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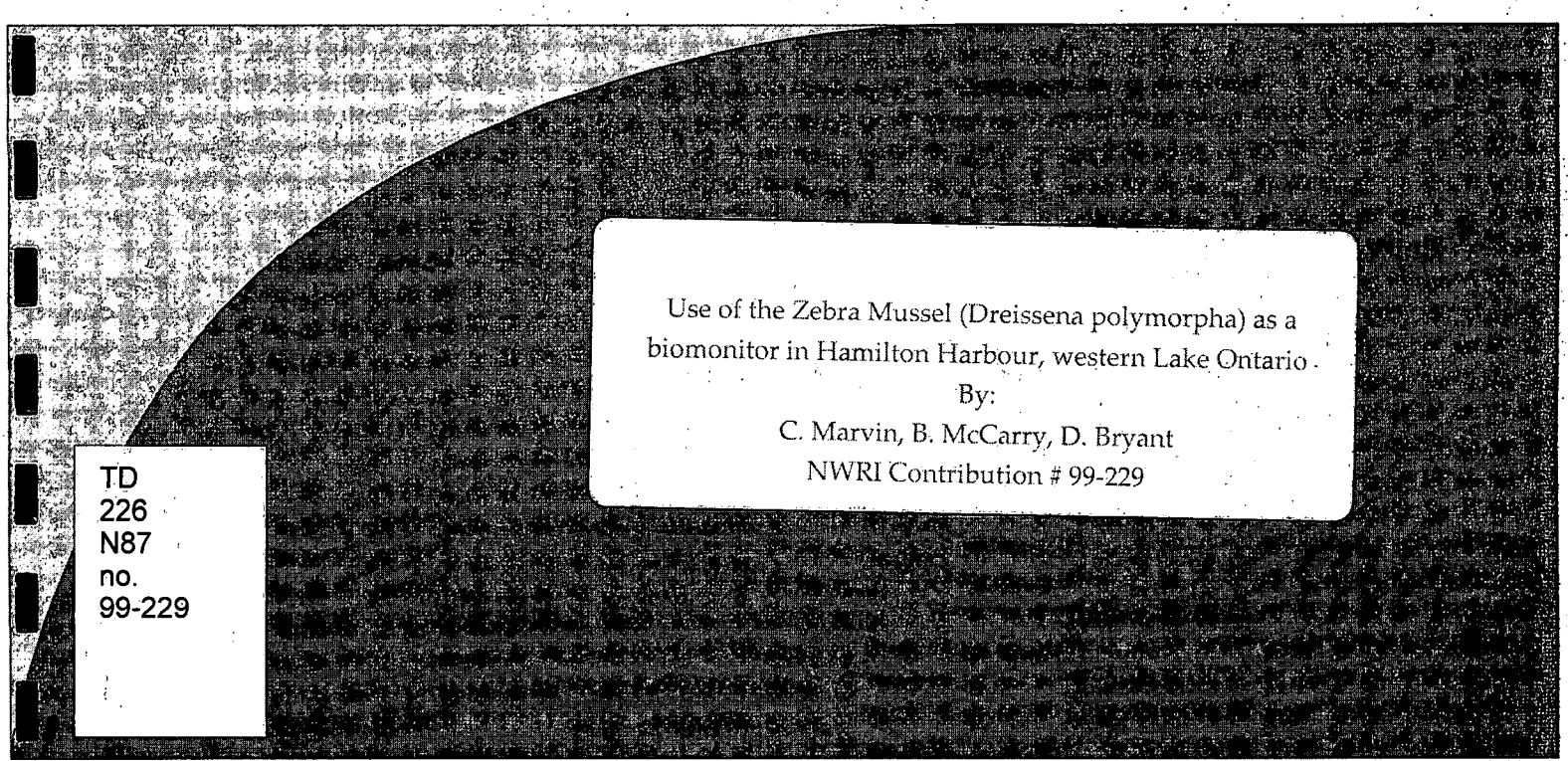

Use of the Zebra Mussel (Dreissena polymorpha) as a biomonitor in Hamilton Harbour, western Lake Ontario

By:
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MANAGEMENT PERSPECTIVE

- **Title:** Use of the zebra mussel (Dreissena polymorpha) as a biomonitor in Hamilton Harbour, western Lake Ontario.

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- **NWRI Publication #:** 99-229
- **Citation:**

- **EC Priority/Issue:** This work was performed under the Great Lakes 2000 Program, to aid in the assessment and remediation of contaminated sediments in areas of coal tar contamination in Hamilton Harbour. This work supports the EC priority of implementing ecosystem initiatives focused on ecosystems of national priority including AOCs in the Great Lakes.

