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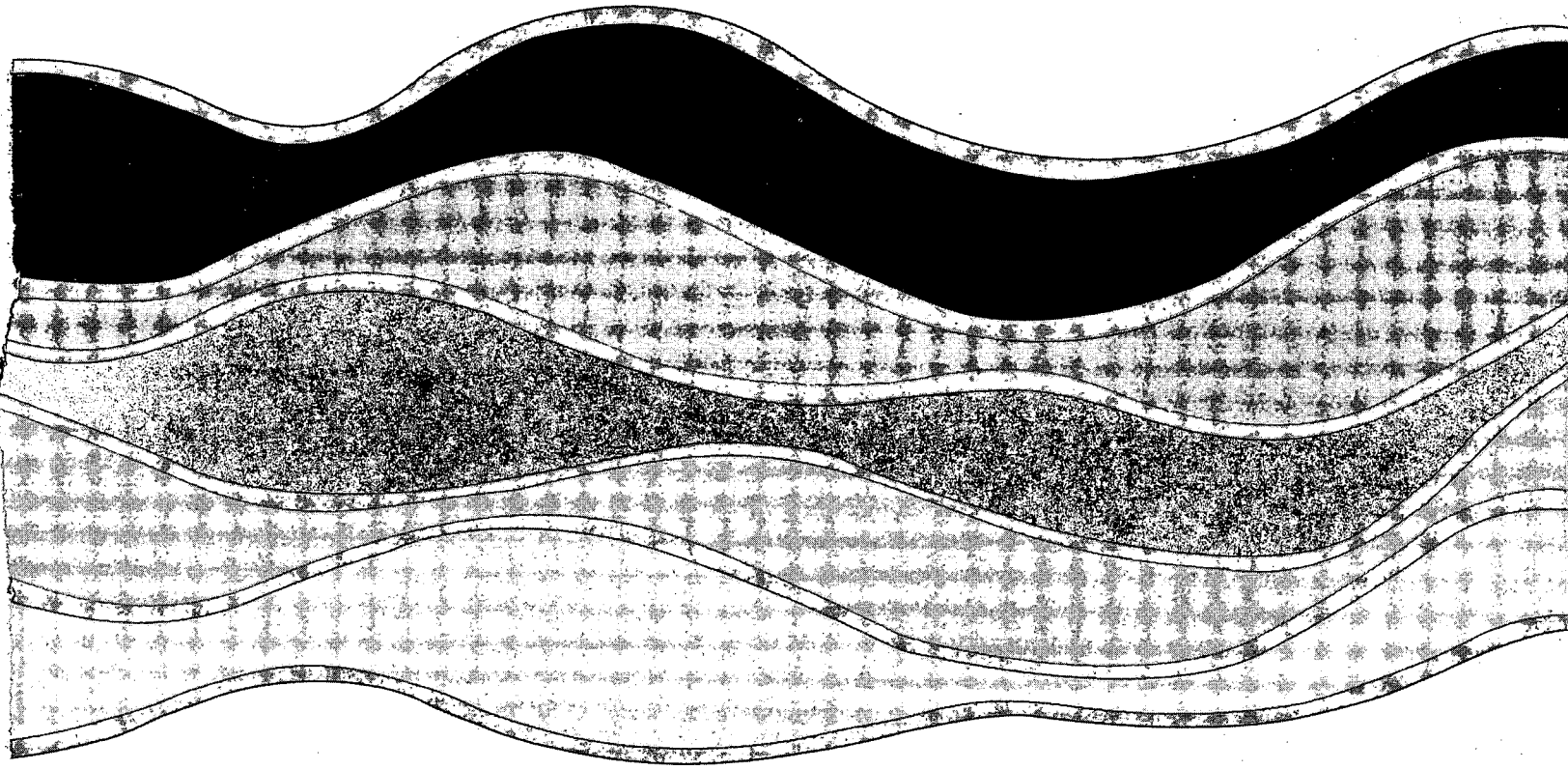

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ALKYLPHENOL POLYETHOXYLATE METABOLITES IN CANADIAN SEWAGE TREATMENT PLANT WASTE STREAMS

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MANAGEMENT PERSPECTIVE

Nonylphenol and its ethoxylates are used as industrial detergents, emulsifiers, dispersants, antifoamers, dyeing assists and pesticide adjuvants in textile manufacturing, petroleum production, leather manufacturing, household/industrial/institutional cleaning and pulp and paper manufacturing. These substances are on the second Priority Substances List (PSL2) of the Canadian Environmental Protection Act (CEPA) to assess their toxicity. Acute and chronic adverse effects have been reported in several terrestrial and aquatic species and there are concerns that organisms exposed to these substances may suffer impaired endocrine function. There are very few data available to determine exposure levels and the risks that these substances may pose to the Canadian environment. This study was undertaken to provide data for use in the assessment process on the occurrence of nonylphenol and related mono- and diethoxylates in the various waste streams of Canadian sewage treatment plants. The results of this study will be used in the PSL 2 assessment. Further studies are being carried out to determine temporal variations in sewage treatment systems, the biological effects of discharged effluents on aquatic biota downstream of sewage treatment plants and the fate of nonylphenol and related ethoxylates when sewage treatment plant sludge is used as an amendment on agricultural soils.

SOMMAIRE A L'INTENTION DE LA DIRECTION

On utilise le nonylphénol et ses éthoxylates dans des produits comme les détergents industriels, les émulsifiants, les dispersants, les antimousses, les adjuvants de teintures et de pesticides, pour la fabrication de textiles, de produits pétroliers, de produits de cuir, pour le nettoyage des domiciles, des usines et des établissements, ainsi que pour la fabrication des pâtes et papiers. Ces substances figurent sur la seconde Liste des substances d'intérêt prioritaire (LSIP-2) de la Loi canadienne sur la protection de l'environnement (LCPA), afin que leur toxicité soit évaluée. On a signalé des effets néfastes aigus et chroniques pour plusieurs espèces terrestres et aquatiques et on craint que les organismes exposés à ces substances ne souffrent de troubles de la fonction endocrine. On ne dispose que de peu de données pour la détermination des teneurs d'exposition et des risques que représentent ces substances pour l'environnement canadien. On a entrepris cette étude afin d'obtenir des données devant servir au processus d'évaluation de l'occurrence du nonylphénol et des ses mono- et diéthoxylates dans divers écoulements résiduaires de stations d'épuration des eaux usées canadiennes. Les résultats de cette étude serviront à l'évaluation des substances de la LSIP-2. D'autres études sont effectuées afin de déterminer les variations temporelles dans les systèmes d'épuration des eaux usées, les effets biologiques des effluents déversés sur le biote aquatique en aval des stations d'épuration des eaux usées et le devenir du nonylphénol et de ses dérivés éthoxylates si l'on utilise les boues des stations d'épuration comme amendements sur des sols agricoles.

ABSTRACT

Nonylphenol polyethoxylates and their refractory metabolites, including nonylphenol, are on the second Priority Substances List (PSL2) to determine if they are "toxic" as defined under the Canadian Environmental Protection Act. This study addresses the need for data on their occurrence in raw sewage, final effluents and sludge in Canada. Samples of raw sewage, final effluent and sludge were collected from 16 wastewater treatment plants across Canada in 1995 and 1996. These samples were analyzed for 4-nonylphenol (4-NP), nonylphenol ethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO) and 4-*tert*-octylphenol (4-*t*-OP). Measurable quantities of these chemicals were found in almost all raw sewage and sludge samples. In the raw sewage, concentrations ranged from < 0.005 to 21 μg/L for 4-*t*-OP, from 0.69 to 155 μg/L for 4-NP, from 2.9 to 43 μg/L for NP1EO and from 0.26 to 24 μg/L for NP2EO. Sludge concentrations (based on dry weight) ranged from < 0.010 to 20 μg/g, from 8.4 to 850 μg/g, from 3.9 to 437 μg/g and from 1.5 to 297 μg/g for 4-*t*-OP, 4-NP, NP1EO and NP2EO, respectively. Of the final effluent samples, 60% contained detectable amounts of 4-*t*-OP and concentrations ranged from < 0.005 to 0.37 μg/L. Almost all of the final effluent samples had detectable levels of 4-NP, NP1EO and NP2EO. The 4-NP concentrations varied from < 0.020 to 13 μg/L, NP1EO was found in the range of 0.072 to 26 μg/L and NP2EO was found in the range of 0.099 to 21 μg/L.

