclic aromatic hydrocarbons (PAH). Fractions which exhibited mutagenic activity contained PAH with molecular masses of 252, 276 and 278 amu; these fractions contained over 80% of the genotoxicity attributable to PAH. Individual compounds identified using Gas Chromatography–Mass Spectrometry analyses in these active fractions included benzo[a]pyrene, indeno[cd]pyrene and dibenz[a,h]anthracene. The YG1025+S9 mutagenic activity profiles were similar for all samples. Mutagenic activity profiles generated using strain YG1024–S9, a TA98-type strain sensitive to compounds characteristic of mobile source emissions, were very different. The mutagenic activities in strain YG1024–S9 were greatest for harbour-suspended sediment samples collected from sites impacted by a major tributary. Suspended sediments collected near areas known to contain high levels of coal tar-contamination in the bottom sediments contained higher levels of genotoxic PAH than suspended sediments collected from other areas of the harbour.

Keywords: Bioassay-directed fractionation; Polycyclic aromatic hydrocarbons; *Salmonella typhimurium*; Suspended sediments; Coal tar

1. Introduction

Hamilton Harbour is an embayment of western Lake Ontario with an approximate surface area of 40 km^2

* Corresponding author. Tel.: +1-905-319-6919; fax: +1-905-336-6430.

E-mail address: chris.marvin@cciw.ca (C.H. Marvin).

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(Fig. 1). The harbour is a receiving body for a watershed of approximately 900 km^2 that includes the cities of Hamilton, Ancaster, Dundas and Burlington with a combined population of approximately 500,000 people. The harbour has been designated as an Area of Concern by the Water Quality Board of the International Joint Commission. Major sources of contaminant influx into the harbour include industrial effluents, roadway runoff and treated municipal sewage. Several species of fish in the harbour including white suckers (*Catostomus commersoni*) and brown bullhead (*Ictalurus nebulosus*) have exhibited increased occurrences of skin and liver neoplasms (Hayes et al., 1990) and two- to threefold increases in frequency of epidermal papillomas (Smith and Ferguson, 1985) compared to fish from other areas of Lake Ontario. Metcalfe et al. (1988, 1990) found organic solvent extracts of sediments from Hamilton Harbour-induced hepatocellular carcinomas in trout using a sac fry microinjection assay. Some areas of the harbour are characterized by sediments that are grossly contaminated by coal tar. Resuspension and transport of material from these areas is commonly thought to be a source of polycyclic aromatic hydrocarbons (PAH) in harbour-wide sediments (Harlow and Hodson, 1988; Murphy et al., 1990).

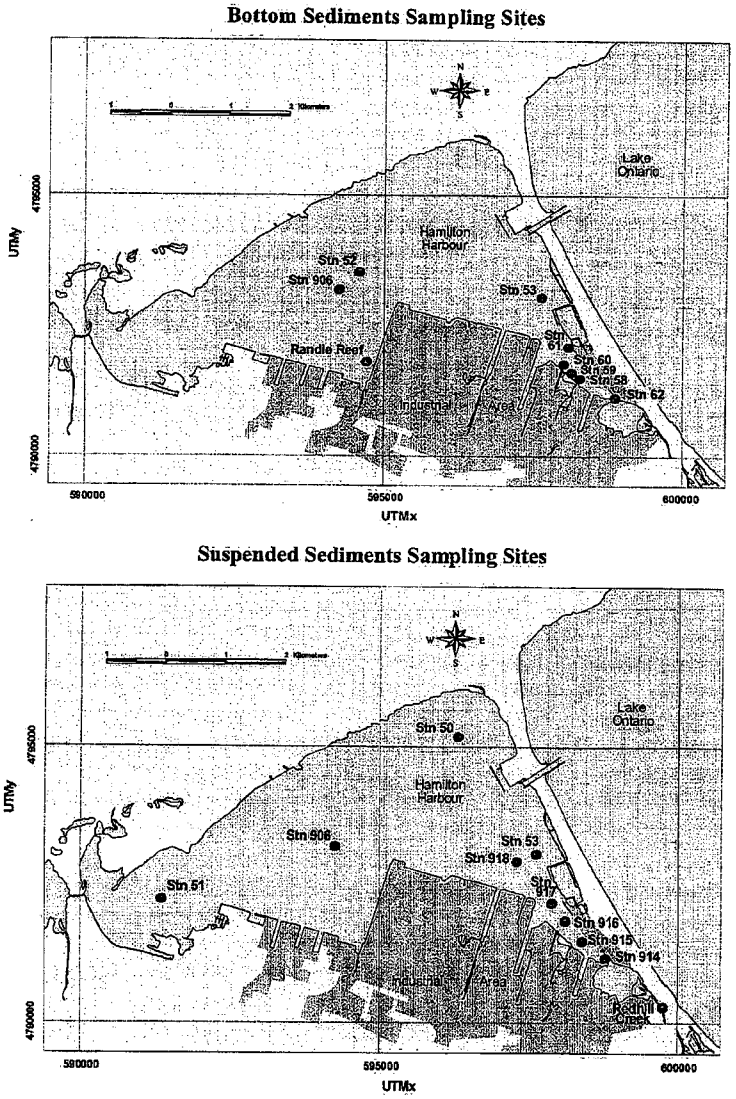

Fig. 1. Maps of Hamilton Harbour showing bottom sediment and suspended sediment sampling sites.