- **Current Status:** This body of work can be segregated into three sub-studies; 1. Methods development for the determination of organic contaminants in mussel tissues; 2. Use of zebra mussels as biomonitors of genotoxic contamination, and; 3. Use of zebra mussels as source apportionment tracers in aquatic systems impacted by coal tar contamination. Data synthesis showed the three sample preparation methods were equally efficient and can be selected on the basis of parameters such as data quality objectives and laboratory resources. Zebra mussels show the potential to be used in monitoring two primary sources of organic contaminants in Hamilton Harbour, coal tar-contaminated sediment and vehicular 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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Use of the Zebra Mussel (*Dreissena polymorpha*) as a Bioindicator for Aromatic Hydrocarbons in Hamilton Harbour

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Three methods for the extraction of polycyclic aromatic hydrocarbons (PAHs) from zebra mussels (*Dreissena polymorpha*) sampled from Hamilton Harbour were compared. Replicate freeze-dried mussel tissue samples were extracted using acid digestion, tissue homogenization (mechanical extraction) and ultrasonication. Each extract was submitted to a cleanup procedure (alumina chromatography and Sephadex LH20 gel chromatography), followed by analysis using gas chromatography-mass spectrometry (GC-MS). The three extraction methods were equally efficient, based on a statistical comparison of mean concentrations of individual PAHs. Mussel extracts, when subjected to bioassays with *Salmonella typhimurium* strain YG1029 (TA100-like) in the presence of an exogenous metabolic activation system (S9), exhibited significant mutagenic responses; these responses varied with the PAH content of the mussel extracts. Sources of PAHs in mussel extracts were determined by examining the profiles of sulfur-containing polycyclic aromatic compounds (thia-arenes). Comparison of the ratios of certain thia-arenes with ratios in source samples enabled identification of vehicular emissions and coal tar-contaminated sediment as two sources of PAH contamination in Hamilton Harbour.

Key words: **polycyclic aromatic hydrocarbons, zebra mussels, *Dreissena*, thia-arenes, Hamilton Harbour, source apportionment**

Introduction

Hamilton Harbour is an embayment of western Lake Ontario with a surface area of approximately 40 km^2. The harbour has been designated as an Area of Concern (AOC) by the International Joint Commission (IJC) due to a number of impairments to beneficial uses, including the presence of contaminants such as PAHs, PCBs, organochlorines and metals (Remedial Action Plan for Hamilton Harbour 1992). Sources of these contaminants are believed to include industrial, vehicular and municipal sources, and urban and rural runoff from a watershed of approximately 600 km^2. There are a number of sites within the harbour associated with industrial contamination, but of primary concern is an area of coal tar-

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contaminated sediment on the south shore of the harbour near Randle Reef. This area is commonly thought to contribute to harbour-wide PAH contamination (Harlow and Hodson 1988; Mayer and Nagy 1992; Murphy et al. 1990). Sediments in this area have been targeted for remediation.

We have previously used whole zebra mussels (*Dreissena polymorpha*) as monitors of PAH contamination in Hamilton Harbour (Marvin et al. 1994). These mussels, whose presence in the harbour is widespread, are sedentary filter feeders. Their processing of particulate material results in the accumulation of non-polar contaminants in their tissues, making them useful as time-integrated samplers of material in the water column. In this paper, we describe a preliminary investigation of the utility of zebra mussels as bioindicators and source apportionment tracers of water-borne PAH contamination in Hamilton Harbour. In the first phase of this study, contaminant body burdens in zebra mussels were determined. Three methods of extraction (acid digestion, tissue homogenization and ultrasonication) were evaluated and compared for efficiency of extraction of PAHs from zebra mussel tissue. The extracts were cleaned up using a two-stage chromatographic procedure, followed by analysis for PAHs by GC-MS. In the second phase of the study, the mutagenic and genotoxic potential of mussel extracts were determined by the *Salmonella typhimurium*/microsome assay using a TA100-like strain with addition of oxidative metabolism (4% S9). These bioassays were used to determine the total genotoxic burden of contaminants being accumulated by zebra mussels.

We have previously shown that we can distinguish between air emissions derived from steel industry operations and vehicular emissions based on differences in profiles of sulfur-containing polycyclic aromatic compounds (thia-arenes) (McCarry et al. 1996). In the final phase of this study, thia-arene profiles of zebra mussel extracts were generated by GC-MS analysis and compared with profiles of two source samples: a diesel particulate reference standard (NIST SRM 1650) and a coal tar reference standard (SRM 1597). The thia-arene profiles of the standards were compared to the zebra mussel profiles to estimate the relative contributions of coal tar-contaminated material and vehicular emissions to the PAH body burdens of the mussels.

Materials and Methods

Initial Sample Preparation

All zebra mussels analyzed in this study were sampled in October 1995 from rock and concrete substrates in an area west of the Lasalle Park Marina on the north shore of Hamilton Harbour, and from a navigation buoy near Randle Reef on the south shore. Mussel aggregates were rinsed in harbour water, sealed in freezer bags, and immediately transported to the laboratory and stored frozen at -70°C. Mussels from Lasalle Park were used in the development of analytical methods and were characterized according to size, percent tissue, percent water and percent shell. Whole

mussels were freeze-dried for 72 h in a Freezemobile model 6201-6220 freeze-dryer (Labconco Corp.). Whole dry mussels were then ground to a 1-mm mesh size with a Willey mill. For the comparison of the three extraction procedures, whole dry mussels were shucked, followed by grinding of the tissue.

