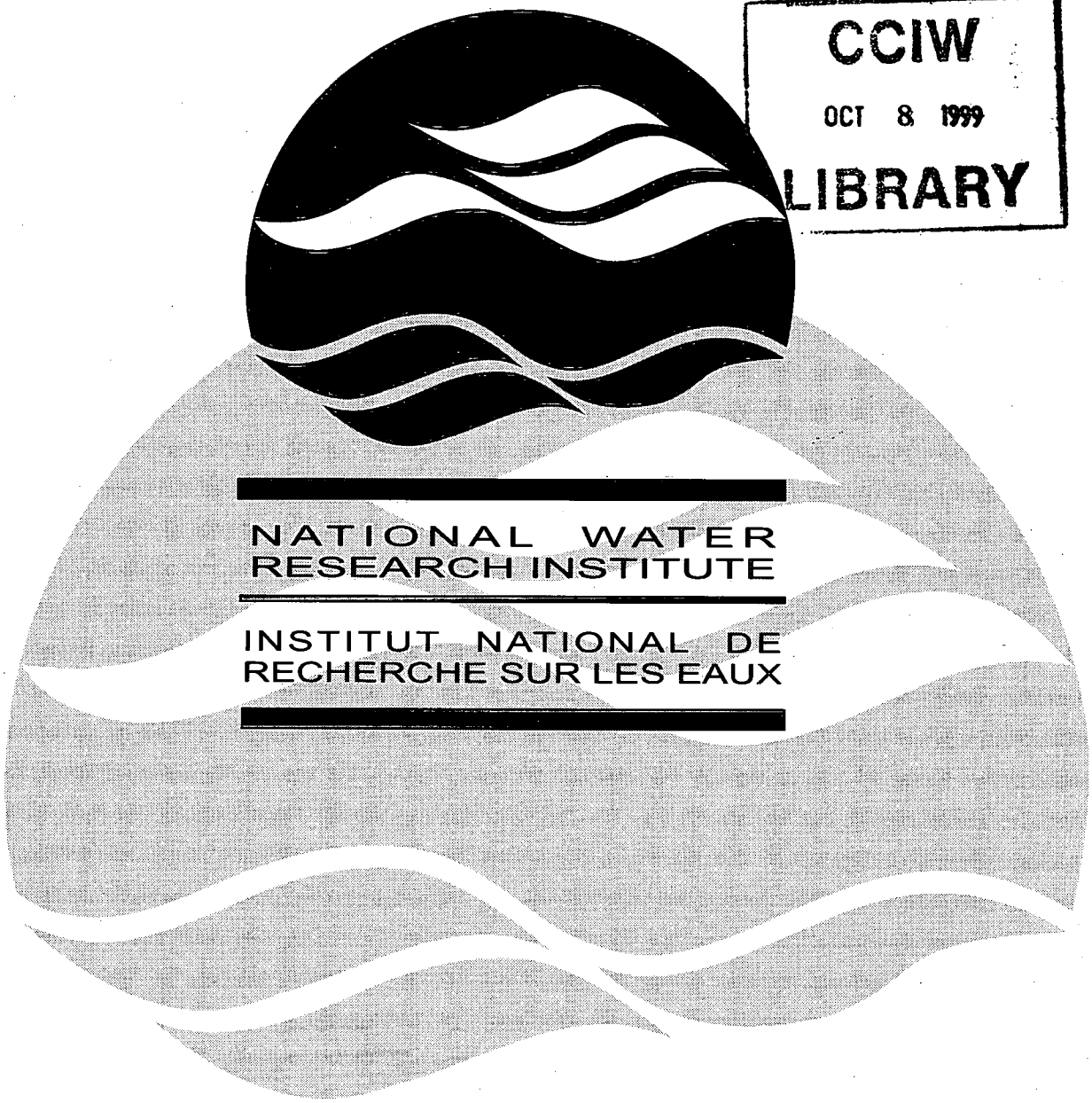

DETERMINIATION OF BISPHENOL A IN MUNICIPAL SEWAGE TREATMENT PLANT EFFLUENT AND SLUDGE BY SOLID-PHASE EXTRACTION AND SUPERCRITICAL FLUID EXTRACTION

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Determination of Bisphenol A in Municipal Sewage Treatment Plant Effluent and Sludge by Solid-phase Extraction and Supercritical Fluid Extraction

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MANAGEMENT PERSPECTIVE

Bisphenol A (BPA) is manufactured in large quantities in North America and used worldwide as building blocks for numerous plastic products. Its environmental occurrence has become a concern recently because it has been shown as one of the more potent anthropogenic endocrine-disrupting chemicals. In the present work, analytical methods have been developed for the determination of BPA levels in municipal sewage effluent and sludge samples. Application of these methods to ca.100 samples collected from sewage treatment plants at various locations in Canada suggests the ubiquitous occurrence of BPA in sewage effluent and sludge. This study is another example of the Institute's continuing effort in research on endocrine-disrupting chemicals in the Canadian environment.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Le bisphénol A (BPA) est produit en grandes quantités en Amérique du Nord et, dans le monde entier, il sert de matière première pour la fabrication de nombreux produits de matières plastiques. On s'inquiète de sa présence dans l'environnement depuis quelque temps parce qu'il a été démontré qu'il s'agit d'un des plus puissants perturbateurs du système endocrinien chez les humains. Au cours de la présente étude, on a développé des méthodes d'analyse pour le dosage du BPA dans des échantillons d'effluents et de boues d'eaux usées municipales. L'application de ces méthodes à environ 100 échantillons recueillis dans des stations d'épuration des eaux usées à divers endroits du Canada semble indiquer que le BPA est omniprésent dans les effluents et les boues d'eaux usées. Cette étude est un autre exemple des travaux en cours à l'Institut pour la recherche d'agents chimiques perturbant le système endocrinien dans l'environnement canadien.