RESUME

Les polyéthoxylates de nonylphénol et leurs métabolites réfractaires, y compris le nonylphénol, figurent sur la seconde Liste des substances d'intérêt prioritaire (LSIP-2) afin que l'on détermine leur « toxicité » au sens de la Loi canadienne sur la protection de l'environnement (LCPA). Cette étude répond au besoin de données sur leur occurrence dans les eaux usées brutes, les effluents finals et les boues au Canada. En 1995 et en 1996, on a recueilli des échantillons d'eaux usées brutes, d'effluent finals et de boues de seize stations d'épuration d'eaux usées de tout le Canada. Dans ces échantillons, on a dosé le 4-nonylphénol (4-NP), l'éthoxylate de nonylphénol (NP1EO), le diéthoxylate de nonylphénol (NP2EO) et le 4-*tert*-octylphénol (4-*t*-OP). On a détecté des quantités mesurables de ces produits chimiques dans presque tous les échantillons d'eaux usées et de boues brutes. Dans les eaux d'égout brutes, les concentrations étaient comprises entre moins de 0,005 et 21 µg/L pour le 4-*t*-OP, entre 0,69 et 155 µg/L pour le 4-NP, entre 2,9 et 43 µg/L pour le NP1EO et entre 0,26 et 24 µg/L pour le NP2EO. Les concentrations des boues (calculées en poids sec) étaient comprises entre moins de 0,010 et 20 µg/g, entre 8,4 et 850 µg/g, entre 3,9 et 437 µg/g et entre 1,5 et 297 µg/g pour le 4-*t*-OP, le 4-NP, le NP1EO et le NP2EO, respectivement. 60 % des échantillons d'effluents finals contenaient des quantités décelables de 4-*t*-OP et leurs concentrations étaient comprises entre moins de 0,005 et 0,37 µg/L. Dans presque tous les échantillons d'effluents finals, on mesurait des concentrations décelables de 4-NP, de NP1EO et de NP2EO. Les concentrations de 4-NP étaient comprises entre moins de 0,020 et 13 µg/L, celles de NP1EO, comprises entre 0,072 et 26 µg/L et celles de NP2EO, entre 0,099 et 21 µg/L.

INTRODUCTION

Alkylphenol polyethoxylates are among the most widely used nonionic surfactants in the world (Talmage, 1994). The most significant commercial alkylphenol polyethoxylate surfactants are octylphenol polyethoxylates, nonylphenol polyethoxylates, dodecylphenol polyethoxylates and dinonylphenol polyethoxylates. Nonylphenol polyethoxylate formulations are employed as detergents, emulsifiers, dispersants, antifoamers, dyeing assists, stabilisers, lubricants, spermicides and pesticide adjuvants. Nonylphenol polyethoxylates (NPnEO, where n = 3 to > 20 ethoxylate units) possess the properties of good wetting, detergency, low foaming, applicability at low water temperatures and low cost. In Canada, the demand for NPnEO surfactants was 6.0 kt in 1989 and was expected to rise to 7.0 kt by 1993 (CPI Product Profiles, 1989). Metcalfe *et al.* (1996) have identified 11 industrial sectors in which NPnEOs are used in Canada, of which 5 sectors are considered to be responsible for the majority of the NPnEO environmental discharges. The major users are textile manufacturing, petroleum production, leather manufacturing, household/industrial/institutional cleaning and the pulp and paper industries.

Nonylphenol polyethoxylates are biodegraded under anaerobic conditions (Stephanou and Giger, 1982; Giger *et al.*, 1984; Ahel *et al.*, 1994), such as those found in most sewage treatment plants, to 4-nonylphenol (4-NP), nonylphenol ethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), nonylphenoxyacetic acid (NP1EC) and nonylphenoxyethoxyacetic acid (NP2EC). The breakdown product 4-NP shows greater aquatic toxicity than the original surfactant (Macek *et al.*, 1975; Rieff *et al.*, 1979; McLeese *et al.*, 1981; Holcombe *et al.*, 1984; Yoshimura, 1986; Granmo *et al.*, 1989; Comber *et al.*, 1993;), and is persistent and lipophilic. The compound 4-*tert*-octylphenol (4-t-OP) is a degradation product of octylphenol polyethoxylate surfactants and may also be found as a minor component in nonylphenol polyethoxylate preparations. Octanol/water partition coefficients (reported as log K_{ow}) for 4-nonylphenol, 4-*tert*-octylphenol and nonylphenol ethoxylate and diethoxylate have been determined experimentally to be in the range of 4.1 to 4.5 (Ahel and Giger, 1993).

Recent studies have found that 4-nonylphenol, 4-*t*-octylphenol, nonylphenol diethoxylate and nonylphenoxyacetic acid mimic the effects of 17β-estradiol by binding to the estrogen receptor and that all four substances are estrogenic to fish, avian and mammalian cells (Soto *et al.*, 1991;

Jobling and Sumpter, 1993; White *et al.*, 1994; Routledge and Sumpter, 1996). Others have noted that these substances have a pronounced effect on the reproductive development of fish, *Daphnia* and rats (Sharpe *et al.*, 1995; Jobling *et al.*, 1996; Gray and Metcalfe, 1997; Baldwin *et al.*, 1997; Shurin and Dodson, 1997). The order of estrogenicity found by White *et al.* (1994) was reported as OP > NP1EC > NP = NP2EO. The estrogenic potency of nonylphenol polyethoxylates also appears to decrease with increasing ethoxylate chain length.

There have been numerous studies on the occurrence of 4-NP and NPnEO in European countries. Most of these studies have focused on monitoring surface waters and sewage treatment plant (STP) effluent and digested sludge. Reported levels in surface waters ranged from < 0.2 to 180 µg/L for 4-NP, < 0.5 to 18 µg/L for NP1EO and < 0.5 to 16 µg/L for NP2EO (Stephanou, 1985; Ahel and Giger, 1985a; Blackburn and Waldock, 1995). Sewage treatment plant effluent concentrations ranged from 0.7 to 21000 µg/L of 4-NP, 0.65 to 133 µg/L of NP1EO, and < 10 to 230 µg/L of NP2EO. Since the log K_{ow} values of these substances are in the range of 4.1 to 4.5, it is expected that they should be adsorbed onto sediments and may be available for uptake and bioaccumulation by benthic organisms. Dried sludge samples from European sewage treatment plants have yielded concentrations of 0.47 to 4000 µg/g of 4-NP, 0.66 to 680 µg/g of NP1EO, and 0.04 to 280 µg/g of NP2EO (Giger *et al.*, 1984; Ahel and Giger, 1985a; Waldock and Thain, 1986; Marcomini and Giger, 1987; Brunner *et al.*, 1988; Wahlberg *et al.*, 1990; Chalaux *et al.*, 1994).

Very few data on the occurrence of NPnEO and its refractory metabolites in the environment have been generated in North America. Naylor *et al.* (1992) found that 60 to 75% of water samples from 30 river sites in the United States had no detectable levels of 4-NP, NP1EO and NP2EO, and that most of the sediment samples collected contained detectable amounts of 4-NP and NP1EO. A recent study by Field and Reed (1996) details the concentrations of carboxylic acid metabolites of NPnEO found in paper mill effluents, municipal STP effluents and natural waters form the Fox River area near Green Bay, Wisconsin. Canadian data on the occurrence and fate of nonionic surfactants are also limited. Rutherford *et al.* (1992) found surfactant concentrations ranging from 5.4 mg/L to 50 mg/L relative to Triton® X-100, a *tert*-octylphenol polyethoxylate formulation, in effluent streams from three textile mills in Atlantic Canada. Bennie *et al.* (1996) reported concentrations of 4-NP, 4-*t*-OP, NP1EO and NP2EO from sediments and natural water

samples from the Laurentian Great Lakes basin and the upper St. Lawrence River. Measurable quantities of 4-NP and 4-*t*-OP were found in 24% of all water samples, while at least 66% of the sediments were found to have detectable levels of all 4 substances. Nonylphenol, octylphenol, octylphenol ethoxylate, nonylphenol ethoxylate and nonylphenol diethoxylate were identified in municipal wastewater and sludge from Vancouver, British Columbia, but were not quantified (Rogers *et al.*, 1986). Lee and Peart (1995) sampled four sewage treatment plants in the Toronto area and found detectable amounts of 4-NP in the effluents and digested sludge. Most of these samples also contained detectable levels of 4-*t*-OP. Concentrations of 4-NP in these digested sludge samples were in excess of 135 μg/g.

