BEHAVIOR OF DEGRADATION PRODUCTS OF ALKYLPHENOL POLYETHOXYLATES TYPE NONIONIC SURFACTANTS (NP, OP, NP1EO and NP1-2EC) IN THE AQUATIC ENVIRONMENTS

2001.3

Environmental Science
Science of Resources and Environment
United Graduate School of Agricultural Science
Tokyo University of Agriculture and Technology

ISOBE Tomohiko
(磯部　友彦)

Doctoral Dissertation

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February, 2001

ISOBE Tomohiko

Abstract in English

Behavior of Degradation Products of Alkylphenol Polyethoxylates Type Nonionic Surfactants (NP, OP, NP1EO and NP1-2EC) in the Aquatic Environments

Environmental Science
Science of Resources and Environment
United Graduate School of Agricultural Science
Tokyo University of Agriculture and Technology

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Alkylphenol polyethoxylates (APnEO), one of the most important classes of nonionic surfactants, are aerobically degraded through carboxylation of the end of ethylene oxide (EO) chain to ethoxy acetic acid and aerobically/anaerobically by shortening of EO chain length to lower ethoxymers and subsequently anaerobically degraded to alkylphenols (APs) by microbial activity in sewage treatment process. APs and some intermediates have not only acute toxicity but also weak endocrine disrupting nature. In the present study, at first, the analytical method using gas chromatography-mass spectrometry (GC-MS) was developed for the determination of the degradation products of APnEO [i.e., nonylphenol (NP), octylphenol (OP), nonylphenol monoethoxylate (NP1EO), nonylphenoxy acetic acid (NP1EC), and nonylphenol monoethoxy acetic acid (NP2EC)] in the environmental samples. The method was then applied to various environmental matrices, i.e. influent and effluent of sewage treatment plants (STPs), river water, and sediment, to investigate distributions and behaviors in the aquatic environments in Tokyo.

The primary and secondary effluent grab samples were taken from five STPs in 1997, and the 24-h composite samples of wastewater were taken from two STPs in 2000. The river water samples were collected from the Tamagawa and Sumidagawa Rivers in 1997. The river water and the surface sediment samples were collected from the Tsurumigawa, Tamagawa, Sumidagawa, Arakawa, Nakagawa, and Edogawa Rivers in 2000. The water samples were filtered with glass fiber filter (GF/F) and both the particulate samples on the filters and the filtrates were analyzed.

Aqueous samples (filtrates) were solid phase extracted using octadecyl silica (tC18, Waters). Solid samples (i.e., filters and sediments) were extracted using Soxhlet extraction with dichloromethane. All the extracts were fractionated using anion exchange column into the

nonionic (NP, OP and NP1EO) and the anionic (NP1EC and NP2EC) fractions. Further purification and fractionation of NP, OP and NP1EO fraction were accomplished by 5% H_2O (w/w) deactivated silica gel column (1cm i.d. x 9cm). NP1EO was trimethylsilylated with N, O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) and NP1EC and NP2EC were methylated with BF_3/MeOH prior to GC-MS analysis. The reproducibilities (RSD) for the filtrate samples were 2 % (n=4), 14 % (n=4), 8 % (n=4), 2 % (n=4), and 3 % (n=2) for NP, OP, NP1EO, NP1EC, and NP2EC, respectively. The recoveries were 91 % (n=4), 81 % (n=3), 99 % (n=4), 98 % (n=4), 127 % (n=3), for NP, OP, NP1EO, NP1EC, and NP2EC, respectively.

The result of the composite samples demonstrated that NP, OP and NP1EO were efficiently removed from the wastewater in the STPs. Their average elimination efficiencies during whole sewage treatment process were 88, 96 and 90 %, respectively. On the other hand, NP1EC and NP2EC were produced during sewage treatment. In the influent, NP1EC and NP2EC concentrations ranged from 0.15 to 1.6 μg/L, whereas in the final effluent, they ranged from 6 to 10 μg/L. Mass balance calculation of nonylphenolic compounds (i.e., NP, NP1EO, NP1EC and NP2EC) indicated that one-third of the nonylphenolic compounds were escaped from sewage treatment and released to the aquatic environments. Furthermore, in the final effluent, NP1EC or NP2EC represented three-quarters of nonylphenolic compounds.

Concentrations of NP, OP, NP1EO, NP1EC and NP2EC in the river water collected in 2000 ranged from 0.02 to 2.9 μg/L, 0.001 to 0.12 μg/L, 0.002 to 3.4 μg/L, 0.47 to 2.8 μg/L and 0.09 to 2.7 μg/L, respectively. The ranges of concentrations were similar to those reported in other countries, and there was no hot spot observed in Switzerland nor in England. NP1EC and NP2EC again accounted for 80 or more percent of total nonylphenolic compounds in most of the river waters. The apparent partition coefficients of NP, OP, and NP1EO between aqueous phase and particulate organic carbon (K'oc) were calculated to be $10^{5.3 \pm 0.5}$, $10^{5.1 \pm 0.5}$ and $10^{4.0 \pm 0.6}$ on the average, respectively. The K'oc values for NP and OP were one order of magnitude higher than those expected from their reported octanol-water partition coefficients (Kow), indicating strong affinity of APs to the aquatic particles. On the estuary survey, the percentages of NP in the particulate phase increased as salinity increased, suggesting shifts of NP in the aqueous phase to the particulate phase in the estuarine water. This may lead to the bottom sediments to be a sink of alkylphenols. NP, OP and NP1EO were also detected in the surface sediments, and the possibility of *in situ* anaerobic formation of APs in the surface sediments was suggested from variation of the NP1EO/NP ratio. On the other hand, no NP1EC and NP2EC were detected in all the sediment samples, indicating carboxylated compounds, especially NP1EC, remain in the aqueous phase in the river water and are diffused widely in the aquatic environments.

Abstract in Japanese

学 位 論 文 要 旨

Behavior of degradation products of alkylphenol polyethoxylates type nonionic surfactants (NP, OP, NP1EO and NP1-2EC) in the aquatic environments.

（非イオン界面活性剤アルキルフェノールポリエトキシレートの分解産物(NP, OP, NP1EO および NP1-2EC)の水環境中での挙動）

磯 部 友 彦

ISOBE Tomohiko

本研究では、非イオン界面活性剤アルキルフェノールポリエトキシレート(APnEO)とその分解産物の水環境中での動態を明らかにすることを目的とした。APnEO の中で最も生産量の多いノニルフェノールポリエトキシレート(NPnEO)に着目し、その分解産物であるノニルフェノール(NP)、ノニルフェノールモノエトキシレート(NP1EO)、ノニルフェノキシ酢酸(NP1EC)、ノニルフェノールモノエトキシ酢酸(NP2EC)などについて、まずその分析法を確立した。次に確立した分析法を用いて下水・河川水・河川堆積物などの環境試料を分析し、これらの物質の環境動態についていくつかの新しい知見を得た。

ノニルフェノールエトキシレート(NPnEO)は、分解されて NP などの難分解性の分解産物を生成し、NP 以外にもノニルフェノールジエトキシレート(NP2EO)や NP1EC などの中間産物についても弱い内分泌攪乱作用が報告されており、生態系への影響が懸念されている。河川水中では NPnEO とその分解産物のほとんどが NP1EC や NP2EC、NP1EO、NP2EO として存在するという報告もあるため、これらの分解産物のモニタリングが重要である。既存の方法では、感度の高い分析法は対象物質が限られ、多成分を分析できる方法は HPLC を使用するため感度が低い等の欠点があった。そのため本研究では、一連の分析操作で包括的にかつ高感度にこれらの物質が分析可能な分析法を開発した。本研究で分析対象にした NP、OP、NP1EO、NP1EC、NP2EC のいずれにおいても、再現性は RSD が概ね 10%以下、添加回収率が 80%以上と良好な結果が得られた。

下水処理場における NP、NP1EO およびオクチルフェノール(OP)の除去率は、2 ヶ所の下水処理場の平均値がそれぞれ 88%、96%、90%であり、下水処理によってこれらの物質が効率良く除去されていることが分かった。それに対して NP1EC および NP2EC は、流入水(0.15～1.6 μg/L)よりも放流水(6～10 μg/L)中の濃度が高く、処理過程で増加していることが観察された。これは親化合物である NPnEO が好気分解を受けてカルボン酸化物が生成されていることを示している。また、放流水中では今回分析した NPnEO の分解産物のうちそれぞれの処理場で 74%、82%が NP1EC+NP2EC であり、主にカルボン酸化物の形態で下水処理場から放出されていることが明らかになった。NP から NP2-18EO までを総計した、ノニルフェノールエトキシレート関連物質として考えると、それぞれ 27%、15%が環境中に放出されていることが分かった。

NP、OP、NP1EO、NP1EC、NP2EC は、東京湾に流入する主要河川水中で広範囲に分布していた。2000 年の調査における河川水中濃度は、NP が 0.02～2.9 μg/L、OP が 0.001～0.12 μg/L、NP1EO が 0.002～3.4 μg/L、NP1EC が 0.47～2.8 μg/L、NP2EC が 0.09～2.7 μg/L であり、大半の地点で NPnEO の分解産物中 8 割以上が NP1EC+NP2EC であった。河川水中では懸濁態の NP1EC および NP2EC は全て検出限界以下でありほとんどが溶存態であったのに対し、NP、OP および NP1EO は懸濁態試料からも有意に検出され、特に NP は OP や NP1EO と比べて懸濁態に存在している割合が高いことが分かった。さらに NP については、多摩川河口において塩分が高くなるに従って懸濁態の割合が上昇することが観察され、塩水との混合の影響を受けて粒子への吸着が進み、粒子が沈降することで堆積物中に蓄積することが示唆された。

女性ホルモン様活性の報告値から、下水放流水、河川水においては今回分析した物質の活性の中で、NP1EC が大部分を占めていることが分かった。このことからも、モニタリングの際には、NP や OP だけでなく、NP1EC や NP2EC についても注意をする必要がある。NP1EC については東京湾や湾外へと汚染が拡散している可能性があり、毒性データや生分解性、分解生成物などに関する情報もほとんどないことから、今後環境動態や生物影響に関する研究が求められる。

Some parts of this study was published in the following papers:

Behaviour and Effect of Nonylphenol in Aquatic Environment; *Journal of Japan Society on Water Environment*, **1998**, *vol. 21*, no. 4, pp. 203-208 (in Japanese).

Determination of Nonylphenol by GC-MS in Environmental samples in Tokyo; *Journal of Japan Society on Water Environment*, **1999**, *vol. 22*, no. 2, pp. 118-126 (in Japanese).

Distribution and Behavior of Nonylphenol, Octylphenol, and Nonylphenol Monoethoxylate in Association with Aquatic Particles and Sedimentary Distributions; *Environmental Science and Technology*, **2001**, *in press.*

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Abbreviation

Alkylphenol polyethoxylates and their degradation products.

APnEO	Alkylphenol polyethoxylates
NPnEO	Nonylphenol polyethoxylates
OPnEO	Octylphenol polyethoxylates
NP1EO	Nonylphenol monoethoxylate
NP2EO	Nonylphenol diethoxylate
NPEC	Nonylphenol ethoxy carboxylic acid
NP1EC	Nonylphenoxy acetic acid
NP2EC	Nonylphenol monoethoxy acetic acid
OP	p-(1,1,3,3-Tetramethylbutyl) phenol (p-t-Octylphenol)
APs	Alkylphenols (meaning NP and OP in this study)

Reagents

Hex.	n- Hexane
DCM	Dichloromethane
MeOH	Methanol
BSTFA	N, O- bis (trimethylsilyl) trifluoroacetamide
BF_3/MeOH	10 % boron trifluoride in methanol (w/w)

1. Introduction

1.1 Surfactants

Surfactants, one of the most widely used groups of chemicals in our life, are utilized for various purposes. They have both hydrophobic and hydrophilic moieties in their chemical structure, which decrease the surface tension between hydrophobic and hydrophilic media. Because of this property, they are widely used as household laundry and kitchen detergents and industrial cleaning agents and emulsifier in textile, pulp and paper mill and plastics industries and agriculture. They are classified into four groups, i.e. anionic, nonionic, cationic and amphoteric, depending on types of the hydrophilic groups. Annual production of surfactants in 1996 is approximately 1.1 million tons in Japan (Ministry of International Trade and Industry of Japan, 1996). Among them, the 48 % is anionic and 40 % is nonionic. Total production of surfactants has not increased dramatically since the early 1980s. However, the production of nonionic surfactants has been gradually increasing, whereas that of anionic surfactants are slightly decreasing. Thus, the share of nonionic surfactants and their potential contribution to water pollution has been increasing (Japan Soap and Detergent Association, 1996).

1.2 Alkylphenol Polyethoxylates

Alkylphenol polyethoxylates (APnEO) are, one of the most important class of nonionic surfactants, and used for industrial, agricultural, and domestic applications. They are comprised of an alkylphenol that is mainly para substituted with branched alkyl moieties, and a hydrophilic ethylene oxide (ethoxylate) chain ether linked at the phenolic oxygen. They have several

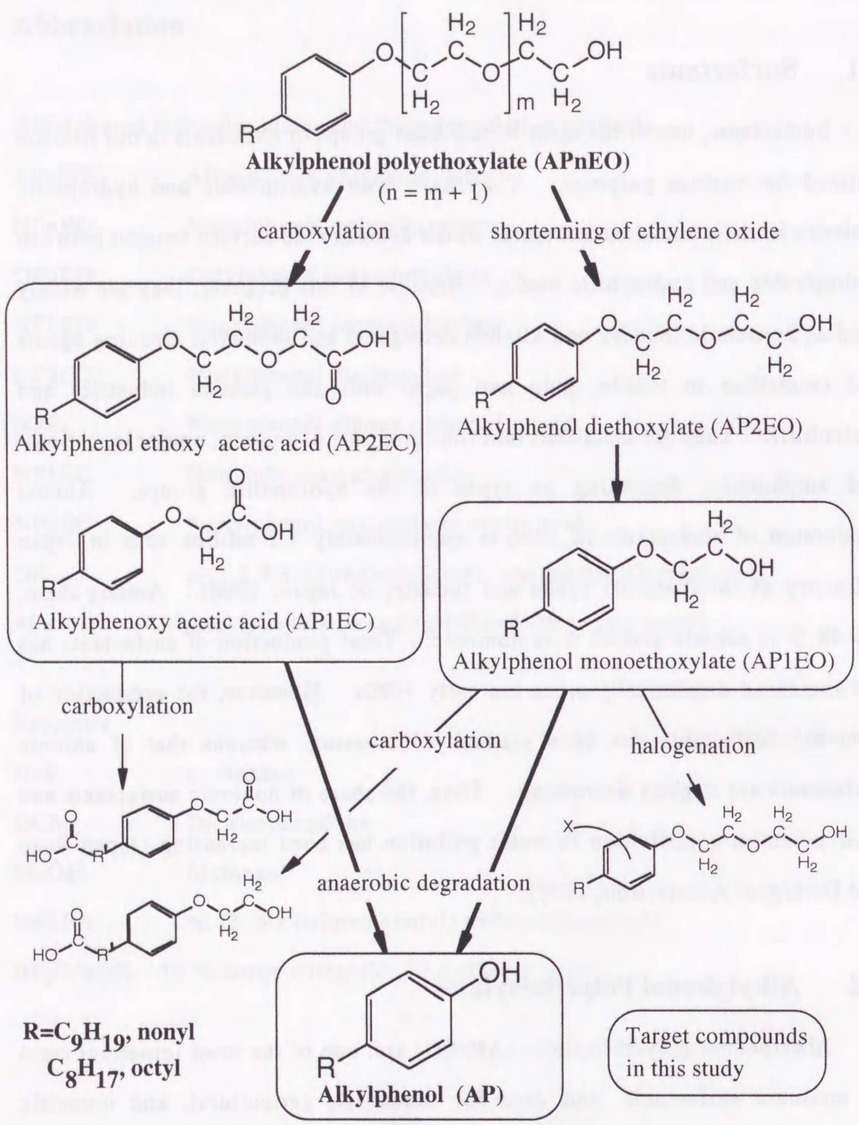


Figure 1.1 Biodegradation pathways of alkylphenol polyethoxylates (APnEO).

homologues with different number of alkyl carbons. Nonylphenol polyethoxylates (NPnEO) account for about 80 % of APnEO, and octylphenol

polyethoxylates (OPnEO) represent most of the remaining 20% (Renner, 1997). Approximately 500 thousand tons of APnEO are produced annually worldwide (Renner, 1997) and 50 thousand tons in Japan (Nakamura, 1998). In Japan, APnEO are used mainly for industrial usage, and minimally used for domestic detergents because major detergent companies do not use them for household detergents.

Because of their usage on a massive scale, these compounds are common constituents of wastewater. Giger *et al.* (1984) found that nonylphenol (NP) is generated through NPnEO degradation during sewage treatments, mainly by anaerobic digestion (Giger *et al.*, 1984). The biodegradation pathways of APnEO are shown in Figure 1.1, which indicates the parent compounds APnEO are degraded to more hydrophobic and persistent compounds. The mechanisms are well documented by Swisher (1987). APnEO are aerobically and/or anaerobically degraded to lower ethoxymer (AP1-2EO) and subsequently anaerobically degraded to alkylphenols (APs) by microbial activity in sewage treatment process. Concurrently, aerobic carboxylation of the end of EO chain results in ethoxy acetic acid (APEC), which was reported to be one of the major degradation products (Ahel *et al.*, 1987). Recent studies indicated that some oligomers which are halogenated at benzene ring (Reinhard *et al.*, 1982; Fujita and Reinhard, 1997; Maki *et al.*, 1998) and/or carboxylated at the end of alkyl moieties are produced (Di Corcia *et al.*, 1998).

1.3 Methodology of Analysis for Alkylphenol Polyethoxylates and Their Degradation Products in the Environmental Samples

The analytical methods for APnEO and their degradation products in recent studies are well documented in some review papers (Aboul-Kassim and Simoneit,

1993; Miszkiewicz and Szymanowski, 1996; Thiele *et al.*, 1997; Lee, 1999). In general, the analytical methods are comprised of extraction from environmental matrices and chromatographic separation and determination. Ahel and Giger (1985) applied solvent sublation, which was developed by Wickbold (Wickbold, 1972), to extract of NPnEO from raw sewage and effluent samples (Ahel and Giger, 1985b). Steam distillation and liquid-liquid extraction using non-polar solvent was used for NP and NP1-2EO extraction (Giger *et al.*, 1981; Stephanou and Giger, 1982), but they are not effective to extract higher EO chain oligomers (NPn≥3EO) due to their lower volatility and higher hydrophillicity. Solid phase extraction was also used to extract APs and related compounds using various resin, such as XAD (Schmitt *et al.*, 1990), graphitized carbon black (GCB) (Di Corcia *et al.*, 1994; Crescenzi *et al.*, 1995), and octadecylsilica (ODS) (Marcomini *et al.*, 1987; Kubeck and Naylor, 1990; Blackburn and Waldock, 1995; Lee and Peart, 1998). Soxhlet or steam distillation was the most common extraction methods for AP, APnEO and APEC from sludge or sediment samples (Giger *et al.*, 1981; Giger *et al.*, 1984; Ahel and Giger, 1985a; Marcomini and Giger, 1987; Lee *et al.*, 1997).

Both normal and reversed phase HPLC with UV or fluorescent detector (FL) have been used by many researchers to detect APnEO and their degradation products because they are applicable to analysis for compounds with wide range of molecular weight and polarity (Ahel and Giger, 1985a; Ahel and Giger, 1985b; Marcomini and Giger, 1987; Kubeck and Naylor, 1990; Jandera and Prokes, 1996). To overwhelm disadvantages (e.g., low resolution and low selectivity) associated with HPLC-UV/FL, LC–MS technique is frequently used in recent studies (Crescenzi*et al.*, 1995; Scullion *et al.*, 1996; Mackay *et al.*,

1997; Maruyama *et al.*, 2000). The electron ionization GC–MS has been most widely used in the analysis of free or derivatized APs and APnEO and APEC with short EO chain (Blackburn and Waldock, 1995; Lee and Peart, 1995; Field and Reed, 1996; Ding and Tzing, 1998). NP mass spectra were reported by (Giger *et al.*, 1981; Bhatt *et al.*, 1992; Wheeler *et al.*, 1997).

1.4 Occurrence and Behavior in the Aquatic Environment

The distributions of APnEO and their degradation products have been documented through many studies in the United States and Europe. Several authors have already reviewed their concentrations in various environmental matrices reported mainly for the past two decades (Naylor *et al.*, 1992; Thiele *et al.*, 1997; Takada and Eganhouse, 1998; Bennie, 1999; Maguire, 1999). Ahel *et al.* reported that concentrations in the Glatt River ranged from <0.3 to 45 μg/L for NP, n.d. to 30 μg/L for NP1EO, <1 to 45 μg/L for NP1EC, and 2 to 71 μg/L for NP2EC (Ahel and Giger, 1985a; Ahel *et al.*, 1994a; Ahel *et al.*, 1996). The recent studies reported that their concentrations in river water in Switzerland have decreased probably due to ban addressed in 1986 (Ahel *et al.*, 1999). In the 30-river study in the United States, NP, NP1EO, and NP2EO concentrations ranged from <0.11 to 0.64 μg/L, from <0.06 to 0.6 μg/L, and from <0.07 to 1.2 μg/L, respectively (Naylor *et al.*, 1992). In England, on the other hand, much higher concentrations of NP (i.e., 330 μg/L) was detected from the river which receive effluent from a sewage treatment plant receiving wastewater from textile-based industries (Blackburn and Waldock, 1995).

NP and lower ethoxymers are concentrated in the sediments and organisms due to their relatively hydrophobic and persistent nature (McLeese *et al.*, 1981;

Granmo *et al.*, 1989; Ekelund *et al.*, 1990; Ahel *et al.*, 1993; Tsuda *et al.*, 1999). There are several reports on the concentrations of NP and NP1EO in sediments and they are mostly in the range of a few-tens μg/g. The half-lives of NP in the sediment were estimated to be 28-104 days (Heinis *et al.*, 1999). There are only limited reports on bioconcentration. The reported bioconcentration factors (BCF) on laboratory experiment for stickleback (*Gasterosteur aculeatus L.*) and mussels (*Mytilus edulis L.*) was 1300 and 3400, respectively (Ekelund *et al.*, 1990). The BCF for fish of several species on field observation was 13-410, 3-300, and 3-330 for NP, NP1EO, and NP2EO, respectively (Ahel *et al.*, 1993).

1.5 Toxicity of Alkylphenol Polyethoxylates and Their Degradation Products

Aquatic toxicity and estrogenic activity of APs and related compounds have been reviewed by some researchers (Naylor, 1995; Nimrod and Benson, 1996; Servos, 1999). APnEO are biodegraded to shorter chain ethoxymers. The toxic effects of APnEO on most organisms generally increases as the length of the EO chain decreases. For example, the 48-h LC50 in Japanese killifish (*Oryzias latipes*) varied from 1400 μg/L for NP to 16600 μg/L for NP16.6EO (Yoshimura, 1986). Weeks *et al.* reported that the 96-h EC50 to *Ceriodaphnia dubia* for NP was 69 μg/L while that for NP1.5EO was 626 μg/L (Weeks *et al.*, 1996). The lowest No Observable Effect Level (NOEL) were 3.9 μg/L (28-d NOEC based on body length of *Mysidopsis bahia* for NP) (Naylor, 1995). Because of their high toxicity on aquatic organisms and persistence in the environment, understanding of the environmental distribution and the fate of APs and lower ethoxymers are important.

There are very few data available in the literature on the aquatic toxicity of APECs. Yoshimura (1986) reported the 48-h LC50 in Japanese killifish (*Oryzias latipes*) was 1400 μg/L for NP1EC (Yoshimura, 1986). Maki *et al.* (1998) reported the 48-h LC50 in *Daphnia magna* was ranged from 770 to 1300 μg/L for NP2EC (Maki *et al.*, 1998).