Epidemiological studies have associated PAH exposure with hepatic neoplasms and related lesions in English sole from Puget Sound (Myers et al., 1991; Stein et al., 1990). Work by Balch et al. (1995) has indicated that PAH in contaminated Hamilton Harbour sediments are potential fish carcinogens. Although PAH have been implicated as causal agents, most studies do not attempt to identify individual compounds as primary contributors to observed impairments. Balch et al. (1995) also indicated that designated priority pollutant PAH may not be responsible for all the observed prevalences of fish tumours. We previously reported the results of chemico/biological investigations of coal tar-contaminated sediments from the Randle Reef area of Hamilton Harbour (Marvin et al., 1993) and from Sydney Harbour, Nova Scotia (Marvin et al., 1994). This work was extended using a bioassay-directed fractionation methodology employing the Ames *Salmonella typhimurium*/microsome assay to identify those compounds responsible for the mutagenic activity (Marvin et al., 1995). Extracts were fractionated into compound classes using an alumina/Sephadex LH20 clean-up procedure coupled with normal phase-high performance liquid chromatography (NP-HPLC). The majority of the mutagenic activity was associated with PAH-containing fractions which displayed high responses in *Salmonella* TA100-type strains with the addition of oxidative metabolism (S9). Separation of the PAH-containing fraction using reversed phase (RP)-HPLC showed the majority of the biological activity coeluted with PAH of molecular masses 252, 276, 278, 302 and 326 amu. Analyses using Gas Chromatography–Mass Spectrometry identified benzo[a]pyrene, the benzofluoranthenes, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene and dibenz[a,h]anthracene as primary mutagens in the sample extracts.

In the present study, we have focussed on Hamilton Harbour and expanded the bioassay-directed investigation to include bottom sediments and suspended sediments from strategic areas of the harbour. The bioassay profiles were compared as a means of identifying probable sources of genotoxic contamination including areas of coal tar-contaminated sediment, tributaries and sewage treatment plants. Sites were sampled in Hamilton Harbour, Windermere Arm and Redhill Creek (Fig. 1). Sample extracts were analyzed using normal phase HPLC and 1-min fractions were bioassayed to produce mutagenic activity profiles in two strains of *S. typhimurium*. Fractions exhibiting mutagenic activity were analyzed by GC–MS. The previous paper in this volume described the analytical chemical characterization of these samples while this paper is focussed on the characterization of the genotoxicity of these samples.

2. Experimental

Samples were collected with the assistance of Murray Charlton of the National Water Research Institute, Environment Canada, Canada Centre for Inland Waters, Burlington, Ontario. Sediment traps were deployed over an 8-month period (October–May) resulting in 5–10 g dry weight accumulations of material per trap tube. Sediment traps were deployed at 2 depths (2 m from surface and 2 m from bottom), with the exception of station 53 where greater water depth allowed deployment of an intermediate trap. Centrifuge samples were collected in October using two Westfalia flow-through centrifuges operating in tandem. Each centrifuge was operated at 5 l/min; total volume of water sampled was 1000 l per site. Centrifuges were mounted on a truck for sampling at station 914 and at Redhill Creek. Sediment samples were dried prior to extraction in a desiccator over $CaCl_2$ (Drierite) until a constant weight was achieved.

Bottom sediments (50–90 g) were dried as described above and extracted in a Soxhlet extractor with dichloromethane (350 ml) for 24 h then for an additional 24 h with methanol. These extracts were combined and an aliquot of the crude extract was weighed after solvent evaporation for calculation of the percentage of organic material extracted. Suspended sediment samples were extracted using a 300 W Dismembrator Model 300 Ultrasonicator with a 0.75 inch diameter titanium tip (Fisher Scientific). Samples ranging from 2 to 7 g were placed in a glass beaker containing 50 ml of dichloromethane and eight consecutive pulses, each of 15 s duration, were applied at full power. An interval of 1 min was maintained between pulses and the beaker was immersed in ice to minimize solvent heating. The resulting suspension was filtered and re-extracted with 50 ml of fresh dichloromethane. The procedure was then repeated with 50 ml of methanol and the extracts were combined.

The extracted material was adsorbed to alumina (3 g) by solvent reduction using a rotary evaporator, whereupon the adsorbed alumina was applied to the top of fresh alumina (6 g) contained in a glass column (1 cm × 30 cm). Hexane (60 ml) was added to the column to elute aliphatics. Non-polar polycyclic aromatic compounds (PAC) were eluted by sequential addition of benzene (50 ml) then dichloromethane/ethanol (70 ml, 99:1 v/v), which were combined to afford a single fraction. Elution of the column with methanol (50 ml) and methanol/water (50 ml, 3:1 v/v) afforded a polar PAC fraction. The non-polar PAC fraction was subjected to an additional clean-up step using a Sephadex LH20 gel column to remove any remaining aliphatic compounds (hexane/methanol/dichloromethane, 6:4:3 v/v, 3 ml/min).

