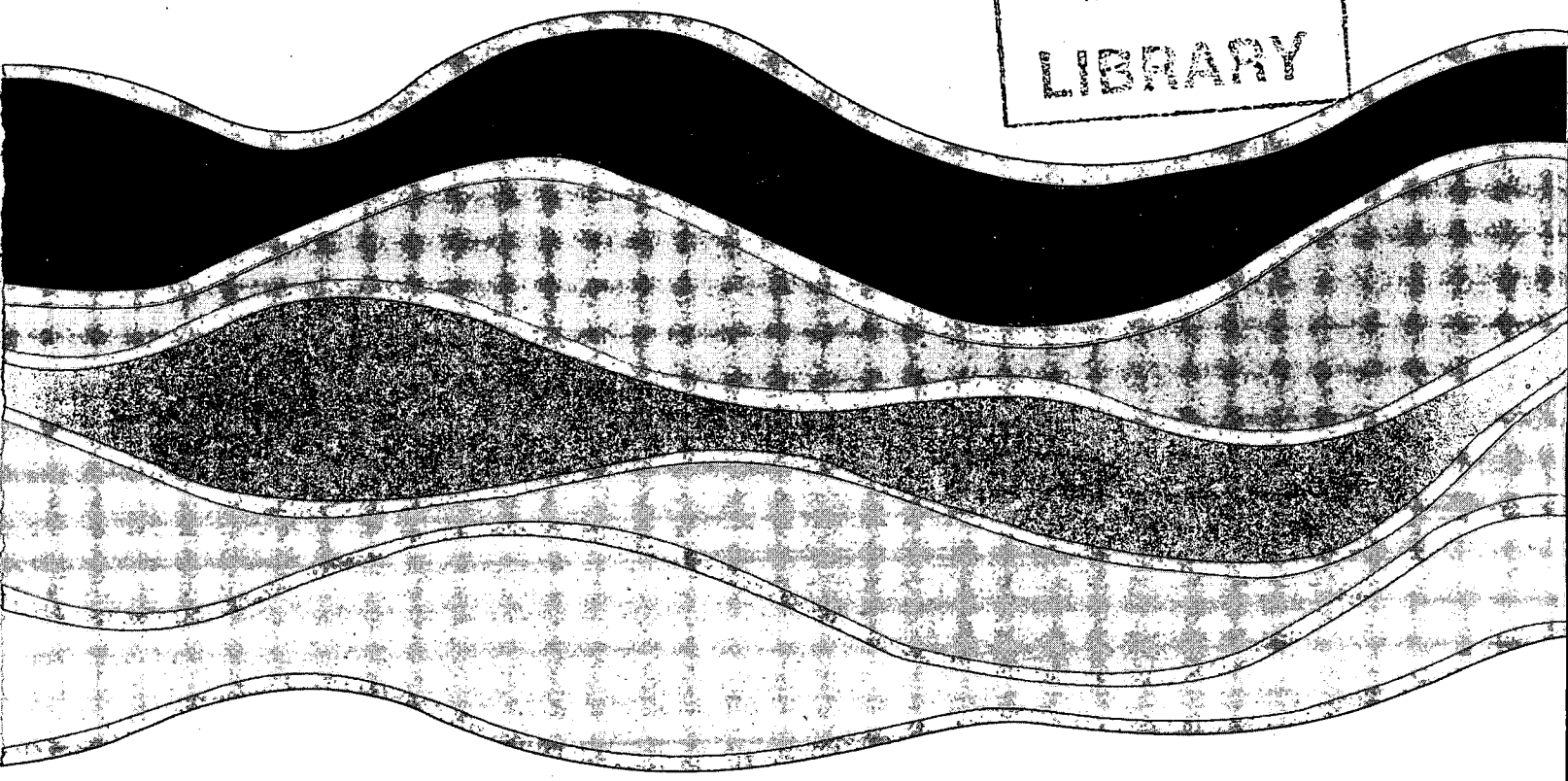
97-88 C.1

NATIONAL WATER RESEARCH INSTITUTE

INSTITUT NATIONAL de RECHERCHE sur les EAUX

CCIW
MAY 8 1997
LIBRARY



SYNTHESIS OF 4-TERT-OCTYLPHENOXYACETIC ACID AND ITS DETERMINATION IN CANADIAN SEWAGE TREATMENT PLANT EFFLUENTS

H.B. Lee, J. Weng, T.E. Peart and R.J. Maguire

NWRI Contribution No. 97-88

Synthesis of 4-*tert*-Octylphenoxyacetic Acid and Its Determination in Canadian Sewage Treatment Plant Effluents

by

Hing-Biu Lee[1], Jianhua Weng[2], Thomas E. Peart[1] and R. James Maguire[1]

[1]Aquatic Ecosystem protection Branch
National Water research Institute
Environment Canada
P.O. Box 5050
Burlington, Ontario L7R 4A6
Canada

[2]Water Quality Research Centre
China Institute of Water Resources and Hydropower Research
P.O. Box 366
Beijing 100044, China

Management Perspective

Residues of non-ionic surfactants such as alkylphenol ethoxylates and their metabolites in the environment have become a concern of late. These residues, readily found in the municipal sewage treatment plant effluents, are not only toxic to certain aquatic organisms but also weakly estrogenic and behave as endocrine disrupters. This report describes the synthesis of a metabolite of 4-*tert*-octylphenol ethoxylates and its determination in sewage effluents. This research supplement our current work on the assessment of the occurrence, fate and pathway of nonylphenol ethoxylates (a PSL 2 substance) in the Canadian environment.