Soto *et al.* found NP have weak estrogenic activity through proliferation test of the human breast cancer cell MCF-7 (Soto *et al.*, 1991). Researchers in UK also reported the estrogenic activity to fish (Harries *et al.*, 1996; Harries *et al.*, 1997). In England, rainbow trout was put in a cage and raised in river water with sewage effluents. They found increase in concentration of vitellogenine, which is yolk protein normally produced by female, in the plasma of male rainbow trout raised in 4 out of 5 investigated rivers. The fact that the affected male rainbow trout was found close to effluent from a sewage treatment plants receiving wastewater from a textile-based industrial area demonstrated that STP discharge would induce endocrine disruption (Harries *et al.*, 1996; Harries *et al.*, 1997). Very high concentration of NP, 330 μg/L, was detected at the site (Harries *et al.*, 1997). Moreover, an *in-vitro* experiment, where a series of concentrations of NP was added to water with male rainbow trout indicated that concentration of vitellogenine in the plasma increased similarly to the observation in the river. Through these investigations, Jobling *et al.* concluded that the lowest concentration of NP and OP required to induce production of vitellogenine in the plasma of a male rainbow trout was approximately 10 μg/L (Jobling *et al.*, 1996).

From recent findings, NP, octylphenol (OP), NP2EO, NP1EC, and NP2EC are known to be estrogenic, while the higher ethoxymers of NPnEO lack

estrogenic activity (Jobling and Sumpter, 1993; White *et al.*, 1994; Jobling *et al.*, 1996; Routledge and Sumpter, 1996). Therefore these compounds have possibility to disrupt endocrine systems in aquatic organisms and in mammals and birds.

1.6 The Purposes of This Study

Many analytical methods for APnEO and their degradation products have been developed by many researchers. However the methods using HPLC with UV or fluorescent detector have disadvantages such as low resolution and low selectivity, as described above. On the other hand, the specific and sensitive methods using GC–MS cover only limited compounds. The broad spectrum and high sensitive analytical method for APnEO and their degradation products is needed. In the present study, at first, the analytical method using gas chromatography–mass spectrometry (GC–MS) was developed for the determination of the degradation products of APnEO [i.e., nonylphenol (NP), octylphenol (OP), nonylphenol monoethoxylate (NP1EO), nonylphenoxy acetic acid (NP1EC), and nonylphenol monoethoxy acetic acid (NP2EC)] in the environmental samples.

The ultimate purpose of this study was elucidation of behavior and fate of degradation products of APnEO in the aquatic environment in Tokyo. Until this study was started, there were almost no information about distribution of these compounds in the aquatic environment except for only a few studies in Japan.

The distributions of alkylphenols (APs) and related compounds have been documented through many studies in United States and Europe as described above. However in Metropolitan Tokyo, one of the most industrialized areas in

the world involving numerous human activities potentially releasing APnEO and their degradation products, only limited information on them is available (Kojima and Watanabe, 1998; Maruyama *et al.*, 2000). The present paper discusses the distribution of NP, OP, nonylphenol monoethoxylate (NP1EO), nonylphenoxy acetic acid (NP1EC), and nonylphenol ethoxy acetic acid (NP2EC) in the aquatic environments in Tokyo. One of the most important factors determining the distributions of APs in the aquatic environments is elimination of APnEO and APs during sewage treatment. Thus, in the present study, NP, OP, NP1EO , NP1EC and NP2EC were measured for the influents and effluents, i.e. raw sewage, primary effluent, secondary effluent and final effluent, from 2 sewage treatment plants (STPs) in Tokyo and their removal efficiencies and mechanisms were discussed.

The association of APs and related compounds with aquatic particles is one of the important processes controlling the fate in the rivers and coastal environments. Although there are many monitoring studies of APs in the aquatic environment, very few studies focused on partitioning of APs between aqueous and particulate phase (Sekela *et al.*, 1999). Some researchers (Kannan *et al.*, 1998) reported that NP was not present in the particulate phase. However, based on its moderate octanol-water partition coefficient (log K_{ow} = 4.48 for NP, 4.12 for OP and 4.17 for NP1EO (Ahel and Giger, 1993)) and on observations of NP in the aquatic sediments (Takada and Eganhouse, 1998) and references therein), it is reasonable to expect that some portion of NP are present in the suspended solids. In the present paper, distribution between aqueous phase and suspended solids was studied for the river water and wastewater effluent samples and significant proportion of NP, OP and NP1EO were found in the suspended solids. The degradation products of APnEO in the particulate matter are finally

deposited to the bottom sediment, which may act as a reservoir and/or ultimate sink of them. The sedimentary distributions of APs and NP1EO in the riverine environment in Tokyo were also demonstrated.

2. Experimental Section

2.1 Materials

The compounds listed below were used as standard materials in this study. 4-Nonylphenol (nonylphenol) and Polyethylene Glycol Mono-4-nonylphenyl Ether (n≈2) (NPn≈2EO) were purchased from Tokyo Chemical Industry co. ltd. (Tokyo) and p-(1,1,3,3-Tetramethylbutyl) phenol (p-t-octylphenol) was from Wako Pure Chemical Industry (Tokyo). Nonylphenoxy acetic acid (NP1EC) and Nonylphenol monoethoxy acetic acid (NP2EC) standard was provided by Hayashi Pure Chemical Industries Ltd. (Osaka). 4-n-nonylphenol-d_4 used as surrogate for NP and OP was also provided by Hayashi Pure Chemical Industries (Osaka). Chemical structures of target compounds are shown in Figure 2.1.

Nonylphenol Tokyo Kasei (Tokyo Chemical Industry co. ltd.); cat no. N 0300

4-Nonylphenol (mixture of compounds with branched side chain); CAS no. 104-40-5, MW = 220.4, d. 0.95, fl. 150°

Octylphenol Wako Pure Chemical Industry; cat no. 158-01252 (95+ %)

p-(1,1,3,3-Tetramethylbutyl) phenol (p-t-Octylphenol); CAS no. 140-66-9, MW = 206, mp. 80-86 °C

NPn≈2EO Tokyo Kasei (Tokyo Chemical Industry co. ltd.); cat no. P 0704

Polyethylene Glycol Mono-4-nonylphenyl Ether *n*≈2; CAS no. 26027-38-3, d. 1.00

NP1EC Hayashi Pure Chemical Industries Ltd.; cat no. 52393 (98.2 %)

Nonylphenoxy acetic acid; MW = 278.4

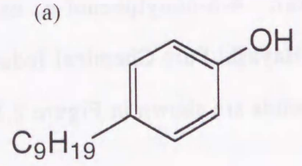
NP2EC Hayashi Pure Chemical Industries Ltd.; cat no. 52394 (99.6 %)

Nonylphenol monoethoxylate acetic acid; MW = 322.4

4-n-NP-d_4 Hayashi Pure Chemical Industries Ltd.; cat no. 52396

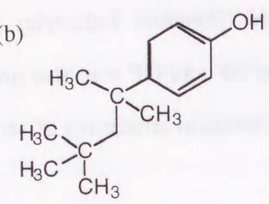
4-n-Nonylphenol-d type; MW = 220

(a)



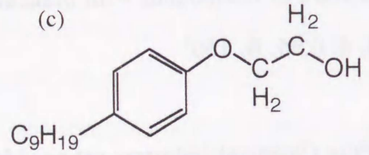
Nonylphenol
(NP)

(b)



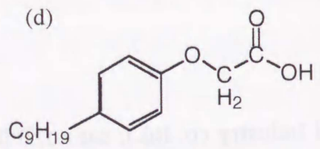
Octylphenol
(OP)

(c)



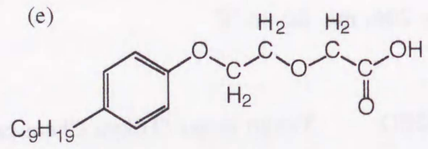
Nonylphenol monoethoxylate (NP1EO)

(d)



Nonylphenoxy acetic acid
(NP1EC)

(e)



Nonylphenol monoethoxy acetic acid
(NP2EC)

Figure 2.1 Chemical structures of the t arget compounds. Nonylphenol (NP, a), octylphenol (OP, b), nonylphenol monoethoxylate (NP1EO, c), nonylphenoxy acetic acid (NP1EC, d), and nonylphenol monoethoxy acetic acid (NP2EC, e). C_9H_{19} is highly branched alkyl chain.

2.2 Study Area

The watershed of Tokyo Bay (Tokyo metropolis, a part of Saitama, Kanagawa, and Chiba Prefectures) is 7600 km^2 and the population is 33 million. Approximately 77 % of domestic wastes and some industrial wastewater generated in the area are transported to municipal STPs and treated prior to their final effluent to the rivers and the Tokyo Bay. The remaining 23 % of domestic wastes (gray water) is directly discharged into streams and rivers without treatment. Wastewater from large-scale industrial plants is treated in the plants and then discharged to the rivers and the bay. The area of the Tokyo Bay is 980 km^2 and the average water depth is 15 m. The Tamagawa and the Sumidagawa Rivers comprise ~30 % of the fresh water inflow to the bay. The Tamagawa River is 140 km long with a normal fresh water inflow of ca. 15 m^3/s. The population and the area of its drainage basin are approximately 3 millions and 1240 km^2. The Tamagawa River has 8 municipal STPs in the drainage, one of which (STW-1; Figure 2.2) was studied in the present study. The population served by the STP-1 was 0.6 millions. The Sumidagawa River has a drainage basin of 610 km^2 and a population of 5.6 million people and is the largest contributor of organic pollution to the bay. Its length is 50 km and the freshwater inflow is approximately 40 m^3/s. There are 10 municipal STPs in the drainage of the Sumidagawa River. The population served by the STPs studied in the present study (STW-3, 4 and 5; Figure 2.2) was 1.7, 0.3 and 1 millions, respectively. Activated sludge treatment followed by mechanical treatment (i.e., settlement) is served in all the plants. The residence time of wastewater in the plants range from 7 to 17 h. The generated sewage sludge is incinerated and land-filled after solidification with cement.

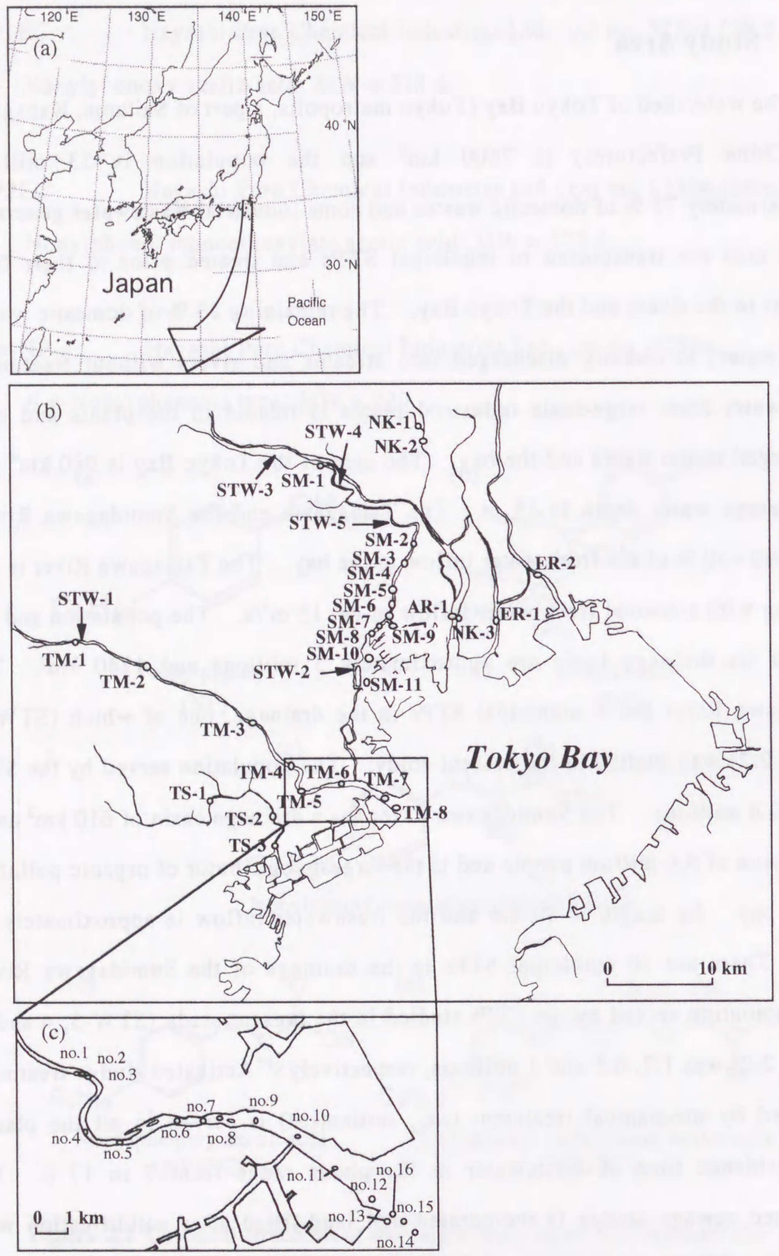


Figure 2.2 Study area and sampling locations. Japan (a), the STPs and river locations (b) and Tamagawa Estuary (c).

2.3 Sampling

Sampling locations are shown in Figure 2.2. The effluent from the primary settling tank (primary effluent) and from the secondary settling pond following aeration tank (secondary effluent) grab samples were taken from five STPs (STW-1-5) in February, August and December, 1997. The wastewater, i.e. raw sewage (influent), primary effluent, secondary effluent and chlorinated effluent (final effluent), were taken as the time proportional 24-h composite sample and sewage sludge grab samples were taken from two STPs (STW-1 and 3) in September and October, 2000 (Figure 2.2-b).

The river surveys were conducted in February, May, August and October, 1997 and July and August, 2000. In 1997, the river water samples were taken at three locations in the Tamagawa River (TM-2, 3 and 6) and three locations in the Sumidagawa River (SM-1, 2 and 6). In 2000, the river waters were taken at 17 locations in the Tamagawa River (TM-1-3 and 6), Sumidagawa River (SM-1-3 and 8), Tsurumigawa River (TS-1-3), Arakawa River (AR-1), Nakagawa River and its tributaries (NK-1-3), and Edogawa River and its tributary (ER-1 and 2) (Figure 2.2-b). To observe the behavior of NP, OP, NP1EO, NP1EC and NP2EC in the estuary, additional sixteen surface water samples (no. 1-16) were collected from the Tamagawa Estuary (Figure 2.2-c).

All the water samples were collected using a stainless steel bucket, stored in a 3L-amber glass bottle, and transported to the laboratory. The samples were filtered within 8 hours after sampling with pre-baked glass fiber filter (GF/F, Whatman). The filtrates were acidified with 4M HCl to pH <1 to depress microbial degradation and stored at 4°C. The filters were stored at –30 °C until analysis.

The surface sediments were collected from the Sumidagawa River (SM-2

and 8), Tamagawa Estuary (TM-4 and 6), Tsurumigawa River (TS-3) and Edogawa River (ER-1 and 2) in December, 1999,August, May, and June, 2000, respectively, using the Ekman dredge. The sediment samples were stored in stainless steel containers with Teflon liner at –30 °C until analysis.

2.4 Analytical Procedures

The scheme of analytical procedure which was developed in this study is shown in Figure 2.3. The details of the analytical method are described below.

2.4.1 Extraction

The acidified aqueous samples (i.e. filtrates) were neutralized to pH 3-5 with 4M sodium hydroxide just before extraction. NP, OP, NP1EO , NP1EC and NP2EC were extracted by solid phase extraction using a glass tube containing 1.0 g of ODS resin (tC18 bulk extraction resin, Waters) at a flow rate of 20 mL/min under nitrogen pressure (0.2-0.5 kg/cm^2). As shown in Figure 2.4, the glass extraction tubes (SPE tubes) were packed by ourselves for every sample. The SPE tubes had been previously washed with 20 mL each of hexane, dichloromethane and methanol and 10 mL of purified water. Purified water was obtained by passed distilled water through a SPE tube. The volume of sample filtrates passed through one SPE tube was 1 L for river water and 200 mL-1 L for wastewater. All the analytes were eluted from the SPE tube with 20 mL of MeOH. Breakthrough was examined using tandem tubes for extraction of a river water sample and no significant amounts of analytes were detected in eluate from the second cartridge. The freeze-dried filter, which trapped particulate matters, was Soxhlet extracted with DCM for at least 10 hr. (10-15 min /cycle). Based on the volume of the samples filtered, the volume-basis concentrations (i.e., μg/L) were calculated for analytes in the particulate phase. Also, before

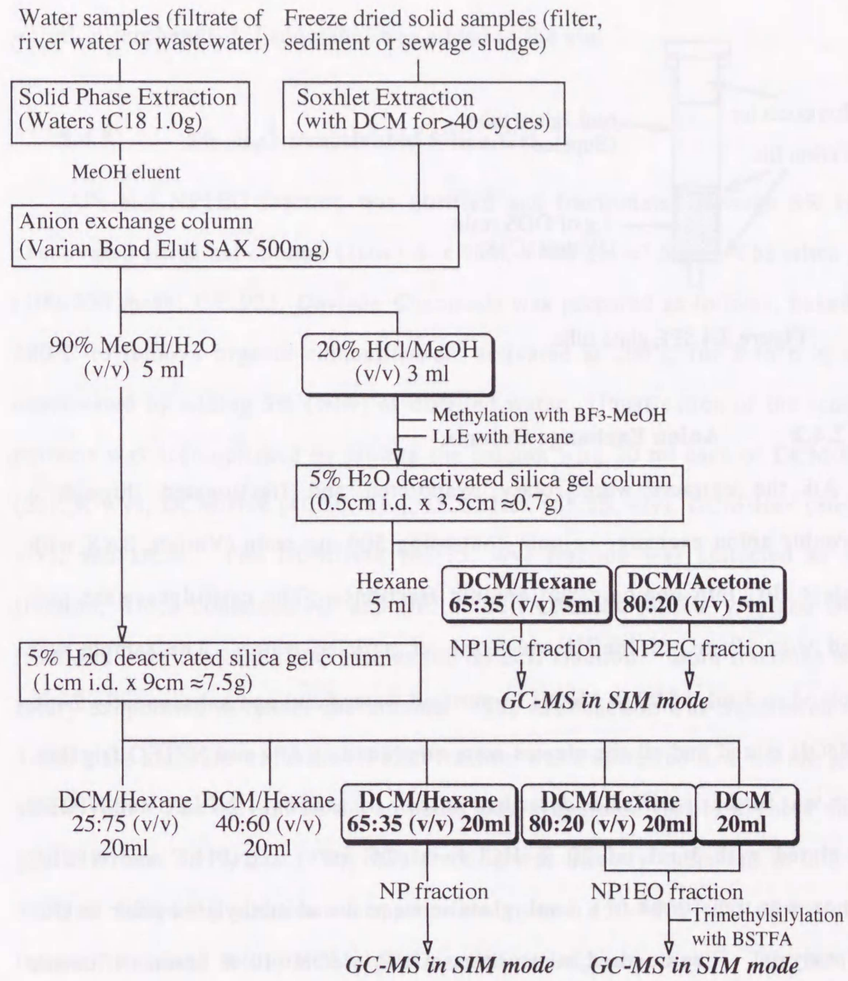


Figure 2.3 The scheme of analytical procedure for NP, OP, NP1EO, NP1EC and NP2EC.

the extraction, the weights of the particles on the filters were measured and, therefore, concentrations in the particulate phase are reported on a weight basis (i.e., μg/g) as well. The freeze-dried sediment samples were Soxhlet extracted with DCM for 12 hr. (10-15 min /cycle).

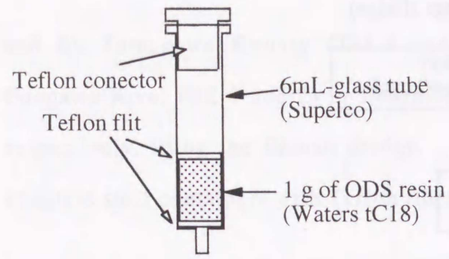


Figure 2.4 SPE glass tube.

2.4.2 Anion Exchange Column

All the extracts were rotary evaporated and fractionated through a disposable anion exchange column containing 500 mg resin (Varian, SAX with stainless flit) into nonionic and anionic fractions. The cartridges were pre-rinsed with 10 mL of MeOH and 5 mL of purified water. The sample was dissolved in 3 mL of 90 % MeOH in purified water (v/v) and subsequently 2 mL of MeOH eluted and all the eluates were combined as APs and NP1EO fraction, which was further purified as described in the next section. NP1EC and NP2EC was eluted with 3 mL of 20 % HCl in MeOH (v/v). NP1EC and NP2EC fraction was transferred to a 4-mL glass screw vial and methylated prior to GC-MS analysis. Five hundred micro liters of BF_3/MeOH (10 % boron trifluoride in MeOH (w/w), Supelco) was added to 20 % HCl in MeOH in the vials, tightly capped, and derivatized for 1 hour at 80 °C. After cooling, methylated NP1EC and NP2EC were liquid-liquid extracted by adding 300-500 μL each of purified water and hexane. The hexane layer was transferred into a 1.5-mL glass screw vial. The extraction was repeated three times, all the hexane extracts were combined, evaporated just to dryness under gentle stream of N_2 gas and appropriate volume (50-1000 μL) of injection internal standard solution (5

μg/mL p-terphenyl-d_{14}/isooctane) was added to the vial.

2.4.3 5% H_2O Deactivated Silica Gel

APs and NP1EO fraction was purified and fractionated through 5% H_2O deactivated silica gel column (1cm i.d. x 9cm, silica gel ≈7.5 g). The silica gel (100-200 mesh; F.C.923, Davison Chemical) was prepared as follows; baked at 380°C to remove organic contamination, activated at 200°C for 5 to 6 h, and deactivated by adding 5% (w/w) of distilled water. Purification of the sample extracts was accomplished by eluting the column with 20 ml each of DCM/Hex (25:75, v/v), DCM/Hex (40:60, v/v), DCM/Hex (65:35, v/v), DCM/Hex (80:20, v/v), and DCM. The DCM/Hex (65:35, v/v) fraction was collected as APs fraction, which contained NP and OP. The DCM/Hex (80:20, v/v) and DCM fractions were combined and used as the NP1EO fraction. Both fractions were rotary evaporated to reduce the volume. The APs fraction was transferred to a 1-mL glass ampoule while the NP1EO fraction was transferred to a 1.5-mL glass screw vial. The solvent in the ampoule was evaporated just to dryness under gentle stream of N_2 gas. The APs fraction was directly subjected to GC-MS analysis following addition of appropriate volume (50-1000 μL) of injection internal standard solution (5 μg/mL anthracene-d_{10}/isooctane). The NP1EO fraction was trimethylsilylated prior to GC-MS analysis. Two hundred micro liters of N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA, Wako Pure Chemicals) was added into the vials containing the extracts. Following 1-h reaction at room temperature, the solvent in the vial was evaporated just to dryness under gentle stream of N_2 gas and appropriate volume (50-1000 μL) of injection internal standard solution (5 μg/mL p-terphenyl-d_{14}/isooctane) was added to the vial.