The HPLC instrument was a Model 1090 liquid chromatograph with diode array detector

(Hewlett-Packard, Mississauga, Ontario). Normal phase HPLC was performed using a 10 μm 25 cm × 9.4 mm i.d. Whatman Partisil M9 PAC column (Whatman, Clifton, NJ). A mobile phase flow rate of 4.2 ml/min was used with the following linear gradient elution program for the strain YG1024–S9 mutation chromatograms (elapsed time, composition of mobile phase): initial, 95% hexane/5% dichloromethane (DCM); 10 min, 95% hexane/5% DCM; 35 min, 70% hexane/30% DCM; 55 min, 30% hexane/70% DCM; 65 min, 100% DCM; 70 min, 100% DCM; 75 min, 100% acetonitrile; 80 min, 100% acetonitrile. Column temperature was 40°C. The following elution program was used for the strain YG1025+S9 mutation chromatograms: initial, 95% hexane/5% DCM; 10 min, 95% hexane/5% DCM; 50 min, 80% hexane/20% DCM; 65 min, 100% DCM; 70 min, 100% acetonitrile; 75 min, 100% acetonitrile.

The bioassay protocol was adapted from Maron and Ames (1983). Bacteria were grown in Oxoid Nutrient Broth #2 (15 ml) with ampicillin (50 μg/ml) and tetracycline (6.25 μg/ml) in a shaker bath for 9 h at 37°C. Extracts were dissolved in 50 μl DMSO and assayed in duplicate, both with and without metabolic activation (4% Aroclor 1254-induced rat liver S9, Maron and Ames, 1983). Revertant colonies were determined using a Biotran II colony counter (New Brunswick Scientific). Two *S. typhimurium* bacterial strains were used (Watanabe et al., 1990, 1991); strains YG1024 and YG1025 are TA98- and TA100-type strains, respectively, which are auxotrophic for histidine, contain plasmid pKM101, and have been modified by addition of plasmid pBR322. Strain YG1024 is a TA98-type strain with multiple copies of plasmid pBR322 containing the gene for the enzyme O-acetyltransferase. The average spontaneous reversion rate for strain YG1024–S9 was 30 ± 15 revertants (rev)/plate ($N = 40$). The positive control was 1,8-dinitropyrene (7.2×10^{-4} μg/plate, 3350 ± 360 rev, $N = 40$). The average spontaneous reversion rate for strain YG1025+S9 (a TA100-type strain) was 160 ± 25 revertants rev/plate ($N = 55$). The positive control was benzo[a]pyrene (1 μg/plate, 900 ± 150 rev, $N = 35$). Procedural blanks were carried through the sample preparation and chromatographic fractionation procedures; none of the blank samples exhibited positive bioassay responses. Doses were expressed in equivalent weights of sediment. For example, if the non-polar aromatic fraction represented 2% by weight of the initial weight of dry sediment, the actual amount of material applied in the assay would be multiplied by 50 to result in a value of equivalent weight of sediment.

3. Results and discussion

Sediment traps were deployed in the fall (October) and collected in the spring (May) at three Environment Canada sites in Hamilton Harbour (stations 50, 51 and 53, Fig. 1). These sites reflected a variety of conditions dictated by water exchange with Lake Ontario and point source and non-point source discharges (Mayer and Nagy, 1992). Bottom sediment samples were collected at three sites in the harbour and at five sites in Windermere Arm in October. Centrifuge samples were collected in October at two depths in the centre of the harbour (station 906, 1 and 24 m), and at a sub-surface depth (0.5–1 m) at five sites extending from the harbour up Windermere Arm. Another centrifuge sample was collected from Redhill Creek at a site upstream of the Hamilton sewage treatment facility outfall.

Samples were dried and extracted ultrasonically with dichloromethane, then methanol. Methanol was used as a second extraction solvent to yield additional polar organic material from the sample matrix. Previous studies with air particulate material have shown that polar compounds extracted by methanol can be responsible for a significant fraction of the biological activity (Legzdins et al., 1995; Bryant et al., 1989). Yields of extracted material ranged from 0.52% to 4.77% of the original mass of dried sediment.

3.1. Bioassays

Both the non-polar and polar aromatic fractions of each sample extract were assayed using two different Ames bioassay conditions to detect different genotoxic endpoints. *S. typhimurium* strain YG1025, a TA100-like strain, exhibits strong response to compounds like PAH (Marvin et al., 1993, 1994, 1995) which cause base pair-substitution mutations; a preparation of rat liver homogenate (S9) derived from Arochlor 1254-treated rats was added to the YG1025 assays to simulate the oxidative metabolic processes in mammalian systems. A second strain, YG1024, a TA98-like strain which is sensitive to frame-shift mutagens, was used without the addition of S9; under these conditions compounds can undergo reductive metabolism. Extracts of particulate material from combustion emissions and urban air have been shown to induce strong responses under these conditions (Legzdins et al., 1995). This pair of bioassay conditions was chosen in order to discriminate between sources of genotoxic compounds in Hamilton Harbour. Strain YG1025+S9 exhibits strong responses to PAH which are found throughout the harbour (Harlow and Hodson, 1988; Mayer and Nagy, 1992; Marvin et al., 1993) and, in this context, are derived primarily from coal tar contamination. Strain YG1024–S9 exhibits strong responses to the products of vehicular and combustion emissions which can enter the harbour from direct atmospheric deposition, from urban runoff via creeks and streams, and from three sewage treatment plants which discharge into the harbour. The polar aromatic fractions of all extracts showed little or no re-

sponse under both bioassay conditions; thus all subsequent discussions will focus only on the responses of the non-polar aromatic fractions.