Sommaire à l'intention de la Direction

Assez récemment, on a commencé à se soucier de la présence dans l'environnement de résidus d'agents tensio-actifs non ioniques tels que les éthoxylates d'alkylphénols et leurs métabolites. Ces résidus, souvent présents dans les effluents des usines d'épuration des eaux usées, sont non seulement toxiques pour certains organismes aquatiques, mais ont aussi de légères propriétés d'oestrogènes et se comportent comme des perturbateurs du système endocrinien. Cet article décrit la synthèse d'un métabolite des éthoxylates du 4-*tert*-octylphénol et leur dosage dans des effluents d'eau d'égout. Cette recherche s'ajoute á nos travaux en cours sur l'évaluation de la présence, du devenir et des voies d'acheminement des éthoxylates de nonylphénols (une substance figurant sur la LSIP2) dans l'environnement canadien.

Abstract

4-*tert*-Octylphenoxyacetic acid (4-tOP1EC) is a metabolite of a non-ionic surfactant derived from the ethoxylation of 4-*tert*-octylphenol (4-tOP). Although the occurrence of 4-tOP1EC was suspected in many Canadian sewage treatment plant (STP) effluents due to the observation of 4-tOP in those samples, its presence has never been reported due to the lack of an authentic standard. In this work, 4-tOP1EC was synthesized by the condensation of 4-tOP and chloroacetic acid in the presence of a base. With this standard, the occurrence of 4-tOP1EC in Canadian STP effluents was confirmed for the first time. The level of this acid ranged from 0.18 to 1.2 μg/L and from 0.45 to 6.8 μg/L, in the primary and final effluents, respectively.

Résumé

L'acide 4-*tert*-octylphénoxyacétique (4-tOP1EC) est un métabolite d'un agent tensio-actif non ionique dérivé de l'éthoxylation du 4-*tert*-octylphénol (4-tOP). Au Canada, on suspectait la présence du 4-tOP1EC dans les effluents de bon nombre d'usines d'épuration des eaux usées car on observait celle du 4-tOP dans les échantillons; cependant, sa présence n'a jamais été signalée faute de disposer d'un véritable étalon. Pour la présente étude, le 4-tOP1EC a été synthétisé par condensation du 4-tOP et de l'acide chloracétique en présence d'une base. Avec cet étalon, il nous a été possible de confirmer pour la première fois la présence de 4-tOP1EC dans les effluents d'usines d'épuration des eaux usées. La concentration de cet acide était comprise entre 0,18 et 1,2 µg/L et entre 0,45 et 6,8 µg/L dans les effluents primaires et terminaux, respectivement.

INTRODUCTION

Non-ionic surfactants such as alkylphenol ethoxylates (APnEO) are widely used in the world for many applications. The more common members, nonylphenol ethoxylates (NPnEO) and octylphenol ethoxylates (OPnEO), have a branched 4-nonyl or 4-octyl alkyl side chain and one to 20 or more ethoxy units. In Canada, NPnEO are mainly used by the pulp and paper industries in the pulping, paper making and deinking processes and by the textile industries in the degreasing, scouring and other processes[1,2]. Although their application in many household products was voluntarily abandoned, high concentrations of NPnEO are found in the effluents of many Canadian municipal sewage treatment plants (STPs) [3]. 4-*tert*-Octylphenol ethoxylates and their chlorinated derivatives have also been identified as contaminants in the Delaware River [4].

Under aerobic and anaerobic sewage treatment conditions, NPnEO degraded into 4-nonylphenol (4-NP), nonylphenol monoethoxylate (NP1EO), diethoxylate (NP1EO), as well as nonylphenoxyacetic acid (NP1EC) and nonylphenoxyethoxyacetic acid (NP2EC) [5]. The occurrence of NP1EC and NP2EC at μg/L levels, particularly in the final STP effluents, has been well documented [5-7]. According to Ahel *et al.*, the level of NP1EC and NP2EC in the final effluent represents as much as 46% of the total nonylphenolics [5]. Recently, the presence of these nonylphenolic compounds in STP, paper mill, textile mill effluents and other environmental samples, has been a concern because these compounds are known as endocrine disrupters [8,9]. Although much lower

in estrogenic potency relative to 17ß-estradiol [10,11] the presence of these compounds has been suspected as a cause for the feminization of male fish downstream of STPs [12].

Albeit to a much smaller extent, the occurrence of a weakly estrogenic 4-*tert*-octylphenol (4-tOP) in Canadian STP effluent has also been reported [3,13]. In many cases, the 4-tOP level in the environment is only 10%, or less, of 4-NP in the same sample. There were, however, no data for the occurrence of 4-*tert*-octylphenoxyacetic acid (4-tOP1EC) in the environment, presumably due to the lack of a commercially available standard. For the same reason, the toxicity and estrogenicity of 4-tOP1EC are unknown, although 4-tOP has been estimated to be over 40 times higher in estrogenic potency than 4-NP or NP1EC using rainbow trout *in vitro* hepatocyte bioassay data [9].