In 1995, Environment Canada added nonylphenol and nonylphenol polyethoxylates to the second Priority Substances List (PSL2) for assessment under the Canadian Environmental Protection Act (Environment Canada, 1995). These compounds are being assessed from a Canadian perspective for their occurrence, transformation, bioavailability, bioaccumulation, and toxicity.

In this work, we are reporting on a preliminary study of the occurrence of 4-nonylphenol, 4-*tert*-octylphenol, nonylphenol ethoxylate and nonylphenol diethoxylate in sewage treatment plant influent, final effluent and sludge from across Canada. Nonylphenol polyethoxylates and the carboxylic acid derivatives of nonylphenol polyethoxylates were not reported in this study due to the lack of commercial availability of certified reference materials at the time during which this preliminary study was carried out. Studies in this area to be reported in the future will include nonylphenol polyethoxylate and carboxylic acid derivative results.

METHODS

Materials

Acetic anhydride, 4-NP and triethylamine were obtained from Aldrich (Milwaukee, WI). The nonylphenol mono- and diethoxylate were purchased as a mixture called POE(1 to 2) nonylphenol from ChemService (West Chester, PA). The POE(1 to 2) nonylphenol mixture consisted mainly of nonylphenol mono-, di- and triethoxylates. The concentrations of each component were determined by preparatory scale high performance liquid chromatography (HPLC)

fractionation of components. Subsequent identification by full scan electron impact gas chromatography-mass spectrometry (EI GC-MS) confirmed the identity of each component. All organic solvents used for extractions and cleanup were pesticide grade and high performance liquid chromatographic determinations were done with HPLC grade solvents. Organic-free water was obtained by purification of reverse osmosis-treated water through a Milli-Q water system (Millipore Canada Ltd., Nepean, Ontario). The anhydrous sodium sulphate, potassium carbonate, silica gel, aluminum foil, glass fibre filters, and disposable pipettes used in the treatment of field samples and laboratory extractions were heated to 450 °C overnight before use. All glassware and filtration equipment was rinsed with organic-free water and pesticide grade solvents before use. *AnalaR*™ grade sulphuric acid and formaldehyde solution (37% to 41% w/v) used for sample preservation as well as the acetic anhydride, which was used for acetylation, were obtained from BDH Inc. (Toronto, Ontario). Non-acid-washed Celite 545 was purchased from Fisher Scientific Co. (Toronto, Ontario) and supercritical fluid extraction grade carbon dioxide was obtained from Air Products (Nepean, Ontario). Samples were stored in bottles and jars that were purchased pre-cleaned to American Society for Testing and Materials standards for organic samples (ASTM, 1986).

Sample Collection and Extraction

Samples of final sewage were collected from 15 municipal sewage treatment plants across Canada in 1995 and 1996. Due to operational constraints, samples of raw sewage were collected from only nine of the fifteen sites. Sludge samples were collected from the same 15 sites. Anaerobically digested secondary sludge was obtained from those plants utilizing secondary and tertiary treatment methods while solids from sedimentation of primary effluent were collected from primary treatment plants.

Two different extraction techniques were used for each sample matrix. Influent and final effluent samples were extracted by *in situ* acetylation for 4-NP and 4-*t*-OP while the nonylphenol polyethoxylates were extracted using liquid-liquid extraction with dichloromethane as the extracting solvent. Sludge samples for 4-NP and 4-*t*-OP were subjected to supercritical fluid extraction and on-line acetylation during the extraction stage. Nonylphenol polyethoxylates in sludge samples were Soxhlet extracted using dichloromethane.

(i) Influent / Final Effluent Samples

Samples were collected in 4 L amber glass pre-cleaned bottles and stored at 4 °C in the dark until returned to the field laboratory or the laboratory in Burlington, Ontario. Later the same day, in the laboratory, samples were pressure-filtered through 1 µm Gelman type A/E glass fibre filters using compressed N_2 and modified pressurized beverage containers, pressure filters and Teflon transfer lines (Fox, 1986). Nonylphenol mono- and diethoxylate samples were preserved in 1 L amber glass pre-cleaned bottles with 10 mL of formaldehyde solution while 4-NP/4-*t*-OP samples were preserved to a pH < 2 with 1 mL of concentrated sulphuric acid in 1 L amber glass pre-cleaned bottles. Samples were stored at 4 °C in the dark until extraction.

Samples to be analyzed for 4-NP and 4-*t*-OP were acetylated *in situ* as outlined by Lee *et al.* (1984). The acid-preserved samples were adjusted to pH 10 using potassium carbonate, and acetylated directly in the extraction vessel with triple-distilled acetic anhydride. The resulting acetates were extracted using hexane. Acetylation and extraction was performed three times and the combined extracts were dried through anhydrous sodium sulphate. Extracts were then concentrated to 1 mL in hexane and analyzed by electron impact gas chromatography-mass spectrometry in the selected ion monitoring (SIM) mode.

Nonylphenol mono- and diethoxylate samples were extracted using liquid-liquid extraction techniques. Sample volumes of 500 mL to 1 L were extracted three times with 100 mL of dichloromethane and the combined extract was dried by passage through anhydrous sodium sulphate, concentrated and partitioned into hexane for normal phase high performance liquid chromatographic analysis.

(ii) Sludge Samples

Samples were collected in pre-cleaned amber 4 L bottles. Sample bottles were only filled to about 3/4 of capacity to allow for gas evolution from the sludge. Sludge samples collected in locations remote to Burlington, Ontario were preserved in the field with formaldehyde solution (20 mL/L of sample). The bottles were stored at 4 °C while awaiting transportation to the laboratory in Burlington for drying, extraction and analysis. In the lab, samples were homogenized, transferred to 1 L glass beakers, air dried at 21 °C, ground using a mortar and pestle and passed through a 30 mesh sieve. The ground material was transferred to pre-cleaned 500 mL glass jars with Teflon-lined screw caps and stored in a freezer at -20 °C until extraction.

For NP1-2EO extraction, 5 g aliquots of sludge were mixed with anhydrous sodium sulphate and Soxhlet extracted with dichloromethane for at least 6 hours at 8 cycles/h. The extracts were dried by passage through anhydrous sodium sulphate, concentrated and partitioned into hexane for normal phase HPLC analysis.

The 4-NP/4-*t*-OP supercritical fluid extraction, cleanup and analysis is described by Lee and Peart (1995). Sludge aliquots of 250 mg to 1 g were added to the extraction thimble of the Hewlett-Packard (HP) 7680T supercritical fluid extractor which already contained Whatman GF/C filter paper and Celite. Triethylamine was then spiked onto the sample. Another layer of Celite was then added, followed by acetic anhydride and another layer of GF/C filter paper. The thimble void volume was then taken up by a suitable length of 1 cm diameter glass rod.

Supercritical carbon dioxide extraction then took place at 80 °C and 5096 psi with a flow rate of 2 mL/min. Extraction times were 15 min static and 10 min dynamic and the variable restrictor nozzle was kept at 50 °C. The acetyl derivatives of the subject compounds were adsorbed on an octadecyl-functionalized silica gel (ODS) trap during the extraction. They were later eluted from the ODS trap with hexane. Extracts were combined, partitioned with 1% potassium carbonate to remove coextracted acetic acid, dried with anhydrous sodium sulphate and concentrated to 1 mL for silica gel column cleanup. Samples were applied to a petroleum ether (30-60 °C) washed Pasteur pipette column containing 5 cm of 5% deactivated silica gel and 5 mm of anhydrous sodium sulphate. Sequential elution followed using (1) 5 mL of 5% dichloromethane in petroleum ether, (2) 10 mL of 50% dichloromethane in petroleum ether and (3) 10 mL of 1% methanol in dichloromethane. Fractions 2 and 3 were saved, solvent exchanged into iso-octane, and concentrated to a final volume of 1 mL for analysis.

Analysis

(i) 4-Nonylphenol and 4-*tert*-Octylphenol

All 4-NP/4-*t*-OP analyses on sample extracts were carried out utilizing EI GC-MS techniques, but two different instruments were used.