Station 906 is located in the centre of the harbour in a deep water depositional area; sediment from this site was selected for initial bioassays because suspended material originating from all areas of the harbour is reported to be ultimately deposited here (Murphy et al., 1990). The mutagenic response of the non-polar aromatic fraction prepared from station 906 bottom sediment, estimated by extrapolation of the linear portion of the dose response curve, was 9100 revertants per gram (rev/g) of sediment in strain YG1025 in the presence of S9 (Fig. 2). The nonlinearity of the dose response curve at the highest doses was characteristic of PAH-rich mixtures (Marvin et al., 1993). The positive response to the station 906 sediment in strain YG1025+S9 indicated the presence of base-pair substitution mutagens such as PAH. Figs. 3 and 4 depict the spatial variations in mutagenic activities (expressed in units of revertants per gram sediment) for non-polar fractions of extracts prepared from suspended sediments (Fig. 3) and bottom sediments (Fig. 4), respectively, in both bacterial strains. Poor responses in strain YG1025+S9 were exhibited at two sites, Redhill Creek and station 914, presumably as a result of the low levels of PAH in these samples (3.4 and 11.6 μg/g, respectively; Table 1). All other sites, which were either further downstream in Windermere Arm or in the harbour proper, exhibited higher PAH contents and correspondingly greater YG1025+S9 responses.

The data showed a wide range of mutagenic responses and of PAH contents; thus, we decided to express the YG1025+S9 response as it related to the total PAH content (sum of concentrations of 16 priority pollutant PAH) of each sediment sample. For example, the station 906 sediment sample discussed above contained 43.6 μg of PAH per gram of sediment (Table 1) which corresponded to 210 revertants per μg of PAH. The mutagenic activity reported for this heavily contaminated Randle Reef sediment was 162,000 rev/g (Marvin et al., 1993); this sediment contained 580 μg/g total PAH which corresponded to 280 revertants per μg (rev/μg) of PAH, a value close to that calculated for the station 906 bottom sediment sample. The values for all sediment samples averaged to 250 ± 125 rev/μg and were generally within a factor of two (Table 1). The similarity of responses in strain YG1025+S9 per μg of PAH indicated that PAH were the compounds primarily responsible for the YG1025+S9 activities exhibited by all samples. Overall, the greater the PAH concentration in the sediment, the greater the response in strain YG1025+S9. Using this bioassay as a genotoxicity endpoint, all of these samples appeared rather similar. Our previous studies have shown that higher mass PAH, such as 252 amu PAH (including benzo[a]pyrene), 276 amu PAH (including indeno[1,2,3-cd]pyrene and benzo[ghi]perylene), 278 amu PAH (dibenz[a,h]anthracene) and PAH of molecular masses 302 and 326 amu were responsible for the majority of TA100-type strain activities of PAH-contaminated sediments (Marvin et al., 1993, 1994, 1995).

Extracts were assayed for frameshift mutagens (e.g., nitro-PAH) using strain YG1024, a strain similar to strain TA98, in the absence of S9. In contrast to results using YG1025+S9, assays in YG1204–S9 exhibited rather different responses compared to the Randle Reef and station 906 sediments (Fig. 4). The response of the station 906 sediment in strain YG1024–S9 (16,900 rev/g) was nearly double the response observed in strain YG1025+S9 (9100 rev/g); furthermore this response was manifest without the addition of oxidative metabolism. For the Randle Reef sediment the mutagenic response in strain YG1024–S9 was only 6400 rev/g sediment compared to 162,000 rev/g with strain YG1025+S9; thus the response in strain YG1024–S9 was 25 times less than the response in YG1025+S9. Clearly, there were direct-acting mutagens present in sediments from the middle of the harbour that were not present in the heavily PAH-contaminated sediments near Randle Reef. The challenge was to identify the source(s) of genotoxic contaminants responsible for this bioassay response. Extracts of sediment trap samples, particularly the station 50 top sample, exhibited strong responses in YG1024–S9 (Fig. 3). Responses of centrifuge sediment extracts in YG1024–S9 were two- to four-fold greater than the corresponding responses in YG1025+S9. The Redhill Creek and station 914 samples exhibited very strong responses in YG1024–S9 while sites farther down Windermere Arm toward the harbour showed less dramatic responses. These data indicated that Redhill Creek

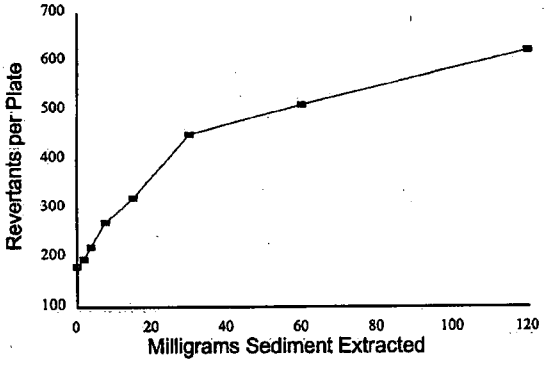

Fig. 2. Dose response curve resulting from a bioassay of station 906 bottom sediment in Salmonella typhimurium strain YG1025 with S9 added. The mutagenic activity of 9100 revertants per gram (dry weight) of sediment was extrapolated from the initial linear portion of the curve.