Several approaches have been used for the determination of NPnEC (n=1 to 4) in effluents. The acids were either solvent extracted or preconcentrated on a solid phase extraction (SPE) cartridge or disk with various adsorbents such as graphitized carbon black [7], octadecylsilane (ODS) modified silica gel [14], or a strong anion exchange resin [15]. For GC/MS determination, the acids were first methylated and then the analysis could either be carried out by electron impact [6] or by chemical ionization mass spectrometry [15]. In the case of LC determination, fluorometric detection [7] as well as atmospheric pressure ionization (API) [7] and particle beam [16] mass spectrometry have been used.

Previously, the synthesis of 4-tOP1EC has been briefly mentioned, yet, the exact reaction conditions and details were lacking [6]. In this work, we describe a detailed procedure for the synthesis of 4-tOP1EC and an analytical method for its

determination in effluent samples. We also report, for the first time, the occurrence of 4-tOP1EC in the effluents of several Canadian STPs.

EXPERIMENTAL

Reagents and chemicals

4-*tert*-octylphenol [4-(1,1,3,3-tetramethylbutyl)phenol)] and chloroacetic acid were products of Aldrich Chemicals. Silica gel (40 - 140 mesh) was purchased from Fisher Scientific. Solvents were distilled-in-glass grade available from Burdick and Jackson.

Synthesis of 4-tOP1EC

4-tOP (4.12 g, 0.02 mole) was dissolved in 10 mL of ethanol in a 125 mL Erlenmeyer flask. A chloroacetic acid (5.67 g, 0.06 mole) solution was prepared in 20 mL of Milli-Q water. After the pH of both solutions was adjusted to 10 with 1 M NaOH, the sodium chloroacetate solution was slowly added to the phenoxide solution, with continuous stirring. The reaction was maintained at pH 10 and a temperature of 55 ± 5 °C for 1 hr. At the end of the reaction, the mixture was acidified with 6N HCl to a pH of 2 or less and a pale yellow solid was formed.

The crude product was then dissolved in 50 mL of diethyl ether and the organic layer was washed with 60 mL of water three times. After each wash, the aqueous layer was discarded. The ether layer was dried over anhydrous sodium sulfate and the solvent was evaporated to yield a yellow oily product. For further purification of 4-

tOP1EC, the acid was recrystallized from petroleum ether (b.p. 30-60°C) three times. The final product was a white, needle-shaped crystal.

Determination of 4-tOP1EC in STP effluents

24-Hr composite effluent samples from a nearby STP and grab samples from several STP across Canada were collected. They were filtered with 0.45μm glass-fibre filters and stored at 4°C in the dark. In cases where the samples were analyzed within 24 hr after collection, no preservative was used. In other cases, the samples were acidified to pH 2 with HCl. An aliquot (250 mL) of the filtered effluent was acidified to pH 2 or less with 3 mL of 1 M HCl. For the extraction of 4-tOP1EC in the effluent, a 3 mL SPE cartridge with an ODS packing material (Cat. no. 5-7063, Supelco, Oakville, ON) was used. The cartridge was first conditioned with 5 mL of acetonitrile, followed by 5 mL of methanol, and 10 mL of water on a SPE manifold. The cartridge was not allowed to dry when methanol and water were used. The effluent was then applied to the cartridge, either manually or automatically by means of a siphon tubing and an adapter (Cat. no. 5-7275, Supelco). A flow rate of ca. 5 mL/min was maintained by adjusting the vacuum. As a safety precaution, the vacuum was never allowed to exceed -20 in Hg. When the extraction was done, the acid was eluted from the cartridge by 10 mL of methanol into a test tube. After evaporation of the methanol just to dryness with a gentle stream of nitrogen in a water bath of 45°C, two mL of a 14% BF_3/methanol reagent were added. The test tube was then securely closed with a screw cap and the mixture was heated at 85°C for 30 min. At the end of reaction, methanol in the mixture was evaporated and three mL of water were added. The methyl ester of 4-tOP1EC formed

was extracted with three 2 mL aliquots of petroleum ether. The extracts were combined, dried, evaporated and exchanged into 1 mL of iso-octane for GC/MS analysis.

GC/MS analysis of 4-tOP1EC methyl ester

Analysis of the methyl ester was performed by a Hewlett-Packard 5890A Series II gas chromatograph equipped with a split/splitless injection port, a 7673A autosampler, and a 5972A Mass Selective Detector. A 30 m x 0.25 mm ID HP-5-MS column with a 0.25 μm film thickness was used. The carrier gas, helium, was maintained at a constant velocity of 38.4 cm/s with an electronic pressure controller. Splitless injection of 1 μL was made and the splitless time was one min. The initial oven temperature, 70°C, was held for one min. It was then ramped at 30°C/min to 160°C, then at 5°C/min to 280°C. The injection port and interface temperatures were 250 and 280°C, respectively. Full scan mass spectral data were collected from m/z 50 to 450, with an electron energy of 70 eV and the electron multiplier voltage set at 200 V above the autotune value.