Analysis of extracts of influent/effluent samples for 4-NP and 4-*t*-OP was done with a Hewlett-Packard 5989A MS Engine in the SIM mode. The Engine was interfaced to an HP 5890 Series II gas chromatograph and all injections were made by an HP 7673A autosampler. Mass

spectrometer conditions, gas chromatograph conditions and data acquisition were controlled by a UNIX-based HP MS ChemStation. Samples were injected in splitless mode onto a 30 m x 0.25 mm id. x 0.25 mm DB-5MS fused silica capillary column (J&W Scientific, Folsom, CA). The injector and GC-MS interface temperatures were both set at 250 °C. EI ion source and quadrupole mass filter temperatures were set at 200 °C and 100 °C, respectively. The GC oven temperature program was as follows: 70 °C for 1 min, 70 °C to 160 °C at 30 °C/min and thence to 240 °C at 5 °C/min. Helium carrier gas flow was about 1 mL/min with a head pressure of 70 kPa. The electron energy was set at 70 eV while the electron multiplier voltage was determined during the instrument tuning process. The quantitative analysis for 4-NP acetates was carried out in the SIM mode by monitoring the ions at *m/z* 107, 121, 135, 163, 191 and 262. The monitored SIM ions for the determination of 4-*t*-OP acetate were *m/z* 135, 177 and 248. Confirmation of 4-NP and 4-*t*-OP was done in the full scan mode while scanning from *m/z* 40 to 300. Calibration standards were produced by acetylation of 10 mg of 4-NP and 4-*t*-OP in 30 mL of potassium carbonate in the presence of acetic anhydride in the same manner as described above for the water samples. The product was diluted to a concentration of 1 mg/mL in hexane for EI GC-MS analysis and quantitation. Sample extracts were quantified by the external standard method using the response factors generated by the acetylated 4-NP and 4-*t*-OP standards. The observation of the *m/z* 262 ion was used for confirmation of the presence of 4-NP while *m/z* 248 was used for 4-*t*-OP confirmation.

Sludge samples were analyzed for 4-NP and 4-*t*-OP using an HP 5972 mass selective detector (MSD) interfaced to an HP 5890 Series II gas chromatograph. Samples were injected via splitless mode using an HP 7673A autosampler onto a 30 m x 0.25 mm id. x 0.25 mm HP-5-MS column (Hewlett-Packard (Canada) Ltd., Mississauga, Ontario). The injector temperature was set at 250 °C while the GC-MSD interface temperature was 280 °C. The GC oven temperature program was the same as described for the HP MS Engine, but the carrier gas flow rate was held constant by electronic pressure sensing at 39.8 cm/sec. The electron multiplier voltage was 2000 V while the electron energy was 70 eV. Confirmation and quantitative analysis were carried out in the same manner as described for water samples.

(ii) Nonylphenol Mono- and Diethoxylates

Analysis for these parameters was performed with normal phase high performance liquid chromatography utilizing fluorescence detection.

The HPLC system consisted of a Hewlett-Packard 1050 Series pump, an HP 1050 Series autosampler and an HP 1046A programmable fluorescence detector interfaced to an HP LC ChemStation. Chromatographic conditions for the separation of the nonylphenol polyethoxylates were derived from the methods published by Ahel and Giger (1985b) and Marcomini and Giger (1987). The samples were chromatographed isocratically on a 100 mm x 2.1 mm i.d. 5 mm particle size HP Hypersil APS normal phase column (Hewlett-Packard (Canada) Ltd., Mississauga, Ontario). The mobile phase was composed of 98% hexane and 2% isopropanol (v/v) and was pumped at a rate of 0.3 mL/min. Sample injection volume was 20 mL. The HP 1046A fluorescence detector used an excitation wavelength of 230 nm and an emission wavelength of 300 nm. Quantitative results were obtained using the external standard method from response factors generated from the POE (1 to 2) nonylphenol standard solution at the 10 ng/mL level. Any samples that generated peak heights above that of the standard peaks were diluted until their response was less than or equal to that of the standard.

Recoveries for all analytes were in excess of 80% for influent, effluent and sludge samples. Reported concentrations have not been corrected for recovery.

RESULTS AND DISCUSSION

The sewage treatment plant locations and their treatment methods at the time of sampling are listed in Table 1. Some of the plants have since undergone major upgrading of their processes and the results shown in this study may not reflect the efficiency of current operating procedures. Several of the municipal STPs have significant inputs from textile manufacturing and processing facilities, namely Cambridge-Galt, Cowansville, Granby, Guelph, Moncton and Truro. Most sites treat wastes from diversified secondary manufacturing processes but the cities of Hamilton, Toronto, Montréal, Winnipeg and Edmonton are highly urbanized and industrial centres.

(i) Raw Sewage Results

The results for raw sewage samples (Table 2) show that measurable levels of 4-NP were found at all nine sites and 4-*t*-OP at eight of the nine sites. Only eight sites were analyzed for NP1EO and NP2EO and these metabolites were present in samples from all eight.

The concentrations of 4-NP in raw sewage ranged from 0.69 µg/L to 156 µg/L and the mean of measurable samples was 43 µg/L. Of the nine sites, the highest values were associated with sewage treatment plants that are known to handle large volumes of textile mill waste effluent. Plants in Cambridge-Galt and Guelph yielded 4-NP concentrations of greater than 100 µg/L in their raw sewage. High concentrations of 4-NP in raw sewage may be indicative of the use of this substance in manufacturing processes or of the biotransformation of the parent surfactants in the sewer systems.

A known degradation product of octylphenol polyethoxylate surfactants, 4-*t*-octylphenol, was found in the raw sewage of eight of the nine sampled plants, indicating that either octylphenol polyethoxylates are being used or 4-*t*-octylphenol is a contaminant found in nonylphenol polyethoxylate surfactants. Concentrations of 4-*t*-OP ranged from < 0.005 µg/L to 21 µg/L and the mean detected concentration was 3.9 µg/L. The highest concentrations of this compound were again associated with the Cambridge-Galt and Guelph STPs.

The values obtained for nonylphenol mono- and diethoxylates in the raw sewage ranged from 0.26 µg/L to 8.5 µg/L with the exception of the Cowansville plant where the concentrations were 43 µg/L for NP1EO and 24 µg/L for NP2EO. That plant also treats large volumes of textile mill waste, but the results are unusual when compared to other plants with similar waste inputs. Mean concentrations of NP1EO and NP2EO in raw sewage were 9.6 and 5.8 µg/L, respectively.

(ii) Final Effluent Results

Final effluent concentrations (Table 3) of 4-NP ranged from < 0.020 µg/L in Regina, a city of 185,000 whose STP utilizes tertiary treatment processes, to 13 µg/L in Burnaby, which, at the time of sampling, utilized a 2-stage primary sedimentation process to treat the waste of its 160,000 inhabitants and its industries. With the exception of the Burnaby plant, there did not appear to be any significant difference in the discharged concentrations of 4-NP between plants with primary, secondary and tertiary treatment. Seven of the nine plants from which both raw and final

effluent were obtained had a reduction in 4-NP levels of greater than 70% over the course of their treatment process. The mean concentration of 4-NP, where detected, was 2.3 µg/L.

Discharges of 4-*t*-OP were not detected at seven of the sixteen STPs and the concentrations at the other plants ranged from 0.030 µg/L in the Burlington STP to 0.37 µg/L in the Charlottetown STP. Of the nine sites with detectable 4-*t*-OP in the final effluent, the mean concentration was 0.13 µg/L.

Nonylphenol mono-and diethoxylates were detected in the final effluents of all sixteen plants. The concentrations of NP1EO ranged from 0.072 µg/L at Granby to 26 µg/L at Cambridge-Galt. For NP2EO, levels varied from 0.099 µg/L at Toronto to 21 µg/L at Guelph. The treatment systems at Guelph and Cambridge were the least efficient at removing these two substances even though the levels in the raw sewage were comparable to other sites. It is possible that these two plants were the most efficient at digesting and degrading the parent surfactants into the shorter ethoxylate-chain metabolites but this may not be desirable since the resultant products may have greater environmental impacts. As Jobling and Sumpter (1993) reported, the estrogenic potency of NP2EO is comparable to that of 4-NP and it is not unreasonable to extrapolate that finding further to suggest that NP1EO has a similar estrogenic potency. Therefore, the higher mono- and diethoxylate concentrations in the final effluents may have weak estrogenic effects on aquatic organisms that are subjected to continuous exposure of the discharged effluents. As well, these short ethoxylate chain compounds will continue to degrade to the more toxic 4-nonylphenol under anaerobic conditions.