Extraction

Tissue homogenization

Dry mussel tissue (0.5 g) was suspended in Corex centrifuge bottles by addition of 50 mL of HPLC grade dichloromethane and extracted using a Janke and Kunkel Ultraturrax T50 tissue homogenizer with an S50 head (Terochem Inc.). The homogenizer was operated at 1000 rpm for four 60-s cycles. The samples were then centrifuged at 4000 rpm and the dichloromethane was decanted and reduced in volume by rotary evaporation.

Ultrasonication

Ultrasonic extraction was performed with a Fisher Model 300 sonic dismembrator apparatus (Fisher Scientific). Dry mussel tissue (0.5 g) was suspended in 50 mL of dichloromethane in a beaker and then subjected to four consecutive 1-min pulses with the sonicator operated at full power. The beaker was partially immersed in ice, and a time interval of 1 min was maintained between ultrasonic pulses to minimize solvent heating. The dichloromethane was decanted and then passed through a 0.45-μm teflon filter. The extraction procedure was then repeated with 50 mL of fresh dichloromethane. After filtration, solvent extracts were combined and reduced in volume by rotary evaporation.

Acid digestion

Dry mussel tissue samples (0.5 g) were placed in glass test tubes and digested overnight (16 h) in concentrated hydrochloric acid (20 mL). Twenty mL of 25% dichloromethane in hexane was added to each tube and extracted on a rotary tumbler for 45 min. The samples were centrifuged at 3000 rpm for 3 min, and the organic layer removed and passed through a cartridge containing a 50:50 mixture of sodium bicarbonate and anhydrous sodium sulphate. Solvent extracts were then reduced in volume by rotary evaporation.

Alumina Cleanup and Gel Chromatography

Organic solvent extracts were subjected to a sequential open-column alumina chromatography and Sephadex LH-20 gel column cleanup as previously described (Marvin et al. 1992). Organic compounds from sample extracts were adsorbed to alumina (1.5 g, Brockman activity 1, 80–200 mesh) by solvent evaporation under reduced pressure, and the resulting material was applied to the top of a column of fresh alumina (3 g activated

at 170°C for 48 h) contained in a glass column (1 cm × 30 cm). Organic compounds were then eluted using solvents of increasing polarity. First, hexane (30 mL) was used to elute aliphatics, and then dichloromethane (35 mL) eluted polycyclic aromatic compounds (PAC). This PAC-containing fraction was subjected to a second chromatographic step using a Sephadex LH-20 (Pharmacia Fine Chemicals) gel column to remove the remaining aliphatic compounds and monobenzenoids. The mobile phase was hexane/methanol/dichloromethane (6:4:3 v/v) with a flow rate of 3 mL/min.

Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GC-MS) analyses were performed using a Hewlett-Packard Model 5890 Series II gas chromatograph equipped with a Hewlett-Packard Model 5971A mass selective detector and an on-column injector (Hewlett-Packard Corp.). The following temperature program was employed: 100 to 300°C at 5°C/min; final time at 300°C, 20 min. The column was a 60 m × 0.32 mm i.d. DB-5 with a 0.25-μm stationary phase film coating (J and W Scientific). The transfer line temperature was maintained at 300°C and the helium carrier gas linear velocity was 30 cm/s. The instrument was operated in selected ion monitoring mode (SIM) and the estimated limits of detection for PAHs under the described chromatographic conditions ranged from 1 to 3 ng/g of dry mussel tissue (5:1 S/N). Benz[a]anthracene-d_{12} was used as a laboratory spike.

Table 1. Concentrations of polycyclic aromatic hydrocarbons in extracts of Hamilton Harbour zebra mussels prepared using three different extraction methods[1]

	Acid Digestion	Mechanical	Ultrasonication
Phenanthrene	211±60	104±38	103±9
Fluoranthene	266±48	185±21	157±4
Pyrene	510±75	426±47	339±37
Benz[a]anthracene	78±7	86±10	67±11
Chrysene	*228±26	201±7	170±19
Benzo[b/j]fluoranthene	103±9	119±3	99±16
Benzo[k]fluoranthene	45±4	48±2	41±10
Benzo[e]pyrene	75±6	74±3	64±9
Benzo[a]pyrene	53±7	56±2	48±7
Indeno[1,2,3-cd]pyrene	68±6	63±5	53±7
Benzo[ghi]perylene	60±6	65±5	64±7
Total PAHs	1697	1427	1205

[1] Concentrations are expressed in ng/g of dry tissue from three replicate analyses. Value marked with an asterisk denotes the presence of an interference.

Both PAHs and thia-arenes were determined concurrently with the described GC-MS method. Identification and quantitation were based on retention time and response factors obtained from the analysis of authentic standards and ratios of target and qualifying ions. The following target and qualifying ions were monitored for quantitation of the PAHs listed in Table 1: phenanthrene (178,179,176), fluoranthene and pyrene (202,203,200), benz[a]anthracene and chrysene (228,226,113), benzo[b/j/k]fluoranthene and benzo[a/e]pyrene (252,250,126), and indeno[1,2,3-cd]pyrene and benzo[ghi]perylene (276,277,138).