2.4.4 Instrumental Analysis

NP, OP, NP1EO, NP1EC and NP2EC were analyzed on HP5972A quadrupole mass spectrometer fitted with HP5890 gas chromatograph or 5973 mass with HP6890 gas chromatograph (Hewlett Packard). GC-MS chromatogram of APs, NP1EO and NP1EC is shown in Figure 2.5, 2.6 and 2.7, respectively. Mass spectra of representative peak in chromatogram of APs, NP1EO and NP1EC is shown in Figure 2.8, 2.9 and 2.10, respectively. A DB-5 (J&W Scientific) or a HP-5 MS (Hewlett Packard) fused silica capillary column (30 m, 0.25 mm i.d., and 0.25 μm film thickness) was used with helium as the carrier gas at 60 kPa. GC-MS operating conditions were 70 eV ionization potential with the MS interface at 310 °C and the electron multiplier voltage at ~2500 eV. The injection port was maintained at 300 °C and the sample was injected with splitless mode followed by purge 1 min after the injection. The column oven temperature for APs analysis was held at 70 °C for initial 2 min, then programmed at 30 °C/min to 180 °C, 2 °C/min to 200 °C, 30 °C/min to 310 °C and held for 10 minutes. The oven temperature for NP1EO was as follows; the initial temperature was 70°C for 2 min, then raised to 200°C at 30 °C/min, to 230 °C at 2 °C/min, to 310 °C at 30 °C/min and held for 10 min. The oven temperature for NP1EC and NP2EC was as follows; the initial temperature was 70 °C for 2 min, then raised 310 °C at 10 °C/min and held for 10 min. A selected ion monitoring method was employed after solvent delay of initial 5 min.

As shown in Figure 2.5, the chromatogram of NP consists of 11 isomer peaks with various branched structures in the nonyl substituent. Furthermore, some of individual NP peaks contain several isomers and do not represent pure

(i.e., one) isomer. On the other hand, OP consists of a single peak due to one specific structure. They were quantified by comparing the integrated peak area by the summed selected ion monitor (m/z= 107 +121 +135 +149 +177 +220) with the peak area of the injection internal standard (anthracene-d_{10}, m/z=188). The peak composition (i.e., concentration of individual peaks) of the NP standard was determined by GC-FID analysis. The quantification was achieved from calibration curves made for individual peaks using standard solution (1, 2, 3, and 5 μg/mL for NP and 0.1, 0.2, 0.3, and 0.5 μg/mL for OP). All the calibration curves for NP and OP showed high linearity ($r^2 \geq 0.99$).

The chromatogram of trimethylsilylated NP1EO (TMS-NP1EO) consists of 10 isomer peaks with various branched structures in the nonyl substituent, as shown in Figure 2.6. They were quantified by comparing the integrated peak area by the summed selected ion monitor (m/z= 117 +135 + 179 +193 +237 +251 +321 +336) with the peak area of the injection internal standard (p-terphenyl-d_{14}; m/z=244). The peak composition of the NP1EO in the NPn≈2EO standard was determined by GC-FID analysis. All the calibration curves made for individual peaks using standard solution (1, 2, 5, 10, 20, 50, and 100 μg/mL) showed high linearity ($r^2 \geq 0.999$).

The chromatogram of methylated NP1EC and NP2EC (Me-NP1EC and Me-NP2EC) consists of 9 and 7 isomer peaks, respectively, with various branched structures in the nonyl substituent, as shown in Figure 2.7. They were quantified by comparing the integrated peak area by the summed selected ion monitor (m/z= 179 + 193 +235 +292 for Me-NP1EC and m/z= 179 + 193 +235 +292 for Me-NP2EC)with the peak area of the injection internal standard (anthracene-d_{10}, m/z=188 for Me-NP1EC and p-terphenyl-d_{14}; m/z=244 for Me-NP2EC). The peak compositions of the Me-NP1EC and Me-NP2EC in the

standard were determined by GC-FID analysis. All the calibration curves made for individual peaks using standard solution (1, 2, 3, 5, 10, and 20 μg/mL) showed high linearity ($r^2 \geq 0.99$).

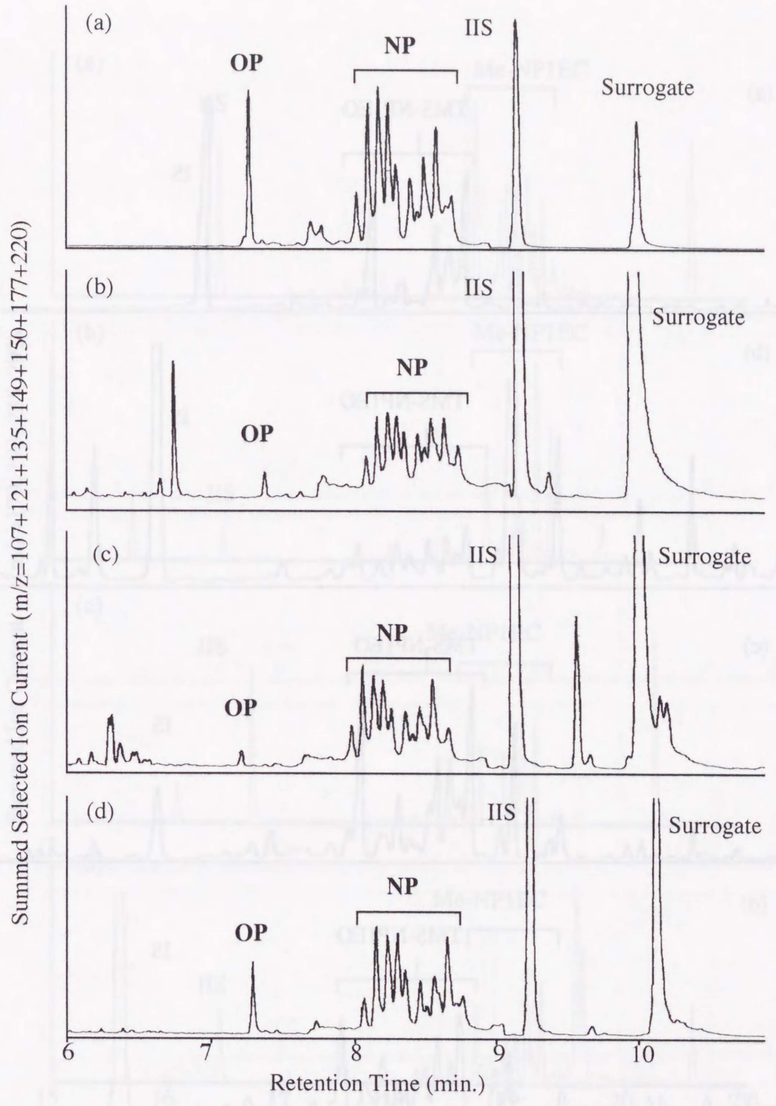


Figure 2.5. GC-MS chromatogram of NP and OP in SIM mode. Standard solution (a), aqueous (b) and particulate (c) phases in the river water and sediment (d) samples. IIS; anthracene-d_{10}, Surrogate; 4-n-NP-d_4.

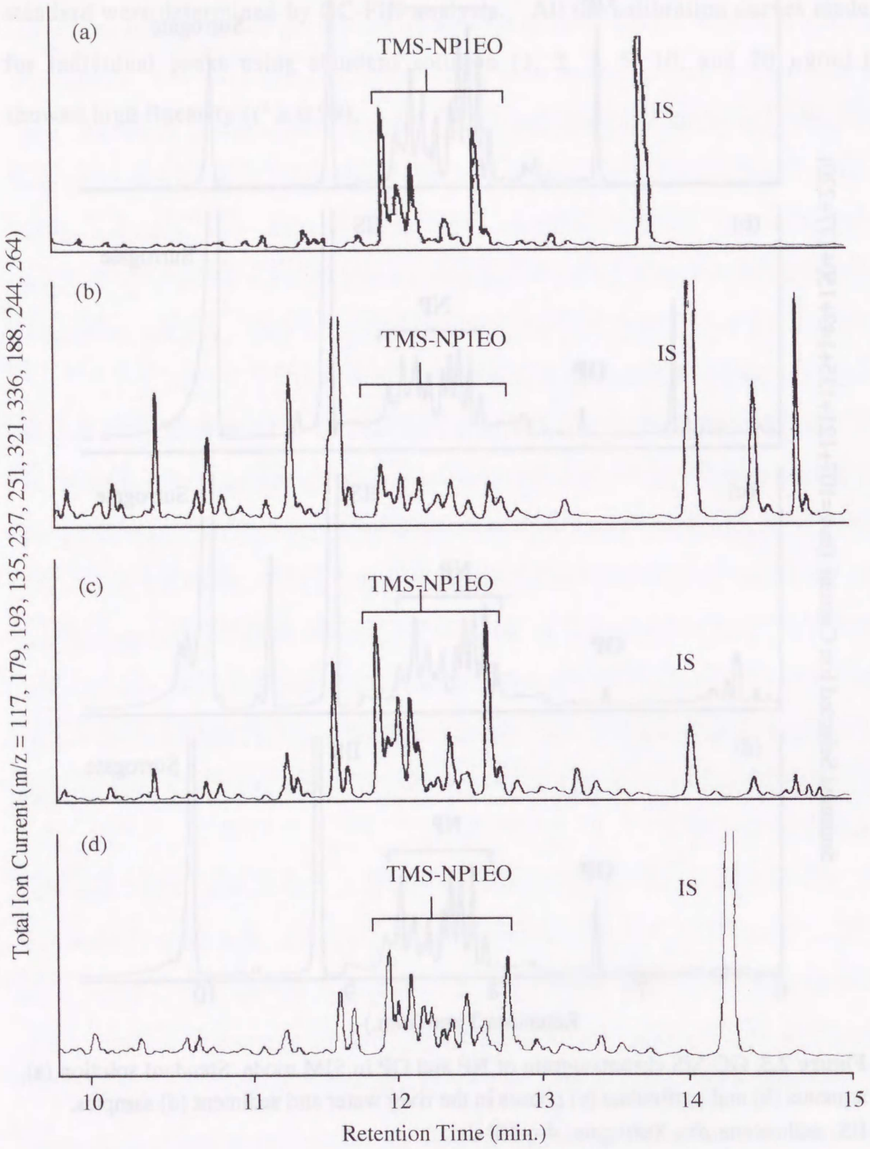


Figure 2.6 GC-MS chromatogram of trimethylsilylated NP1EO in SIM mode. Standard solution (a), aqueous (b) and particulate (c) phases in the river water and sediment (d). TMS-NP1EO; trimethylsilylated NP1EO, IS; p-terphenyl-d_{14}.

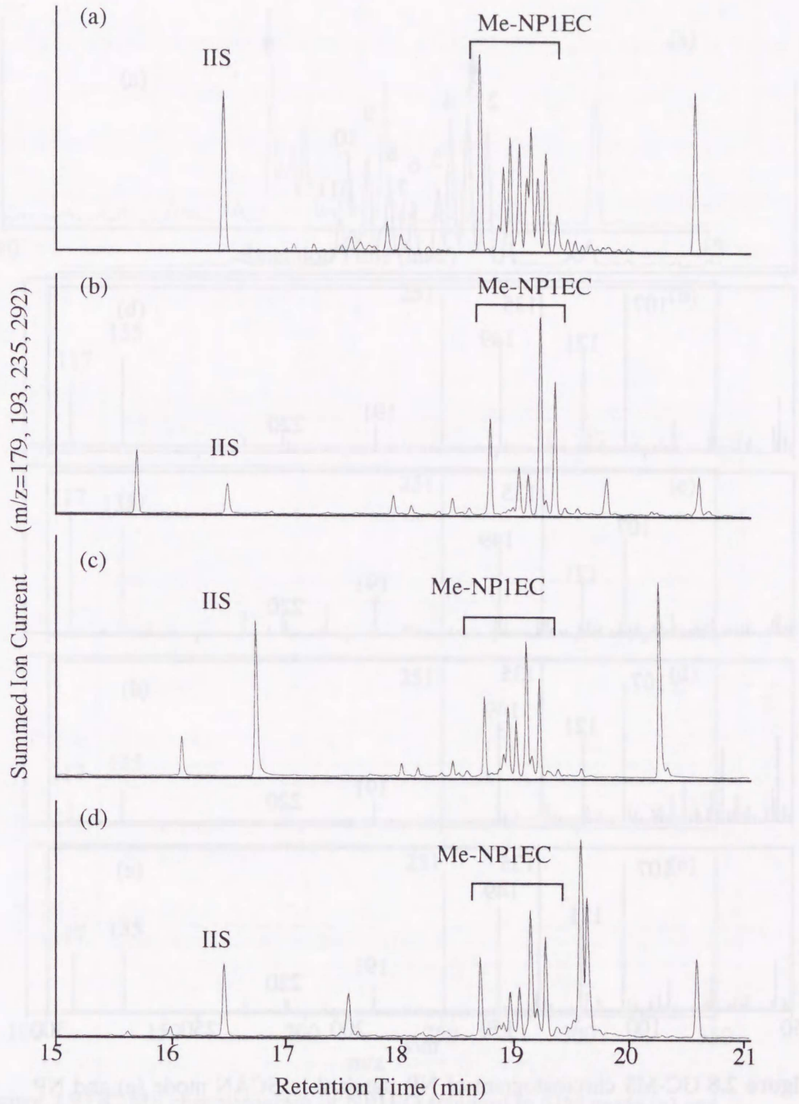


Figure 2.7 GC-MS chromatograms of methylated NP1EC in SIM mode. Standard (a), sewage effluent (b), river water (c) and activated sludge (d). Me-NP1EC; methylated NP1EC, IIS; anthracene-d_{10}.

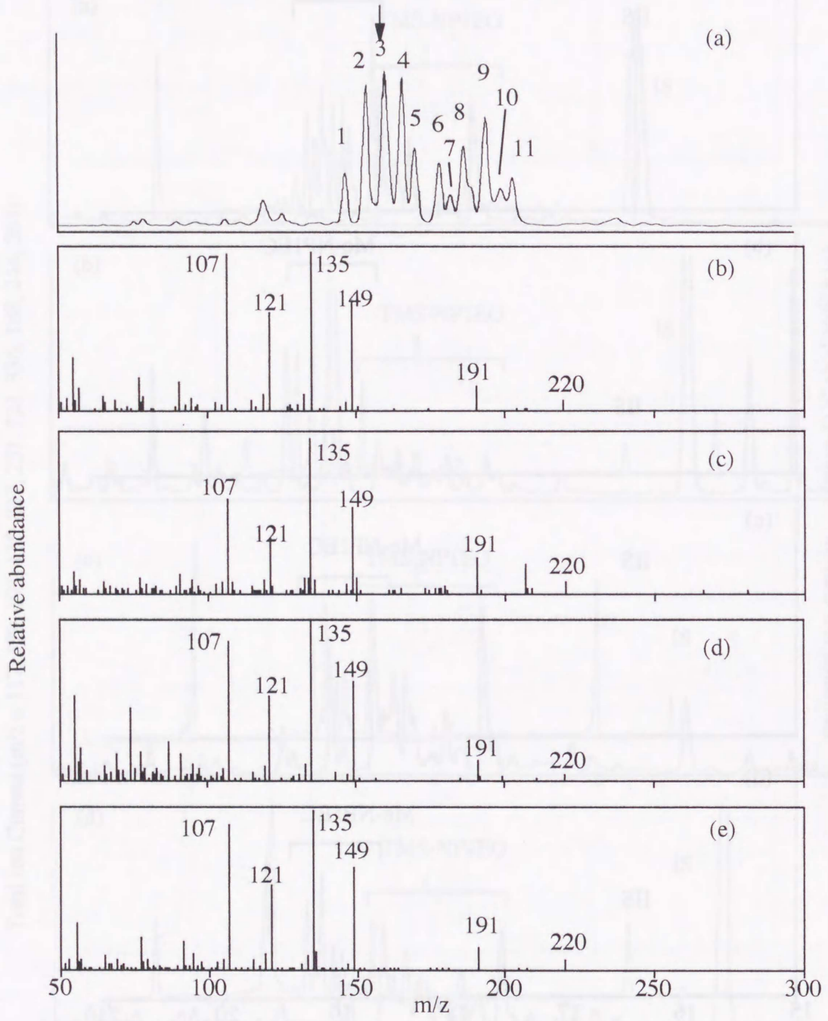


Figure 2.8 GC-MS chromatogram of NP standard in SCAN mode (a) and NP mass spectra of the representative peak (indicated by arrow) of standard solution (b), river water (c), primary effluent (d) and sediment (e).

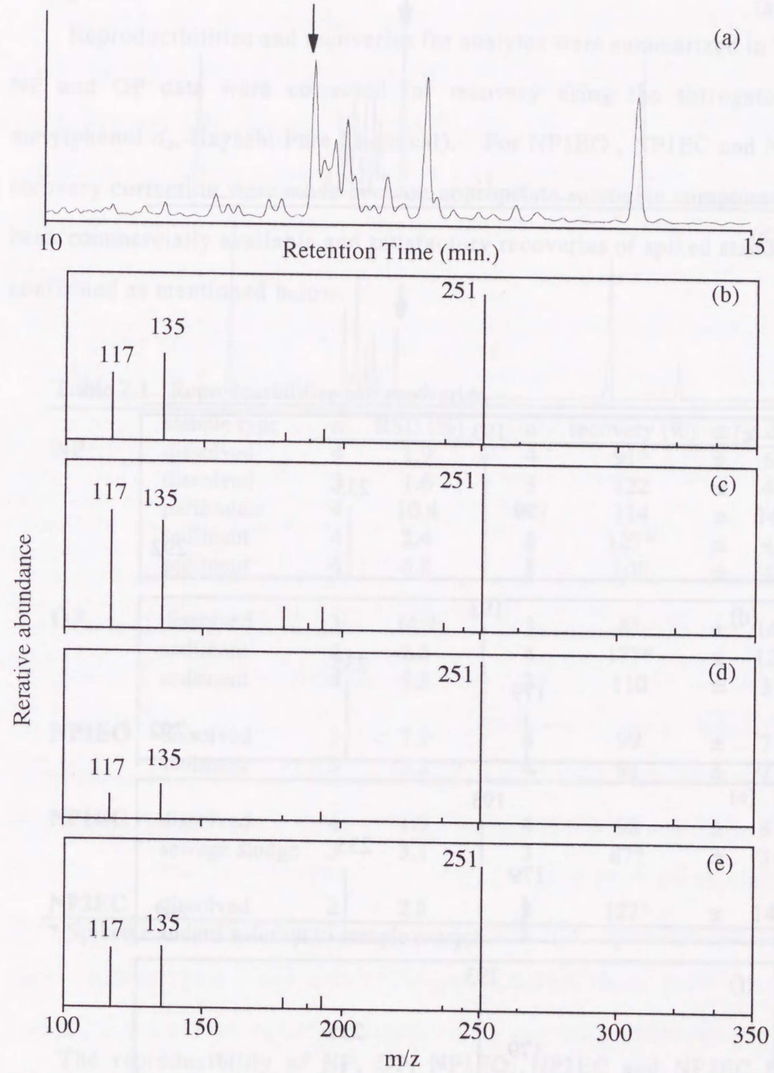


Figure 2.9 GC-MS chromatogram of NP1EO standard in SIM mode (a) and NP1EO mass spectra of the representative peak (indicated by arrow) of standard solution (b), aqueous (c) and particulate (d) phases in the river water and sediment (e).

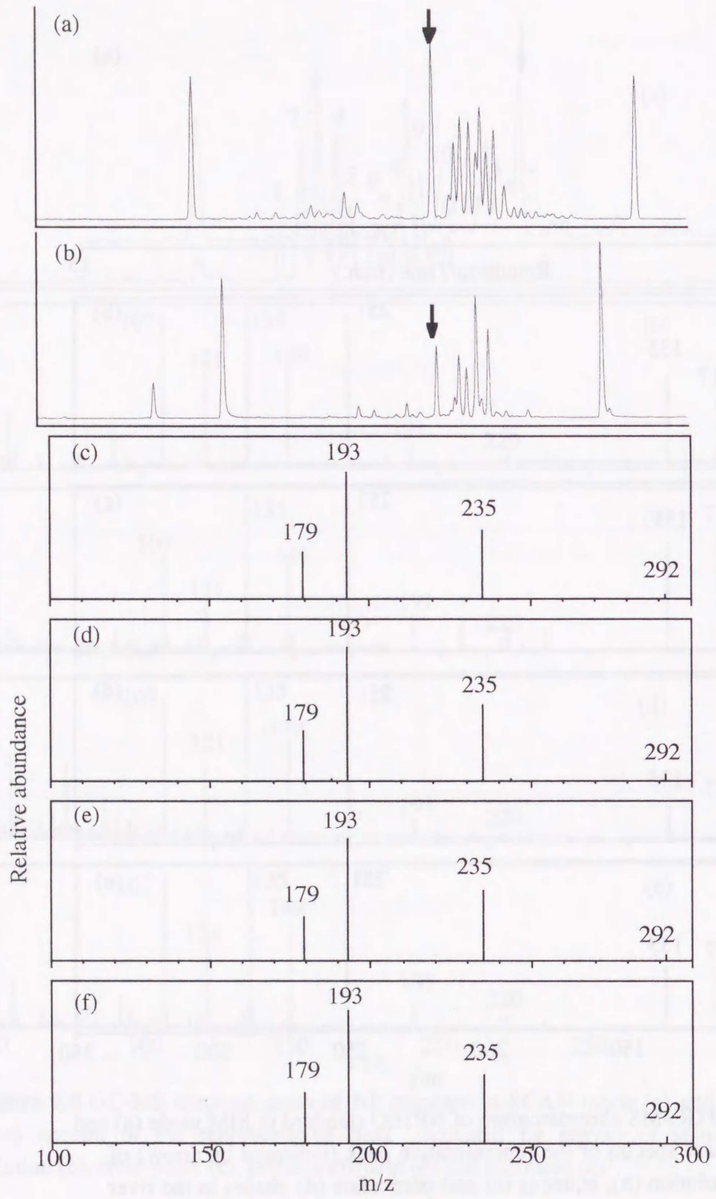


Figure 2.10 GC-MS chromatogram of NP1EC standard (a) and river water (b) in SIM mode and methylated NP1EC mass spectra of representative peak (indicated by arrow) of standard solution (c), final effluent (d), river water (e) and sewage sludge (f).

2.4.5 Analytical Precision

Reproducibilities and recoveries for analytes were summarized in Table 2.1. NP and OP data were corrected for recovery using the surrogate (i.e., n-nonylphenol-d_4, Hayashi Pure Chemical). For NP1EO , NP1EC and NP2EC no recovery correction were made because appropriate surrogate compounds had not been commercially available and satisfactory recoveries of spiked standards were confirmed as mentioned below.

Table 2.1 Reproducibilities and recoveries.

	sample type	n	RSD (%)	n	recovery (%)	±	s.d.
NP	dissolved	4	1.9	4	91*	±	6
	dissolved	3	1.6	3	122	±	4
	particulate	4	10.4	4	114	±	14
	sediment	4	2.4	4	127*	±	4
	sediment	4	4.8	3	108	±	4
OP	dissolved	3	14.2	3	81	±	14
	sediment	4	2.3	4	177*	±	12
	sediment	4	5.3	3	110	±	3
NP1EO	dissolved	3	7.9	4	99	±	7
	sediment	3	3.2	4	91	±	7
NP1EC	dissolved	4	1.9	4	98	±	8
	sewage sludge	3	3.1	3	87*	±	3
NP2EC	dissolved	2	2.8	3	127*	±	14

* Spiked standard solution to sample extract

The reproducibility of NP, OP, NP1EO, NP1EC and NP2EC for water samples was examined through replicate analysis of the river water or sewage effluent filtrates and the relative standard deviation (RSD) was 2 % (n=4), 14 % (n=3), 8 % (n=3), 2 % (n=4) and 3 % (n=2), respectively. The recovery was checked through replicate analysis of the filtrates spiked with NP, OP, NP1EO,

NP1EC and NP2EC standard solution, and was calculated at 122±4 % (n=3), 81±14 % (n=3), 99±7 % (n=4), 98±8 % (n=4) and 127±14 % (n=3), respectively. The reproducibility of NP, OP, and NP1EO for sedimentary samples was examined through replicate analysis of a sediment sample and RSD was 4.8 % (n=4), 5.3 % (n=4) and 3.2 % (n=3), respectively. Their recovery for sediment analysis was checked through replicate analysis of the sediment samples spiked with NP, OP, and NP1EO standard solution just before Soxhlet extraction. The recovery for NP, OP, and NP1EO was 108±4 % (n=3), 110±3 % (n=3), 91±7 % (n=4), respectively. The reproducibility and recovery of NP1EC for solid sample analysis were checked using activated sludge collected from STW-1 and 3 in 1997. The reproducibility of NP1EC was examined through triplicate analysis and RSD was estimated to be 3 %. The recovery was checked through triplicate analysis of the sludge samples spiked with NP1EC standard solution to extracts and it was 87±3 % (n=3). The reproducibility and recovery of NP2EC for solid sample analysis were not performed. Since reproducibility and recovery of NP1EC for sludge sample and those of NP2EC for water samples were sufficient as above, it was assumed that those of NP2EC for solid sample analysis would be also sufficient.