Concentrations of nonylphenol polyethoxylate metabolites in final effluents were quite variable relative to their corresponding raw sewage values. This is probably a reflection of the varied inputs to the systems and the efficiencies of treatment processes that were used to remove these substances. Plants with only primary treatment tended to be less efficient. The comparison between influent and effluent in four primary treatment plants is shown in Figures 1 and 2. The figures show that in Moncton, a facility with primary clarification and sedimentation only, the final effluent concentrations were not significantly different than those concentrations found in the raw sewage. However, Granby and Cowansville, two other facilities with primary treatment, were able to decrease emissions by at least a factor of 2 and, in most parameters, by an order of magnitude. Figures 3 and 4 illustrate the comparison between the raw sewage and final effluent concentrations

of the analytes in four secondary and tertiary treatment plants. Some plants also discharged higher concentrations of the degradation products than were found in the raw sewage. This indicates that biotransformation of the parent surfactants was continuing very efficiently during the treatment processes and the hydraulic retention time of the treatment process was insufficient to remove the newly produced contaminants. The increased concentrations of NP1EO and NP2EO in the final effluent at the Guelph facility are an example of this. The tertiary treatment systems of the Cambridge-Galt and Guelph STPs were the most efficient at removing traces of 4-NP and 4-*t*-OP while the efficiencies of secondary treatment processes at Burlington and Hamilton were > 85% as well.

Concentrations of nonylphenol polyethoxylate metabolites in raw sewage and final effluent are comparable to those reported by Schaffner *et al.* (1982), Stephanou and Giger (1982), Stephanou (1985), Ahel *et al.* (1987) and Brunner *et al.* (1988). These authors reported 4-NP levels in raw sewage and final effluents ranging from < 2 μg/L to 69 μg/L and NP1-2EO levels from < 10 μg/L to 198 μg/L.

The fate and degradation of nonylphenol polyethoxylate metabolites in waters receiving sewage treatment plant final effluent discharges is an area of research that has not been fully explored from a Canadian perspective. In areas where STP discharges make up a significant portion of the flow of a creek or river, the effects of the discharge may be deleterious to the resident fish populations and aquatic biota. Harries *et al.* (1997) have suggested that estrogenic responses from the various estrogenic substances in STP final effluents may be additive; therefore, substances present in concentrations too low to have an individual estrogenic effect may act in concert to affect the reproductive success of fish populations. Significant plasma vitellogenin concentrations have been induced in 2-year old male rainbow trout (*Oncorhynchus mykiss*) when exposed to 20 μg/L of 4-nonylphenol or 5 μg/L of 4-*tert*-octylphenol (Jobling *et al.*, 1996). A concentration of 30 μg/L of 4-NP also inhibited testicular growth in male rainbow trout in the same study. Gray and Metcalfe (1997) found that 50% of male Japanese medaka (*Oryzias latipes*) developed testis-ova when exposed to 50 μg/L of 4-NP for 3 months. A 33 day study, using survival as the endpoint, indicates that the NOEC for exposure of fathead minnows (Pimiphales promelas) to 4-nonylphenol is 7.4 μg/L (Environment Agency, 1997). Stimulation of vitellogenin gene expression, gene transcription and growth of human breast cancer cell lines has been reported

for 4-nonylphenol, 4-tert-octylphenol, nonylphenol diethoxylate and nonylphenoxycarboxylic acid by White *et al.* (1994). Concentrations as low as 10^{-7}M (~21 µg/L) of 4-*t*-OP or 10^{-6}M (~220 µg/L) of 4-NP stimulated vitellogenin gene expression and transcription of the reporter gene EREBLCAT. The morphological development of tail spines and swimming setae of *Daphnia magna* were affected by 4-NP at concentrations as low as 10 µg/L (Shurin and Dodson, 1997) and Comber *et al.* found that the 21 day NOEC for *Daphnia magna*, based on live offspring per surviving parent, was 24 µg/L using 4-NP. The Federal Environmental Agency (Umweltbundesamt) of Germany has calculated a predicted no effect concentration (PNEC) for 4-nonylphenol of 0.1 µg/L in water (Gies *et al.*, 1997), while the European Union has proposed PNECs of 0.74 µg/L for fresh water, 0.039 µg/L for salt water and 0.39 µg/L for fresh water and salt water combined (Environment Agency, 1997).

(iii) Sludge and Primary Solids Results

Table 4 summarizes the concentrations of the four analytes in the primary solids and digested sludge from the sixteen studied sites. Only one N.D. (not detected) result was recorded for any of the analytes at any site. This occurred at the Cowansville STP where 4-*t*-OP was not detected in the primary solids. Due to the hydrophobic nature of the nonylphenol polyethoxylate metabolites produced during sewage treatment processes, it is expected that the majority of these substances would be associated with the sludge. Therefore much higher concentrations of 4-NP, NP1EO, NP2EO and 4-*t*-OP are to be expected in the sludge than those found in the final effluent.

Significant concentrations of 4-NP were measured in most samples. Concentrations of 4-NP ranged from 8.4 µg/g to 850 µg/g with a mean concentration of 225 µg/g. The highest values were found in secondary digested sludge samples from Hamilton and Cambridge-Galt. The 4-NP determination was the only sludge analysis in which there were significant differences between sites with primary treatment only and those with secondary or tertiary treatment. The secondary/tertiary sites, on average, tended to have 4-NP concentrations about 3 times higher than the average level found in primary solids. This phenomenon in secondary and tertiary plants is due to the significant bacterial degradation of nonylphenol polyethoxylates that has been previously reported by several authors. The high levels of 4-NP in sludge may have implications with respect to the disposal of the sludge.

Concentrations of 4-*t*-OP in primary sedimentation solids and secondary digested sludges were found in the range of < 0.010 μg/g at the Cowansville site to 20 μg/g at the Guelph site, with a mean (where detected) of 7.1 μg/g. The nonylphenol mono- and diethoxylates were found in samples from each of the sixteen sites. Values ranged from 3.9 μg/g to 437 μg/g for NP1EO and 1.5 μg/g to 297 μg/g for NP2EO. The levels found in the secondary digested sludge from Regina and Edmonton were highest for NP1EO and NP2EO, respectively. The mean values for all sites were 100 μg/g and 52 μg/g for NP1EO and NP2EO, respectively.

Samples of primary solids and sludge contained contaminant levels of three to four orders of magnitude higher than their corresponding influents and effluents. The levels which we found in these Canadian sewage treatment plant solids are comparable to those reported by Giger *et al.* (1984), Brunner *et al.* (1988) and Marcomini *et al.* (1989).

Many sewage treatment plants dewater their sludge after which it may be landfilled, burned to produce energy or used as an amendment for agricultural soils. Use of sludge as an amendment applied to agricultural soil has caused some controversy regarding the fate and bioavailability of these contaminants. There is no information regarding the toxicity of nonylphenolics to soil dwelling organisms, however, the European Union has derived a PNEC of 0.069 mg/kg for 4-NP in soil (Environment Agency, 1997). This PNEC was calculated using the assumption that soil-dwelling organisms are similar to water column organisms in their 4-NP sensitivity. The Danish and Swedish governments have set very strict guidelines on the allowable concentrations of 4-nonylphenol in sludge used for agricultural purposes. Sweden limits the nonylphenol concentration in sludge used as fertilizer to 50 mg/kg (based on dry weight). Winther-Nielsen *et al.* (1997) discussed the recent amendment of the Danish Ministry of Environment and Energy Order No. 823 which sets the limits for the concentrations of organic contaminants, including nonylphenols (in Denmark, this term includes 4-nonylphenol and the mono- and diethoxylates of nonylphenol), in sludge to be distributed on agricultural land. The nonylphenol limit has been set at 50 mg/kg (based on dry matter) until June, 2000 after which the limit will decrease to 10 mg/kg. That paper also suggests that the degradation of 4-NP, NP1EO and NP2EO in sludge applied to soil at 15°C is very slow and that the contaminants persist at the essentially the same concentration for 28 days. A recent German study by Kujawa *et al.* (1997), however, has concluded that 4-NP in sewage sludge applied to agricultural soil degrades completely within 30 days. Thus the fate and bioavailability of

these refractory metabolites in sludge applied to agricultural soil is not clear at this time. This indicates that further research in this area is required especially since the persistence of these contaminants in soil will be mitigated by the physicochemical properties of the soil and the sludge (Beck *et al.*, 1996), as well as environmental (weather) factors and various anthropogenic factors.

This was a preliminary study of the occurrence of alkylphenol polyethoxylate surfactant metabolites in sewage treatment plant waste streams. Further studies are in progress to determine temporal variations in well-managed Canadian secondary and tertiary treatment systems as well as the biological effects of discharged sewage treatment plant effluents on aquatic life downstream of these facilities. A sludge-amended soil study is also being carried out to determine the fate of nonylphenol polyethoxylates and their refractory metabolites under Canadian agricultural conditions.