ABSTRACT

Methods for the determination of bisphenol A (BPA) residues in municipal sewage and sludge samples have been developed. BPA in wastewater samples was enriched with a C_{18} solid phase extraction cartridge, eluted with acetone, and converted into its pentafluoropropionyl derivative. For sludge samples, BPA was acetylated and extracted by supercritical carbon dioxide. In both cases, BPA-d_{16} was used as a surrogate to monitor the extraction efficiency. Final analyses of derivatized sample extracts were carried out by gas chromatography/mass spectrometry operating in the electron impact mode. For water samples, the mean recoveries and standard deviations were 89±6%, 94±4%, and 85±7% at fortification levels of 1, 0.1, and 0.025 μg/L, respectively, with a method detection limit of 0.006 μg/L. For solid waste samples, the mean recoveries and standard deviations were 93±5% and 92±6% at fortification levels of 2.5 and 0.25 μg/g, respectively, and the method detection limit was 0.05 μg/g. For the Canadian samples under investigation, concentrations of BPA ranged from 49.9 to 0.031 μg/L in sewage influent and effluent and from 36.7 to 0.104 μg/g in sludge.

RÉSUMÉ

On a développé des méthodes pour le dosage des résidus de bisphénol A (BPA) dans les échantillons d'eaux usées et de boues d'installations municipales. On a enrichi le BPA des échantillons d'eaux usées à l'aide d'une cartouche d'extraction en phase solide de type C_{18}, puis on l'a élué avec de l'acétone et converti en dérivé pentafluoropropionyle. Dans le cas des échantillons de boue, on a acétylé le BPA et on l'a extrait avec du dioxyde de carbone supercritique. Dans les deux cas, on a utilisé du BPA-d_{16} comme substitut pour surveiller l'efficacité de l'extraction. Pour les analyses finales des dérivés des extraits tirés des échantillons, on a utilisé la combinaison chromatographie gazeuse/spectrométrie de masse en mode de bombardement électronique. Pour les échantillons d'eau, les taux de récupération moyens et les écart-types étaient de 89 ± 6 %, de 94 ± 4 % et de 85 ± 7 % à des teneurs d'enrichissement de 1, de 0,1 et de 0,025 µg/L, respectivement, et la limite de détection de la méthode était de 0,006 µg/L. Dans le cas des échantillons de matières solides, les taux de récupération moyens et les écart-types étaient de 93 ± 5 % et de 92 ± 6% à des teneurs d'enrichissement de 2,5 et de 0,25 µg/g, respectivement, et la limite de détection de la méthode était de 0,05 µg/g. Pour les échantillons canadiens à l'étude, les concentrations de BPA étaient comprises entre 49,9 et 0,031 µg/L dans les influents et les effluents d'eaux usées, et entre 36,7 et 0,104 µg/g dans les boues.

INTRODUCTION

Bisphenol A (BPA or 4,4'-isopropylidenediphenol) is manufactured in large quantities in the United States from the acid-catalyzed condensation of phenol with acetone. In recent years, the demand for BPA has increased steadily, from 0.73 billion kg in 1995 to 0.82 billion kg in 1998. By 2002, the projected demand will exceed 1 billion kg, representing a 5% annual growth [1]. BPA is the building block of many resins. About 63% of BPA produced today is used worldwide for the manufacture of polycarbonate plastic resins, 27% for epoxy resins, and the remaining 10% for miscellaneous products such as flame retardants (mainly tetrabromobisphenol A), unsaturated polyester, polysulfone, polyetherimide and polyarylate resins [1].

Aquatic toxicity studies of BPA indicated an acute toxicity (EC_{50}/LC_{50}) varying from 1 mg/L for algae (*Skeletonoma costatum*) to 15.5 mg/L for invertebrates (*Daphnia magna*) [2]. Several biodegradation studies demonstrated rapid breakdown of BPA under acclimated conditions. In one study, >90% degradation was observed within four days in a BPA chemical plant discharge and two other surface water samples [2]. Another study showed that BPA biodegraded rapidly (in a few hours) through the action of a gram-negative, aerobic bacterium (strain MV1) and nearly 60% of the carbon in BPA was mineralized to CO_2 [3]. This novel bacterium used BPA as a sole source of carbon and energy and was isolated from a sludge enrichment taken from a wastewater treatment plant at a plastic manufacturing facility. In contrast, studies performed under unacclimated conditions indicated less than 1% degradation in 28 days [4,5].

Recently, there has been a great deal of attention on the estrogenic activities of BPA, which were first identified by Dodds and Lawson [6]. These findings were later

confirmed by the work of Bitman and Cecil [7], Bond et al. [8], and Krishnan et al. [9]. In the last case, BPA was found to induce progesterone receptors in cultured human breast cancer cells (MCF-7) at a potency 5,000 times less than 17β-estradiol (E_2). BPA also binds to estrogen receptors with an affinity 2,000 times less than that of E_2, and is thus one of the more potent anthropogenic estrogen mimics [10]. As a result, there have been some concerns about the possibility of BPA leaching out from food packaging materials and beverage containers where polycarbonate plastic resins and epoxy resins have been used in food contact applications [11].

BPA may find its way into the environment through the thermal degradation of many plastic products, or from the discharge of BPA manufacturing or processing plants. Few analytical methods are available for the determination of BPA in environmental samples. A liquid chromatographic method with electrochemical detection was earlier developed for the determination of BPA in air [12]. More recently, a GC/MS method has been reported for the detection of BPA in water samples [13]. However, with a relatively high method detection limit of 0.6 μg/L, no BPA was found in any of the sea water and spring water samples tested [13]. No procedure has been published in the open literature for the extraction of BPA in solid waste samples. For these reasons, very few data on the occurrence and fate of BPA in sewage and sludge samples are presently available.

In order to establish a database on the occurrence of BPA and evaluate its environmental fate in Canada, new and more sensitive analytical methods are required. In the present study, methods based on established solid phase extraction (SPE) and supercritical fluid extraction (SFE) techniques in combination with gas chromatography/mass spectrometry (GC/MS) are described for the determination of BPA

in municipal sewage and sludge samples. This work is another example of our continuing research effort on the identification, occurrence, and fate of endocrine- disrupting chemicals in sewage samples [14-17].