The procedural blank ran several times with samples and usually 1.5 ng of NP, 0.3 ng of OP, 4 ng of NP1EO, 1.3 ng of NP1EC, and 3 ng of NP2EC were found. Quantification limits were set at 10 times as the procedure blank values. Therefore, the limits of quantification were 15 ng/L, 3 ng/L, 40 ng/L, 13ng/L, and 30 ng/L for NP, OP, NP1EO, NP1EC and NP2EC, respectively, when 1 L of water sample was analyzed, and 15 ng/g, 3 ng/g, 40 ng/g, 13 ng/g and 30 ng/g for NP, OP, NP1EO, NP1EC and NP2EC, respectively when 1 g of sediment sample was analyzed.

3. Results and Discussion

3.1 Development of Analytical Procedure for Degradation Products of Alkylphenol Polyethoxylates

To improve reliability of analytical method, several examinations were conducted as below.

3.1.1 NP, OP and NP1EO analysis

Stability of NP and OP the Water Samples

To examine stability during preservation of NP and OP in the water samples which acidified and stored at 4 °C, river water samples were analyzed before and after the storage. The examination was made for two sets of river water samples. The first sample was taken from NK-1 in August 18. The sample was extracted and analyzed before and after 23 days of storage at 4 °C. In the sample without storage, 2.1 and 0.087 μg/L of NP and OP were detected, while 1.5 and 0.098 μg/L in the sample stored for 23 days. Twenty eight % of NP was lost during 23 days of preservation, and OP was increased by 12%, but no appropriate explanation is made here. The second sample was taken from TS-3 in May 25, and the storage period was 96 days at 4 °C. NP and OP concentrations in the pre-storage extract were 0.031 and 0.0018 μg/L and in the second extract were 0.022 and 0.0016 μg/L, indicating 29 and 15 % were lost during 96 days of preservation, respectively. Because filtrates were extracted usually within a week, the APs decrease over the storage period was ignored in the present study.

Adsorption to the Glass Ware

To examine adsorption loss of APs to the glass apparatus during filtration, a filtrate sample was re-filtered and analyzed. The detected NP and OP concentrations in the first filtrate sample were 0.19 and 0.017 μg/L (n=4) while those of the re-filtered sample were 0.20 and 0.023 ng/L (n=4). Thus no decrease in APs concentrations in the double-filtered sample was confirmed (Figure 3.1),.though appropriate explanation for OP increase between the first and the second filtration was not clear. The recovery of NP standard spiked to distilled water was approx. 60 % (n=2, data not shown). However, when spiked

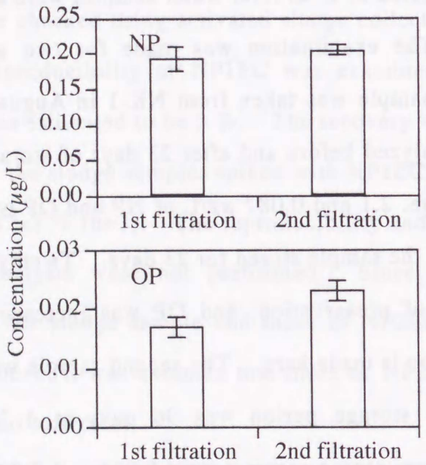


Figure 3.1 APs concentrations in the single- and double-filtered samples.

to the primary effluent of a STP, the NP recovery was 121 % (n=3). These results suggests that the loss of the target compounds due to adsorption to glass apparatus is insignificant when environmental sample is analyzed, which could be explained by matrix effect.

Stability of TMS-NP1EO Derivatization

The stability of trimethylsilylated NP1EO (TMS-NP1EO) was examined. The relationship between time intervals after trimethylsilylation and detected amounts was shown in Figure 3.2. No significant decrease in concentration was observed 16 hours after the derivatization.

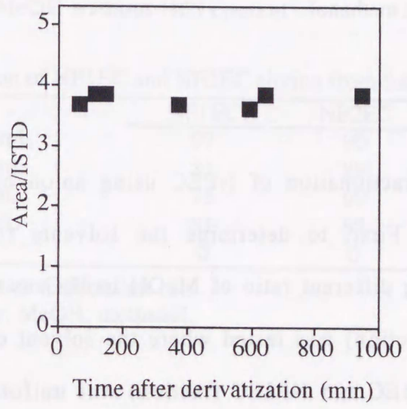


Figure 3.2 Stability of trimethylsilylated NP1EO.

3.1.2 NP1EC and NP2EC analysis

Solid Phase Extraction

The condition for extraction of NPEC from water sample using SPE column was examined. In the test 1, the standard solution was added to SPE column using distilled water and it was eluted by 20 mL of MeOH. In the test 2, after adding NPEC standard solution using distilled water, it was cleaned by 10 mL of 20 % MeOH/H_2O, then eluted by 20 mL of MeOH. In the test 3, the standard solution was added using 40 % MeOH/H_2O instead of distilled water and then it was eluted by 20 mL of MeOH. No remarkable loss was observed in all the 3 tests (Table 3.1). In addition, every column used in the tests was extracted by DCM to confirm no significant residual NP1EC and NP2EC on the column.

Table 3.1 Condition of SPE using tC18 extraction tube.

subject	mL	wash	mL	elution	mL	recovery (%) NP1EC	NP2EC
DW	5			MeOH	20	110	118
DW	5	20% MeOH/DW	10	MeOH	20	92	114
40% MeOH/DW	10			MeOH	20	105	112

No significant NPEC in following DCM eluate was confirmed.
DW; distilled water, MeOH; methanol.

SAX Fractionation

The condition for fractionation of NPEC using anion exchange column (SAX) was examined. First, to determine the solvents for fractionation, recoveries of NPEC using different ratio of MeOH in H_2O as the first fraction (i.e., APs and NP1EO fraction) was tested where the solvent condition for the second fraction (i.e., NP1EC and NP2EC fraction) was uniformed to 3 mL of 20 % HCl/MeOH (Table 3.2). Regardless of the ratio of MeOH in H_2O, satisfactory recoveries were obtained, except for 100% MeOH which showed a significant loss. In order to simplify the procedure afterward, it is preferable to use less H_2O in the first fraction. Therefore 3 mL of 90 % MeOH/H_2O was employed.

Table 3.2 Condition of NP1EC and NP2EC extraction using SAX.

		recovery (%) NP1EC	NP2EC
DW		93	97
DW		93	107
25% MeOH/DW	3mL	91	94
50% MeOH/DW	3mL	96	104
80% MeOH/DW	3mL	97	99
90% MeOH/DW	3mL	98	104
95% MeOH/DW	3mL	99	106
MeOH	5mL	84	52

5 mL of 20%HCl/MeOH was used for elution.
DW; distilled water, MeOH; methanol.

Then, the condition for further fractionation from SAX was examined (Table 3.3). The extraction efficiency was tested using different ratio of HCl in MeOH after addition of standard solution onto SAX. The results showed that the loss was minimized when 3mL of 20 % HCl/MeOH was used and therefore the fractionation condition was set. Moreover, it was confirmed that NPEC does not elute in MeOH without HCl content.

Table3.3 Condition of NP1EC and NP2EC elution from SAX

	NP1EC	NP2EC
20% HCl/MeOH 5mL	97	90
10% HCl/MeOH5mL	81	89
5% HCl/MeOH 5mL	71	89
1% HCl/MeOH 5mL	81	85
MeOH 10mL	0	0

DW was used for all transferration.
DW; distilled water, MeOH; methanol.

Mini-Silica Column

Peak separations of NP in GC-MS chromatogram were sometimes reduced when followed by methylated NP2EC injection. To solve this problem, the step to fractionate NP1EC and NP2EC using 5 % H_2O deactivated silica gel column chromatography (namely mini-silica column) was investigated. The same silica gel used to fractionate NP and NP1EO as described above was filled in a pasteur pipette (0.5 cm i.d. and 3.5 cm length silica gel ≈0.7 g), and 0.5 cm of sodium sulfate anhydrous was put on top. NPEC fraction was further fractionated using this mini-silica column. As Table 3.4 shows, 5 mL of 65 % DCM/Hex. Fraction for NP1EC, and 5 mL of 20 % Acetone/DCM fraction for NP2EC were suggested to be collected and analyzed.

Table 3.4 Fractionation of NP1EC and NP2EC using mini-silica column.

	solvent	volume (mL)	NP1EC	NP2EC
series 1	65% DCM/Hex	5	114	−
	20% Ace/DCM	5	−	106
series 2	80% DCM/Hex	5	106	+
	40% Ace/DCM	5	−	78

The reproducibility and recovery of NPEC described in the previous section were the result of this method of using mini-silica column. In the latter half of this study, the fractionation of NP1EC and NP2EC was skipped since NP was analyzed using different GC-MS and thus separation of NP2EC from NP1EC was not necessary. Peak separations of NP in GC-MS was affected by methylated NP2EC but not for other compounds. Though the cause of this problem is unclear, special caution is needed when NP is analyzed together with NPEC.

Application to the River Water and Sewage Sludge

To confirm whether this method for NPEC analysis is applicable to routine analysis, NP1EC in the 12 filtrate samples were analyzed. These samples were

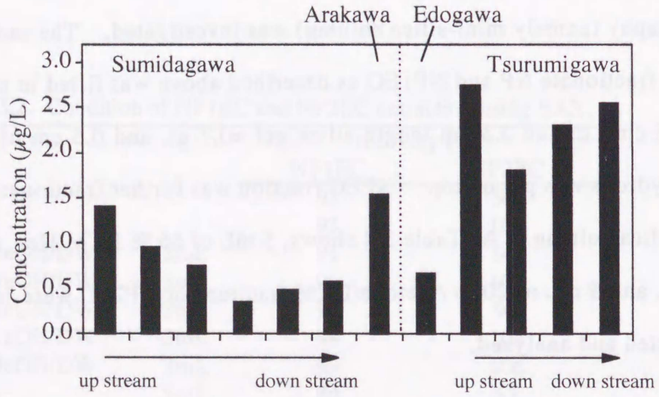


Figure3.3 NP1EC concentations in the river water (preliminary research).

collected as a preliminary investigation from the Tsurumigawa, Sumidagawa, Kyu-Edogawa, and Arakawa in May and June, 2000. As shown in Figure 3.3, the concentrations detected in the river waters were varied from 0.37 to 2.7 μg/L. The range was higher in the Tsurumigawa River than that in the other rivers and the Sumidagawa River had the seaward decreasing trend. These trends are similar to those observed in later study described in below.

In addition, to confirm the applicability of the method to analyze solid sample, activated sludge obtained from STW-1 and STW-3 were analyzed. As shown in Figure 3.4, concentration up to x10^3, weight basis, was observed when compared with the concentration in final effluent sample collected from STW-1 on the other day.

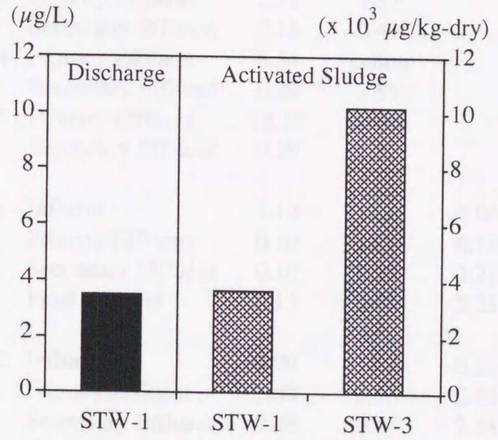


Figure 3.4 NP1EC concentration in the final effluent and activated sludge (preliminaly research).

3.2 Occurrence and Elimination of Degradation Products of Alkylphenol Polyethoxylates in Sewage Treatment Plants

3.2.1 Occurrence and Elimination

The concentrations of NP, OP, NP1EO, NP1EC and NP2EC in the wastewater collected from various stages of sewage treatment, i.e. influent, primary effluent, secondary effluent and final effluent, are listed in Table 3.5 and those in the sewage sludge are in Table 3.6.

Table 3.5 Concentrations of degradation products of alkylphenol polyethoxylates in the wastewater.

Date			Concentration in the wastewater (μg/L)				
			NP	NP1EO	NP1EC	NP2EC	OP
17.Feb.97	STP-1	Primary Effluent	2.49				0.906
		Secondary Effluent	0.53				0.484
	STP-2	Primary Effluent	17.24				1.075
		Secondary Effluent	1.03				0.105
6.Aug.97	STP-1	Primary Effluent	2.04	0.16			0.535
		Secondary Effluent	0.54	0.21			0.220
	STP-2	Primary Effluent	16.38	contm			1.173
		Secondary Effluent	0.41	2.01			0.073
28.Aug.97	STP-3	Primary Effluent	6.10	0.97			0.922
		Secondary Effluent	0.08	0.26			0.017
	STP-4	Primary Effluent	3.99	0.14			0.867
		Secondary Effluent	0.11	0.36			0.037
	STP-5	Primary Effluent	21.23	contm			1.817
		Secondary Effluent	0.49	0.71			0.158
3.Dec.97	STP-3	Primary Effluent	2.58	1.37			0.401
		Secondary Effluent	0.15	0.49			0.053
	STP-4	Primary Effluent	5.54	contm			1.087
		Secondary Effluent	0.29	2.85			0.127
	STP-5	Primary Effluent	18.17	contm			1.548
		Secondary Effluent	0.39	1.53			0.080
22.Sep.00	STP-1	Influent	1.12	11.4	0.04	0.50	0.033
		Primary Effluent	0.80	11.8	0.18	0.51	0.022
		Secondary Effluent	0.10	0.22	2.21	1.67	0.003
		Final Effluent	0.13	0.63	2.25	2.12	0.005
12.Oct.00	STP-2	Influent	0.99	11.2	0.36	0.10	0.086
		Primary Effluent	1.00	6.56	0.44	0.30	0.065
		Secondary Effluent	0.49	0.48	2.55	2.50	0.004
		Final Effluent	0.12	0.32	2.89	1.85	0.005

contm; contaminant interference.

Table 3.6 Concentrations of degradation products of alkylphenol polyethoxylates in the sewage sludge.

Date			Concentration in the sewage sludge (μg/g dry)				
			NP	NP1EO	NP1EC	NP2EC	OP
22.Sep.00	STP-1	Activated Sludge	1.71	14.3	0.13	0.42	0.077
		Raw Sludge	1.31	contm	0.12	0.25	0.044
12.Oct.00	STP-2	Activated Sludge	0.90	5.63	0.23	1.09	0.078
		Raw Sludge	1.63	contm	0.19	0.55	0.118

contm; contaminant interference.

APs Elimination in the secondary treatment

Figure 3.5 represents NP and OP concentrations in the primary and secondary effluents. The concentrations of NP in the secondary effluents ranged from 0.08 to 1.24 μg/L. The range is much lower than those reported for STPs in Switzerland (i.e., 2.2-44 μg/L, Ahel *et al.*, 1994a) when the usage of NPnEO for household detergents was legal. This is consistent with the fact that usage of NPnEO for household detergents in Japan is minimal. The range in 2000 is lower than that in 1997. Having no evidence supporting this, it is possible that consumption of APs in Japan has been decreasing. On the other hand, OP concentrations in the wastewater were twenty times lower than those of NP. This is similar to other studies (Lee and Peart, 1995; Bennie *et al.*, 1998).

The concentration range in Tokyo is also slightly lower than those reported in some other countries (Di Corcia *et al.*, 1994; Naylor, 1995; Lee and Peart, 1998). This is probably due to the higher removal efficiency of NP in STPs in Tokyo. The elimination efficiency for NP during the secondary treatment (Figure 3.6a) was estimated to be an average of 89 % with a range of 51-99 %. These values are derived from comparing the concentrations in the secondary effluents with those in the primary effluents. The calculations for 1997 data are

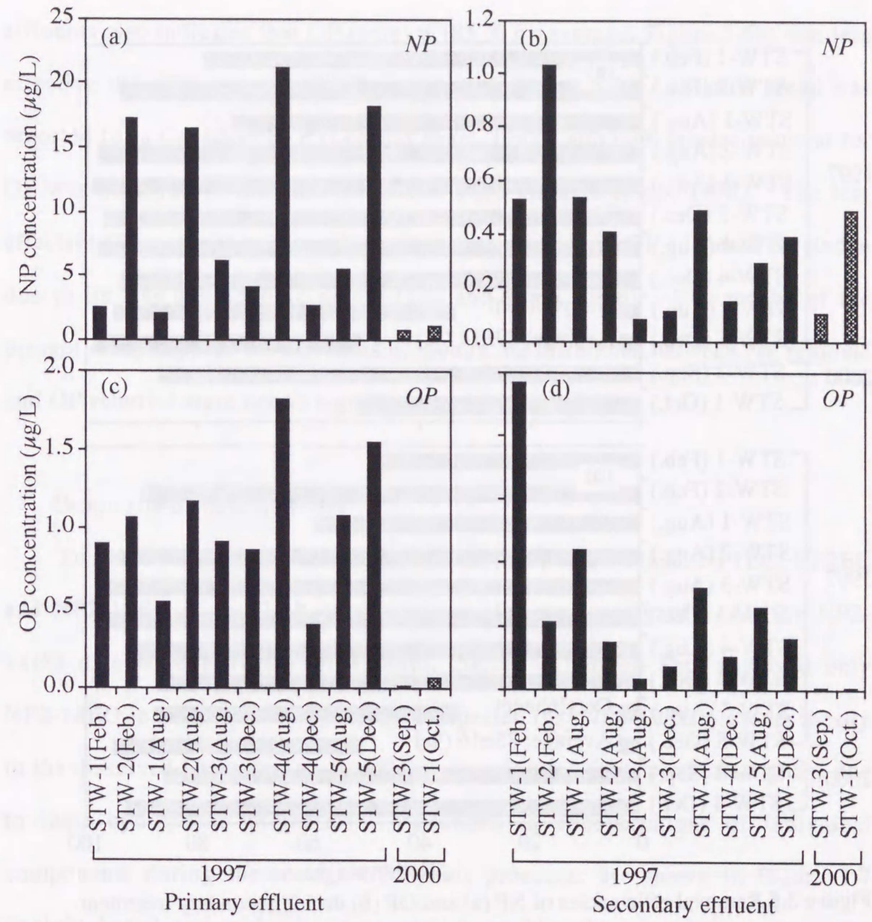


Figure 3.5 NP (a, b) and OP (c, d) concentrations in the primary (a, c) and secondary (b, d) effluents in the sewage treatment plants.

based on grab samples and, therefore, the calculated efficiencies should carry some error due to diurnal variation in APs concentrations (especially in the primary effluents). However, the 24-h composite samples gave 88 and 51 % of the removal efficiency for NP. Because the removal efficiencies calculated from the grab samples are in the range of, or higher than, the composite samples, these values are still valid though they might be overestimated. Since NP is

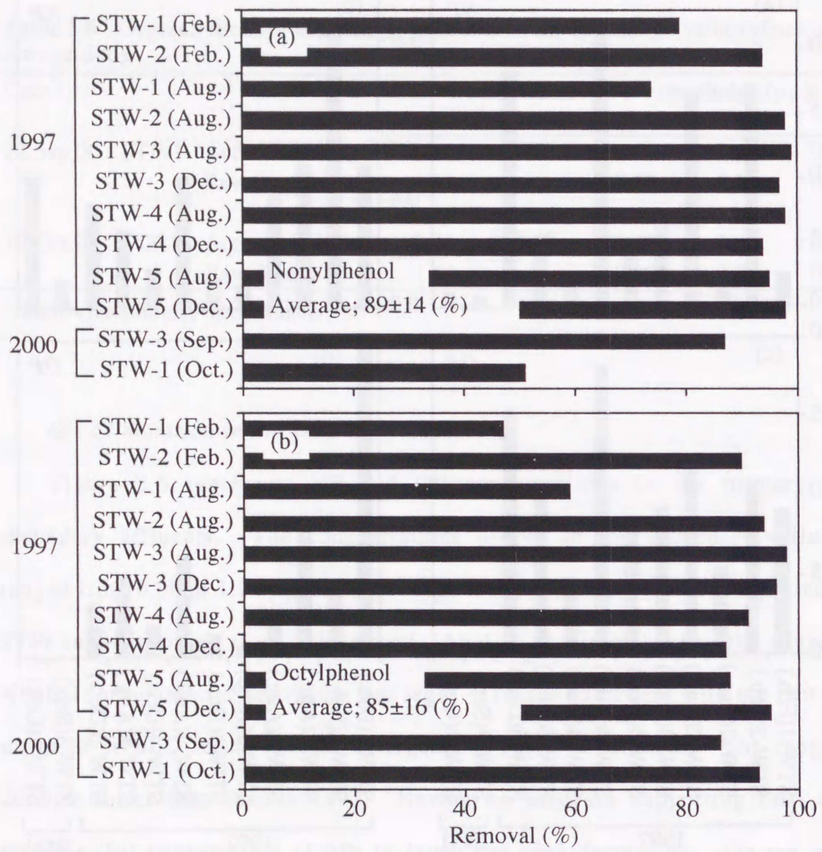


Figure 3.6 Removal efficiencies of NP (a) and OP (b) during secondary treatment.

also produced by the anaerobic degradation of NPnEO in the plant, the actual elimination of this compound should be higher. The removal in STPs in Tokyo seems to be as efficient as that in Italy (85 %, Di Corcia *et al.*, 1994) and Canada (88 %, Bennie *et al.*, 1998), and slightly less efficient than the United States (97 %, Naylor, 1995). Lower concentrations of NP in Japanese STPs than European STPs may be partially due to that NPnEO are mainly used as industrial surfactants and rarely used for household applications in Japan.

The comparison of APs concentrations between the primary and secondary

effluents also indicates that OP removal (85 % on average, Figure 3.6b) was less effective than NP removal (89 % on average, Figure 3.6a). Similar trend was reported for a Canadian STP (Lee and Peart, 1998), although greater removal for OP was observed in the other Canadian STPs (Bennie *et al.*, 1998). The less effective OP removal was ascribed to smaller amounts of OP adsorbed by sludge due to its less hydrophobic nature (Lee and Peart, 1998). The results of the present study support the mechanism, though the difference between NP removal and OP removal were not so significant.

Composite Sample Analysis

To obtain more quantitative information, NP, OP, NP1EO, NP1EC, NP2EC and NP2-18EO in the 24-h composite samples were analyzed. All the NP2-18EO data were provided from (Sato, 2001). Although Sato analyzed only NP2-18EO in the dissolved phase, it is expected that NP2-18EO presents mostly in the dissolved phase and no significant contribution from particulate phase due to their hydrophilic properties. Variations in concentrations of individual components during the sewage treatments processes are shown in Figure 3.7 (weight basis) and molar basis expression enables the comparison among the components as shown in Figure 3.8. NP, OP, NP1EO and NP2-18EO were removed efficiently from wastewater during sewage treatment, while NP1EC and NP2EC were increased in their concentrations during the treatments. The calculation clearly demonstrates that NP1EC and NP2EC were generated during treatment.