Standard unsubstituted mass 184 amu thia-arenes analyzed included dibenzothiophene, naptho[1,2-b]thiophene, naphtho[2,1-b]thiophene and naphtho[2,3-b]thiophene. The following target and qualifying ions were monitored for quantitation of the thia-arenes: dibenzothiophene and the naphthothiophene isomers (184,152,139), monomethyl derivatives (198,197), and dimethyl and ethyl derivatives (212,211).

Bioassays

Salmonella typhimurium bacterial strain YG1029 was used in this study. This strain is a TA100-type strain auxotrophic for histidine containing pKM101 and modified by addition of plasmid pBR322 containing multiple copies of the gene for the activating enzyme O-acetyltransferase (Watanabe et al. 1991). The protocol for the assay was modified from Maron and Ames (1983). Bacteria were grown for 10 h in Oxoid nutrient broth (15 mL) in the presence of ampicillin (50 μg/mL) and tetracycline (6.25 μg/mL). Organic extracts were dissolved in 50 μL of DMSO and assayed with metabolic activation in the form of a 4% Aroclor 1254-induced rat liver S9 homogenate. After incubation at 37°C for 48 h, the number of revertant colonies was determined with a Biotran colony counter (New Brunswick Scientific). Dose-response curves were generated by assaying a range of five concentrations of extract assayed in duplicate. Mutagenic activities of extracts were extrapolated from the linear portion of the dose-response curves. The positive control was 1-μg/plate of benzo[a]pyrene which resulted in an average reversion rate of 1030±270 rev/μg based on 10 replicate experiments. The average spontaneous reversion rate (background rate) for YG1029+S9 was 140±20 revertants. Reagent blanks were assayed for each sample set and checked against the spontaneous reversion rate.

Reference Materials

The diesel particulate standard reference material (SRM 1650) and the coal tar standard reference material (SRM 1597) were obtained from the National Institute of Standards and Technology (Gaithersburg, MD). The coal tar reference standard was obtained in liquid form and was used unaltered. The diesel reference material was obtained as a solid and was extracted using ultrasonication in dichloromethane followed by the described cleanup procedure. The two standard reference materials were

quantitatively characterized with the described GC-MS method. Levels of dibenzothiophene in SRM 1650 (diesel) and SRM 1597 (coal tar) were calculated to be 14 μg/g and 22 μg/g, respectively, while the sum totals of the three naphthothiophene isomers were 3 μg/g and 23 μg/g, respectively. Mossner and Wise (1999) reported values of 18 μg/g and 19 μg/g for dibenzothiophene and the three naphthothiophene isomers, respectively, in SRM 1597. For comparison, levels of selected PAHs reported by NIST for SRM 1650 and SRM 1597 were 71 μg/g and 101 μg/g, respectively, for phenanthrene, 48 μg/g and 235 μg/g for pyrene, and 1.2 μg/g and 95.8 μg/g for benzo[a]pyrene. The diesel standard reference material (SRM 1650) was also characterized biologically and exhibited a bioassay response of 22,000 rev/mg of particulate.

Results and Discussion

Methods Development

Bottom sediment PAH levels near Lasalle Park are typically low (0 to 17 μg/g total PAH [Remedial Action Plan for Hamilton Harbour 1992]) compared with some sites on the south shore near Randle Reef that are highly contaminated by coal tar (500 μg/g to greater than 1500 μg/g [Marvin et al. 1993; Murphy et al. 1990]). Mussels at Lasalle Park were presumably exposed to particulate material circulating in the harbour water column. Compartmental distributions by weight of mussels from Lasalle Park were estimated from six replicate subsamples consisting of approximately 40 individual mussels (approximately 60 g wet weight) and were as follows: percent tissue, 3.3±0.4%; percent water, 58.5±0.9%; and percent shell, 38.2±1.2%. Mussels were exclusively *Dreissena polymorpha*, ranging in size from 1.9 cm to 3.0 cm.

The freeze-drying protocol for whole mussels was modified from a procedure originally designed for the preparation of vegetation samples. Strict temperature control was maintained to maximize recovery of volatile PAHs. The vacuum chamber was maintained at -5°C for the first 24 h of the procedure and then increased to 5°C for the duration of the drying period (approximately 48 h). We have found that freeze-dried samples, both whole mussels and mussel tissue, enable determination of accurate sample weights and can be easily ground and homogenized. Large masses of whole mussels can be reduced to volumes that can be stored in standard 250-mL amber glass jars.

The tissue homogenizer (mechanical) extraction procedure was modified from a National Oceanic and Atmospheric Administration (NOAA) protocol for extraction of organic contaminants from marine mussels (MacLeod et al. 1985). The ultrasonic extraction procedure was modified from a protocol developed for extraction of PAHs from sediments and air particulate material (Marvin et al. 1992). The acid digestion procedure was performed according to a standard Ontario Ministry of the Environment (1983) protocol. The ultrasonication and tissue homogeniza-

tion extractions could each be accomplished in approximately 25 min for each sample. The ultrasonication and tissue homogenization techniques could also be applied to ground whole mussels, but dry mussel tissue samples were chosen for this comparative study as the acid digestion procedure was not applicable to whole mussels.