EXPERIMENTAL

Chemicals and reagents

BPA (99+%), BPA-d_{16} (98 atom % D), pentafluoropropionic anhydride (PFPA, 99%), acetic anhydride, and formaldehyde (37% solution in water) were obtained from Aldrich Chemical Company. Acetic anhydride was triple-distilled before use. All other chemicals were used without further purification. Stock solutions of BPA (1000 μg/mL) and BPA-d_{16} (500 μg/mL) were prepared in acetone. Working solutions of these phenols at lower concentrations were also prepared in acetone by serial dilution. Celite and anhydrous potassium carbonate were the products of Fisher Scientific.

Distilled-in-glass grade solvents including acetone, dichloromethane (DCM), ethyl acetate, *iso*-octane, methyl *tert*-butyl ether (MTBE), and petroleum ether (b.p. 30-60°C) were purchased from Caledon or Burdick and Jackson. Supercritical carbon dioxide (SFE grade) without helium head pressure was manufactured by Air Products.

Sample collection and preservation

Composite (24-hr) samples of raw sewage and final effluent in 1 L aliquots were collected in late 1998 and early 1999. For sewage treatment plants located in southern Ontario, samples were kept at 4°C in the dark without any preservative as they were extracted within 24-hr after collection. For samples collected from plants elsewhere, 10

mL of the 37% formaldehyde solution were added to each sample at the time of collection. They were kept cold in transit (by overnight courier) and kept at 4°C in the dark at the laboratory. Grab samples of raw and digested sewage sludge were unpreserved. They were air-dried, pulverized, passed through a 100-mesh sieve, and kept at room temperature.

(*Caution*: Because of the presence of bacteria, viruses, and parasites that may pose a health hazard to workers, protective equipment must be used for the collection and handling of sewage samples. Workers should also have proper immunization as recommended by the local health authorities.)

Extraction, cleanup, and derivatization procedures

(1) Solid-phase extraction of effluent samples

Each sewage sample was filtered through a 47-mm Whatman GF/C filter with a pore size of 1.2 μm connected to an all-glass funnel support assembly. In this process, a filter aid such as Celite 545 was used to minimize plugging of the filter. An aliquot of the sample (typically 250 mL) was measured, 500 ng of BPA-d_{16} was added as a surrogate, and the mixture was acidified with 1 N HCl to pH 3.

Prior to extraction, each C_{18} SPE cartridge (ENVI-18, Supelco, 505706) was conditioned with 5 mL of acetone, 5 mL of methanol, and 10 mL of pH 3 water on an SPE manifold (Supelco Visiprep DL 5-7044) as per manufacturer's instructions. The filtered sample was then applied to the cartridge via a siphon tube and an adaptor (Supelco 5-7275). An average flow rate of 10 mL/min was maintained by adjusting the vacuum to ca. -15 in Hg. When the extraction was completed, the cartridge was dried under vacuum

for 5 min. A 5 mL aliquot of acetone-water (1:4, v/v), in 2 equal fractions, was used to rinse the cartridge, and the washes were discarded. BPA was removed from the cartridge by eluting with 10 mL of acetone.

The acetone extract was gently evaporated, by a stream of nitrogen on a water bath of 40°C, to 200 - 300 µL, at which point the residue was mainly water. BPA was then back extracted into three 2 mL aliquots of ethyl acetate. The combined ethyl acetate layer was filtered through a 3 cm Celite column prepared in a disposable Pasteur pipet. (*Caution*: The use of anhydrous sodium sulfate at this point could lead to a lower recovery due to adsorption.) The extract was evaporated to 200 µL before it was applied to a cleanup column packed with 5 cm of 5% deactivated silica gel in a disposable Pasteur pipet that had been prewetted with 3 mL of petroleum ether. The column was then eluted with 10 mL of 1+2 (v/v) acetone/hexane for the removal of BPA. (Note: The silica gel cleanup step can be omitted for less contaminated samples such as those from some surface waters.)

The acetone/hexane extract was evaporated to dryness. The phenol was derivatized with 50 µL of PFPA in the presence of 50 µL of ethyl acetate at room temperature. After 20 minutes, 3 mL of petroleum ether and 3 mL of 1% K_2CO_3 were added to remove excessive reagent and acids. The mixture was vortexed and the upper layer was removed and extraction of the aqueous layer was repeated twice with 3 mL aliquots of petroleum ether. The combined organic layer was passed through a column of anhydrous sodium sulfate in a Pasteur pipet, evaporated, and exchanged into1 mL of iso-octane for GC/MS analysis. Calibration standards of the PFP derivative of BPA were

prepared by reacting known amounts of the authentic standard with PFPA as described above.

(2) Supercritical carbon dioxide extraction of sludge

BPA in sludge was extracted and acetylated by an SFE procedure similar to that developed for the determination of nonylphenol in sewage sludge [14], by the use of a Hewlett-Packard 7680T extractor. Prior to extraction, 50μL of a 10 μg/mL BPA-d_{16} solution was spiked to a 100 to 250 mg sample as a surrogate standard. Acetic anhydride (200 μL) and triethylamine (30 μL) were also added to the sludge to facilitate the in situ acetylation. The sample was extracted at 80°C with non-modified supercritical carbon dioxide of a density of 0.79 g/mL (pressure 37 MPa). The static and dynamic extraction times were 15 and 10 min, respectively, and the flow rate of CO_2 was 2 mL/min during the dynamic extraction. At the end of the extraction, the BPA diacetate adsorbed on the ODS trap was eluted with 1.7 mL and 1.0 mL of acetone in two rinses.

The combined sludge extract in acetone was evaporated to dryness and then 3 mL of petroleum ether and 3 mL of 1% K_2CO_3 were added. The mixture was shaken in a vortex mixer for 1 min and the upper organic layer was removed. A centrifuge was used when an emulsion formed. Extraction of the aqueous layer was repeated twice, with 3 mL aliquots of petroleum ether. The combined organic layer was passed through an anhydrous sodium sulfate column and evaporated to ca. 1 mL.

The concentrated extract containing BPA diacetate was cleaned up on a 5 cm 5% deactivated silica gel column prepared in a disposable Pasteur pipet. After wetting the column with 3 mL of petroleum ether, the extract was applied to the column, and the

derivatized phenol was eluted with 10 mL of DCM. The latter was evaporated and exchanged into 1 mL of iso-octane for GC/MS analysis.