For the quantitative analysis of 4-tOP1EC in STP effluents, an aliquot of 20 μg of the acid was methylated as described above. The methyl derivative was then diluted in iso-octane to give calibration solutions at 250 and 25 pg/μL. The mass spectrometer was operating in the selected ion monitoring (SIM) mode and ions at m/z 207 (quantitation ion), 208 and 278 (confirmation ions) were monitored.

RESULTS AND DISCUSSION

Synthesis and purification of 4-tOP1EC

Based on the methods reported for the synthesis of NP1EC, there are at least two approaches that can be used to prepare 4-tOP1EC. The first one involves the oxidation of 4-tOP1EO by a strong oxidizing agent such as Jones reagent (potassium dichromate in sulfuric acid) [14,17]. Alternatively, it can be achieved by the condensation 4-tOP with chloroacetic acid in the presence of a base [6,15]. In this work, the latter approach was adopted because of the availability and purity of starting materials.

For a faster reaction and higher synthetic yield, it was necessary to have the condensation carried out at an elevated temperature and the chloroacetic acid reagent present in excess. At a reaction temperature of ca. 55°C, the yield was more than double in comparison to the room temperature reaction. Because of its high water solubility, the excess chloroacetic acid was easily removed by washing the crude product with water. Purification of the product on a 30 x 1 cm i.d. activated silica gel column using chloroform as an eluant was also attempted. Six 100 mL fractions of chloroform were collected. This tedious procedure was later replaced by repeated recrystallization of the product since the former required testing of the purity of the 4-tOP1EC in each fraction.

Although the overall yield of the acid was only ca. 30% (1.55 g), the emphasis of this synthesis was the quality rather than the yield of the product since the aim was to obtain a pure analytical standard. The purity of the final product was estimated, by full scan GC/MS, to be >98%.

Mass spectral properties of 4-tOP1EC ester derivatives

The total ion current chromatogram of the methylated 4-tOP1EC exhibited a single peak at 12.36 min and its electron impact mass spectrum is shown in Figure 1A. While the molecular ion, M^+, at m/z 278 was weak, a base peak at m/z 207 was observed. The latter species was consistent with a resonance stabilized α,α–dimethylbenzylic structure, resulting from the loss of a neopentyl group from the alkyl side chain of the molecular ion, i.e. $(M-C_5H_{11})^+$. Other fragmentation ions at m/z 179, 147, 117, and 91 were also observed in low abundance for 4-tOP1EC methyl ester.

The pentafluorobenzyl (PFB) ester derivative of 4-tOP1EC was also prepared by reaction with pentafluorobenzyl bromide and potassium carbonate according to a procedure previously established for substituted phenoxyacetic acid herbicides [18]. The mass spectrum of this derivative (Figure 1B) indicated a weak molecular ion at m/z 444, a base peak at m/z 373 for $(M-C_5H_{11})^+$, as well as the pentafluorobenzyl ion, $C_6F_5CH_2^+$, at m/z 181. The formation of this PFB ester further confirmed the phenoxyacetic acid structure of 4-tOP1EC. Also, with either electron capture detection or negative ion chemical ionization mass spectrometry, the electron capturing properties of this ester can be exploited if a lower detection limit is required.

Recovery of 4-tOP1EC from spiked water samples

Owing to their similar chemical properties, 4-tOP1EC in acidified effluents was initially extracted by DCM using a procedure developed for NP1EC and NP2EC [6]. However, the mean recovery of 4-tOP1EC from spiked distilled water by this liquid-liquid extraction procedure was only 57 ± 7% (n=4) at 4 μg/L. In contrast, near quantitative

recovery of this acid was obtained by SPE using an ODS cartridge, with recoveries of 97 ± 6 % (n=6) at 4 μg/L and 87 ± 6% (n=6) at 0.4 μg/L. Since this procedure also generated >90% recovery for NP1EC, the SPE technique was deemed to be a better alternative than liquid-liquid extraction for the preconcentration of these carboxylic acid metabolites in effluents. Based on a 250 mL sample and a concentration factor of 250, the method detection limit for 4-tOP1EC is 0.05 μg/L.

Determination of 4-tOP1EC in STP effluents

A brief survey of 4-tOP1EC in several Canadian STP effluents indicated that the acid was readily detected in every sample analyzed in this work (Table 1). Its concentrations ranged from 0.18 to 1.2 μg/L and from 0.45 to 6.8 μg/L, in the primary and final effluents, respectively. For comparison, the levels of NP1EC in the same samples were higher as expected and they varied from 2.4 to 17.7 μg/L and from 4.4 to 703 μg/L in the primary and final effluents, respectively. Similar to NP1EC, the levels of 4-tOP1EC in the final effluents were between 11 and 2000% higher than those found in the primary effluents collected from the same plant at the same time. As shown in Table 1, 24-hr composite samples were collected from one STP (plant A) in four different occassions. Lower concentrations of 4-tOP1EC and NP1EC were found in the January and February samples when the ambient temperatures were lower. Very high levels of the two acids were found in the final effluents collected from STP E and F; these plants are known to receive effluents from the nearby textile industries.