The elimination efficiencies for NP during the entire sewage treatment process were estimated to be 89 % and 88 % for STW-3 and 1, respectively,

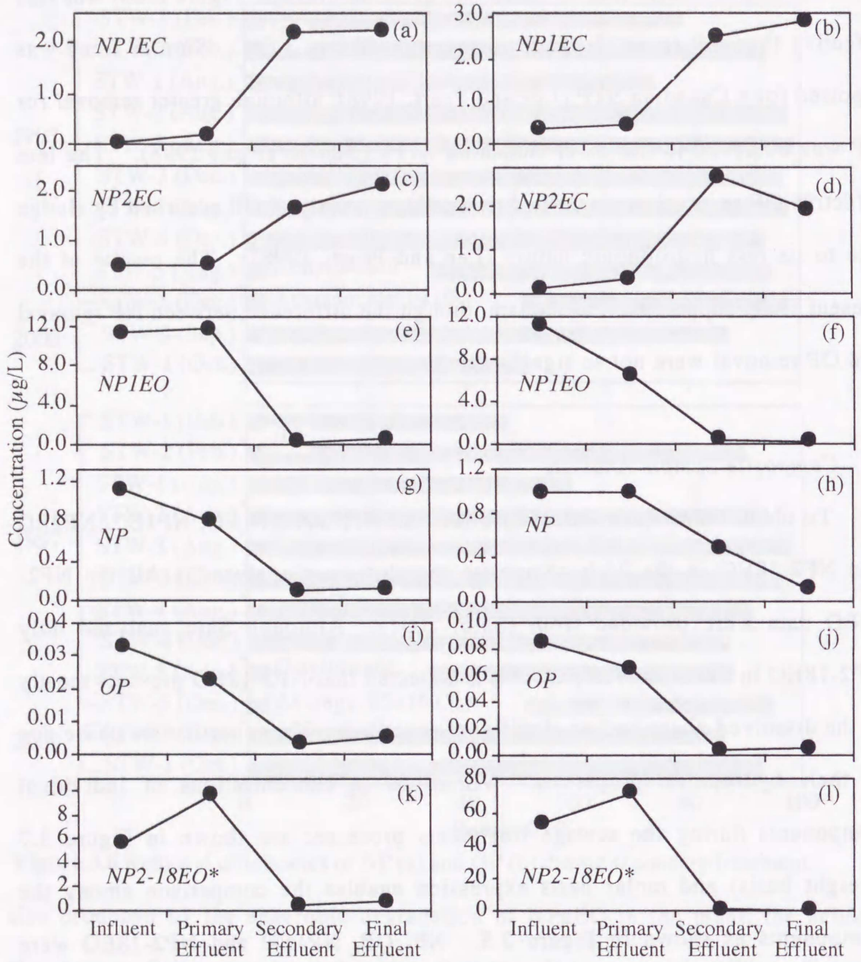


Figure 3.7 Variation in concentrations of NP1EC (a, b), NP2EC (c, d), NP1EO (e, f), NP (g, h), OP (i, j) and NP2-18EO (k, l) during sewage treatment in the STW-3 (a, c, e, g, i, k) and STW-1 (b, d, f, h, j, l) in 2000.
* NP2-18EO data from Sato (2001)

whereas those during the secondary treatment were estimated to be 88 and 51 % (Table 3.7). The former estimations were calculated by comparing the concentrations in the influents with those in the final effluents, and the latter by comparing the concentrations in the secondary effluents with those in the

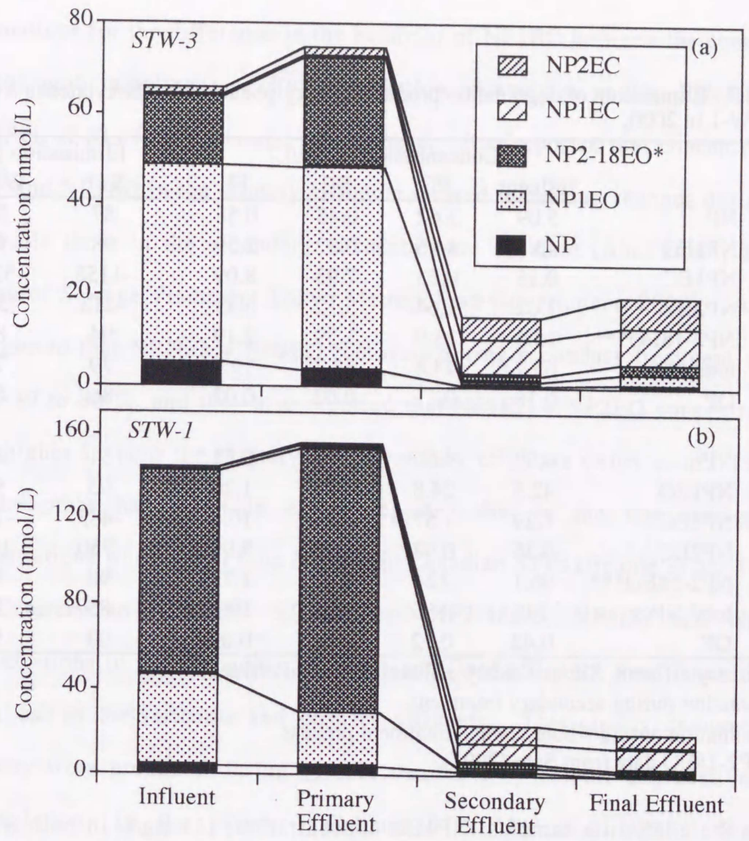


Figure 3.8 Variation in concentrations of total nonylphenolic compounds in STW-3 (a) and STW-1 (b) in 2000.
* NP2-18EO data from Sato (2001)

primary effluents. OP was eliminated as efficiently as NP in the STPs (85 and 94 % in STW-3 and 1). This was different from the calculation from the grab samples, where the OP removals during secondary treatment (85% on average) were less effective than the NP removals (89% on average) as described above. Although these inconsistency might be caused by analysis of the grab samples (e.g., diurnal variation), (Bennie *et al.*, 1998) reported OP was removed more efficiently than NP in the other Canadian STPs.

Table 3.7 Elimination of degradation products of alkylphenol polyethoxylates in STW-3 and STW-1 in 2000.

		Concentration (nmol/L)				Elimination (%)	
		Influent	PE	SE	FL	ST*	WT**
STW-3	NP	5.09	3.62	0.45	0.58	87	89
	NP1EO	43.3	44.6	0.82	2.39	98	94
	NP1EC	0.15	0.63	7.93	8.09	-1158	-5297
	NP2EC	1.62	1.64	5.12	6.49	-213	-302
	NP2-18EO***	15.5	23.9	1.39	2.19	94	86
	total NPcs	65.7	74.4	15.7	19.7	79	70
	OP	0.16	0.11	0.02	0.02	86	85
STW-1	NP	4.52	4.56	2.22	0.53	51	88
	NP1EO	42.5	24.8	1.83	1.20	93	97
	NP1EC	1.29	1.57	9.17	10.4	-483	-703
	NP2EC	0.35	0.97	8.25	5.99	-750	-1624
	NP2-18EO***	96.1	124	2.22	1.77	98	98
	total NPcs	145	156	23.7	19.9	85	86
	OP	0.42	0.32	0.02	0.02	93	94

PE; primary effluent, SE; secondary effluent, FE; final effluent.
* Elimination during secondary treatment.
** Elimination during whole sewage treatment process.
*** NP2-18EO data from Sato (2001).

In the composite samples, NP1EO concentrations (11 μg/L in both STPs) were much higher than NP in the influents and efficiently eliminated during sewage treatment (97 and 94 %) in both STPs (Table 3.5 and 3.7). This observation was quite different from the results for the grab samples analysis. In the grab samples (Table 3.5), NP1EO concentrations (0.14-1.37 μg/L) were much lower than that of NP (2.0-21 μg/L) in the primary effluents, while in the secondary effluents, they were roughly the same level (0.21-2.96 μg/L for NP1EO, 0.29-1.0 μg/L for NP). Possible explanation for this observation is the production of NP1EO through aerobic breakdown of NPnEO during secondary treatment. The elimination efficiencies of NP1EO during secondary treatment were either low or minus (i.e., generated during treatment). Possible

explanations for the difference in the behavior of NP1EO between the composite and the grab samplings are diurnal variation of analytes and/or difference in condition of STPs such as water temperature. It is reported that concentrations of NP and NP1EO in the primary effluents showed the strong changes during the day while those in the secondary effluents were constant (Ahel *et al.*, 1994a; Bureau of Sewage Treatment Tokyo Metropolitan Government, 2000). Ahel *et al.* reported that NP1EO + NP2EO removals during secondary treatment varied from -19 to 80 %, and they also reported that NP1EO + NP2EO concentrations were higher in both the primary and secondary effluents (Ahel *et al.*, 1994a). On the other hand, Bennie *et al.* reported that in the raw sewage NP concentrations were higher than NP1EO in Canadian STPs (Bennie *et al.*, 1998).

Contrary to NP, OP, NP1EO and NP2-18EO, NP1EC and NP2EC concentrations in the secondary effluents and final effluents were much higher than those in the influents and primary effluents. This clearly demonstrated that they were produced during aerobic treatment by aerobic degradation (i.e. carboxylation) in the plants. Calculated NP1EC and NP2EC removal efficiencies during the whole sewage treatment ranged from -302 to -5300% (Table 3.7). This phenomenon is consistent with other researches (Ahel *et al.*, 1987; Ahel *et al.*, 1994a; Bennie *et al.*, 1998). Ahel *et al.* reported that NP1EC and NP2EC concentrations in the secondary effluents were from 2 to 7 times higher than those in the primary effluents and were the most predominant compounds among NPnEO and degradation products in the effluent from the STPs in Switzerland (Ahel *et al.*, 1994a).

3.2.2 Phase Distribution of NP, OP and NP1EO in the STPs

The phase distribution of NP, OP and NP1EO in the sewage effluents is summarized in Figure 3.9-11. In the influents and primary effluents 52-91 % of NP (Figure 3.9), 41-81 % of OP (Figure 3.10) and 58-84 % of NP1EO (Figure 3.11) were found in the particulate phase, while in the secondary effluents and final effluents 6-64 % of NP, 4-32 % of OP and 2-21 % of NP1EO were in the particulate phase. The smaller proportion of particulate NP, OP and NP1EO in the secondary effluents and final effluents is explained by that most of the

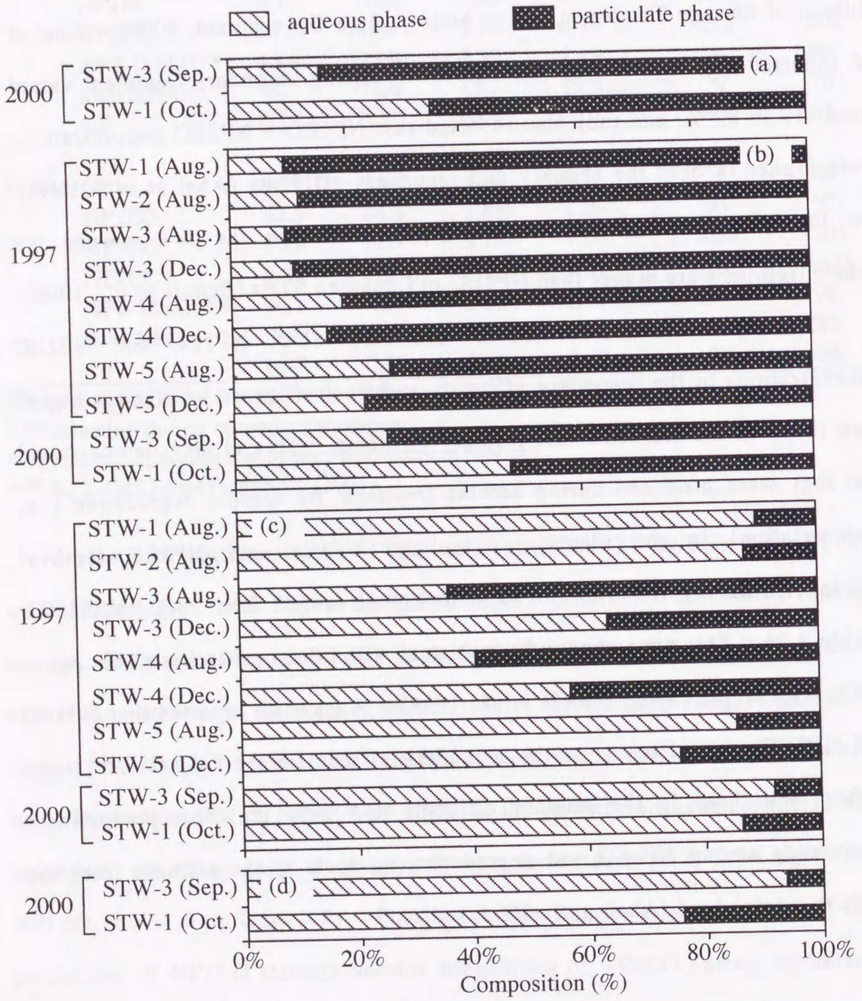


Figure 3.9 Phase distribution of NP in the influent (a), primary effluent (b), secondary effluent (c) and final effluent (d) from STPs.

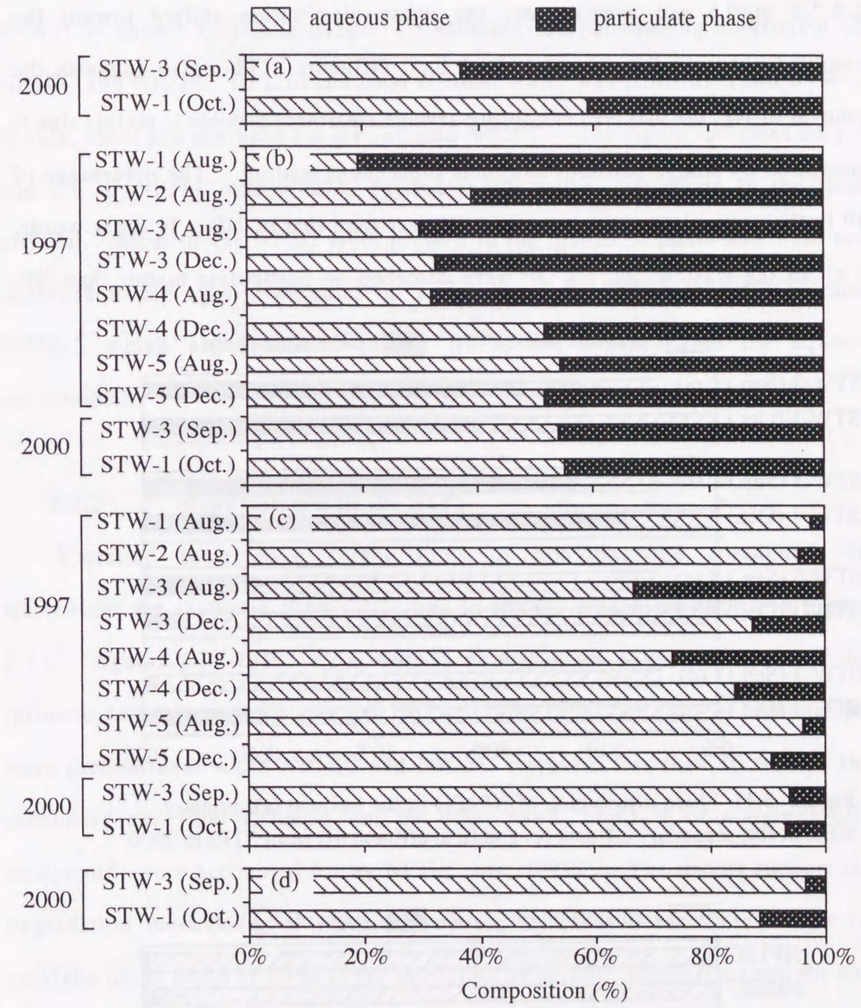


Figure 3.10 Phase distribution of OP in the influent (a), primary effluent (b), secondary effluent (c) and final effluent (d) from STPs.

particulate matter is removed in the settling tank (secondary clarifier) by precipitation and that the effluents from the settling tank (i.e., the secondary effluents and final effluents) contain only small amounts of suspended solids (2.8-7.2 mg/L) and consequently the phase distribution shifted toward the dissolved phase in the secondary and final effluents. This also suggests the removal of NP, OP and NP1EO during sewage treatment process is mainly due to adsorption by sludge particles and their subsequent settling. The percentage of OP in the particulate phase was always lower than that of NP. In other words, in all of the wastewater less OP were adsorbed on particulate matter than NP.

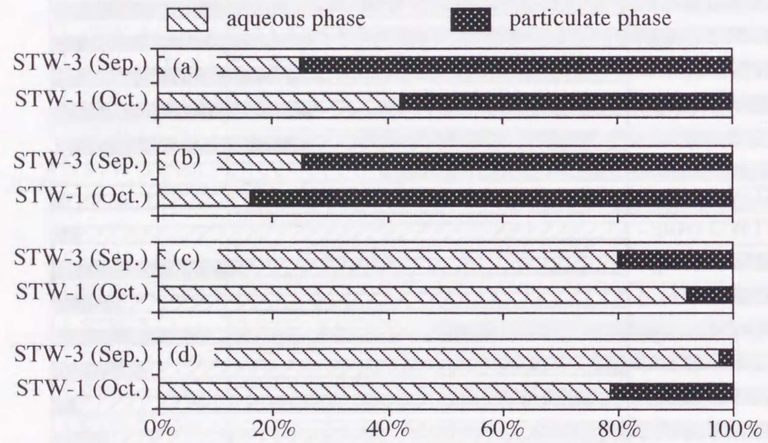


Figure 3.11 Phase distribution of NP1EO in the influent (a), primary effluent (b), secondary effluent (c) and final effluent (d) from STPs in 2000.

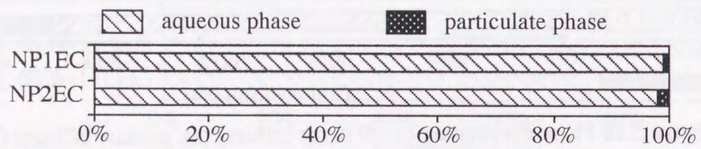


Figure 3.12 Phase distribution of NP1EC and NP2EC in the final effluent grab sample from STW-1 taken in July, 2000.

This is consistent with less hydrophobic nature of OP than NP. Because NP1EO have almost the same hydrophobicity as OP, the percentage of NP1EO in the particulate phase was similar to that of OP.

The phase distribution of NP1EC and NP2EC in the final effluent from STW-1 is shown in Figure 3.12. To estimate the partitioning coefficient of NP1EC and NP2EC, 10 L of the final effluent water was collected from STW-1 in July, 2000 and analyzed for NP1EC and NP2EC. The figure indicates only 1 and 2% of NP1EC and NP2EC existed in the particulate phase in the final effluent. Most of the NPEC were present in the dissolved phase and were not adsorbed to the aquatic particles in the wastewater. This means NP1EC and NP2EC, which are generated in the STPs, are released into the aquatic environment.

3.2.3 Mass Balance in the STPs

Variation in relative compositions of nonylphenolic compounds in wastewater are shown in Figure 3.13 and in sewage sludge are shown in Figure 3.14. Again, NP2-18EO data were provided from (Sato, 2001). In the influents and primary effluents, NP1EO and NP2-18EO (i.e., parent compounds) were predominant while NP1EC and NP2EC represent less than 10 %. In the secondary and final effluents, on the other hand, 74-83 % of nonylphenolic compounds were accounted for by NP1EC and NP2EC. The results support the degradation mechanism of NPnEO proposed by many researchers. That is, ethylene oxide chain of NPnEO are shortened by aerobic degradation and the end of chain are partially carboxylated in the STPs. This observation consists with (Ahel *et al.*, 1994a), which reported that 82 % of NPnEO and their degradation

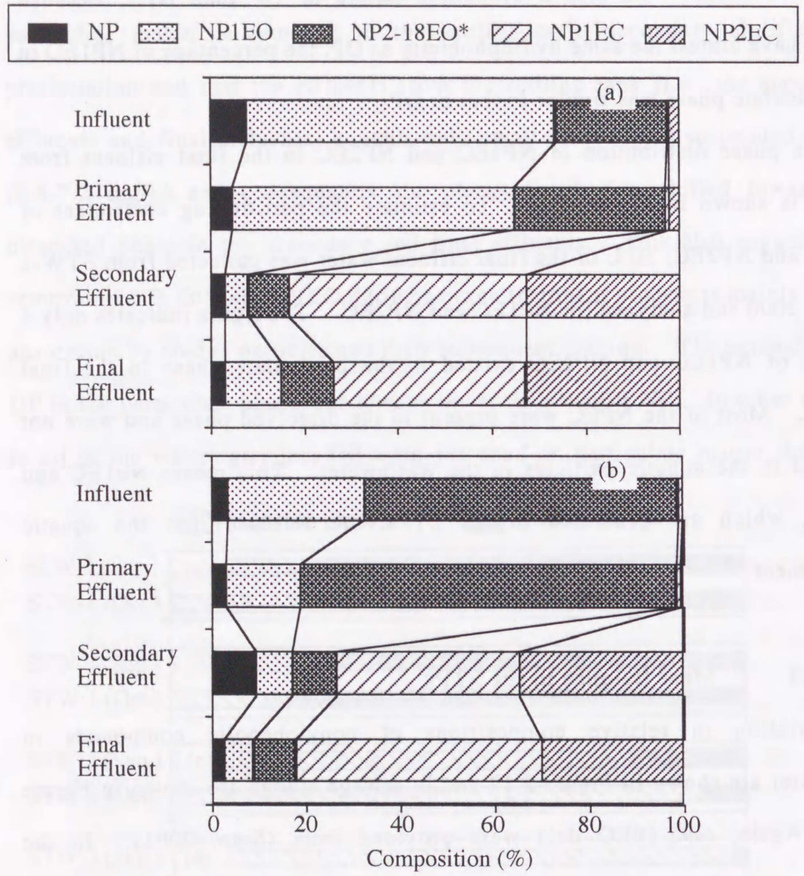


Figure 3.13 Variation in composition of nonylphenolic compounds in STW-3 (a) and STW-1 (b) in 2000.
* NP2-18EO data from Sato (2001)

products in the primary effluents existed as NPnEO (n≥3) whereas 28 % in the secondary effluents. NP1EC + NP2EC accounted for approx. 80 % of NPnEO and their degradation products in the effluent from STPs. In the sewage sludge, most of the nonylphenolic compounds were present as NP and NP1EO. This also means most of the NP1EC and NP2EC were not adsorbed to the suspended particles as described before. Although NP2-18EO in the sludge were not

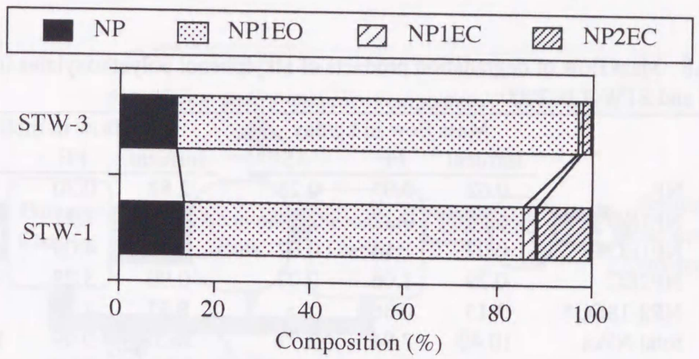


Figure3.14 Variation in composition of nonylphenolic compounds in activated sludge collected from STW-3 and STW-1.

analyzed, they are expected not to contribute significantly to nonylphenolic compounds in the sludge.