Calibration standards for the acetate derivative of BPA were prepared by acetylating known amounts of the authentic standard (no solvent) with acetic anhydride (200 μL) and pyridine (10 μL) at 60°C for 30 min. Although a silica gel column cleanup was not needed, the reaction products were washed with 1 % K_2CO_3 as described before for the PFP derivatives.

(3) Accelerated solvent extraction (ASE) of sludge

ASE of BPA from sludge samples was carried out with a Dionex ASE 200 extractor. Typically, 0.25 g of a sample and the surrogate standard, placed in a 22-mL stainless steel extraction cell, were extracted with a 1+1 (v/v) mixture of DCM and acetone in 3 cycles. The oven temperature was 100°C and the pressure was 12.7 MPa or 1800 psi. The oven heat-up time was 5 min and the static time was 10 min. Flush volume was 75% of the extraction cell volume.

An aliquot of the extract was evaporated to dryness and redissolved in 200 μL of ethyl acetate. It was then cleaned up on a 5% deactivated silica gel column and derivatized with PFPA as described above for effluent samples.

Quantification procedure by gas chromatography/mass spectrometry

GC/MS analyses of BPA derivatives were carried out with a Hewlett-Packard 6890 gas chromatograph equipped with a 5973 Mass Selective Detector operating in the electron impact mode. A 30 m x 0.25 mm ID HP-5-MS column with a 0.25 μm film thickness was used for chromatographic separation. GC conditions for the PFP

derivatives were: initial oven temperature, 70°C; initial time, 1.0 min; programming rates, from 70°C to 210°C at 30°C/min, and then to 240°C at 2°C/min. A post-analysis baking at 270°C for 4 min was also applied to the column. Carrier gas (helium) flow rate was held constant at 1.1 mL/min with a linear velocity of 39 cm/s. For the acetate derivatives, the conditions were: initial oven temperature, 70°C with a 1-min hold; programming rates, from 70°C to 200°C at 30°C/min, and then to 260°C at 5°C/min with a 4-min hold at the final temperature. Constant carrier gas (helium) flow rate was set at 0.9 mL/min with a linear velocity of 35 cm/s. In both cases, splitless injections (1 μL) were made by a Hewlett-Packard 7683 autosampler with a splitless time of 1 min.

The detector was tuned with perfluorotributylamine (PFTBA) using the autotune program. The electron energy was 70 eV and the electron multiplier was operating at 200 V above the autotune value with the high energy dynode on. Temperatures for the MS source and quadrupole were 230°C and 150°C, respectively. Full scan mass spectra were recorded from m/z 50 to 550. The following ions were used for quantitative selected ion monitoring (SIM) work on the PFP derivatives: m/z 520 and 505 (BPA) and m/z 534 and 516 (BPA-d_{16}). In the case of the acetate derivatives, the ions of m/z 312 and 228 (BPA) and m/z 326 and 242 (BPA-d_{16}) were used.

Fortified samples were quantitated, by external standard method, against a derivatized standard containing the same amount of BPA as the spiked samples. Sewage effluent and sludge samples were quantitated also by external standard method using a 2-point standard curve (50 and 250 pg/μL). Sample extracts with BPA concentrations above 250 pg/μL were diluted and rerun.

RESULTS AND DISCUSSION

Pentafluoropropionyl and acetyl derivatives of BPA and their MS properties

In this work, pentafluoropropionyl (PFP) and acetyl derivatives have both been used for the determination of BPA. In addition to better chromatography, another advantage of derivatizing BPA prior to GC/MS analysis is higher detector selectivity. The latter was achieved by shifting the more volatile derivatives to shorter retention times and concurrently monitoring characteristic ions at higher masses. Combination of the two would generally lead to reduced interference in the final analysis. This approach is particularly beneficial for the PFP derivatives of BPA and its labeled analog, of which intense characteristic ions above m/z 500 were monitored (ca. 300 over the parent compound) at relatively low elution temperatures.

The PFP derivative was used for the effluent samples because it offered better sensitivity than the acetyl derivative. The latter was selected for sludge samples so that the method would be backward compatible with a previously developed, in situ acetylation SFE procedure for the determination of nonylphenol in the same type of matrix. If another extraction technique (e.g. Soxhlet, etc.) was selected for use with sludge samples, an off-line derivatization procedure with PFPA may be used instead.

PFPA reacted readily with the unlabeled and labeled BPA at room temperature and, in both cases, a single product was formed. The relative standard deviation (as determined by the peak area in full-scan GC/MS runs) of a triplicate derivatization of BPA at the 10 μg level was 5.4%. Solutions of the BPA derivatives in isooctane were stable for at least 4 weeks at -20°C in the dark.

The EI mass spectrum for the PFP derivative of BPA (Figure 1A) displayed a molecular ion (M^+) at m/z 520, indicating that both phenolic groups were acylated. Other

characteristic ions observed included m/z 505 or $(M-CH_3)^+$ (base peak), m/z 281, $(M-239)^+$ or $(M-C_6H_4OCOC_2F_5)^+$, as well as m/z 265, a fragment arising from a neutral loss of CH_4 from the m/z 281 species. Similarly, the PFP derivative of the labeled BPA (Figure 1B) displayed characteristic ions at m/z 534 (M^+), 516 $(M-CD_3)^+$ (base peak), 291 $(M-C_6D_4OCOC_2F_5)^+$ or $(M-243)^+$, and 271 $(M-243-CD_4)^+$.

Acetylation of BPA and BPA-d_{16} also produced, in each case, a single, stable product with a diacetate structure, as indicated by an M^+ occurring at m/z 312 and 326, respectively. Other characteristic ions of BPA diacetate (Figure 2A) included $(M-CH_2CO)^+$ at m/z 270, $(M-CH_2CO-CH_2CO)^+$ at m/z 228, and $(M-CH_2CO-CH_2CO-CH_3)^+$ (base peak) at m/z 213. Similarly, characteristic ions at m/z 281, 242, and 224 (base peak) derived from the same fragmentation pattern were observed for the acetyl derivative of the labeled compound (Figure 2B). It should be noted that while the $(M-CH_3)^+/(M-CD_3)^+$ ion was the base peak in the EI spectrum for the PFP derivative of BPA/BPA-d_{16}, the same fragment was barely observable for the acetyl analogs.