GC/MS total ion chromatograms of the extracts for typical STP primary and final effluents depicting the presence of the carboxylic acids are shown in Figure 2A

and 2B, respectively. The methyl ester of 4-tOP1EC has a retention time of 12.36 min. Two other groups of peaks with retention times from 13.8 to 15.1 min and from 18.6 to 20.0 min are attributed to methyl esters of NP1EC and NP2EC, respectively.

ACKNOWLEDGEMENT

We thank Mr. D. Bennie for the collection of some effluent samples.

REFERENCES

[1] C. Metcalfe, L. Hoover, and S. Sang, "Nonylphenol Ethoxylates and Their Use in Canada", a World Wildlife Fund Canada Report, December 1996.

[2] "An Assessment of Nonylphenol Ethoxylates and Their Use in Canada", a draft report prepared by Environment Canada.

[3] D.T. Bennie, C.A. Sullivan, H.B. Lee, T.E. Peart, and R.J. Maguire, unpublished results.

[4] L.S. Sheldon and R.A. Hites, *Sci. Total Environ.*, 11 (1979) 279.

[5] M. Ahel, W. Giger, and M. Koch, *Wat. Res.*, 28 (1994) 1131.

[6] M. Ahel, T. Conrad, and W. Giger, *Environ. Sci. Technol.*, 21 (1987) 697.

[7] A. Di Corcia, R. Samperi, and A. Marcomini, *Environ. Sci. Technol.*, 28 (1994) 850.

[8] E. J. Routledge and J. P. Sumpter, *Environ. Toxicol. Chem.*, 15 (1996) 241.

[9] A.C. Nimrod and W.H. Benson, *Crit. Rev. Toxicol.*, 26 (1996) 335.

[10] A. M. Soto, H. Justicia, J.W. Wray, and C. Sonnenschein, *Environ. Health Perspectives*, 92 (1991) 167.

[11] R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, and M.G. Parker, *Endocrinology*, 135 (1994) 175.

[12] B. Hileman, *Chem. Engineer. News*, January 31 (1994) 19.

[13] H.B. Lee and T.E. Peart, *Anal. Chem.*, 67 (1995) 1976.

[14] A. Marcomini, A. Di Corcia, R. Samperi, and S. Capri, *J. Chromatogr.*, 644 (1993) 59.

[15] J.A. Field and R.L. Reed, *Environ. Sci. Technol.*, 30 (1996) 3544.

[16] L.B. Clark, R.T. Rosen, T.G. Hartman, J.B. Louis, I.H. Suffet, R.L. Lippincott, and J.D. Rosen, *Intern. J. Environ. Anal. Chem.*, 47 (1992) 167.

[17] M. Reinhard, N. Goodman, and K. E. Mortelmans, *Environ. Sci. Technol.*, 16 (1982) 351.

[18] H.B. Lee, T.E. Peart, J.M. Carron, and H. Tse, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 835.

Table 1. Levels of 4-tOP1EC and NP1EC (μg/L) in various STP effluents.

	4-tOP1EC			NP1EC	
STP, date	Influent	Primary	Final	Influent	Primary	Final
A, Oct 96	0.42	0.45	3.1	5.1	2.7	22.0
A, Jan 97	--[a]	0.68	3.4	1.5	3.5	8.4
A, Feb 97	0.70	0.72	0.80	1.6	2.9	4.4
A, Mar 97	1.1	1.2	2.4	8.3	9.0	27.9
B, Jun 96	NA[b]	0.30	0.45	NA	7.3	7.2
C, Jun 96	NA	0.39	0.70	NA	3.5	22.4
D, Jun 96	NA	0.18	0.91	NA	7.8	12.9
E, Jun 96	NA	0.22	4.6	NA	17.7	703
F, Jun 96	NA	0.61	6.8	NA	2.4	24.3