Three replicate analyses of dry mussel tissue were performed using each of the extraction methods. Organic solvent extracts were subjected to an open-column alumina and Sephadex LH20 gel column cleanup procedure, and then analyzed for PAHs by GC-MS in selected ion monitoring (SIM) mode. Figure 1 shows a typical GC-MS SIM chromatogram resulting from the analysis of a zebra mussel extract prepared by using ultra-

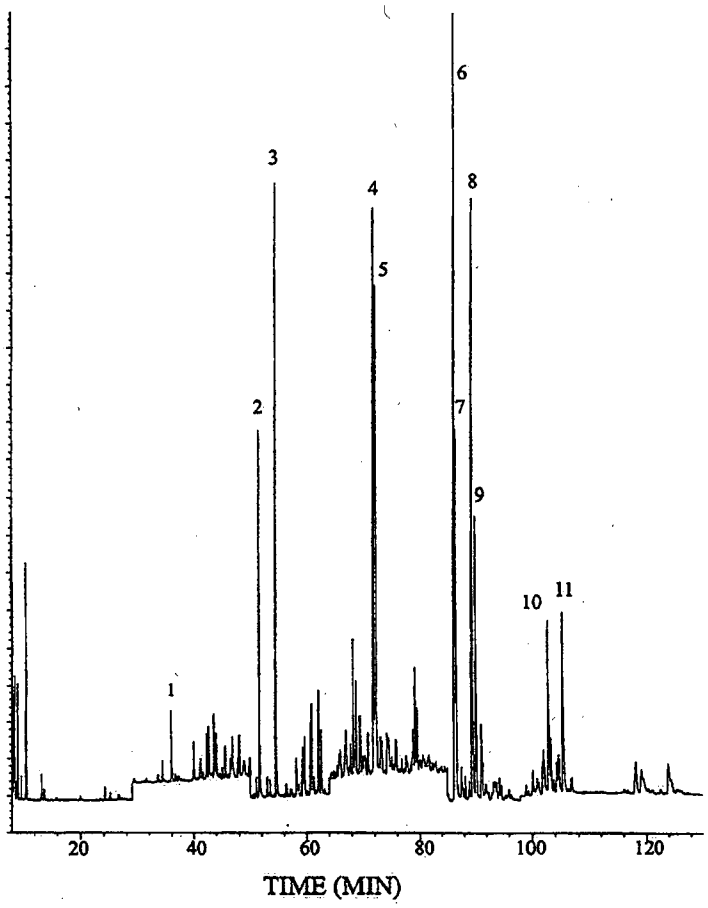

Fig. 1. GC-MS selected ion monitoring chromatogram of an extract of zebra mussels sampled from Lasalle Park, Hamilton Harbour. The peaks are numbered to correspond to the PAHs listed in Table 1.

sonic extraction. Chromatograms from the analyses of extracts from the ultrasonication and tissue homogenization procedures were relatively free from interfering compounds, which enabled accurate identification and quantitation of PAHs. However, the acid digestion procedure afforded larger quantities of co-extracted material in the solvent extracts compared to the other methods. This material was not completely removed by the cleanup procedure, and several peaks in the acid digest extracts acted as interferences in the determination of some PAHs, particularly chrysene (data not shown).

Table 1 lists the PAHs determined in the mussel extracts for each of the three extraction methods and their concentrations expressed in ng/g of dry mussel tissue. The total PAH concentrations, expressed as the sum of the 12 individual compound concentrations, ranged from 1200 ng/g for ultrasonic extraction to 1700 ng/g for acid digestion. There were no differences in the efficiencies of the three extraction methods, based on a comparison of the mean concentrations of individual PAHs quantitated in three replicate samples (t test, $p>0.05$). The reproducibility of the three methods was evaluated by performing an f test ($p>0.05$) on the standard deviations of each of the individual PAHs; no differences in the individual values were found. For the acid digestion and tissue homogenization procedures, the between-run variation was greatest for phenanthrene (Table 2). The average between-run variations were 12% for acid digestion, 9% for tissue homogenization and 13% for ultrasonication.

Genotoxicity of Mussel Extracts

Short-term bioassays such as the *Salmonella typhimurium* microsome assay (Ames assay) can be used as indicators of contamination in complex environmental mixtures by providing evidence of mutagenic and/or potentially carcinogenic compounds. These short-term tests can provide a means for assessing the potential genetic hazard associated with chemical exposure and are a complement to more expensive assays and epidemiological studies. The Ames *Salmonella* bacteria strains are engineered with a non-functional gene for the production of histidine, an essential amino acid. Exposure to a mutagen introduces a genetic change in the bacterial DNA and results in the histidine gene reverting to functional status and subsequent growth of bacterial colonies in an agar medium. The number of bacterial colonies, as determined with a colony counter, is proportional to the potency and quantity of the mutagen present. In addition, a rat liver homogenate, containing P_{450} monooxygenase enzymes induced by exposure of the rat to an Aroclor PCB mixture, can be added to the test mixture to simulate metabolic processes in mammalian systems. Certain compound classes, such as PAHs, require oxidative metabolism to transform them from relatively inert substances into metabolic by-products, thereby manifesting their responses. Extensive epidemiological studies have implicated PAHs as causal agents of liver carcinogenesis in aquatic biota (Myers et al. 1990; Stein et al. 1990).