Table 3.8 demonstrates the mass flow of degradation products of alkylphenol polyethoxylates in the STW-3 and 1, which is expressed by both molar and weight basis. The load of nonylphenolic compounds from STW-3 and STW-1 to the aquatic environment is estimated to be 2.9 kg/day (10 mol/day) and 1.0 kg/day (3.5 mol/day), respectively. Most of the excess sludge from the primary and secondary clarifier is incinerated and used to make bricks in Tokyo. Consequently, it is likely that no NPnEO and their degradation products are released into the environment from the sewage sludge. Mass flux of nonylphenolic compounds in STPs is illustrated in Figure 3.15. As the figure indicates, 27 and 15 % of the nonylphenolic compounds entering the STPs escaped from sewage treatment and released to the rivers. NP1EC and NP2EC represented three-quarters of total nonylphenolic compounds in the final effluents. Also 44 and 77 % of the nonylphenolic compounds entering the STPs disappeared in the aeration tank, respectively. The possible mechanisms for

Table 3.8 Mass flow of degradation products of alkylphenol polyethoxylates in STW-3 and STW-1 in 2000.

		Mass flow in kg/day			Mass flow in mol/day		
		Influent	FE	AS	Influent	FE	AS
STW-3	NP	0.62	0.07	0.28	2.82	0.30	1.27
	NP1EO	6.34	0.32	2.34	24.01	1.21	8.87
	NP1EC	0.02	1.14	0.02	0.08	4.09	0.08
	NP2EC	0.29	1.06	0.07	0.90	3.28	0.21
	NP2-18EO*	3.13	0.36	n.a.	8.57	1.11	n.a.
	total NPcs	10.40	2.94	2.71	36.38	9.99	10.43
	OP	0.018	0.002	0.013	0.090	0.012	0.061
STW-1	NP	0.20	0.02	0.10	0.90	0.09	0.44
	NP1EO	2.23	0.06	0.60	8.45	0.21	2.28
	NP1EC	0.07	0.51	0.02	0.26	1.84	0.09
	NP2EC	0.02	0.34	0.12	0.07	1.06	0.37
	NP2-18EO*	10.72	0.10	n.a.	19.13	0.31	n.a.
	total NPcs	13.25	1.03	0.84	28.81	3.52	3.18
	OP	0.017	0.001	0.008	0.083	0.004	0.041

AS; activated sludge, total NPcs; nonylphenolic compounds analyzed in this study, FE; final effluent, n.a.; not analyzed.
* NP2-18EO data from Sato (2001).

this disappearance are ultimate degradation (i.e., mineralization) and transformation to compounds which are not analyzed in this study. There are some reports on the formation of NPnEO-related compounds which are halogenated on the benzene ring (Reinhard *et al.*, 1982; Fujita and Reinhard, 1997; Maki *et al.*, 1998) or are carboxylated at the end of alkyl chain (Di Corcia *et al.*, 1998). To further understand the mass balance of nonylphenolic compounds, the quantitative measurement of those compounds in the STPs is needed.

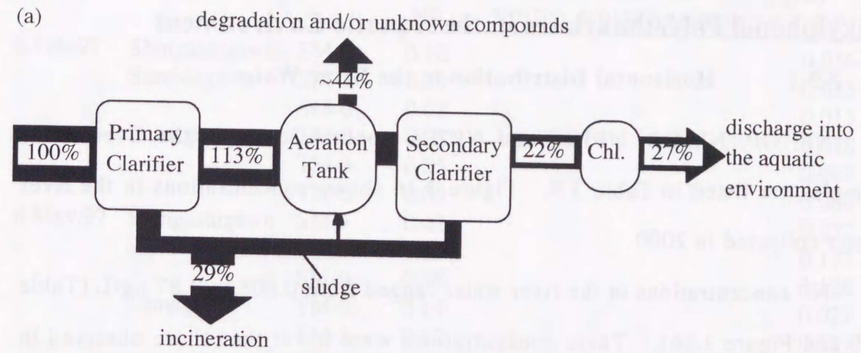


Figure 3.15 Calculated mass flow of nonylphenolic compounds in STW-3 (a) and STW-1 (b) (molar basis) in 2000.
Chl.; chlorination.

3.3 Distribution and Behavior of Degradation Products of Alkylphenol Polyethoxylates in the Aquatic Environment

3.3.1 Horizontal Distribution in the River Water

NP, OP, NP1EO, NP1EC and NP2EC concentrations in the river water samples are listed in Table 3.9. Figure 3.16 shows concentrations in the river water collected in 2000.

NP concentrations in the river water ranged from 0.008 to 2.87 μg/L (Table 3.9 and Figure 3.16). These concentrations were lower than those observed in European rivers (Ahel and Giger, 1985a; Ahel *et al.*, 1994b; Blackburn and Waldock, 1995; Ahel *et al.*, 1996; Schaffner *et al.*, 1999) and were similar to the range observed in the United States rivers (Naylor *et al.*, 1992) with one exception (NK-1). NK-1 might be received industrial wastewater with no or poor treatment. Lower concentrations of NP in Japanese rivers than that in European rivers (e.g., Ahel *et al.*, 1994b) may be due to that NPnEO are mainly used as industrial surfactants and rarely used for household applications in Japan. OP concentrations (0.0004-0.18 μg/L) were one order of magnitude lower than NP concentrations, similar to observations in other countries (Blackburn and Waldock, 1995; Bennie *et al.*, 1997) and observations in the STPs as described above.

In the Sumidagawa River, the downstream trend of APs concentration was similar between 1997 and 2000 surveys (Figure 3.17). Both NP and OP were once increased in the middle reaches, and decreased toward the river mouth. The concentration ranges of APs, however, were different. The range in 2000 was obviously lower than that in 1997, though the sampling location was not exactly same. This suggests APs production and/or consumption has decreasing trend.

Table 3.9 Concentrations of degradation products of alkylphenol polyethoxylates in the river water samples.

Date	River	Site	Concentration in the river water (μg/L)				
			NP	NP1EO	NP1EC**	NP2EC**	OP
6.Feb.97	Shingashigawa	SM-1	0.18				0.036
	Sumidagawa	SM-2	0.24				0.053
		SM-6	0.08				0.013
	Tamagawa	TM-2	0.05				0.018
		TM-3	0.05				0.007
		TM-6	0.05				0.006
8.May.97	Shingashigawa	SM-1	0.42				0.077
	Sumidagawa	SM-2	1.08				0.177
		SM-6	0.68				0.126
	Tamagawa	TM-2	0.14				0.027
		TM-3	0.12				0.071
		TM-6	0.12				0.043
4.Aug.97	Shingashigawa	SM-1	0.37	0.12*			0.104
	Sumidagawa	SM-2	0.66	0.22*			0.148
		SM-6	0.43	0.23*			0.085
	Tamagawa	TM-2	0.12	0.067*			0.051
		TM-3	0.13	0.035*			0.031
		TM-6	0.10	0.035*			0.017
30.Oct.97	Shingashigawa	SM-1	0.26	0.43*			0.125
	Sumidagawa	SM-2	0.32	0.81*			0.053
		SM-6	0.28	0.46*			0.079
	Tamagawa	TM-2	0.10	0.14*			0.050
		TM-3	0.17	0.16*			0.061
		TM-6	0.09	0.042*			0.039
13.Jul.00	Tsurumigawa	TS-1	0.052*	0.18*	2.59*	2.69*	0.0037*
		TS-2	0.085*	0.26*	2.82*	1.50*	0.0046*
		TS-3	0.053*	0.15*	1.71*	1.05*	0.0039*
	Tamagawa	TM-1	0.014*	0.095*	0.77*	0.81*	0.0007*
		TM-2	0.008*	0.070*	0.60*	0.70*	0.0004*
		TM-3	0.017*	0.064*	0.67*	0.70*	0.0007*
		TM-4	0.025*	0.075*	0.63*	0.47*	0.0009*
30.Aug.00	Shingashigawa	SM-1	0.06	0.0003***	2.00	2.63	0.0013
	Sumidagawa	SM-2	0.12	0.27	2.31	1.70	0.0030
		SM-3	0.13	0.24	1.76	1.27	0.0048
		SM-4	0.06	0.17	0.57	0.28	0.0024
	Arakawa	AR-1	0.093*	0.001*	1.67	1.55	0.0021
	Denugawa	NK-1	2.87	3.38	0.92	0.17	0.118
	Ayasegawa	NK-2	0.55*	0.026*	1.32	0.78	0.0350
	Nakagawa	NK-3	0.054*	0.002*	1.27	0.90	0.0037
	Kyu-Edogawa	ER-1	0.045*	0.001*	1.10	0.67	0.0022
	Edogawa	ER-2	0.021*	0.001*	0.47	0.09	0.0016

* Particulate phase was not analyzed
** Concentration in the particulate phase was below the detection limit.
*** Aqueous phase was not analyzed.

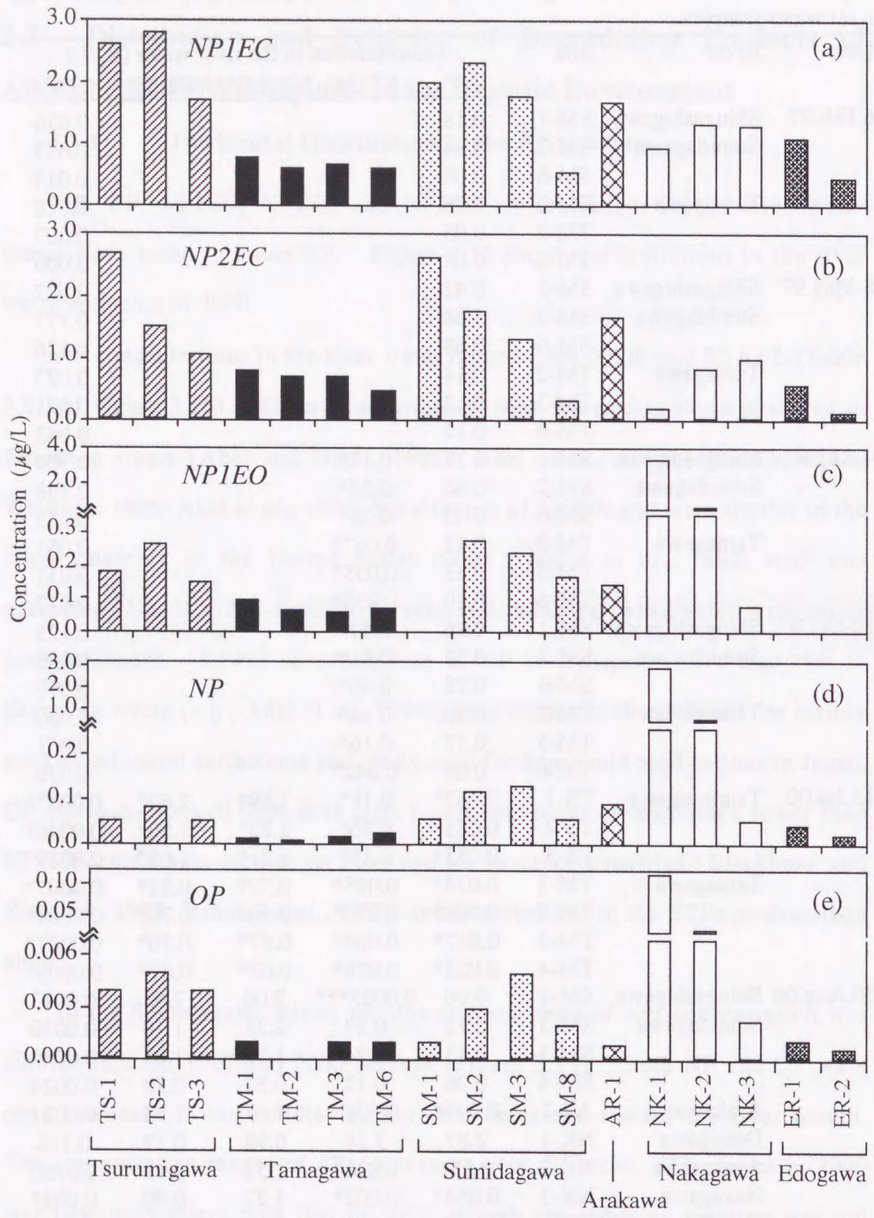


Figure 3.16 Concentrations of NP1EC (a), NP2EC (b), NP1EO (c), NP (d) and OP (e) in the river water.

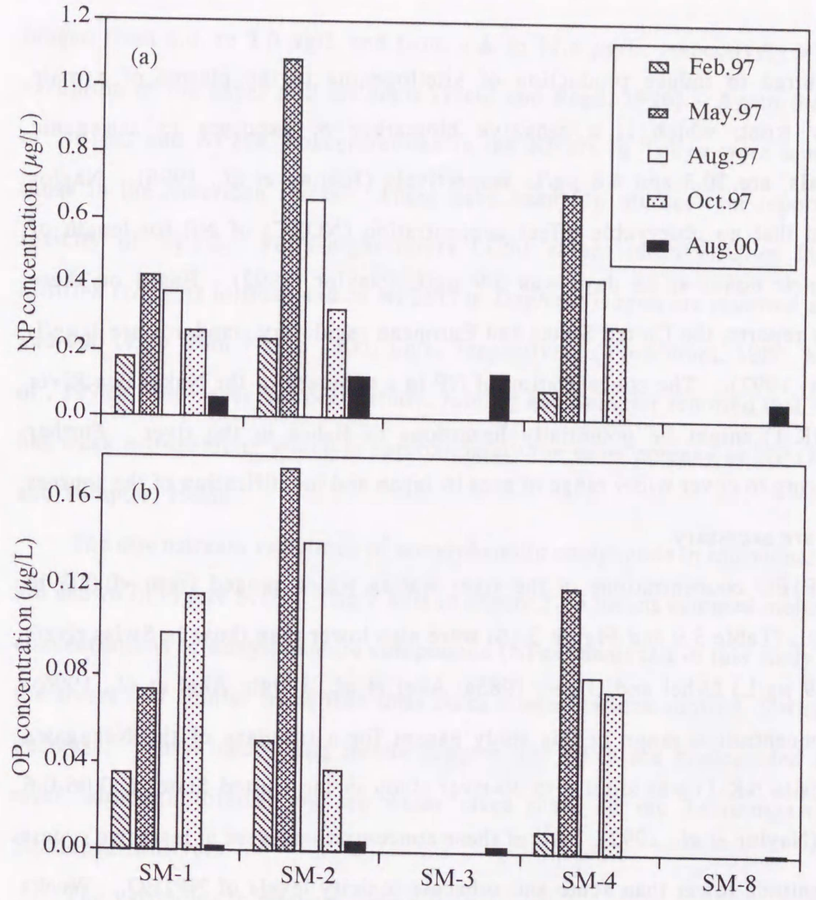


Figure 3.17 NP (a) and OP (b) concentrations in the river water collected from the Sumidagawa River.

Observed APs concentrations in the river waters were 1-3 orders of magnitude lower than reported acute toxicity levels (hundreds μg/L; Comber *et al.*, 1993). Recently, however, it was reported that much lower concentrations of APs disrupt endocrine systems of aquatic organisms (Soto *et al.*, 1991; Jobling and Sumpter, 1993; Jobling *et al.*, 1996). The lowest concentrations of NP and

OP required to induce production of vitellogenine in the plasma of a male rainbow trout, which is a sensitive biomarker of exposure to estrogenic chemicals, are 20.3 and 4.8 μg/L, respectively (Jobling *et al.*, 1996). Naylor reported that no observable effect concentration (NOEC) of NP for length of *Mysidopsis bahia* at 28 days was 3.9 μg/L (Naylor, 1992). Based on these toxicity reports, the United States and European regulatory standards are 1 μg/L (Renner, 1997). The concentration of NP in a tributary of the Nakagawa River (site NK-1) might be potentially hazardous to fishes in the river. Further monitoring to cover wider range of area in Japan and identification of the sources of APs are necessary.

NP1EO concentrations in the river waters which ranged from <0.003 to 3.38μg/L (Table 3.9 and Figure 3.16) were also lower than those in Swiss rivers (n.d.-69 μg/L) (Ahel and Giger, 1985a; Ahel *et al.*, 1994b; Ahel *et al.*, 1996). The concentration range in this study except for a tributary of the Nakagawa River (site NK-1) was similar to 30-river study in the United States (<0.06-0.6 μg/L) (Naylor *et al.*, 1992). All of these concentrations were at least two orders of magnitude lower than acute and subacute toxicity levels of NP1EO. Weeks *et al.* reported that 96-h EC 50 and 7-d LC 50 of NP1EO for *Ceriodaphnia dubia* were 626 and 319 μg/L, respectively (Weeks *et al.*, 1996). There is no information about chronic or estrogenic effect thresholds for organisms to our knowledge.

NP1EC and NP2EC concentrations in the river waters ranged from <0.09 to 2.8 μg/L (Table 3.9 and Figure 3.16). Ahel *et al.* has reported on the occurrence of NP1EC and NP2EC in the Glatt River, ranged from <1 to 45 μg/L and 2 to 71 μg/L, respectively (Ahel *et al.*, 1994b; Ahel *et al.*, 1996). NP1EC and NP2EC concentrations in the United States rivers which were reported by Field and Reed

ranged from n.d. to 2.0 μg/L and from n.d. to 11.8 μg/L, respectively with one exception of the paper mill effluents (Field and Reed, 1996). Again the range of NP1EC and NP2EC concentrations in the Rivers in Tokyo were similar to those in the American rivers. There have been few studies that report acute toxicity of NPEC. Forty-eight hours LC50 values of NP1EC in Japanese killifish (*Oryzias latipse*) and of NP2EC in *Daphnia magna* are reported at 9600 and 990 (vary from 770 to 1300) μg/L, respectively (Yoshimura, 1986; Maki *et al.*, 1998). However, in recent study, Jobling and Sumpter reported that NP1EC has weak estrogenicity which is approximately the same potency as NP (Jobling and Sumpter, 1993).

The downstream variations of nonylphenolic compounds in individual rivers are shown in Figure 3.18. The Y axis in Figure 3.18 means summed molar basis concentrations of nonylphenolic compounds (NPcs) analyzed in this study. All the rivers had similar trend that total NPcs concentrations showed downstream decrease. These decreasing trends suggest that NPcs are biodegraded in the river, although dilution by sea water takes place in the Tsurumigawa and Sumidagawa Rivers.

The variations in relative composition of nonylphenolic compounds (NPcs) in the river water are shown in Figure 3.19. In general, NP1EC and NP2EC were predominant in the river water. They are produced during aerobic sewage treatment and even in the aquatic environment from nonylphenol polyethoxylates. Ahel *et al.* have found during a study of the Glatt River in Switzerland that contribution of NP1EC+NP2EC to the nonylphenolic compounds increased from 51 % to 85 % during 35 km of downstream transport in the river (Ahel *et al.*, 1994b). In our survey, only Nakagawa River showed downstream increasing trend in the contribution of NP1EC+NP2EC. NP and NP1EO were predominant

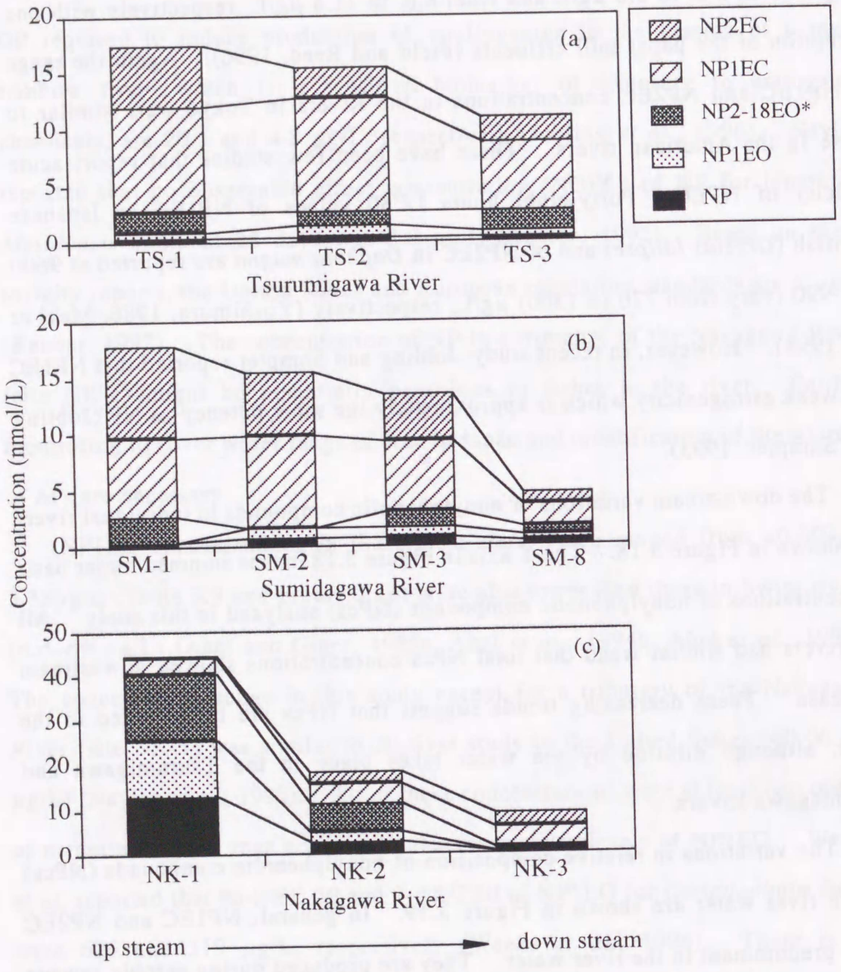


Figure 3.18 Variation in concentrations of total nonylphenolic compounds in the Tsurumigawa (a), Sumidagawa (b), and Nakagawa (c) Rivers.
* NP2-18EO data from Sato (2001).

in a tributary of the Nakagawa River (site NK-1) and their contribution decreased downstream with increasing contribution of NPEC. Because this trend was similar to variation in the STPs described above, it is quite likely that untreated wastewater was discharged directly to the river and was aerobically degraded in

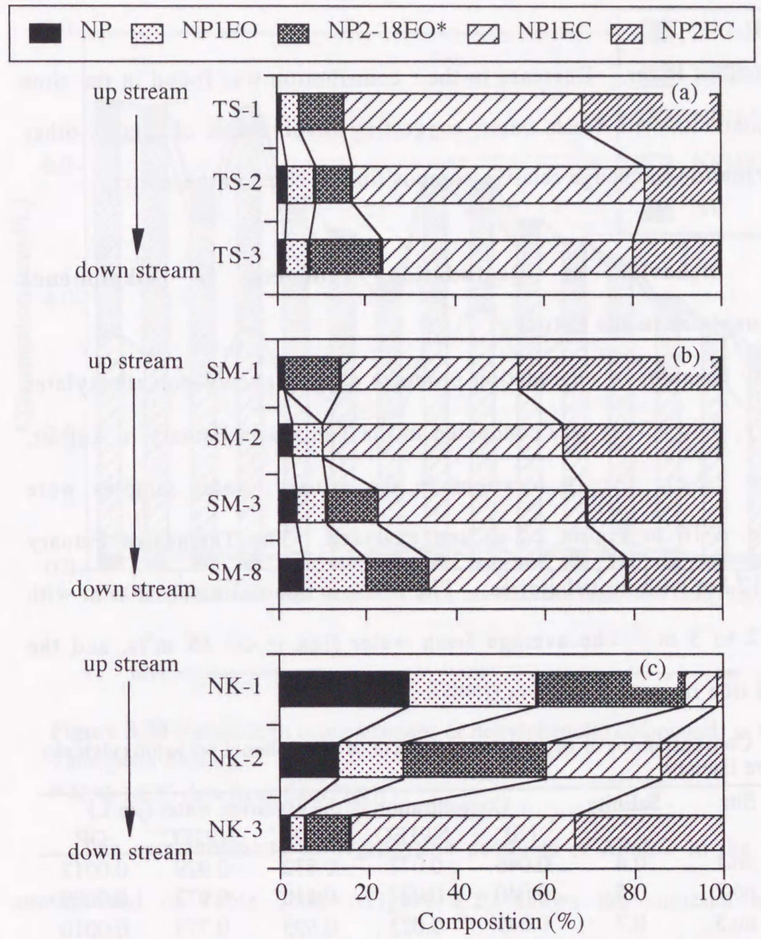


Figure 3.19 Variation in composition of nonylphenolic compounds in the river water. Tsurumigawa (a), Sumidagawa (b), and Nakagawa (c) Rivers.
*NP2-18EO data from Sato (2001).

the river water. In the other rivers (i.e., the Tsurumigawa, Tamagawa and Sumidagawa Rivers), however, such downward trend in compositional change was not consistently observed. As Figure 3.19 indicates, the proportion of NPEC, especially NP2EC, was slightly decreasing downstream in the Tsurumigawa and Sumidagawa Rivers. This observation was different from

result of the Glatt River. Decrease in their contribution was found in the sites where seawater mix with fresh water, suggesting involvement of factors other than biodegradation to change relative composition of NPcs in the estuary.