Detector linearity and quantitation limit

Linearity of the Mass Selective Detector response to the PFP derivative of BPA was assessed by injecting standard solutions in isooctane of the following concentrations: 1000, 250, 100, 25, and 2.5 pg/μL. In SIM mode with two ions monitored (m/z 505 and 520), the detector response was linear (r^2=0.998) over the range from 2.5 to 250 pg injected. The quantitation limit was better than 0.4 pg injected (S/N ratio 10:1). For the acetate derivative of which ions of m/z 312 and 228 were monitored, the detector response was linear (r^2=0.999) over the range from 10 to 500 pg injected. The

quantitation limit was 2.7 pg injected (S/N ratio 10:1). The detector sensitivity for BPA diacetate could have been improved if the ion m/z 213 (base peak, Figure 2A) had been used in the SIM runs. However, it was not used owing to the observation of interference from this ion in the analysis of sludge extracts.

Validation of the preconcentration procedures

(1) SPE of water samples

Recovery of BPA from distilled water using the SPE procedure was obtained, in replicates at three spiking levels (Table 1). At concentrations of 1, 0.1, and 0.025 μg/L, the mean recovery of BPA was between 85 and 94 %. The mean recovery of BPA-d_{16} at the constant spiking level of 2 μg/L was 91%. Based on the results obtained at the lowest spiking level (0.025 μg/L), the calculated method detection limit [18] at 0.006 μg/L (MDL=S x t where S=0.0018 μg/L, and t=3.14 for 7 replicates) was 100 times better than that (0.6 μg/L) reported earlier [12]. Recovery experiments have not been performed on sewage effluents, due to the lack of a field sample with a low BPA blank. However, replicate extraction of some wastewater samples generated results with good precision (Table 2). Consistent recovery (a range from 87 to 107%) of the surrogate, BPA-d_{16}, for all effluent samples (n=47) also implied good accuracy of the procedure.

The performance of the present SPE procedure was also evaluated against a classical solvent extraction procedure using MTBE. In this case, the aqueous solubility of BPA at room temperature (22 to 24°C) was determined by replicate extraction of a saturated solution (after dilution) using these two procedures. In the literature, a solubility of 120-300 mg/L has been cited for BPA. In this work, the solubility of BPA was 253 ±

14 mg/L (n=4) as determined by the SPE technique and 257 ± 9 mg/L (n=3) as determined by the solvent extraction technique. Since the numbers are basically the same within experimental errors, it was concluded that SPE is as effective as solvent extraction for the preconcentration of dissolved BPA in aqueous samples.

(2) SFE of solid samples

Although the technique has not been widely used, the in situ acetylation of phenols under supercritical carbon dioxide extraction conditions is a very efficient method for the determination of chlorinated phenols in sediment and soil [19,20], and of alkylphenols in sewage sludge [14]. In these cases, phenolic compounds are extracted by supercritical carbon dioxide and at the same time acetylated in the presence of small amounts of acetic anhydride and triethylamine. At the beginning of this work, we attempted the extraction of BPA from sewage sludge by applying the procedure developed previously for the determination of 4-nonylphenol and 4-*tert*-octylphenol in the same matrix. While the results were encouraging as BPA was converted into its diacetylated derivative, the recoveries for BPA and the deuterated surrogate in spiked samples were only 45 to 80%. Longer extraction times and different extraction temperatures (60 or 100°C) did not improve the yield. Although the unextracted BPA could be recovered by a second extraction with fresh reagents, it was more conveniently accomplished by using excess acetic anhydride (200 μL) at the start of the extraction. When spiked to a previously solvent extracted sediment sample, the mean % recoveries of BPA were over 90% at fortification levels of 2.5 and 0.25 μg/g (Table 1). The calculated method detection limit was 0.05 μg/g (0.016 μg/g x 3.14).

The efficiency of the SFE procedure has also been compared to a solvent extraction procedure using an accelerated solvent extractor (ASE). In the latter case, a generic procedure designed for the extraction of priority pollutants (bases, neutral, and acids) was used [21]. Based on the results of replicate extraction of two sewage sludge samples (Table 2), it was concluded that both SFE and ASE produced comparable results for the determination of BPA in sludge samples.

Cleanup and procedure blank

Due to the high level of contamination in sewage samples, cleanup steps were necessary to reduce interference in the final analysis of the target contaminant. For influent and effluent samples, removal of the more polar coextractives such as dyes and pigments was achieved by elution of the ODS cartridge with a 1+4 mixture of acetone and water prior to the elution of BPA with acetone. The BPA extract was further cleaned up by a 5% deactivated silica gel column before chemical derivatization. The same column was also used for the cleanup of the SFE extracts of sludge samples. Procedure blanks were determined with organic-free water (SPE) and high-purity celite (SFE). No BPA was detected in those cases.

Applications to environmental samples

These newly developed methods have been applied to the determination of BPA in municipal wastewater and sludge samples in Canada since late 1998. To date, BPA has been detected in all 24-hr composite influent and effluent samples collected from sewage treatment plants with both primary (sedimentation) and secondary (activated sludge)

treatment facilities. While the levels of BPA in these wastewater samples ranged from 49.9 to 0.031 μg/L (n = 47 from 14 sewage treatment plants across Canada), they were generally below 1 μg/L in the influent and below 0.3 μg/L in the effluent. These results also indicated that the % reduction (from influent to effluent) of BPA ranged from 37 to 94% among the treatment plants. BPA has also been detected in all grab samples of raw and digested sewage sludge (n=51) collected from 18 plants, with an overall concentration range from 36.7 to 0.104 μg/g on a dry weight basis. A few examples of BPA levels are listed in Table 3. Total ion current chromatograms of derivatized sample extracts are shown in Figures 3A (influent) and 3B (sludge). The same procedures are also being applied to the monitoring of BPA in industrial wastewater as well as pulp and paper mill effluents and sludge. A detailed description of the occurrence and fate of BPA in the Canadian environment will be reported elsewhere.