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Table 1: Study Sites and Effluent/Sludge Treatment

Location	Effluent Treatment	Sludge Treatment
Burlington, Ontario	2° - activated sludge	anaerobic digestion
Burnaby, British Columbia	1°	2-stage sedimentation
Cambridge-Galt, Ontario	2° - activated sludge 3° - sand filtration	anaerobic digestion
Charlottetown, Prince Edward Island	1°	2-stage sedimentation
Cowansville, Québec	1°	sedimentation
Edmonton, Alberta	2° - activated sludge	anaerobic digestion
Granby, Québec	1°	sedimentation
Guelph, Ontario	2° - with rotating biological contactors 3° - sand filtration	anaerobic digestion
Hamilton, Ontario	2° - activated sludge	anaerobic digestion
Moncton, New Brunswick	1°	sedimentation
Montréal, Québec	1° - $FeCl_3$ coagulation	sedimentation
Regina, Saskatchewan	2° - activated sludge 3° - nutrient removal	anaerobic digestion
Saint John, New Brunswick	2° - with rotating biological contactors	anaerobic digestion
Toronto, Ontario	2° - activated sludge	anaerobic digestion
Truro, Nova Scotia	1°	sedimentation
Winnipeg, Manitoba	2° - activated sludge with pure O_2 reactors	anaerobic digestion

Table 2: Raw Sewage Results (µg/L - ppb)

Sewage Treatment Plant	4-t-OP Concentrations (µg/L)	4-NP Concentrations (µg/L)	NP1EO Concentrations (µg/L)	NP2EO Concentrations (µg/L)
Burlington, Ontario	1.8	28	3.6	0.88
Cambridge-Galt, Ontario	21	156	3.2	1.3
Cowansville, Québec	N.D.	62	43	24
Granby, Québec	0.12	2.3	4.3	6.5
Guelph, Ontario	6.3	119	5.3	0.26
Hamilton, Ontario	1.2	12	5.9	0.59
Moncton, New Brunswick	0.14	0.69	2.9	4.3
Toronto, Ontario	0.56	5.5	N.A.	N.A.
Truro, Nova Scotia	0.28	2.9	8.5	8.4
Limit of Detection	0.005 µg/L	0.020 µg/L	0.020 µg/L	0.020 µg/L
Frequency of Occurrence (total # of sites)	8 (9)	9 (9)	8 (8)	8 (8)
Mean (where detected)	3.9 µg/L	43 µg/L	9.6 µg/L	5.8 µg/L
Range	< 0.005 to 21 µg/L	0.69 to 155 µg/L	2.9 to 43 µg/L	0.26 to 24 µg/L

Table 3: Final Effluent Results (µg/L - ppb)

Sewage Treatment Plant	4-t-OP Concentrations (µg/L)	4-NP Concentrations (µg/L)	NP1EO Concentrations (µg/L)	NP2EO Concentrations (µg/L)
Burlington, Ontario (secondary treatment)	0.030	0.60	0.15	0.16
Burnaby, British Columbia (primary treatment)	N.D.	13	5.3	2.4
Cambridge-Galt, Ontario (tertiary treatment)	N.D.	1.4	26	12
Charlottetown, Prince Edward Island (primary treatment)	0.37	2.5	3.3	1.7
Cowansville, Québec (primary treatment)	N.D.	0.37	1.5	2.1
Edmonton, Alberta (secondary treatment)	N.D.	0.55	6.9	4.8
Granby, Québec (primary treatment)	0.054	0.62	0.072	0.67
Guelph, Ontario (tertiary treatment)	N.D.	1.9	14	21
Hamilton, Ontario (secondary treatment)	0.18	1.6	1.3	0.54
Moncton, New Brunswick (primary treatment)	0.13	1.8	3.0	4.4
Montréal, Québec (primary treatment)	0.12	1.2	2.5	1.7
Regina, Saskatchewan (tertiary treatment)	N.D.	N.D.	1.11	0.46
Saint John, New Brunswick (secondary treatment)	0.049	0.45	0.26	0.38
Toronto, Ontario (secondary treatment)	0.060	1.7	0.092	0.099
Truro, Nova Scotia (primary treatment)	0.18	1.8	1.5	5.4
Winnipeg, Manitoba (secondary treatment)	N.D.	4.8	1.0	1.8
Limit of Detection	0.005 µg/L	0.020 µg/L	0.020 µg/L	0.020 µg/L
Frequency of Occurrence (total # of sites)	9 (16)	15 (16)	16 (16)	16 (16)
Mean (where detected)	0.13 µg/L	2.3 µg/L	1.7 µg/L	3.8 µg/L
Range	< 0.005 to 0.37 µg/L	< 0.020 to 13 µg/L	0.072 to 26 µg/L	0.099 to 21 µg/L

Table 4: Sludge Results (µg/g - ppm, based on dry weight)

Sewage Treatment Plant	4-t-OP Concentrations (µg/g)	4-NP Concentrations (µg/g)	NP1EO Concentrations (µg/g)	NP2EO Concentrations (µg/g)
Burlington, Ontario	5.9	229	29	2.6
Burnaby, British Columbia	9.7	469	54	26
Cambridge-Galt, Ontario	15	329	167	42
Charlottetown, Prince Edward Island	3.0	45	71	20
Cowansville, Québec	N.D.	11	26	43
Edmonton, Alberta	5.3	159	332	297
Granby, Québec	1.1	8.4	14	69
Guelph, Ontario	20	850	36	23
Hamilton, Ontario	16	609	25	1.5
Moncton, New Brunswick	0.70	15	70	14
Montréal, Québec	5.3	113	128	34
Regina, Saskatchewan	8.1	198	437	93
Saint John, New Brunswick	0.87	11	3.9	20
Toronto, Ontario	7.9	272	70	26
Truro, Nova Scotia	0.52	37	70	93
Winnipeg, Manitoba	6.7	239	62	34
Limit of Detection	0.010 µg/g	0.10 µg/g	0.015 µg/g	0.015 µg/g
Frequency of Occurrence (total # of sites)	15 (16)	16 (16)	16 (16)	16 (16)
Mean (where detected)	7.1 µg/g	225 µg/g	100 µg/g	52 µg/g
Range	< 0.010 to 20 µg/g	8.4 to 850 µg/g	3.9 to 437 µg/g	1.5 to 297 µg/g