3.3.2 Behavior of Degradation Products of Alkylphenol Polyethoxylates in the Estuary

To study behavior of degradation products of alkylphenol polyethoxylates in the estuary, field survey was conducted in the Tamagawa Estuary in August, 2000 (Figure 2.2-c). At 16 locations in the estuary, water samples were collected (no. 1-16 in Figure 2.2-c) and analyzed. The Tamagawa Estuary extends ca. 12 km with no tributaries. The width is approximately 200 m with depth from 2 to 5 m. The average fresh water flux is ca. 15 m^3/s, and the astronomical tide range is 0.5-1.5 m.

Table 3.10 Concentrations of degradation products of alkylphenol polyethoxylates in the Tamagawa Estuary.

Date	Site	Salinity	Concentration in the estuarine water (μg/L)				
			NP	NP1EO	NP1EC	NP2EC	OP
30.Aug.00	no.1	0.4	0.046	0.077	0.832	0.929	0.0012
	no.2	0.5	0.040	0.027	0.816	0.972	0.0009
	no.3	0.7	0.043	0.023	0.695	0.773	0.0010
	no.4	1.4	0.033	0.021	0.930	0.910	0.0007
	no.5	2.3	0.034	0.030	0.698	0.732	0.0009
	no.6	4.2	0.023	0.017	0.653	0.640	0.0006
	no.7	5.1	0.059	0.019	0.541	0.539	0.0007
	no.8	8.1	0.068	0.013	0.850	0.731	0.0009
	no.9	8.3	0.034	0.066	0.964	0.652	0.0009
	no.10	12.1	0.028	0.012	0.836	0.732	0.0006
	no.11	13.1	0.065	0.043	0.831	0.666	0.0006
	no.12	14.4	0.052	0.041	0.793	0.565	0.0007
	no.13	19.0	0.029	0.021	0.704	0.508	0.0007
	no.14	26.7	0.029	b.d.	0.471	0.255	0.0006
	no.15	18.5	0.037	0.024	0.718	0.560	0.0007
	no.16	19.9	0.039	0.007	0.605	0.404	0.0009

b.d.; below the detection limit.

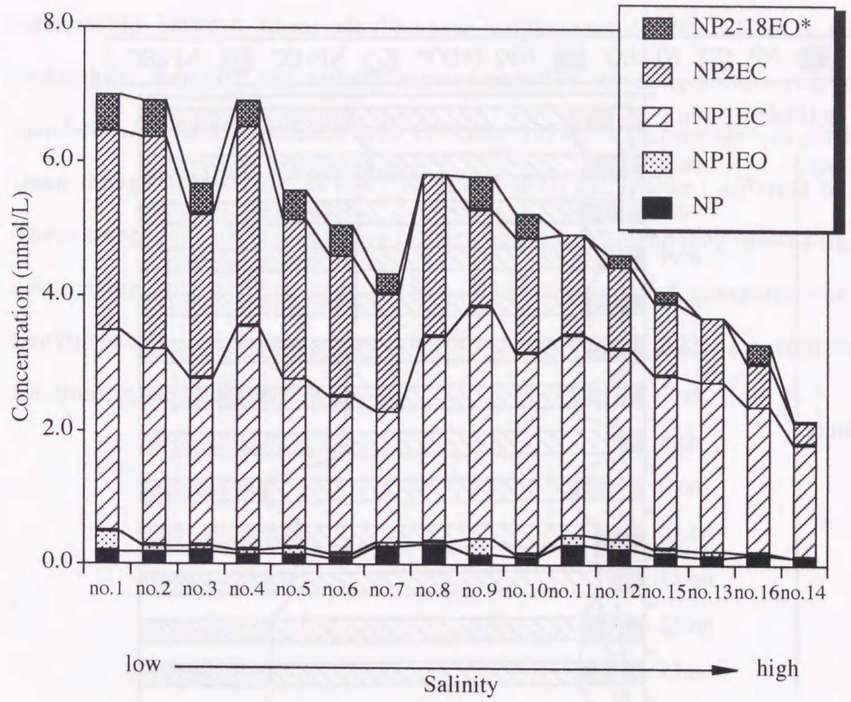


Figure 3.20 Variation in concentrations of nonylphenolic compounds in the Tamagawa Estuary.
* NP2-18EO data from Sato (2001)

The concentrations of degradation products of APnEO in the estuary are summarized in Table 3.10. Figure 3.20 shows the summed molar basis concentrations of nonylphenolic compounds (NPcs) including NP2-18EO (Sato, 2001) in the estuary. As salinity increased, NPcs concentration decreased. The variations in relative composition of nonylphenolic compounds (NPcs) in the estuarine water are represented in Figure 3.21. Again NP1EC and NP2EC were predominant throughout the estuary. Additionally, relative proportion of NP2EC decreased with increase in salinity, while that of NP1EC was increased. This suggests the carboxylic group of NP2EC degraded to NP1EC in the estuary.

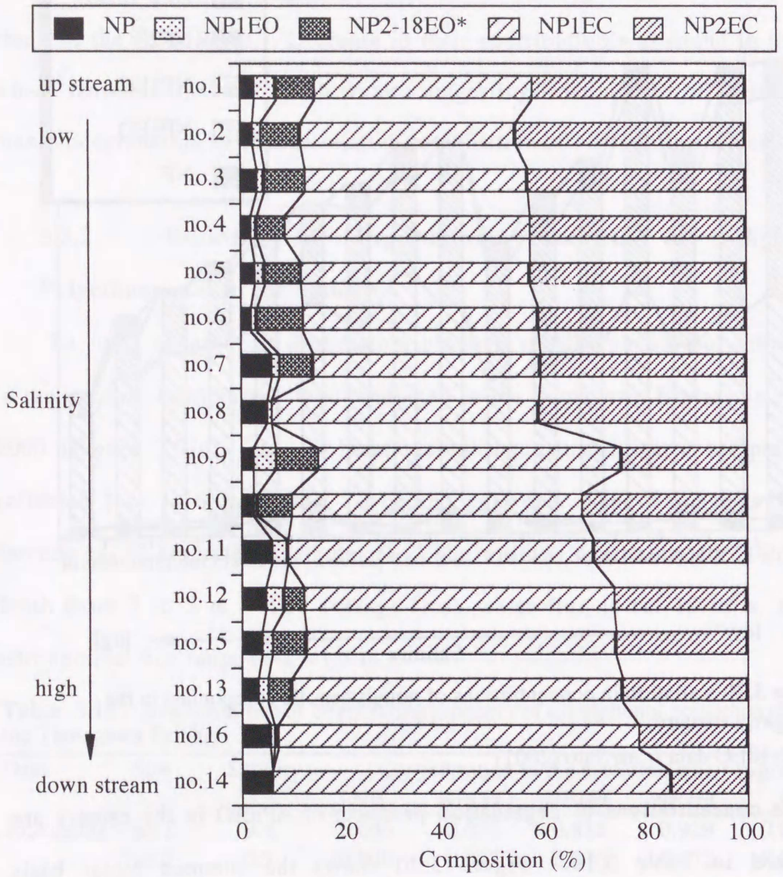


Figure 3.21 Variation in relative composition of nonylphenolic compounds in the Tamagawa Estuary.
* NP2-18EO data from Sato (2001)

Consequently, most of the NPcs entering Tokyo Bay was NP1EC.

Figure 3.22 shows the relationship between the concentrations of NP, OP, NP1EO, NP1EC and NP2EC in the aqueous phase versus salinity. In the dissolved NP-salinity diagram, all the data (dotted curve) were plotted below the theoretical mixing line (solid line). This negative curvature indicates that NP is removed from estuarine water by some mechanisms. It is reported that the

relationship between linear alkylbenzene sulphonates (LAS), which is more hydrophilic than NP, and salinity showed negative curvature from a two end-member LAS-salinity dilution line (Takada, 1989). OP also showed similar trend though not as clear as NP. NP1EC and NP2EC showed different trend. These compounds decreased where salinity was 1 to 5 ‰, and they showed linear relationship with salinity over 10 ‰. When the summed concentrations of nonylphenolic compounds were shown as in Figure 3.23, no consistent relationship was observed.

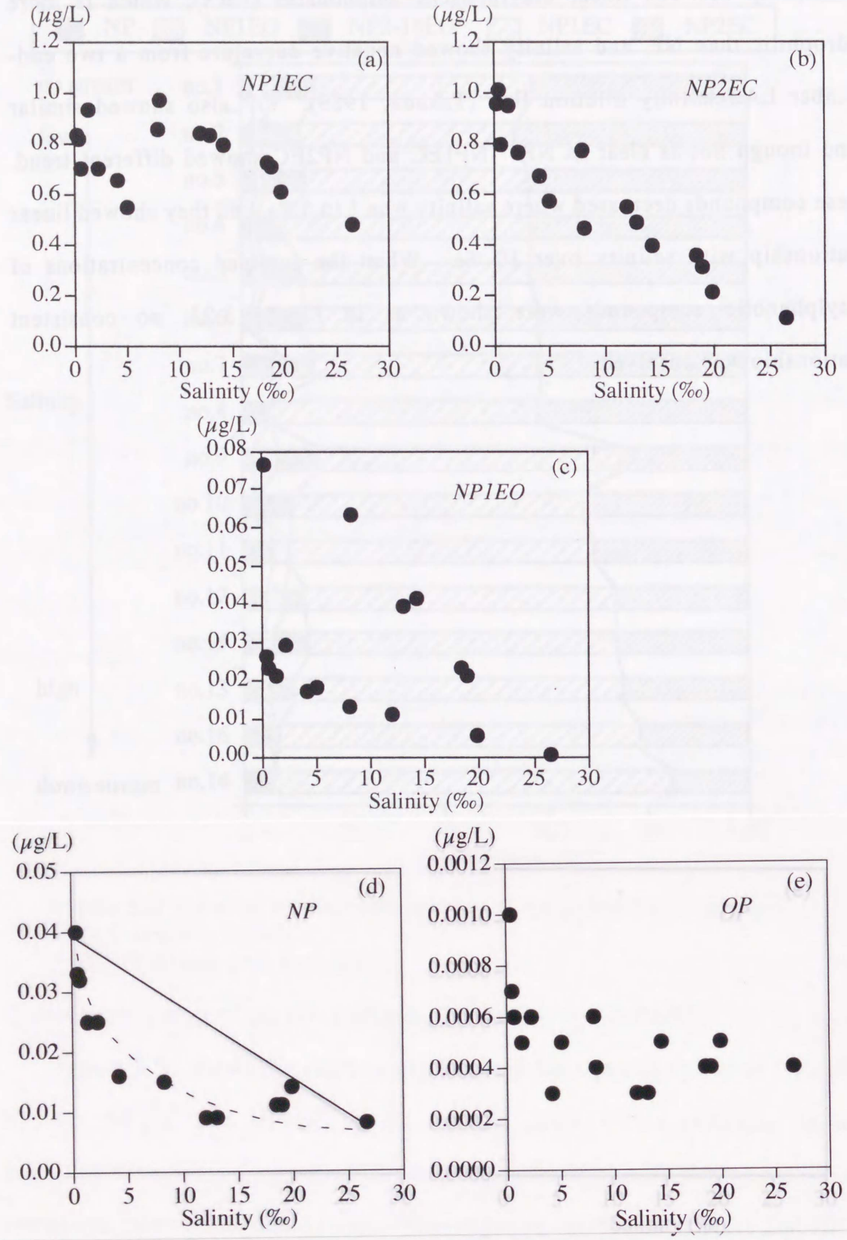


Figure 3.22 Relationship between salinity and NP1EC (a), NP2EC (b), NP1EO (c), NP (d) and OP (e) concentrations in the aqueous phase in the Tamagawa Estuary.

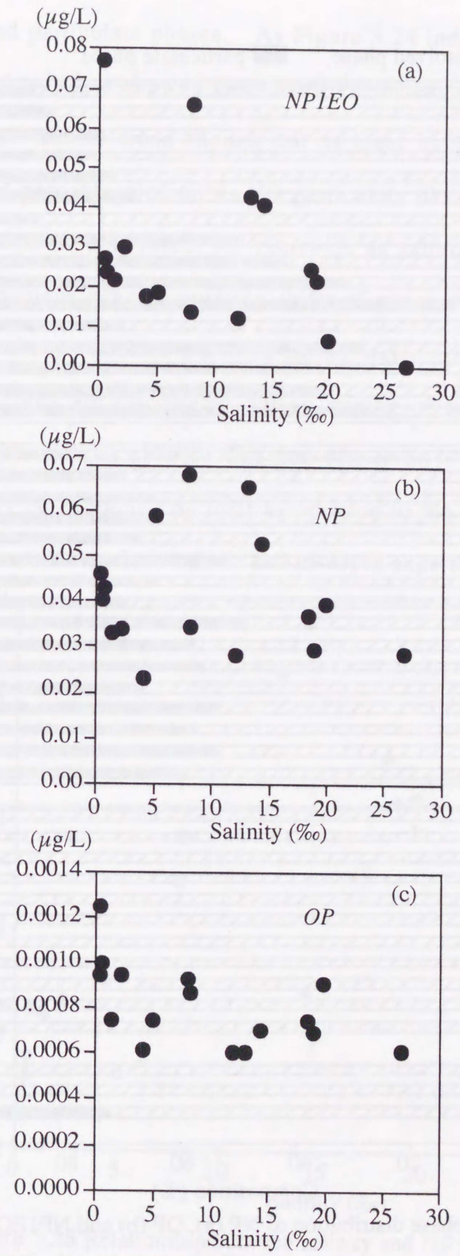


Figure 3.23 Relationship between salinity and total concentrations of NP1EO (a), NP (b) and OP (c) in the Tamagawa Estuary.

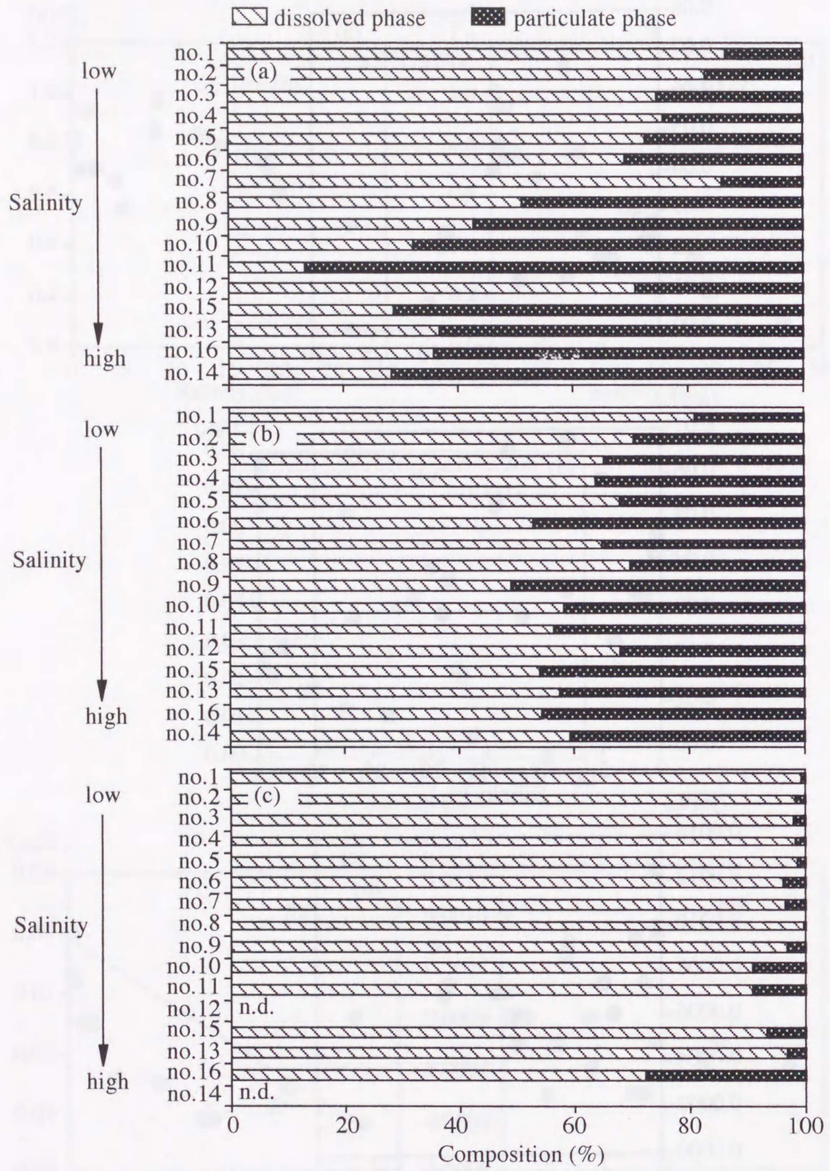


Figure 3.24 Variation in phase distribution of NP (a), OP (b) and NP1EO (c) in the Tamagawa Estuary.

n.d.; particulate phase was not detectable.

Figure 3.24 shows the phase distribution of NP, OP and NP1EO between aqueous and particulate phases. As Figure 3.24 indicates, percentage of NP in the particulate phase showed clear seaward increasing trend. OP and NP1EO also showed similar trend though not as clear as NP. Relationship between salinity and percentage of NP in the particulate phase (Figure 3.25) shows that the proportion of particulate NP increases as salinity increases up to ~15 ‰. These phenomena suggest possible shifts of phase distribution of NP, OP, and NP1EO to the particulate phase in saline water. It is well documented that organic colloids are flocculated during mixing of freshwater with seawater in estuaries (Sholkobitz, 1976). The non-conservative behaviors of NP, OP, and NP1EO may be explained by their association to the flocculated particles in the saline water. More observations and in-depth experiments are necessary.

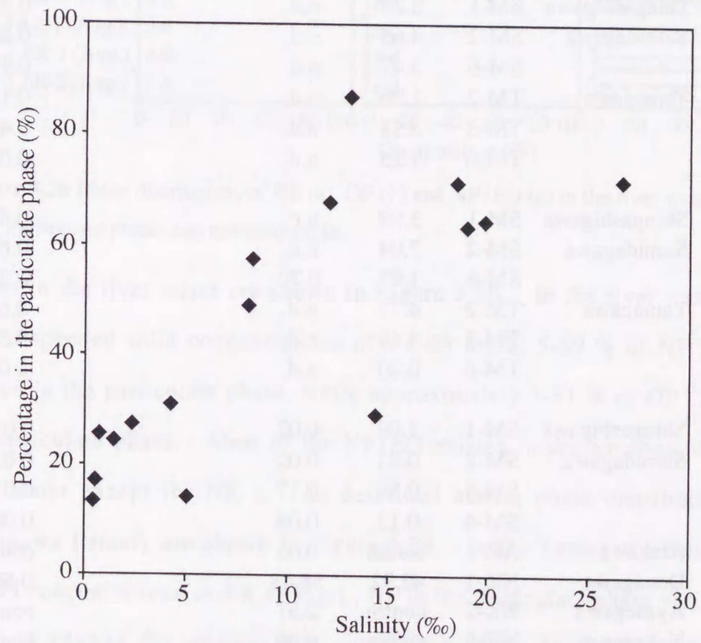


Figure 3.25 Relationship between salinity and NP percentage in the particulate phase in the Tamagawa Estuary.

3.3.3 *In-situ* Partitioning of NP, OP and NP1EO between Aqueous and Particulate Phases

The present study clearly demonstrated that significant amounts of NP, OP and NP1EO were present in the particulate phase in the river water samples, as shown in Table 3.11. In this study, the aqueous phase means filtrate passed through the GF/F filter whereas the particulate phase means suspended solid trapped by the filter. Phase distributions between aqueous and particulate

Table 3.11 Concentrations of degradation products of alkylphenol polyethoxylates in the particulate phase.

Date	River	Site	Concentration in the particulate phase (μg/g dry)				
			NP	NP1EO	NP1EC*	NP2EC*	OP
4.Aug.97	Shingashigawa	SM-1	3.28	n.d.			0.23
	Sumidagawa	SM-2	4.65	n.d.			0.25
		SM-6	3.47	n.d.			0.19
	Tamagawa	TM-2	1.76	n.d.			0.15
		TM-3	5.58	n.d.			0.41
		TM-6	1.25	n.d.			0.09
30.Oct.97	Shingashigawa	SM-1	3.99	n.d.			0.58
	Sumidagawa	SM-2	7.04	n.d.			0.62
		SM-6	1.97	0.70			0.25
	Tamagawa	TM-2	4.77	n.d.			0.69
		TM-3	4.49	n.d.			0.52
		TM-6	0.20	n.d.			0.03
30.Aug.00	Shingashigawa	SM-1	1.09	0.02			0.031
	Sumidagawa	SM-2	0.83	0.02			0.020
		SM-3	0.80	0.17			0.015
		SM-4	0.13	0.04			0.003
	Arakawa	AR-1	contm	0.05			contm
	Denugawa	NK-1	43.92	58.43			0.501
	Ayasegawa	NK-2	contm	2.51			contm
	Nakagawa	NK-3	contm	0.09			contm
	Kyu-Edogawa	ER-1	contm	0.01			contm
	Edogawa	ER-2	contm	0.01			contm

* Concentration in the particulate phase was below the detection limit.
contm; contaminant interference, n.d.; not detectable.