Conclusions

We have developed new analytical methods for the determination of BPA in sewage effluent and sludge samples. The use of SPE and SFE techniques in the preconcentration steps has been shown to be time- and solvent-efficient. They have also produced quantitative recoveries for BPA at residue levels. Analysis of the PFP and acetyl derivatives of BPA by GC/MS is more sensitive and selective than existing methods without chemical derivatization. These methods have been successfully applied to ca. 100 samples and the results have suggested that this estrogenic compound is ubiquitous in Canadian municipal wastewater and sludge.

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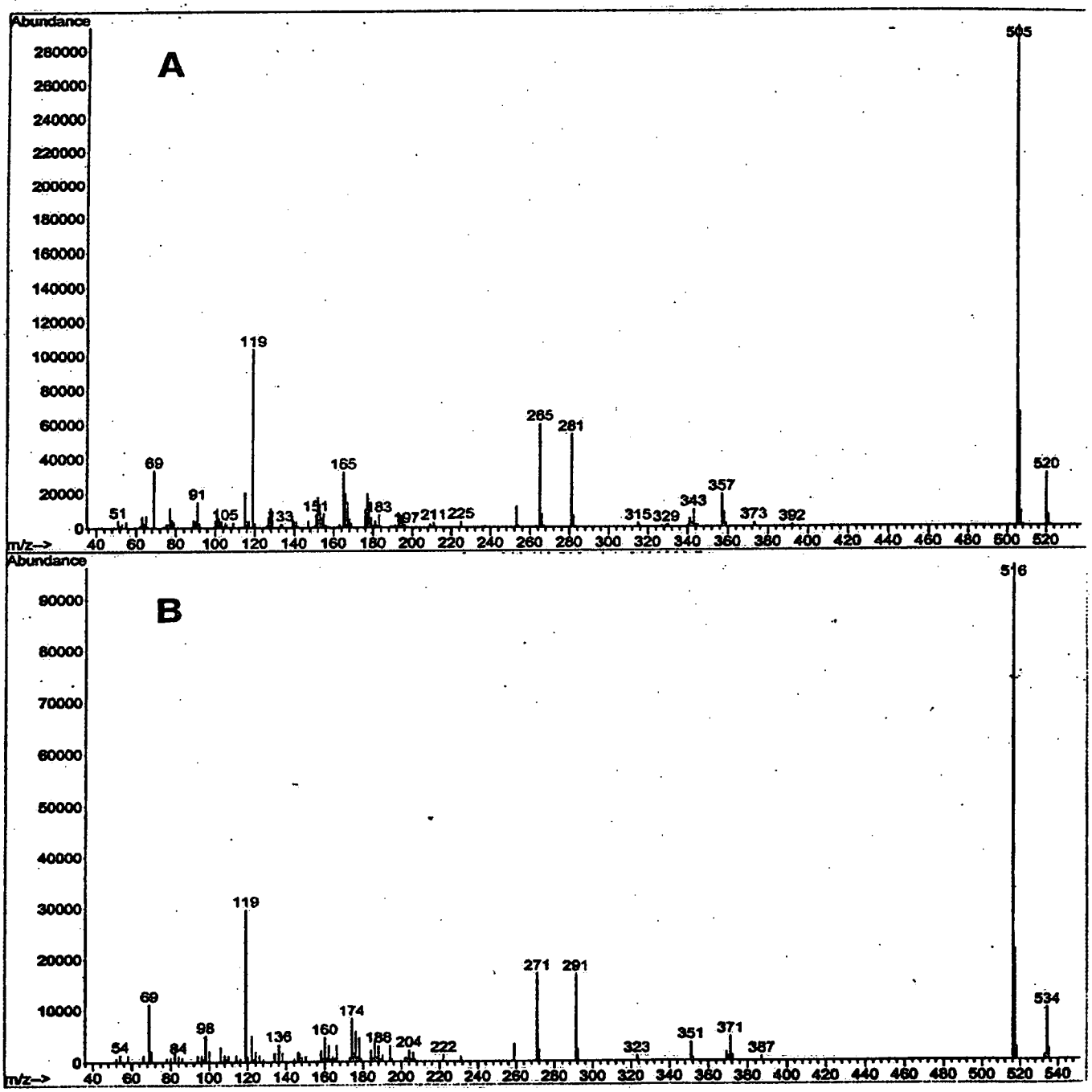
FIGURE CAPTIONS

Figure 1. Full-scan mass spectra of the PFP derivatives of BPA (A) and BPA-d_{16} (B).

Figure 2. Full-scan mass spectra of the acetyl derivatives of BPA (A) and BPA-d_{16} (B).

Figure 3. Extracted ion chromatograms of the extracts of an influent from Burlington (A) and a sludge from Toronto (B), with BPA levels at 0.193 μg/L and 12.5 μg/g, respectively. Peaks: 1=BPA-PFP, 2=BPA-d_{16}-PFP, 3=BPA-diacetate, 4=BPA-d_{16}-diacetate.