The *Salmonella typhimurium* strain used in this study (YG1029) is

related to the Ames TA-100 strain and has had its cell wall modified to permit freer flow of large molecules in and out of the cell. Strain YG1029 also contains two genes located on multicopy plasmids that confer additional sensitivity to chemical events that damage DNA and code for the activating enzyme O-acetyltransferase, which is important for converting (activating) compounds such as nitro-PAH that directly affect DNA to produce mutational events. Strain YG1029 was chosen for this study as we have found the O-acetyltransferase strains to be optimal for general determination of mutagenic activity in complex environmental mixtures. Assays were conducted exclusively in the presence of oxidative metabolism (S9), as our previous studies did not detect any direct-acting activity in extracts of Hamilton Harbour zebra mussels (Marvin et al. 1994).

Whole dry mussels, including the shells, were ground and processed by tissue homogenization extraction followed by the described cleanup procedure. Extracts of zebra mussels were subjected to bioassays using *Salmonella typhimurium* strain YG1029 with the addition of a rat liver supernatant (4% Aroclor 1254-induced). Figure 2 shows the dose-response curves exhibited by the mussel extracts assayed with strain

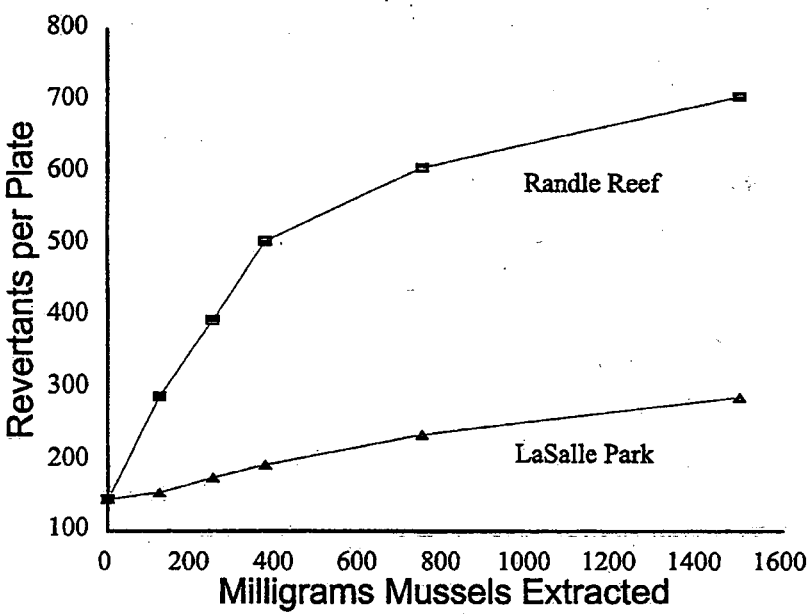

Fig. 2. Dose-response curves exhibited by extracts of zebra mussels sampled from two sites in Hamilton Harbour when assayed with Salmonella typhimurium strain YG1029 with the addition of 4% rat liver S9. The doses are expressed in milligrams of whole dry zebra mussels extracted. Mutagenic activities (± standard deviation) of 1160±135 revertants/mg and 120±30 revertants/mg whole wet mussels extracted were extrapolated from linear segments of the curves.

YG1029+S9. Criteria for a positive response in the assay are a positive dose-response relationship and a number of revertant colonies at the highest dose double that of the spontaneous reversion rate. The mutagenic activities of 1160±135 revertants/mg and 120±30 revertants/mg whole dry mussels extracted for the Randle Reef and Lasalle Park Marina mussels, respectively, were calculated from the linear portions of the dose-response curves. The non-linear nature of the curves at the highest doses is typical of PAH extracts of complex environmental mixtures. Total PAH concentrations (sum of concentrations of PAH listed in Table 1) of the Randle Reef and Lasalle Park mussels were approximately 23.8 μg/g and 0.9 μg/g dry mussel tissue, respectively. The ratio of the calculated mutagenic activities between the Randle Reef mussels and the Lasalle Park mussels was 9.7:1, while the ratio in the levels of PAHs in mussels from the two sites was 26.4:1. We have previously observed poor correlations between mutagenic responses and mussel tissue PAH concentrations, presumably due to the presence of other mutagens, including PAHs in the extracts (Marvin et al. 1994). However, the positive responses exhibited by these extracts, particularly in the extract of mussels from Randle Reef, show that compounds accumulated by zebra mussels in Hamilton Harbour are genotoxic and that these compounds are possibly bioavailable to other aquatic organisms exposed to material circulating in the harbour water column.