Figure 1: Primary Treatment - Raw Sewage Comparison

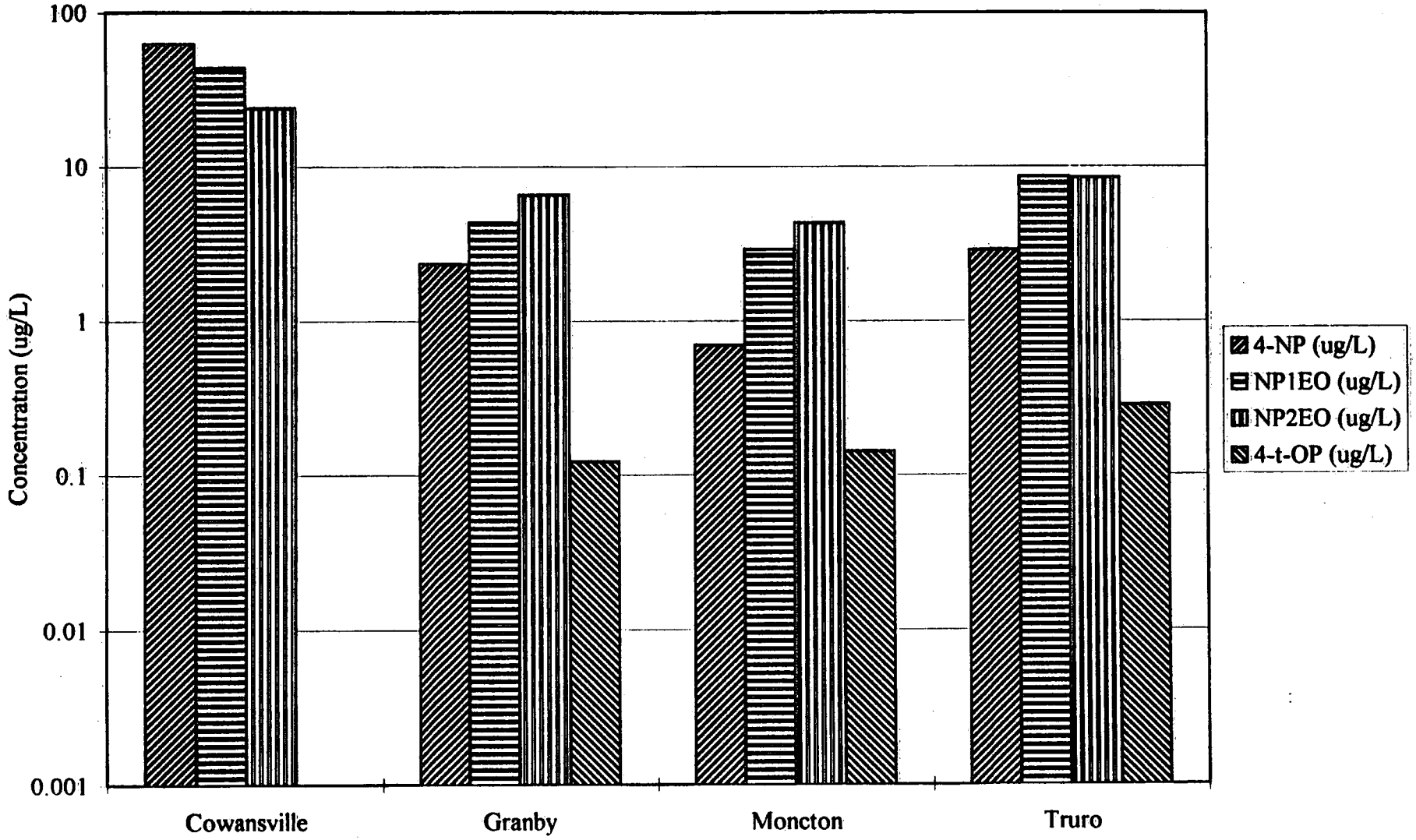


Figure 2: Primary Treatment - Final Effluent Comparison

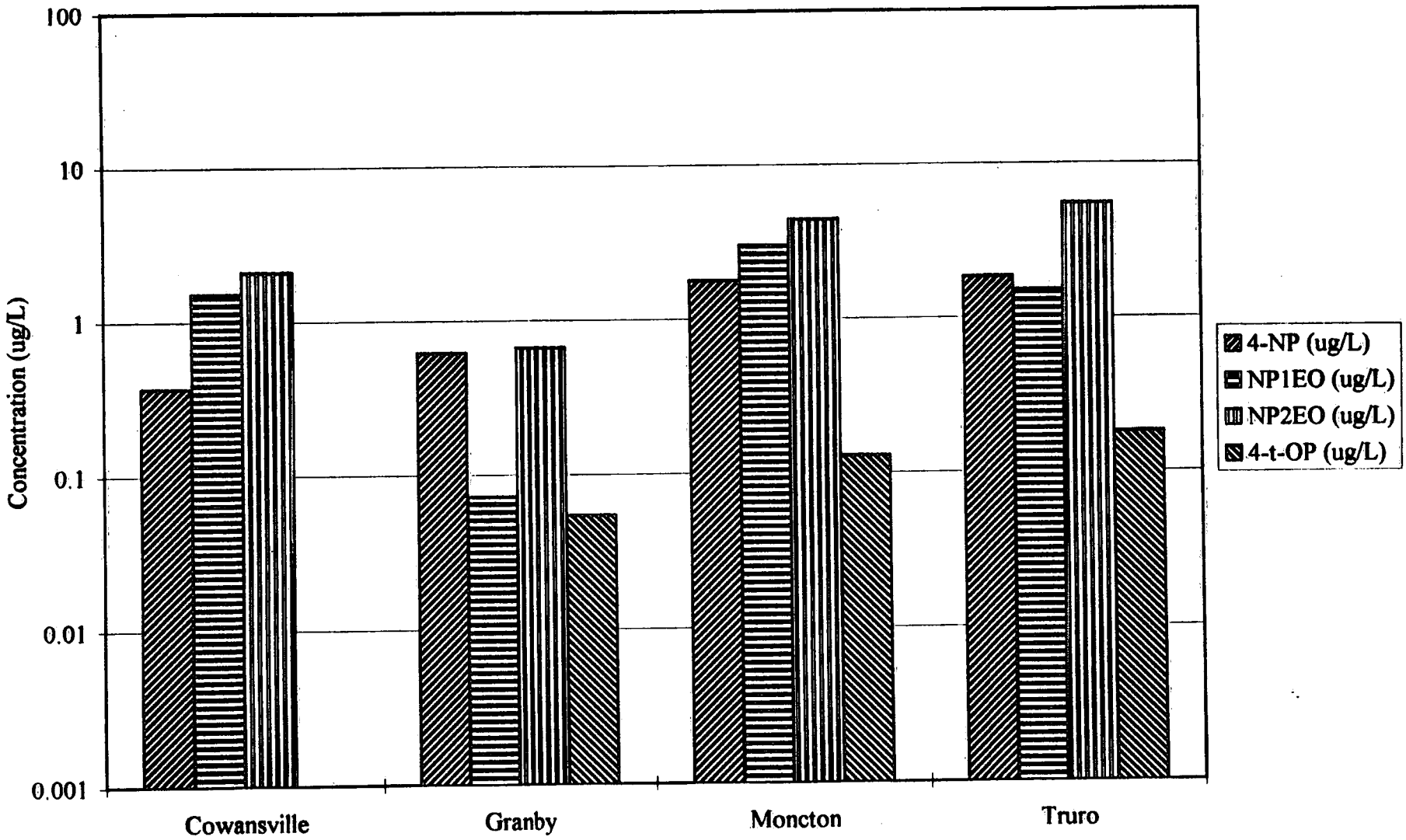


Figure 3: Secondary/Tertiary Treatment - Raw Sewage Comparison