Figure 1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285
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91
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151
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211
225
315
329
373
392
B
Abundance
m/z-->
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291
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174
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136
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188
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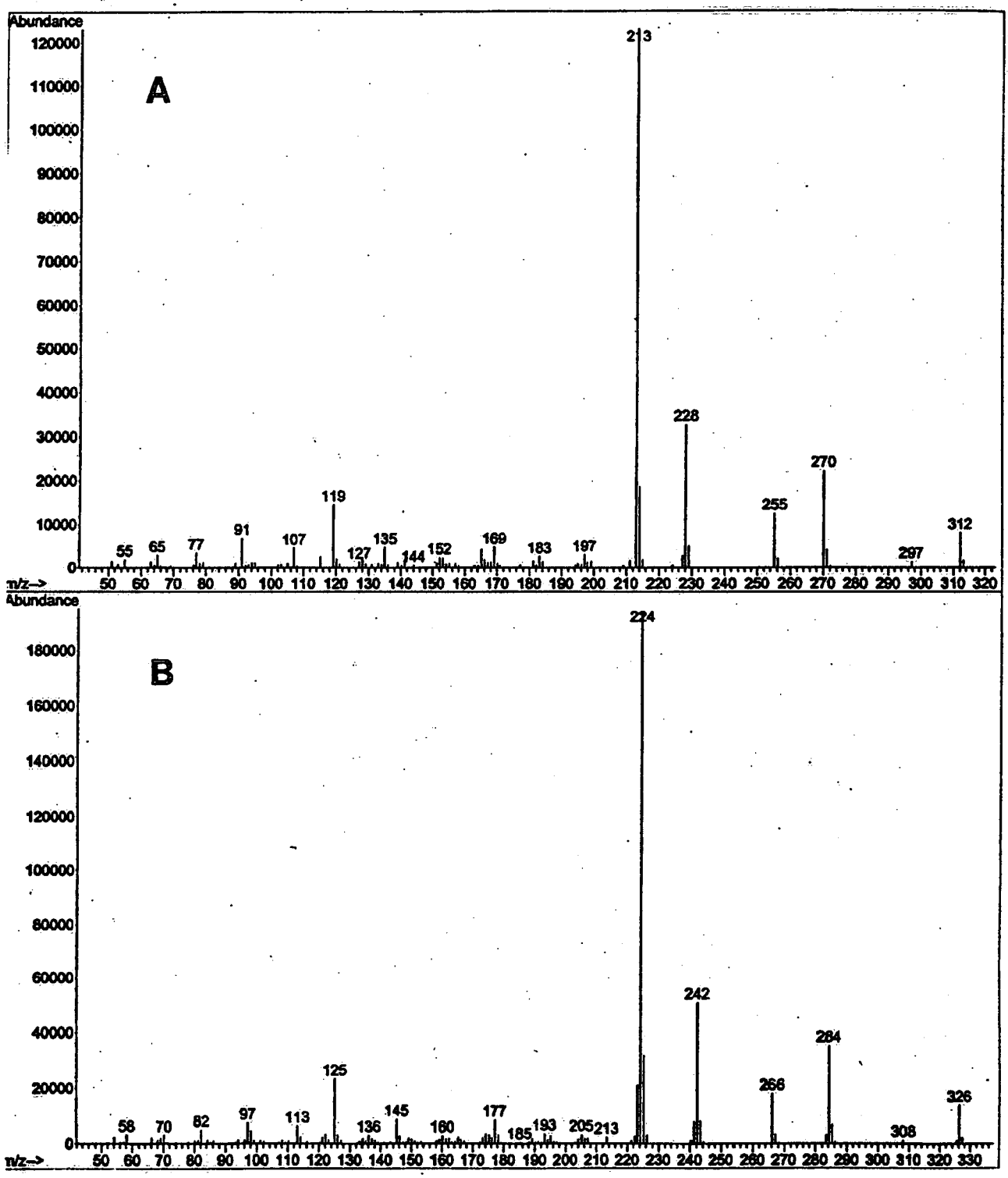
222
54
84
323
351
371
387

Figure 2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144
152
m/z-->
B
Abundance
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242
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308
m/z-->

Figure 3

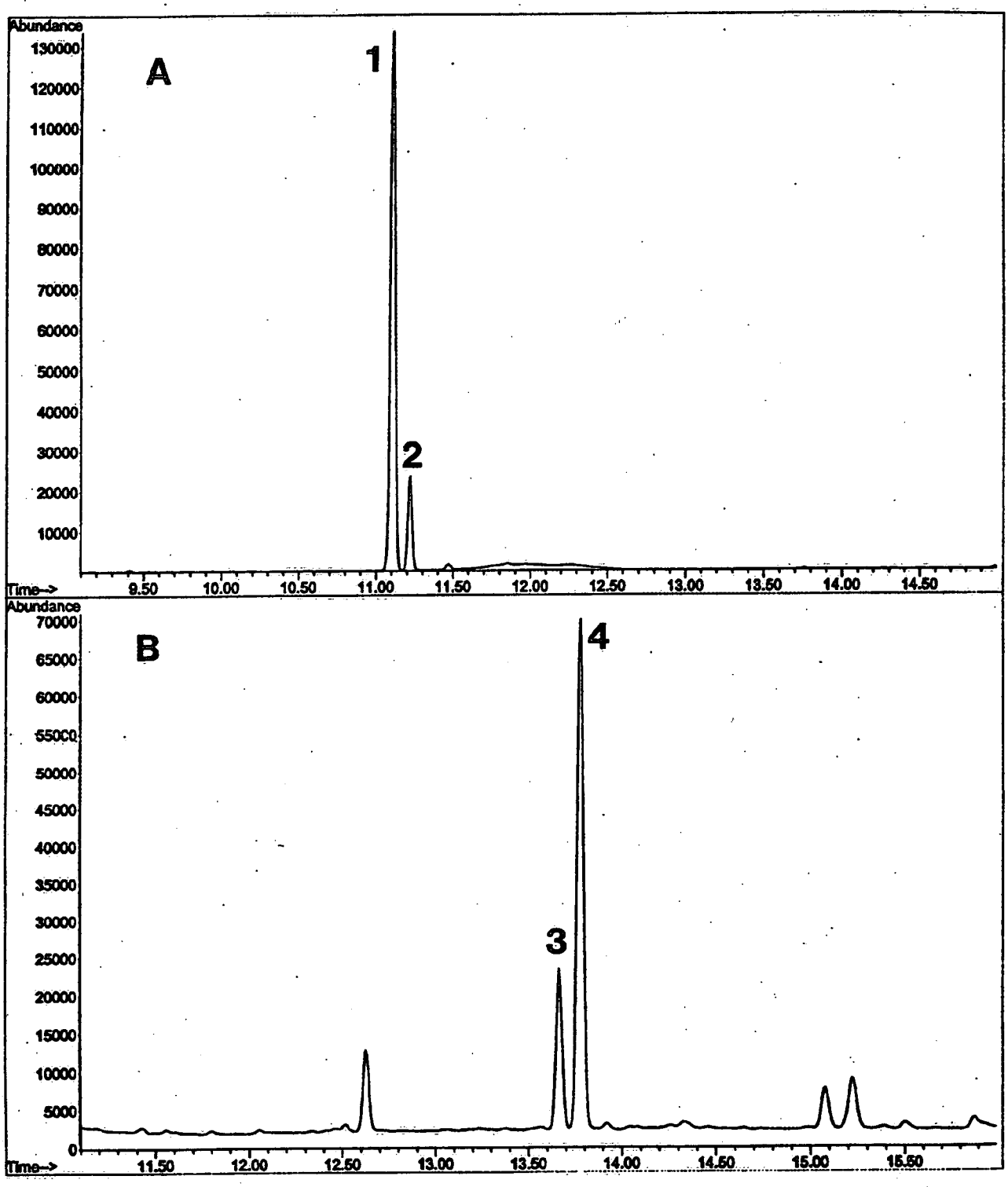

Table 1. Recovery of BPA and BPA-d_{16} from fortified samples.