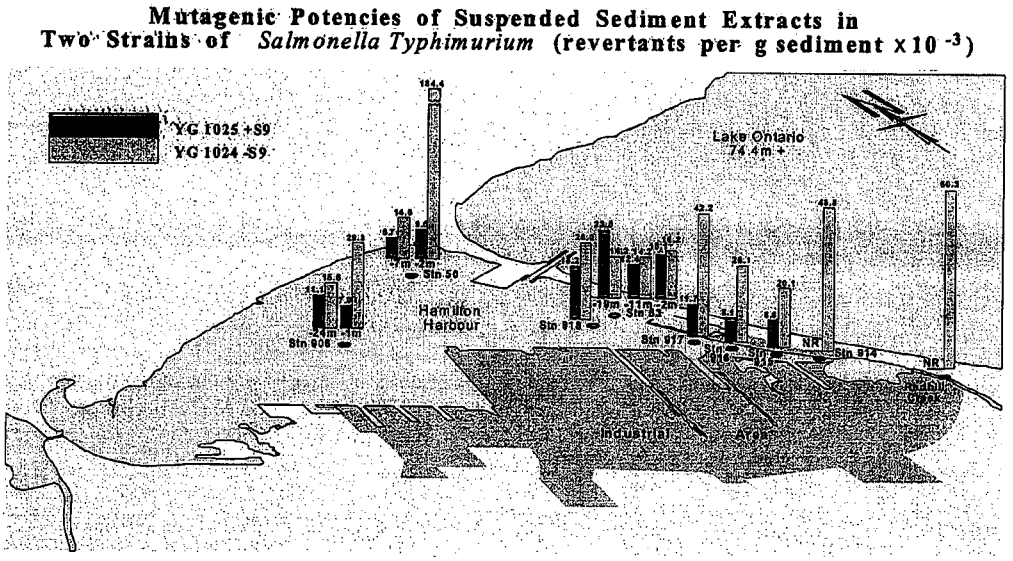

Fig. 3. Spatial representation of mutagenic activities of Hamilton Harbour sediment trap and Westfalia centrifuge sediment samples. Mutagenic activities are expressed in revertants $\times 10^{-3}$ per gram of dry sediment. 'NR' denotes no response.

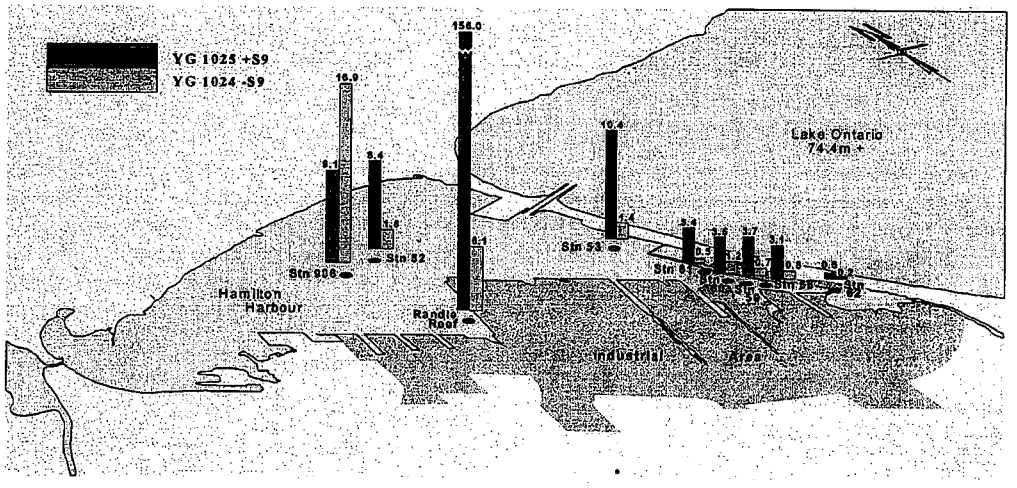

Fig. 4. Spatial representation of mutagenic activities of Hamilton Harbour bottom sediment samples. Mutagenic activities are expressed in revertants $\times 10^{-3}$ per gram of dry sediment.

was a source of direct-acting genotoxic material to the harbour.

Bottom sediments from Windermere Arm appeared to be less heavily impacted by the creek; these bottom sediments exhibited rather low ratios of YG1024–S9:YG1025+S9 activities (Table 1). In general, the predominance of YG1025+S9 activity in bottom sediments contrasted with suspended sediments where YG1024–S9 activity was predominant. In general, YG1024–S9 activities of bottom sediments in Windermere Arm were lower than the activities of harbour bottom sediments and all suspended sediment samples. Reducing conditions in bottom sediments in Windermere Arm and throughout the harbour could favour reduction of chemical substances; thus many direct-acting frameshift mutagens would be reduced in these sediments resulting

Table 1
Mutagenic activity data in strains YG1024–S9 and YG1025+S9 for Hamilton Harbour centrifuge sediments, sediment trap and bottom sediment samples

Station	Ratio of responses (YG1024–S9:YG1025+S9)	YG1025+S9 (per μg PAH)	Total PAH (μg/g dry)
Centrifuge samples			
Redhill Creek	Large[a]	No response	3.4
914	Large[a]	No response	11.6
915	2.4	251	35.0
916	3.2	238	34.0
917	3.6	245	47.7
918	1.4	538	33.8
906 – 1 m depth	3.7	345	22.9
906 – 24 m depth	1.4	278	40.0
Sediment trap samples			
53 Top	1.2	207	66.2
53 Middle	1.1	349	35.8
53 Bottom	0.5	534	49.2
50 Top	19.4	206	46.0
50 Bottom	2.2	236	28.4
Bottom sediment samples			
62 Bottom	0.3	85	7.2
58 Bottom	0.2	146	21.3
59 Bottom	0.2	156	23.7
60 Bottom	0.3	121	29.0
61 Bottom	0.1	106	32.0
52 Bottom	3.5	176	47.6
53 Bottom	2.5	227	45.9
906 Bottom	1.9	210	43.6
Randle Reef	0.04	280	580
Average (± S.D.)		250 ± 125	

[a] Denotes values that could not be calculated since no response was observed in YG1025+S9; these values will be large as a result.

in decreased YG1024–S9 responses, compared to suspended sediments.