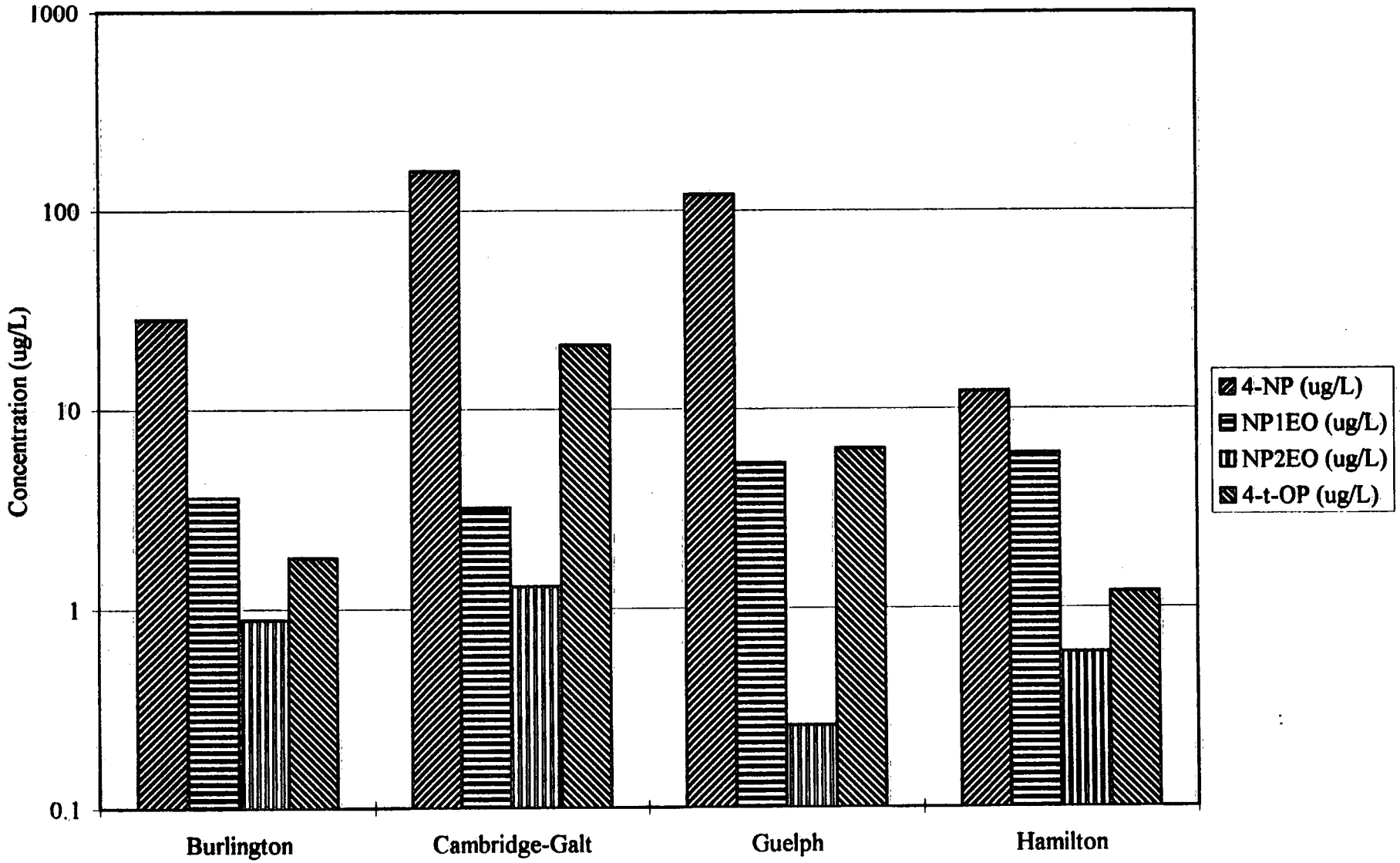
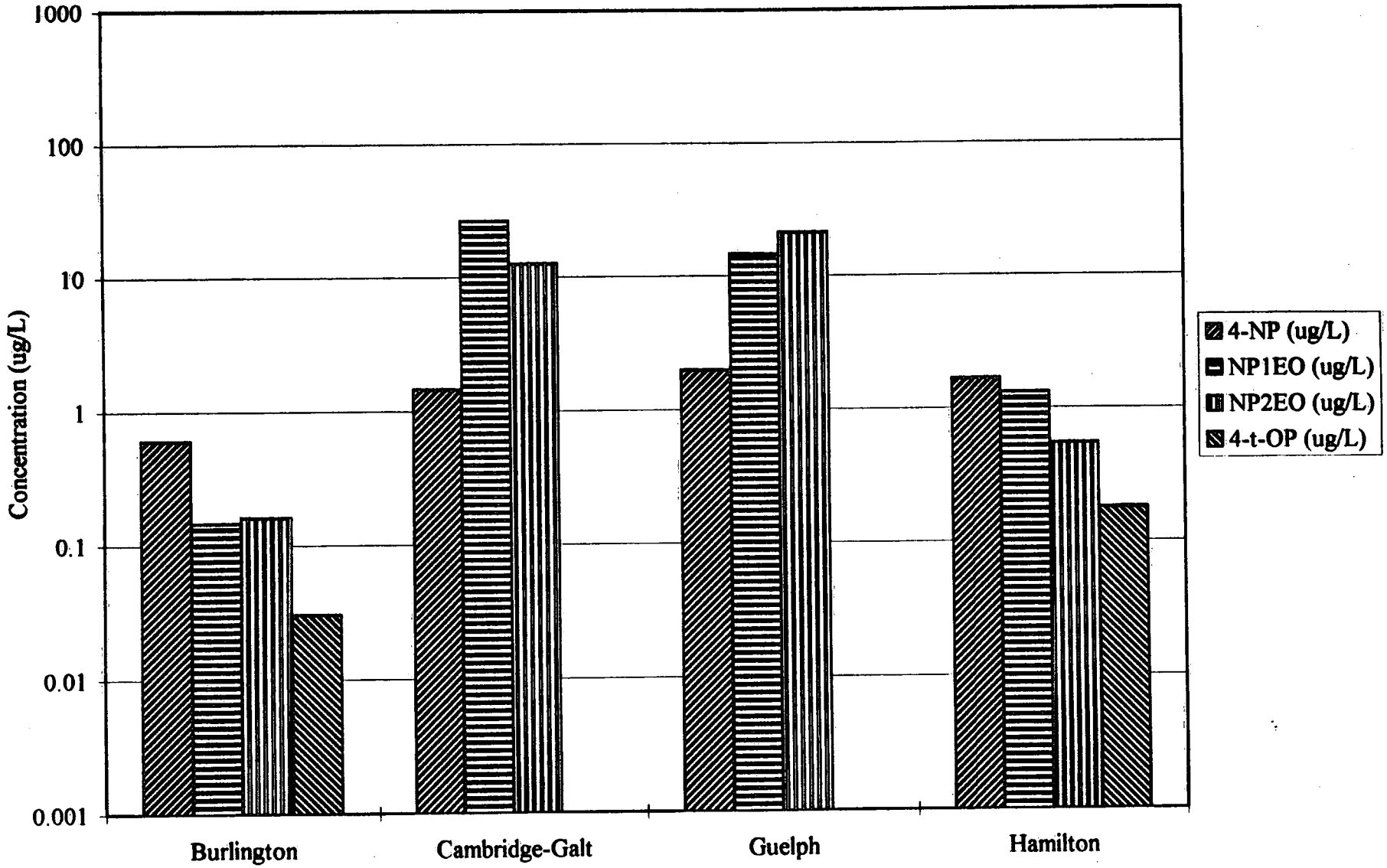
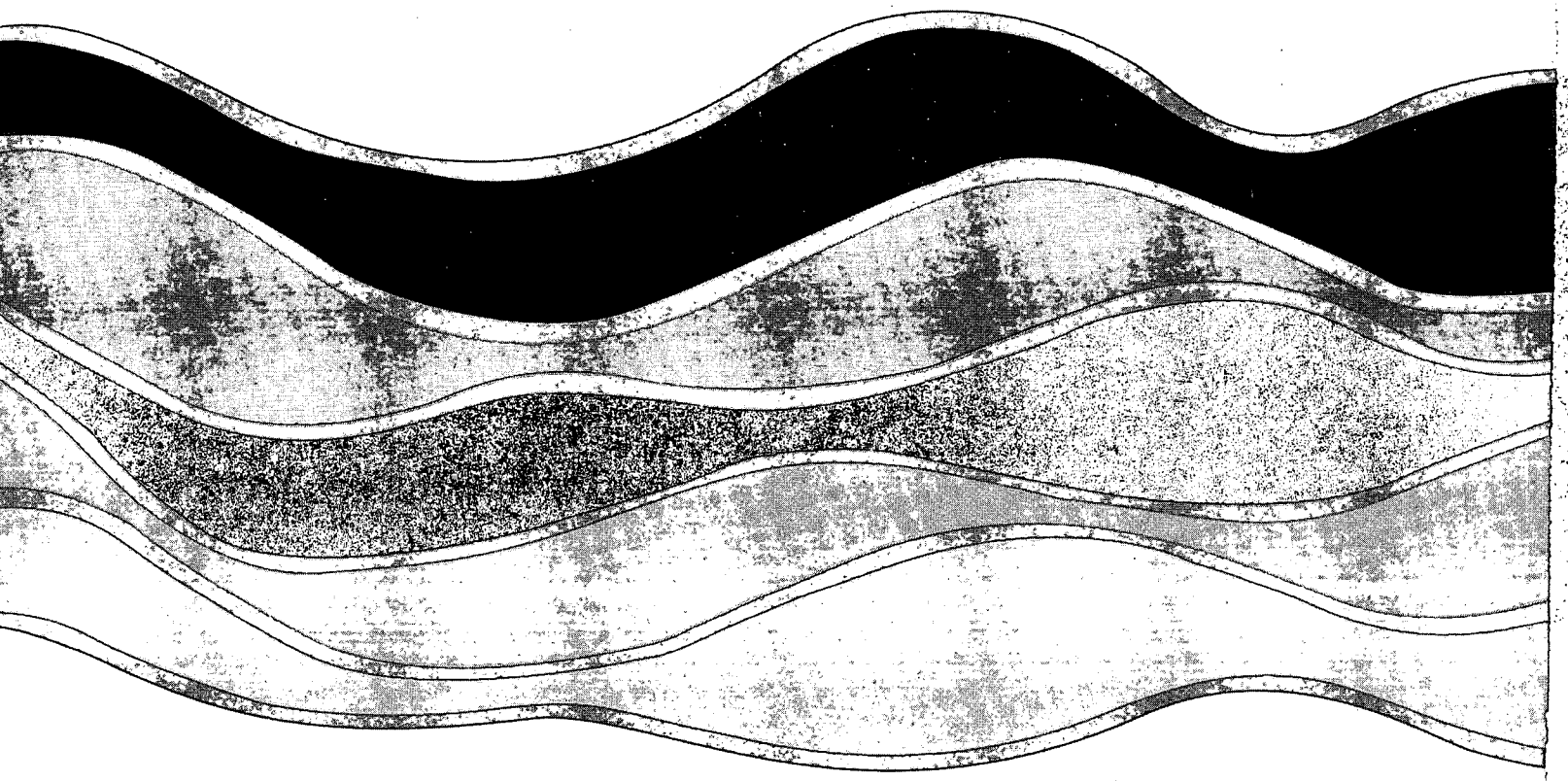


Figure 4: Secondary/Tertiary Treatment - Final Effluent Comparison





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