Sample	No. of replicates	BPA spiking level	BPA recovered, mean (%)	S.D.	BPA-d_{16} recovered, mean (%)	S.D.
Distilled water	4	1.0 µg/L	0.89 µg/L (89%)	0.063 µg/L	1.81 µg/L (90%) [a]	0.055 µg/L
Distilled water	4	0.10 µg/L	0.094 µg/L (94%)	0.0044 µg/L	1.90 µg/L (95%) [a]	0.096 µg/L
Distilled water	7	0.025 µg/L	0.021 µg/L (84%)	0.0018 µg/L	1.74 µg/L (87%) [a]	0.088 µg/L
Sediment	7	2.5 µg/g	2.33 µg/g (93%)	0.125 µg/g	4.80 µg/g (96%) [b]	0.24 µg/g
Sediment	7	0.25 µg/g	0.23 µg/g (92%)	0.016 µg/g	4.73 µg/g (94%) [b]	0.28 µg/g

[a] Surrogate spiking level 2 µg/L
[b] Surrogate spiking level 5 µg/g

Table 2. Mean levels of BPA and % recovery of BPA-d_{16} in field samples by various extraction procedures. No. of replicate extraction = 3.

Sample [a]	BPA found, mean ± S.D.	Mean % recovery ± S.D. of BPA-d_{16}
A (influent by SPE)	4.99 ± 0.04 µg/L	105 ± 2 % [c]
B (effluent by SPE)	0.277 ± 0.003 µg/L	103 ± 2 % [c]
C (sludge by SFE)	1.51 ± 0.09 µg/g [b]	88 ± 7 % [d]
C (sludge by ASE)	1.36 ± 0.02 µg/g [b]	98 ± 2 % [d]
D (sludge by SFE)	36.7 ± 1.6 µg/g [b]	93 ± 5 % [d]
D (sludge by ASE)	35.8 ± 1.8 µg/g [b]	97 ± 3 % [d]

[a] Samples A and B were collected from Vancouver, BC, C from Toronto, ON, and D from Guelph, ON. The above samples were selected to validate the procedures since they covered a wide range of BPA levels found in field samples.

[b] On a dry weight basis.

[c] Surrogate spiking level 2 µg/L.

[d] Surrogate spiking level 5 µg/g.

Table 3. Levels of BPA in municipal sewage treatment plant samples.[a]

Plant [b]	Sampling date	Influent, µg/L	Final effluent, µg/L	Raw sludge, µg/g [c]	Digested sludge, µg/g [c]
Burlington	99/1/11	0.193	0.031	N/A [d]	0.316
Calgary A	99/3/02	0.278	0.147	N/A	0.789
Calgary B	99/3/02	0.219	0.035	N/A	0.795
Galt	99/5/13	0.954	0.201	N/A	1.59
Toronto A	99/5/13	0.232	0.048	0.197	0.268
Toronto B	99/5/31	2.44	0.112	8.73	12.5
Toronto C	99/5/31	0.405	0.183	N/A	1.13
Toronto D	99/6/01	0.889	0.223	1.72	N/A

[a] All influent and effluent are 24-hr composite samples. All sludge are grab samples.
[b] There are four sewage treatment plants in Toronto and two in Calgary.
[c] On a dry weight basis.
[d] N/A: Sample not available.

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YOUR COPY

September 15, 1999

Dear Sir/Madam:

I am pleased to present you with a copy of the Aquatic Ecosystem Protection Branch (AEPB) annual report. It gives an overview of the research carried out in 1998-99 by AEPB and lists our most recent publications.

We hope that our report will be useful to you and your organization.

Yours sincerely,

R.J. Maguire

Branch Director

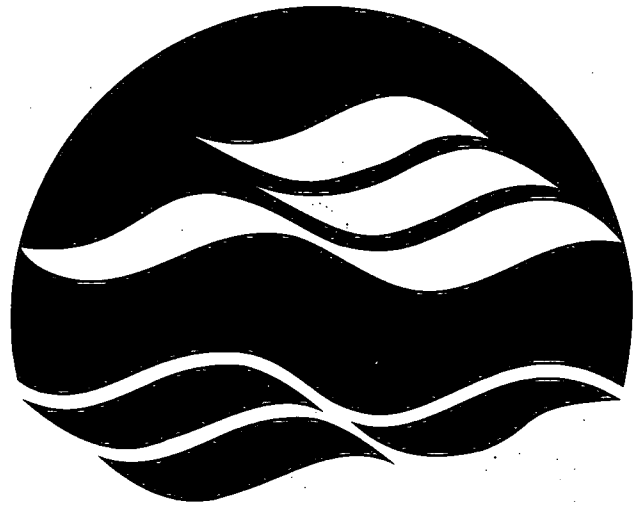
le 15 septembre 1999 Original on File

Monsieur/Madame,

Veuillez trouver ci-joint le rapport annuel de la Direction de la protection des écosystèmes aquatiques. Le rapport dresse un bref aperçu de la recherche effectuée en 1998-99 par la Direction. Vous y trouverez aussi la liste des publications récentes de nos chercheurs. J'espère que ce rapport vous sera utile.

Je vous prie d'agréer l'expression de mes sentiments les meilleurs.

Le directeur de la Direction



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