Table 1 lists the ratios of bioassay responses for each extract, i.e., the ratios of YG1024–S9:YG1025+S9 activities; within Table 1 are listed two extreme values. The ratio for Randle Reef sediment was the lowest (0.04) while the ratios for the station 914 and Redhill Creek centrifuge sediments were 'large' and for the station 50 top sample was 19.4. The Randle Reef sediment was heavily contaminated with coal tar and exhibited a high PAH content and a strong response in a TA100-type strain in the presence of oxidative enzymes. Thus, in Hamilton Harbour a low YG1024–S9:YG1025+S9 ratio was indicative of genotoxic contamination dominated by PAH. In contrast, the Redhill Creek and station 914 centrifuge sediments which had very low PAH contents exhibited no response above background in a TA100-type strain in the presence of oxidative metabolism; however, these samples exhibited very strong responses in a TA98-type strain without oxidative metabolism. Thus, the YG1024–S9:YG1025+S9 ratios for these two samples, listed in Table 1 as large although they are formally infinite values, were indicative of combustion emission products which are PAH derivatives. With these two sediment types as 'frames of reference', other sediments could be qualitatively described as composites contaminated by these two (or perhaps more) genotoxic sources. The data in Table 1 and Fig. 3 indicated that Redhill Creek was a source of YG1024–S9 activity to the harbour and that this activity supplemented the YG1025+S9 activity present in the harbour as the creek effluent mixed with harbour water as it flowed down Windermere Arm. The Station 50 top sample also showed a very large response in YG1024–S9 and a large ratio of genotoxic responses (19.4). This sample was collected near the mouth of another major tributary, Indian Creek. This sample contained 46 μg PAH per gram sediment and exhibited 210 YG1025+S9 revertants per gram sediment.

3.2. Mutation chromatograms

In order to obtain a more detailed understanding of the chemical substances responsible for observed genotoxic responses, some samples were further characterized using a combination of NP-HPLC and bioassays to afford mutation chromatograms. During the course of an NP-HPLC separation, 85 1-min fractions were col-

lected and each subfraction was bioassayed in strains YG1025+S9 and YG1024–S9. Fractions exhibiting positive responses were re-assayed in singlicate at three concentrations to afford dose response curves so as to provide more accurate estimates of mutagenic activities, rather than relying on single dose data alone. The resulting dose-corrected histograms of net revertants (number of revertants minus spontaneous revertants) exhibited by each subfraction were plotted to afford a mutation chromatogram. Fig. 5 shows the YG1025+S9 mutation chromatogram of the station 906 bottom sediment with the accompanying NP-HPLC UV absorption chromatogram. Polar compounds eluting after 70 min on this gradient were not collected. Each active sub-fraction (response greater than 200 net revertants) was analyzed by GC–MS (data not shown). The fractions corresponding to the peak of activity eluting between 23 and 26 min contained the following known mutagens; benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene and benzo[a]pyrene. Compounds identified in the active sub-fractions collected between 27 and 30 min were indeno[1,2,3-cd]pyrene, benzo[ghi]perylene and dibenz[a,h]anthracene. The mutation chromatogram showed the following relative mutagenic responses: 276 > 252 > 278 > 228 amu PAH. These compounds were responsible for over 80% of the sample genotoxicity attributable to PAH. These data were similar to results from fractions prepared from extracts of coal tar-contaminated sediments from Randle Reef (Marvin et al., 1993) and Sydney Harbour, Nova Scotia (Marvin et al., 1994, 1995); the highest activities in YG1025+S9 co-eluted with PAH ranging in molecular mass from 252 to 276 amu.. These data show conclusively that the YG1025+S9 response of the station 906 sediment was primarily due to the presence of homocyclic PAH in this extract.

The comparison of mutation chromatograms was used to investigate possible sources of genotoxic contamination. In Fig. 6, the YG1025+S9 mutation chromatograms derived from the station 906 bottom sediment and station 917 centrifuge sediment were compared; the PAH contents of these sediments were very similar (43.6 and 47.7 μg/g, respectively). These profiles are very similar although the two sites are well removed from each other (Fig. 1). Not only were the retention times of the bioactive zones of the chromatograms essentially identical, but the PAH profiles and the bioassay responses (expressed in revertants per gram of sediment) were also very similar. These similarities leads us to conclude that the PAH identified as mutagens in station 906 sediment were the same compounds responsible for the activity observed in sub-fractions of the station 917 centrifuge sample.