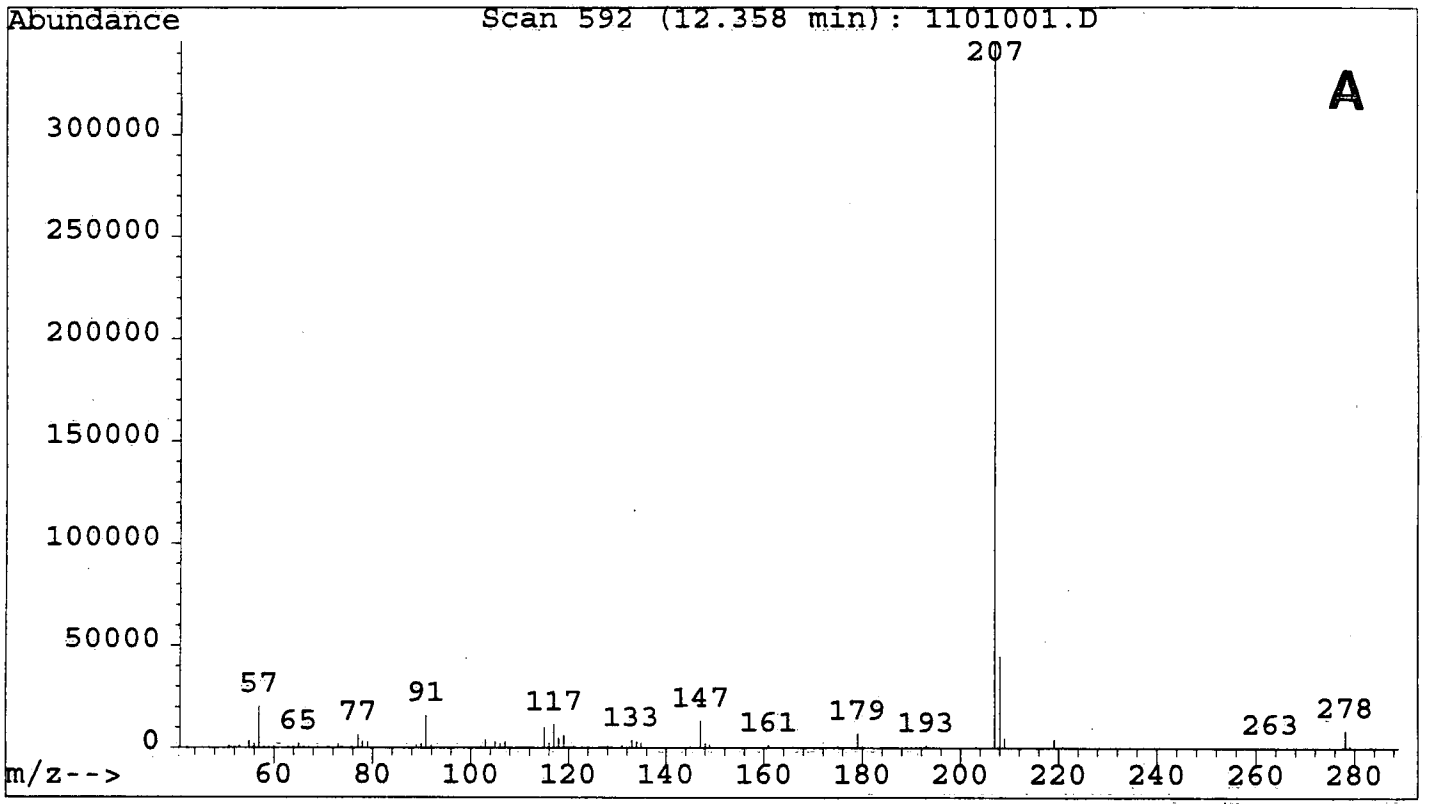
[a] No result due to contamination of extract.
[b] Sample not collected.

List of Figures

Figure 1. Mass spectra of 4-*tert*-octylphenoxyacetic acid methyl (A) and pentafluorobenzyl (B) esters.

Figure 2. Total ion current chromatograms of the 4-tOP1EC and NP1EC/NP2EC methyl esters in a primary (A) and final (B) STP effluent.

Figure 1.


Abundance
Scan 592 (12.358 min): 1101001.D
207
A
300000
250000
200000
150000
100000
50000
0
57
65
77
91
117
133
147
161
179
193
263
278
m/z-->
60
80
100
120
140
160
180
200
220
240
260
280