Thia-arene Profiling of Mussel Extracts

The primary use of zebra mussels in our laboratory has been as in situ bioindicators of suspended particulate material in the water column in Hamilton Harbour. The sedentary nature of zebra mussels, combined with their mechanism of filter feeding, may result in data that better indicate temporal water and suspended sediment quality compared with routine grab sampling that provides only time-specific data. The ability to implicate sources of contamination in the harbour using PAHs as a source apportionment tool is confounded by the multitude of sources of PAHs and by the similarity of the profiles of PAHs from these sources. We have previously shown that sulfur-containing polycyclic aromatic compounds (thia-arenes) are useful tracer compounds for distinguishing between coal-derived emissions and petrogenic-derived emissions in Hamilton air particulate (McCarry et al. 1996). Unlike PAHs, which are produced during combustion of carbon-based fuels, thia-arenes are produced only by combustion of fuels containing sulfur such as coal and petroleum fuels. Since vehicular emissions and steel industry emissions are two major sources of PAH contamination in the harbour, we are investigating thia-arene profiles as source apportionment tracers of water-borne contamination.

Thia-arene analyses were performed on extracts of whole dry mussels using GC-MS in selected ion monitoring (SIM) mode. The ions monitored in the retention time range of 34 to 50 min were selected based on typical important ions for mass spectral characterization of unsubstituted

and substituted thia-arenes. Unsubstituted thia-arenes typically exhibit intense molecular ions (m/z 184) and often lose sulfur (M-32, m/z 152) and a CHS unit (M-45, m/z 139). In addition to these characteristic ions exhibited by the unsubstituted compounds, the substituted thia-arenes also exhibited elimination of a hydrogen (M-1, m/z 197 and m/z 211).

Figures 3 and 4 show the reconstructed ion chromatograms from the analysis of a coal tar standard reference material (SRM 1597) and zebra

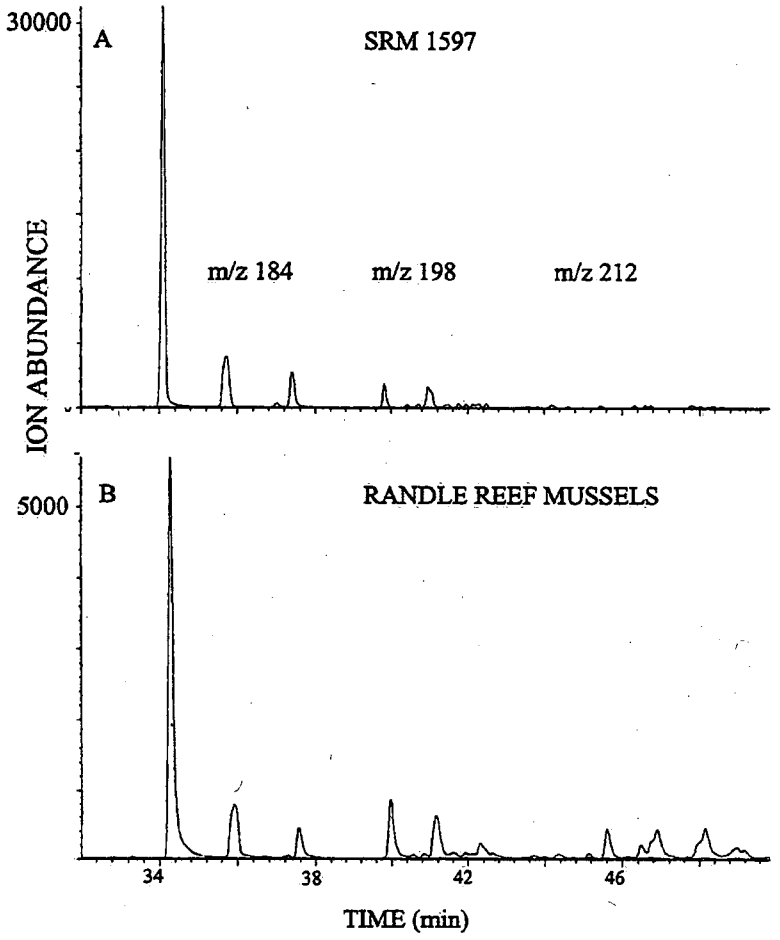

Fig. 3. Reconstructed ion chromatograms of m/z 184, m/z 198 and m/z 212 ions corresponding to thia-arenes in (A) a coal tar standard reference material (SRM 1597) and (B) zebra mussels sampled from Randle Reef, Hamilton Harbour. The predominant ions correspond to the dibenzothiophene isomers (m/z 184, 34–38 min), the monomethyl derivatives (m/z 198, 38–44 min), and the dimethyl/ethyl derivatives (m/z 212, 44–50 min).