A series of NP-HPLC mutation chromatograms were also produced using strain YG1024–S9. In order to obtain profiles of the compounds responsible for the direct-acting activitiy observed in samples extracts, the gradient elution program used in the generation of YG1024–S9 mutation chromatograms was modified from the elution program used for the generation of the

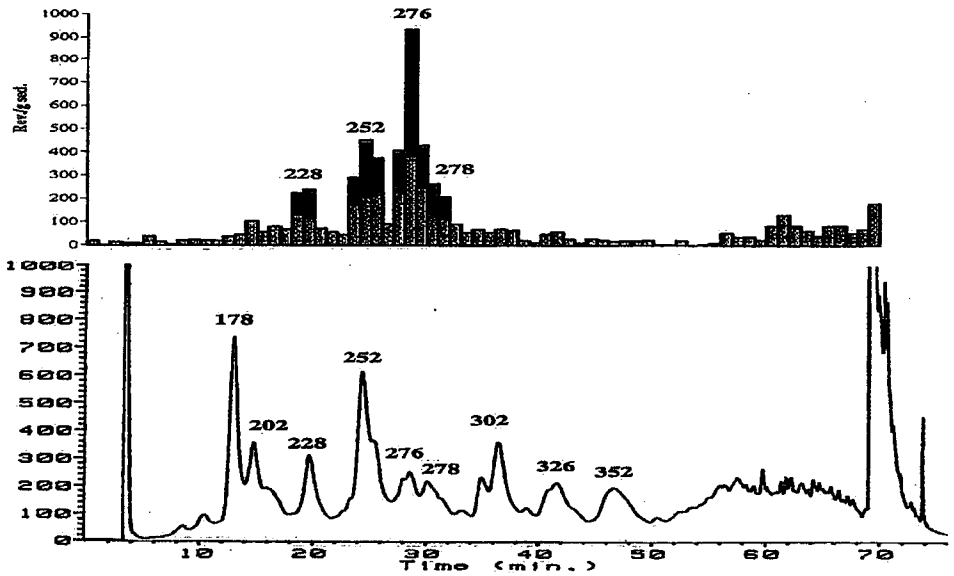

Fig. 5. Mutation chromatogram of station 906 bottom sediment produced by assaying one-minute normal phase HPLC fractions using strain YG1025 with 4% S9 added. The responses are expressed as revertants per gram of sediment. The original single-dose data is shown in grey; the dose-corrected data is shown in black. Peaks are labelled to identify the molecular masses of the homocyclic PAH.

YG1025+S9 mutation chromatograms. The PAH were eluted more rapidly allowing for better separation of later eluting polar PAC. The YG1024–S9 mutation chromatogram for the station 906 bottom sediment is shown in Fig. 7. The majority of the bioassay activity eluted much later than the homocyclic PAH. Sub-fractions exhibiting the greatest responses eluted after 74 min with lesser active sub-fractions between 41 and 47 min. The material eluting after 74 min consisted of very polar components that were eluted by a 100% acetonitrile wash of the normal phase column. As a result, there was little chromatographic separation of these very polar compounds.

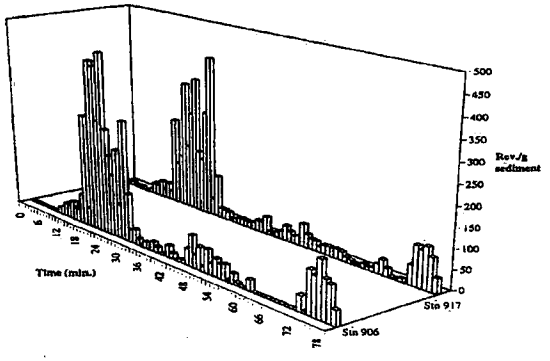

Fig. 6. Comparison of mutation chromatograms of station 906 bottom sediment and station 917 centrifuge sediment samples using strain YG1025 with 4% S9 added.

A comparison of the YG1024–S9 mutation chromatograms from the Redhill Creek station, station 914, station 917 and station 906 bottom sediment is shown in Fig. 8. The profiles of the three centrifuge sediment samples are very similar but contrast sharply with the profile of the station 906 bottom sediment. The majority of mutagenic activity exhibited by the centrifuge samples eluted in the 40–55 min range while less active sub-fractions eluted after 70 min. While there was mutagenic activity which eluted after 74 min, the majority of the YG1024–S9 response eluted between 40 and 55 min. The UV absorption in the region of this large response was very low, indicating that the compounds responsible for this response are potent mutagens. We have not identified the compounds responsible for these bioassay responses. However, we know that dinitropyrenes and dinitrofluoranthenes elute in the 44–50 min range using this gradient elution programme while mononitro arenes elute in the 28–38 min range. These data provide evidence of a source or sources of direct-acting mutagens into the harbour via Redhill Creek, and presumably via other tributaries. For example, the highest YG1024–S9 activity was observed in the station 50 top sediment trap sample (Fig. 3), a site which is impacted by discharges from a tributary (Indian Creek) and a STP outfall.