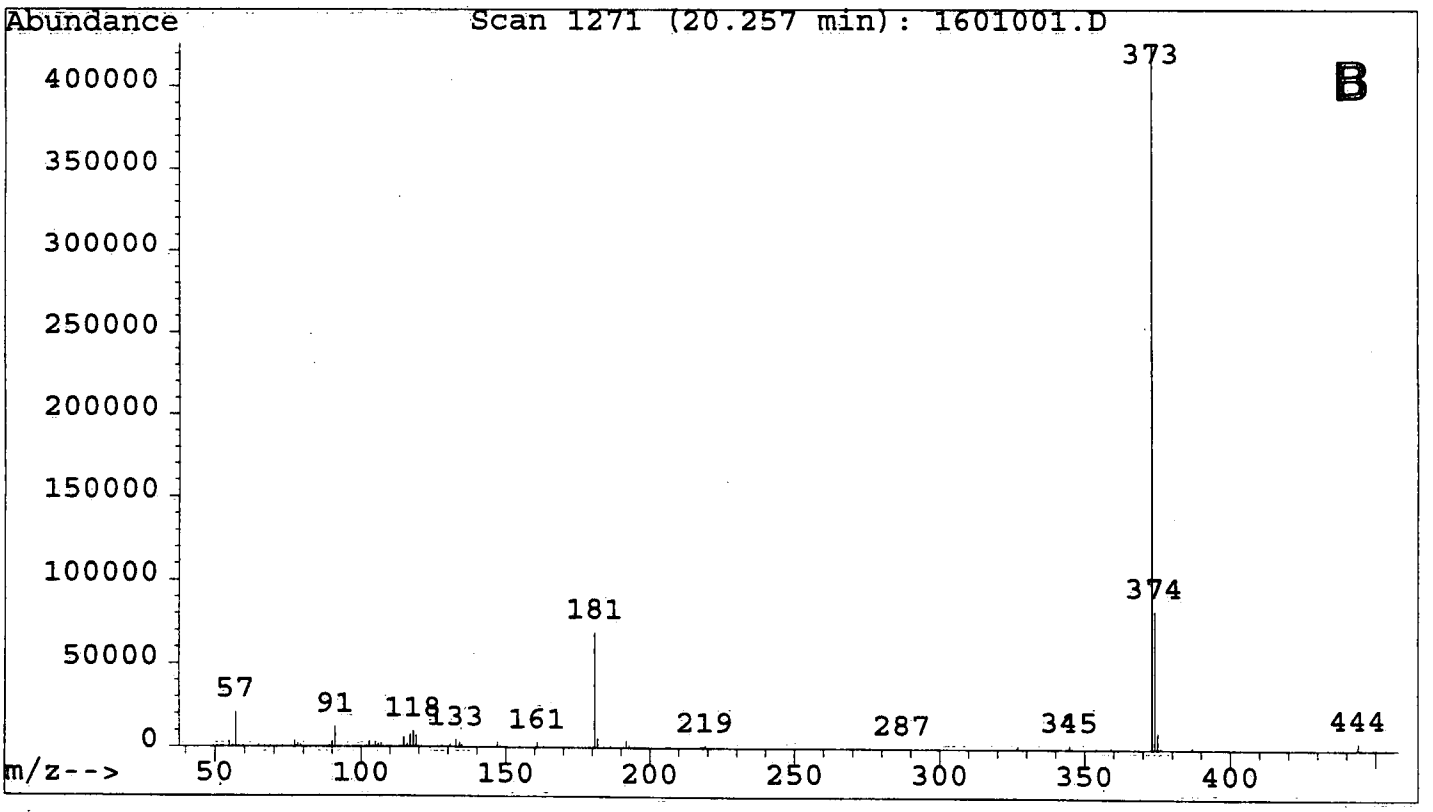
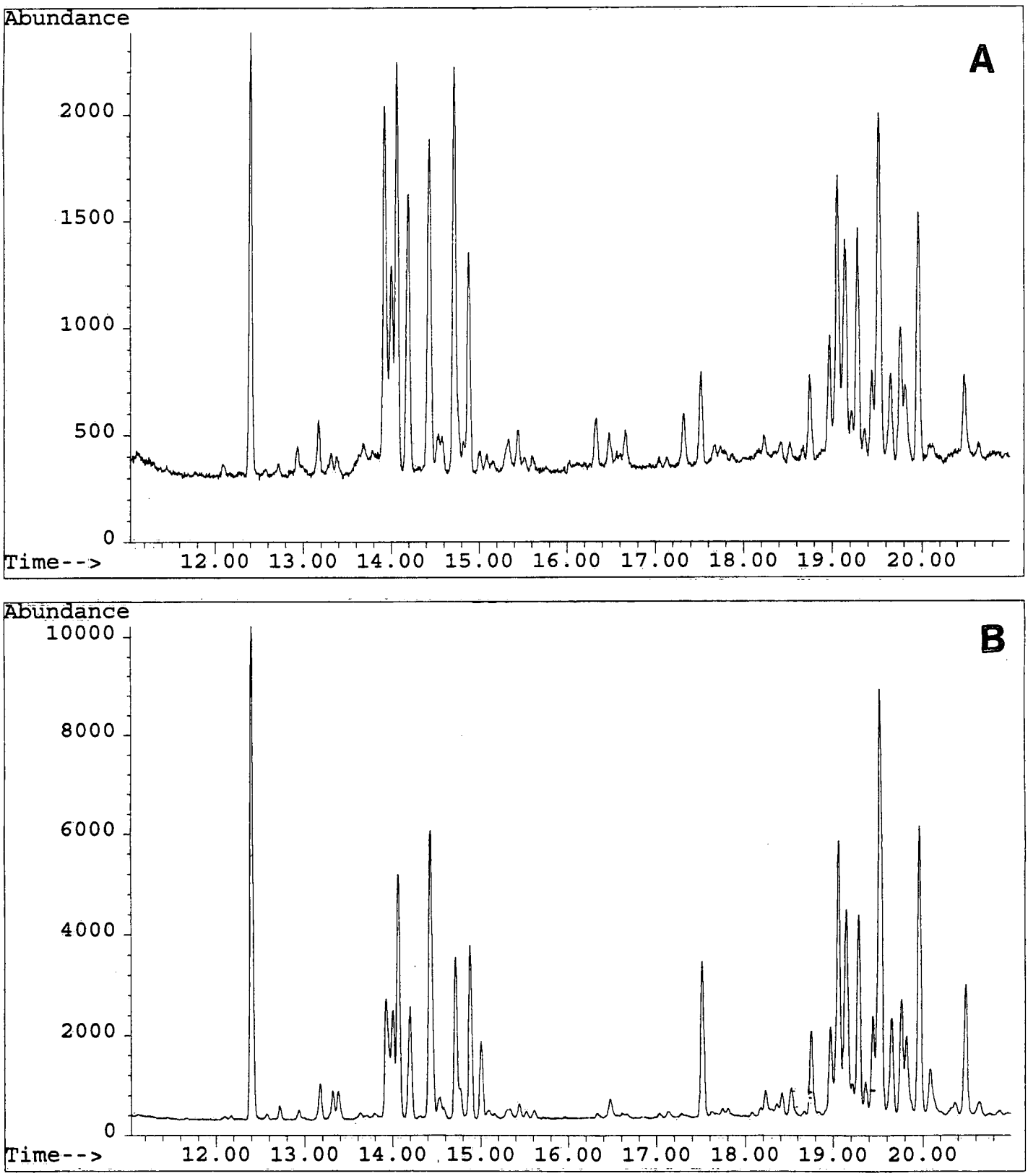
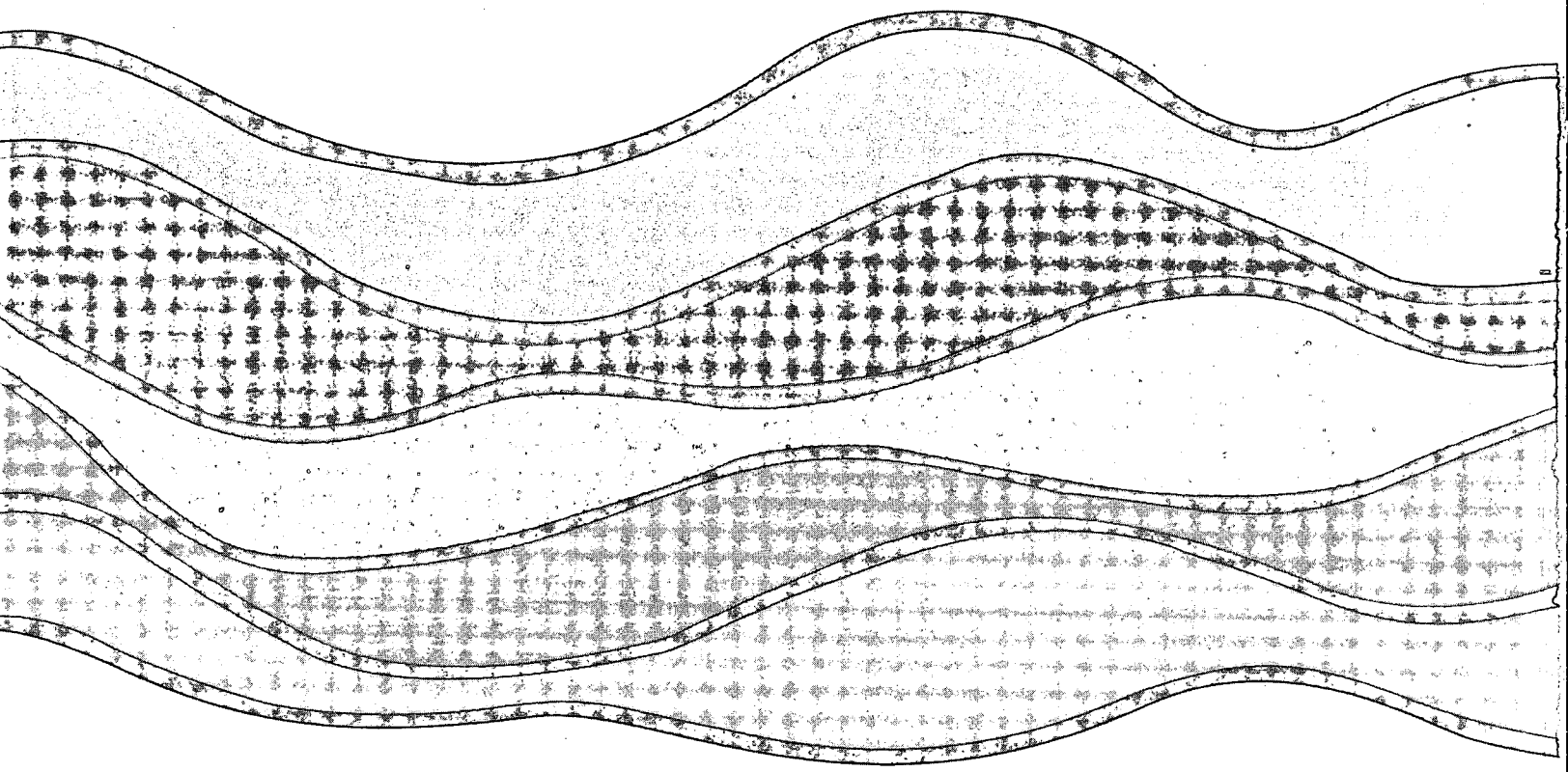

Abundance
Scan 1271 (20.257 min): 1601001.D
373
B
400000
350000
300000
250000
200000
150000
100000
50000
0
374
181
57
91
118
133
161
219
287
345
444
m/z-->
50
100
150
200
250
300
350
400

Figure 2.


Abundance
A
2000
1500
1000
500
0
Time-->
12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00
Abundance
B
10000
8000
6000
4000
2000
0
Time-->
12.00 13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00



NATIONAL WATER RESEARCH INSTITUTE
P.O. BOX 5050, BURLINGTON, ONTARIO L7R 4A6

Environment Canada Environnement Canada

Canada

INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX
C.P. 5050, BURLINGTON (ONTARIO) L7R 4A6

Think Recycling!

Pensez à recycler !