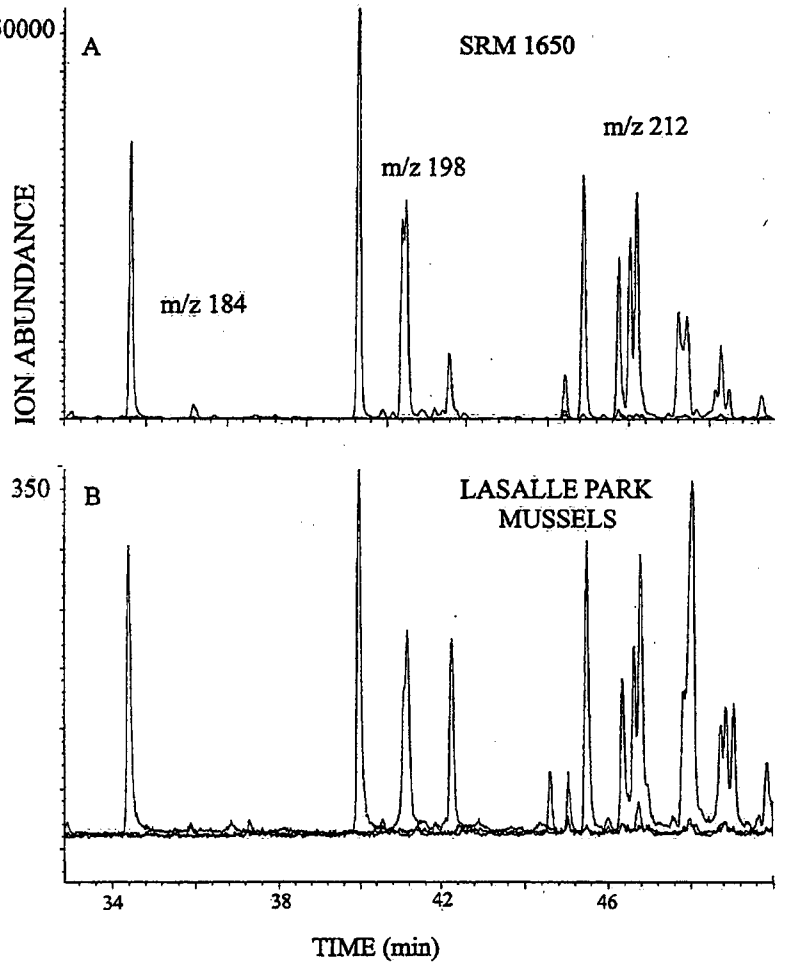

Fig. 4. Reconstructed ion chromatograms of m/z 184, m/z 198 and m/z 212 ions corresponding to thia-arenes in (A) a diesel particulate standard reference material (SRM 1650) and (B) zebra mussels sampled from Lasalle Park, Hamilton Harbour. The predominant ions correspond to the dibenzothiophene isomers (m/z 184, 34–38 min), the monomethyl derivatives (m/z 198, 38–44 min), and the dimethyl/ethyl derivatives (m/z 212, 44–50 min).

mussels from Randle Reef (Fig. 3), and a diesel particulate standard reference material (SRM 1650) and zebra mussels from Lasalle Park (Fig. 4). The m/z 184 ions correspond to the molecular ions of dibenzothiophene and the naphthothiophene isomers, and the m/z 198 and m/z 212 ions correspond to the monomethyl and dimethyl/ethyl derivatives, respectively. In the chromatogram of the diesel particulate (SRM 1650, Fig. 4A)

the ion abundances of the alkyl derivatives equal or exceed those of the parent compounds (m/z 184), which is in striking contrast to the chromatogram of the coal tar standard (SRM 1597) where the ion abundances of the alkyl derivatives are much lower than those of the parent compounds (Fig. 3A).

In both Fig. 3 and 4, the thia-arene profiles of the mussel extracts are very similar to the respective source samples, providing strong evidence as to the primary sources of contamination at these two sites. At Randle Reef, contamination appears to have originated predominately through resuspension of coal tar-contaminated sediment; the intensities of the m/z 198 and m/z 212 ions in the Randle Reef mussel extract were greater than in the coal tar reference standard, indicating a 5 to 10% contribution of PAHs from petrogenic sources. At Lasalle Park, the PAH contamination was derived primarily from vehicular emissions via roadway runoff and/or direct air deposition; the thia-arene profiles provided no evidence that the Lasalle Park mussels were exposed to coal tar-contaminated suspended particles.

Conclusions

All three methods for extraction of PAHs from dry mussel tissue samples were efficient. Extraction by ultrasonication or tissue homogenization, combined with the described cleanup procedure, afforded extracts that were free of interfering compounds and enabled accurate identification and quantitation of analytes. Extracts prepared using acid digestion contained interferences that made the determination of some PAHs difficult. Extracts of mussels from both sites sampled exhibited positive responses in the *Salmonella*/microsome assay. The ratios of the Randle Reef mussel extract:Lasalle Park mussel extract mutagenic activities was roughly 10:1, while the ratio of the total PAH body burdens was approximately 25:1. Thia-arene profiles of mussel extracts provided evidence of two different sources of contamination at two sites in Hamilton Harbour: vehicular emissions and coal tar-contaminated sediment. These data also showed that contaminants in harbour sediments are not necessarily sequestered from the aquatic environment. Ultimately, with greater spatial sampling of the harbour, this methodology might be useful for time-integrated monitoring of water quality and suspended sediment quality before, during and after a sediment remediation project.

Acknowledgments

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