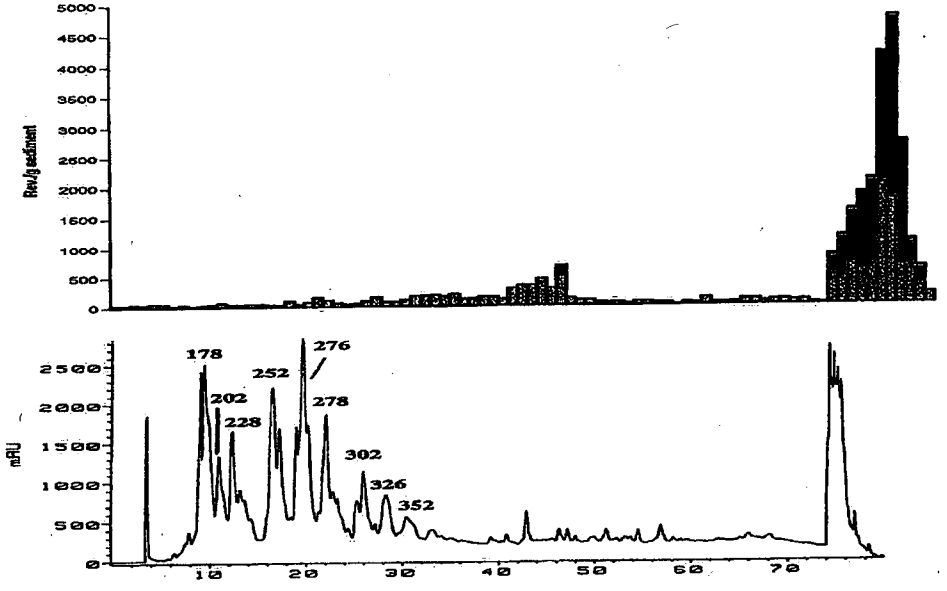

Fig. 7. Mutation chromatogram of station 906 bottom sediment sample produced by assaying one-minute normal phase HPLC fractions using strain YG1024 without 4% S9. The responses are expressed as revertants per gram of sediment. The original single-dose data are shown in grey; dose-corrected data are shown in black. Peaks in the normal phase HPLC chromatogram are labelled to identify the molecular masses of the homocyclic PAH in each peak. The gradient elution program was modified from that used for generation of YG1025+S9 mutation chromatograms.

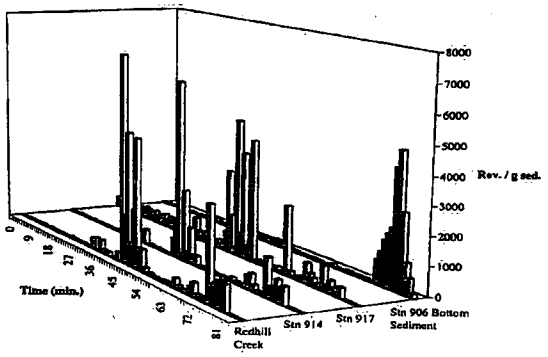

Fig. 8. Comparison of mutation chromatograms of Redhill Creek centrifuge, station 914 centrifuge, station 917 centrifuge and station 906 bottom sediment samples using strain YG1024 without 4% S9.

Creek sources may thus represent a substantial and heretofore largely unrecognized genotoxic burden to the harbour. Metabolic processes in the water column were potentially responsible for conversion of mutagens eluting in the 40–55 min range of the Redhill Creek/Windermere Arm centrifuge samples into more polar compounds which may be the mutagens eluting in the 70–85 min range of the station 906 bottom sediment sample.

Work is currently underway to identify compounds in the active sub-fractions of the YG1024–S9 mutation chromatograms. There is a striking resemblance between the YG1024–S9 sediment profiles and profiles obtained from Hamilton rerspirable air particulate extracts (Legzdins et al., 1995). In the air particulate extracts, strong bioassay responses were observed in fractions eluting at 47, 49 and 55 min using the same normal phase column and gradient elution conditions. Thus, there is evidence that the YG1024–S9 response in the sediment and suspended sediment extracts may be due to atmospheric emissions entering the harbour via runoff from roadways or the watershed. Compounds which elute in this region include dinitro-PAH and various keto- and aza-PAH.

4. Conclusions

Bioassay data obtained using *S. typhimurium* strain YG1025+S9 showed PAH were a significant source of genotoxic contamination in Hamilton Harbour. We determined that PAH responsible for the observed YG1025+S9 activity in sediments and suspended sediments were primarily the 5- to 7-membered ring PAH with molecular masses between 252 and 302 amu, including the known mutagens and carcinogens benzo[a]pyrene, indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene. These compounds required oxidative metabolism to manifest their biological activities. The similarities in the PAH profiles and mutagenic activities per unit mass of PAH in the harbour sample extracts showed these contaminants to be common in samples collected throughout the Hamilton Harbour.

In contrast to the coal tar-contaminated Randle Reef sediment, sediment trap and centrifuge sample extracts exhibited strong genotoxic responses in strain YG1024–S9 in addition to their responses in strain YG1025+S9. These direct-acting mutagenic activities were not correlated with PAH content and implicated Redhill Creek as a source of genotoxic contamination to the harbour. The chromatographic profiles of the YG1024–S9 active NP-HPLC fractions showed that the active compounds were polar PAC that are potent mutagens which require reductive metabolic activation.

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Environment Canada Environnement Canada

Canada

Canada Centre for Inland Waters
P.O. Box 5050
867 Lakeshore Road
Burlington, Ontario
L7R 4A6 Canada

National Hydrology Research Centre
11 Innovation Boulevard
Saskatoon, Saskatchewan
S7N 3H5 Canada

St. Lawrence Centre
105 McGill Street
Montreal, Quebec
H2Y 2E7 Canada

Place Vincent Massey
351 St. Joseph Boulevard
Gatineau, Quebec
K1A 0H3 Canada

Centre canadien des eaux intérieures
Case postale 5050
867, chemin Lakeshore
Burlington (Ontario)
L7R 4A6 Canada

Centre national de recherche en hydrologie
11, boul. Innovation
Saskatoon (Saskatchewan)
S7N 3H5 Canada

Centre Saint-Laurent
105, rue McGill
Montreal (Québec)
H2Y 2E7 Canada

Place Vincent-Massey
351 boul. St-Joseph
Gatineau (Québec)
K1A 0H3 Canada