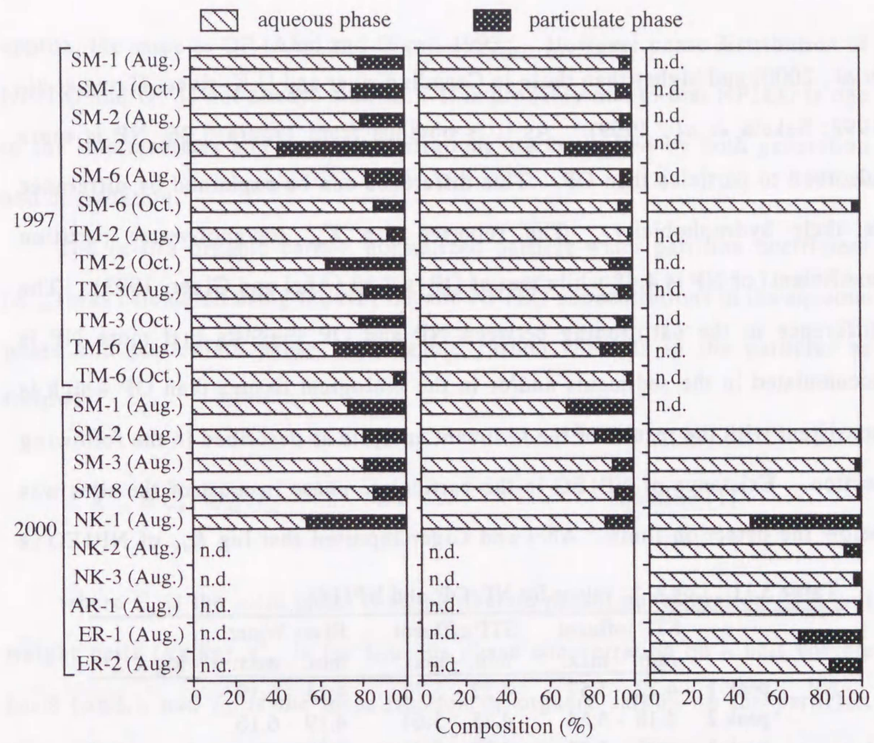


Figure 3.26 Phase distribution of NP (a), OP (b) and NP1EO (c) in the river water samples. n.d.; Particulate phase was not detectable.

phases in the river water are shown in Figure 3.26. In the river water samples with suspended solid concentrations of 4.2-65 mg/L, 5-59 % of NP (23±14 %) existed in the particulate phase, while approximately 1-31 % of OP (11±9 %) in the particulate phase. Most of the NP1EO existed in the aqueous phase in the river water except for NK-1. As described above, phase distributions in the Tamagawa Estuary are shown in Figure 3.24. In the Tamagawa Estuary which had SS concentrations of 4.8-31 mg/L, NP in the particulate phase varied from 13 to 86 % (44±24 %) whereas OP varied from 19 to 51 % (38±8 %). These proportions were similar to the values reported in an American estuary (Van Ry

et al., 2000) and higher than those in Canadian river and U.K. rivers (Long *et al.*, 1998; Sekela *et al.*, 1999). As it is obvious from Figure 3.26, NP is more adsorbed to particles than OP. This difference can be explained by difference in their hydrophobicity. The reported log K_{ow} (octanol-water partition coefficient) of NP is 4.48 while that of OP is 4.12 (Ahel and Giger, 1993). The difference in the partitioning between NP and OP suggests that more NP is accumulated in the sediments and/or in the biological tissues than OP which is consistent with the results of the sediment analysis as described in the following section. Existence of NP1EO in the particulate phase in most of the sites was below the detection limit. Ahel and Giger reported that log K_{ow} of NP1EO is

Table 3.11 Log K'_{oc} values for NP, OP, and NP1EO.

		STP influent min. - max.	STP effluent min. - max.	River Water min. - max.	log K_{oc}*
NP	peak 1	4.22 - 5.57	4.22 - 5.73	4.13 - 6.19	
	peak 2	4.18 - 5.54	4.85 - 5.64	4.19 - 6.16	
	peak 3	4.11 - 5.52	4.27 - 5.82	4.23 - 6.07	
	peak 4	4.13 - 5.51	4.17 - 5.58	4.17 - 6.04	
	peak 5	4.13 - 5.54	4.08 - 5.55	3.69 - 6.24	
	peak 6	4.21 - 5.50	4.09 - 5.47	4.12 - 6.04	
	peak 7	4.27 - 5.45	4.06 - 5.43	3.93 - 6.09	
	peak 8	4.10 - 5.47	4.17 - 5.90	4.03 - 6.09	
	peak 9	4.28 - 5.51	4.33 - 5.48	4.36 - 6.14	
	peak 10	3.85 - 5.74	4.76 - 5.54	4.03 - 6.10	
	peak 11	4.08 - 5.41	4.12 - 5.45	4.06 - 6.11	
	total	4.16 - 5.53 (n=8)	4.33 - 5.54 (n=9)	4.12 - 6.07 (n=33)	3.81
OP		3.54 - 5.02 (n=8)	3.55 - 5.12 (n=9)	3.86 - 5.73 (n=33)	3.52
NP1EO		4.72 - 5.63 (n=4)	4.50 - 5.37 (n=4)	2.59 - 5.25 (n=23)	3.56

STP influent; influent and primary effluent.
STP effluent; secondary and final effluent.
* Calculated from reported K_{ow} (Ahel *et al*., 1993) using eqation (2) (Schwarzenbach *et al*., 1993).

approx. the same as OP (Ahel and Giger, 1993). However phase distribution of NP1EO and OP is not always similar. It is probably due to that NP1EO is one of the intermediates and their concentrations are controlled by both generation and degradation.

The *in-situ* organic carbon normalized particle-water partition coefficient (K'_{oc}) was calculated using the NP, OP and NP1EO concentrations in the aqueous phase and particulate phase and organic carbon contents in the particles as follows:

$$K'_{oc} = C_s / C_{aq} / f_{oc} \qquad \text{equation (1)}$$

where C_s is the solid phase (i.e., particulate phase) concentration on a unit weight basis (μg/kg), C_{aq} is the aqueous phase concentration on a unit volume basis (μg/L), and f_{oc} is the mass fraction of organic carbon on the particles. Since POC was not measured for the samples collected in 2000, it was assumed that 20 % of suspended solid was organic carbon (i.e. f_{oc} = 0.2). The log K'_{oc} values calculated from the river water samples are 4.12-6.07 (n=21), 3.86-5.73 (n=26), and 2.59-5.25 (n=23) for NP, OP, and NP1EO, respectively (Table 3.12). These ranges are close to those observed in the Canadian river (4.7-5.6 for NP) (Sekela *et al.*, 1999). Despite the possible influence from recovery-correction of NP and OP concentrations and estimated POC for samples in 2000, the result was almost identical to the data in 1997. Johnson *et al.* measured organic carbon normalized partition coefficients (K_{oc}) of octylphenol through laboratory batch techniques using suspended and bed-sediments collected from English rivers and measured log K_{oc} ranged from 3.52 to 5.59 (Johnson *et al.*, 1998). The log K'_{oc} values for OP were within the range of the K_{oc} determined by the

laboratory experiment.

The partitioning of organic compounds between water and organic matter is generally controlled by hydrophobicity. Schwarzenbach *et al.* (1993) found that there is a linear correlation between K_{ow} and K_{oc} (i.e. organic carbon normalized particle-water partition coefficient) on a laboratory sorption experiment using natural sediments as follows:

$$\log K_{oc} = 0.82 \times \log K_{ow} + 0.14 \qquad \text{equation (2)}$$

If isotherm and equilibrium partitioning are assumed, K'_{oc} of nonpolar compounds can be predicted from K_{ow}, which is referred to as predicted K_{oc}. The predicted log K_{oc} values calculated from reported K_{ow} values of NP, OP and NP1EO (4.48, 4.12 and 4.17) using equation (2) are 3.53, 3.24 and 3.28, respectively. As shown in Figure 3.27, the K'_{oc} values of NP and OP observed in the river water samples were one order of magnitude higher than the predicted K_{oc}. The K'_{oc} of NP1EO was also higher than the predicted K_{oc}, though the difference was not as much as that of NP and OP. This indicates that APs partition more to the particulate phase than expected because of their hydrophobicities. This has also implied that the particulate APs could play a significant role in their transport in the aquatic environments and their incorporation into bottom sediments and could be one of the most important sinks and their fates.

In the above discussion, the K'_{oc} for NP was calculated in terms of "sum of all the isomers". Although NP consists of many isomers with various branched structures in the nonyl substituent, many researchers determine sum of all the isomer peaks as "p-nonylphenol". As shown in Figure 2.5 and 2.8, NP consists

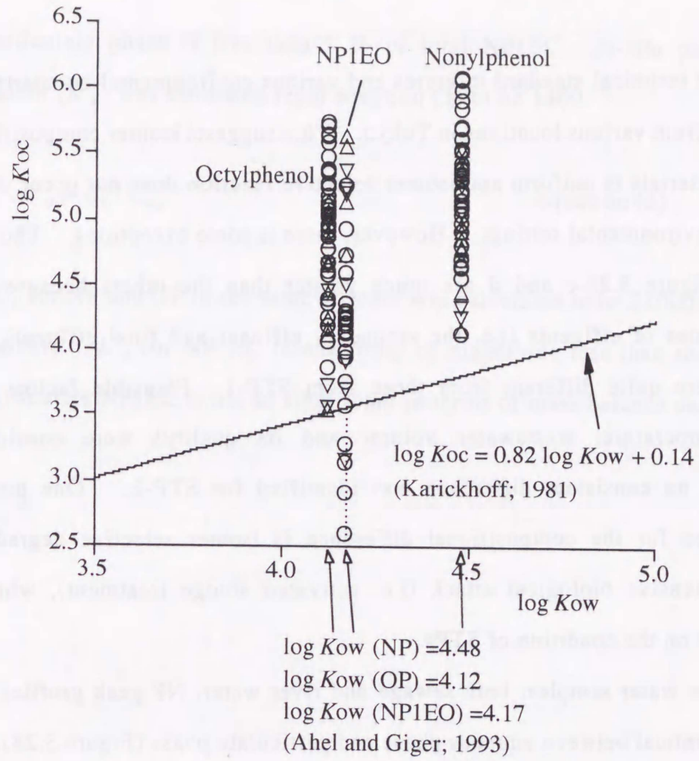


Figure 3.27 Relationship between octanol-water partition coefficients (*K*ow) and organic carbon-water partition coefficients in the environmental samples (*K*'oc).

of 11 peaks on the gas chromatogram. Alkyl group in nonylphenol is propylene trimer and therefore, NP consists of more than 11 isomers. Some researchers reported that some of the peaks consist of several isomers (Bhatt *et al.*, 1992; Wheeler *et al.*, 1997; Yamashita *et al.*, 1999). Wheeler *et al.* found 22 para-isomers in commercial p-nonylphenol using a 100-m capillary GC column (Wheeler *et al.*, 1997). Recently, Yamashita *et al.* indicated that estrogenic activity is different among isomers, which underscores the need to determine the specific NP isomers in environmental samples (Yamashita *et al.*, 1999). As shown in Figure 3.28, peak composition of NP in GC-MS was fairly constant

among the technical standard mixtures and various environmental compartments collected from various locations in Tokyo. This suggests isomer composition of source materials is uniform and isomer selective reaction does not occur during various environmental settings. However, there is some exceptions. The error bars in Figure 3.28-c and d are much greater than the others because peak compositions of effluents (i.e. the secondary effluent and final effluent) from STP-2 were quite different from those from STP-1. Plausible factors (i.e., water temperature, wastewater volume and its quality) were considered. However, no consistent difference was identified for STP-2. One possible explanation for the compositional difference is isomer selective degradation during intensive biological attack (i.e. activated sludge treatment), which is dependent on the condition of STPs.

In the water samples, both sewage and river water, NP peak profiles were almost identical between aqueous phase and particulate phase (Figure 3.28). To discuss quantitatively, K'_{oc} was calculated for each peak (Table 3.12). Because some of the peaks contain several isomers, the K'_{oc} calculated for individual peaks do not represent K'_{oc} for individual isomers but for individual mixture of isomers. No significant differences in K'_{oc} were observed among isomer peaks, suggesting that partitioning behavior is similar between isomers. This could be one of the major reasons why the isomer peak distribution is constant throughout the various environmental compartments. This may suggest that risk assessment of NP can be accomplished in terms of sum of all the isomers.

To estimate the partitioning coefficient of NP1EC, 10 L of the final effluent water from STW-1 was filtrated and NP1EC concentration in the particulate phase was determined. The particulate NP1EC concentration was 0.02 μg/L (or 3.7 μg/g) whereas it was 2.6 μg/L in the aqueous phase. This means NP1EC in

the particulate phase is less than 1 % of total NP1EC. *In-situ* partitioning coefficient (K'_d) was estimated from equation (3) to be 1400.

$$K'_d = C_s / C_{aq} \qquad \text{equation (3)}$$

K'_d for NP and OP in the same effluent was calculated to be 21000 and 5300, respectively. K'_d for NP1EC is one order of magnitude less than that for NP and particulate NP1EC is not so significant in terms of mass balance calculation.

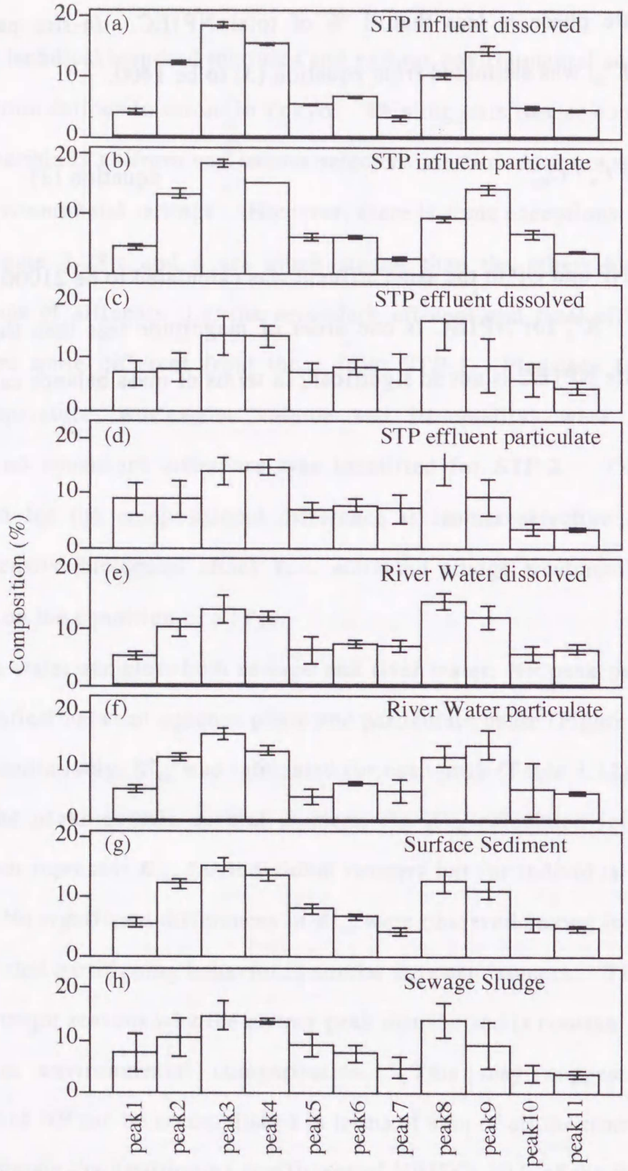


Figure 3.28 Individual peak composition of NP in the dissolved (a) and particulate (b) phases in the influents (influent and primary effluent), dissolved (c) and particulate (d) phases in the effluents (secondary and final effluent) of STPs, dissolved (e) and particulate (f) phases in the river waters, surface sediments (g), and sewage sludge (h).

3.3.4 Accumulation in the Surface Sediment

Occurrence in the Surface Sediments

Significant concentrations of NP, OP and NP1EO were found in the sediments from Tokyo metropolitan area, but NP1EC and NP2EC were not detected in all the sediment samples as well as the particulate phase (Table 3.13). Nonylphenolic compounds (NPcs) concentrations in the surface sediments from the Tsurumigawa, Tamagawa, Sumidagawa and Edogawa Rivers are shown in

Table 3.13 Concentrations of degradation products of alkylphenol polyethoxylates in the river sediments.

Date	River	Site	Concentration in the sediments (μg/g dry)				
			NP	NP1EO	NP1EC	NP2EC	OP
19.Nov.97	Sumidagawa	SM-2	5.49	3.47			0.520
		SM-4	1.97				0.229
		SM-5	6.76	0.27			0.230
		SM-6	3.58	0.21			
		SM-7	2.59	0.01			0.370
		SM-8	8.41	0.74			0.670
		SM-9	10.4	b.d.			0.650
		SM-11	0.52	0.04			0.050
28.Aug.98	Tamagawa	TM-4	0.537	0.22			0.022
		TM-5	1.144	0.97			0.052
		TM-6	0.318	0.06			0.015
		TM-7	0.487				0.017
		TM-8	0.033	b.d.			0.003
22.Dec.99	Sumidagawa	SM-10	3.07				0.049
		SM-11	0.34				0.005
25.May.00	Tsurumigawa	TS-3	0.545	0.250	b.d.	b.d.	0.014
6.Jun.00	Sumidagawa	SR-4	0.765	0.457	b.d.	b.d.	0.025
		SR-2	0.776	0.366	b.d.	b.d.	0.021
	Kyu-Edogawa	ER-1	1.816	0.220	b.d.	b.d.	0.040
	Edogawa	ER-2	0.190	0.053	b.d.	b.d.	0.002
3.Aug.00	Tamagawa	no.2	0.030	0.040	b.d.	b.d.	0.002
		no.6	0.112	0.116	b.d.	b.d.	0.005

b.d.; below the detection limit.

Figure 3.29. The concentrations of analytes in the sediments were higher in the Tsurumigawa, Sumidagawa and Kyu-Edogawa (site ER-1) Rivers than those in the Tamagawa and Edogawa (site ER-2) Rivers.

NP concentrations in the surface sediments from the Sumidagawa and Tamagawa Rivers and Tokyo Bay are shown in Figure 3.30. The concentration range of NP in the river sediments (0.03-13.0 μg/g-dry) was similar to those reported for American (Naylor, 1995), Canadian (Bennie *et al.*, 1997), U.K. (Blackburn *et al.*, 1999), and Korean (Khim *et al.*, 1999) rivers. In Canadian rivers high values for sedimentary NP were reported in close proximity to the

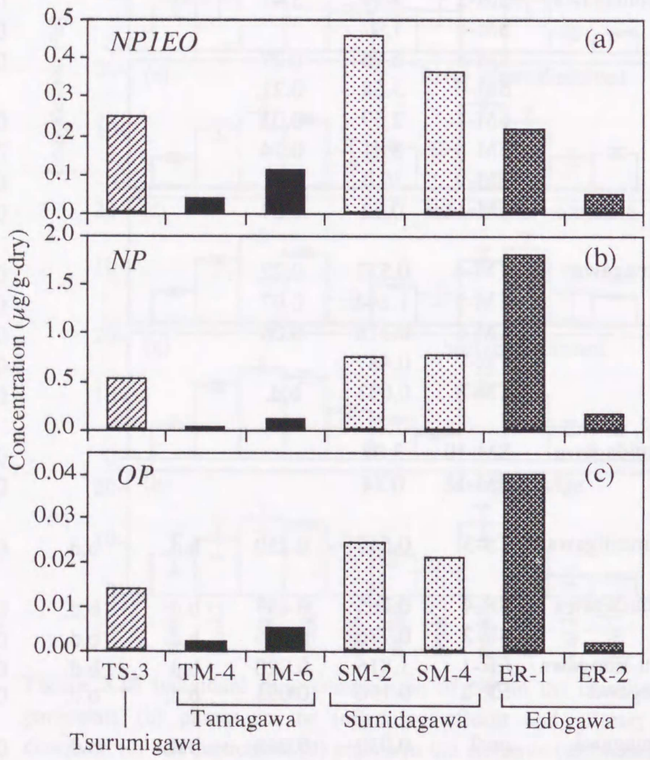


Figure 3.29 Concentrations of NP1EO (a), NP (b), and OP (c) in the river sediments.

discharge points of sewage effluents (Bennie *et al.*, 1997) and the distributions of NP were localized to areas close (e.g., 1 km) to the outfalls (Bennett and Metcalfe, 1998; Bennett and Metcalfe, 2000). In the Sumidagawa River, however, relatively high concentrations (0.52-13.0 μg/g-dry) of NP were detected in a long reach (~10 km). The freshwater flow and tidal current, especially during flood, possibly mix the surface sediments horizontally. A large proportion of the wastewater effluent may also attribute to the ubiquitously high concentrations of APs in the riverine sediments. Effluents from the STPs take 52 % of freshwater flow in the Sumidagawa River in normal flow conditions. In addition, industrial effluents from large-scale factories supply APs. On the other hand, once APs are transported to the coastal zone, there appears to be a seaward decrease. NP concentrations in the surface sediments from Tokyo Bay ranged from 0.12 to 0.64 μg/g-dry, which was one order of magnitude lower than that in the Sumidagawa River sediments. Blackburn *et al.* also reported the seaward decrease in NP concentrations in the English estuaries (Blackburn *et al.*, 1999).

Although there are few data on toxicity of sedimentary alkylphenolic compounds to benthic organisms, the reported lowest effect concentration is 26 μg/g for subacute toxicity of NP to shrimp (Naylor, 1995). In the previous study, the maximum NP concentration observed in the surface sediment from the Sumidagawa River (i.e., 13.0 μg/g-dry at SR-9911) was in the same order of magnitude as the concentration which may induce the subacute adverse effects on benthic organism. On the other hand, Hashimoto *et al.* found reproductive abnormalities on male flounder from Tokyo Bay (Hashimoto *et al.*, 2000). Further research is needed to investigate whether the observed levels of NP are significantly disrupting the endocrine systems of benthic organisms in Tokyo

Bay.

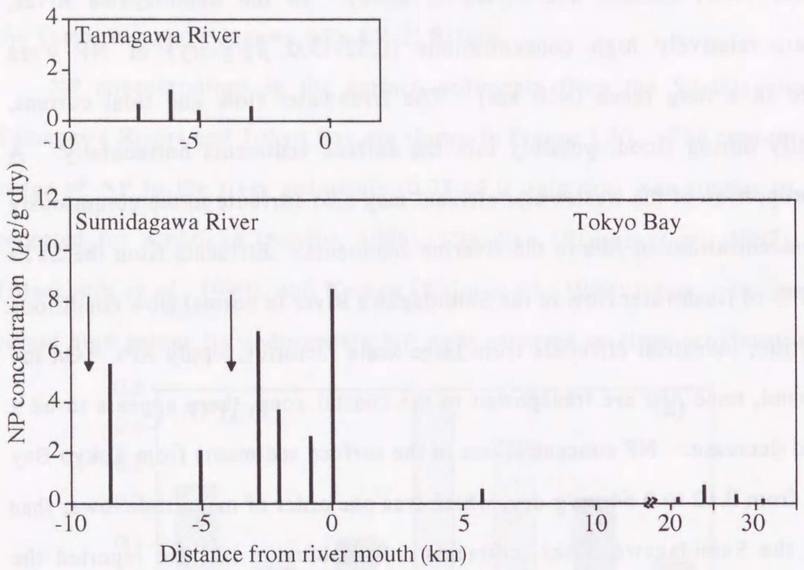


Figure 3.30 NP concentrations in the surface sediment samples from the rivers in Tokyo and Tokyo Bay. Arrows indicate the discharge points of STPs effluents.

Possibility of Formation of APs in the Sediments

It has been proved that AP1EO is transformed to APs during the anaerobic digestion of sewage sludge in the STPs (Giger *et al.*, 1984). So far, however, there has been no report demonstrating the anaerobic transformation of NPnEO to NP in the river environment. The observations in the present study may be explained as a result of the transformation. APs are accumulated in the river sediments. Especially, NP concentrations in the sediment from the Sumidagawa (0.76-0.78 μg/g-dry), Tsurumigawa (0.54 μg/g-dry) and Kyu-Edogawa (site ER-1, 1.8 μg/g-dry) Rivers were higher than those in the

Tamagawa River (0.03-0.11 μg/g-dry), as shown in Table 3.13 and Figure 3.29 and 30. Deeper (~3 m) water, slower stream, and invasion of seawater in addition to higher inputs of organic matter make those rivers anaerobic in its bottom environment, whereas shallow (~1 m) streams in the Tamagawa River supply oxygen into the river and keep it constantly aerobic. The anaerobic condition in the Sumidagawa , Tsurumigawa, and Kyu-Edogawa Rivers, the sediments were confirmed by high contents of organic carbon and strong odor of hydrogen sulfide during the sampling.

As shown in Table 3.13, the sedimentary NP concentrations in the Sumidagawa River (0.76-0.78 μg/g-dry) was in the same range as those in the suspended particles in the river water (0.13-1.1 μg/g-dry). This was different from the observation in U.K. rivers (Long *et al.*, 1998) where NP concentrations in the bottom sediments were one order of magnitude lower than those in the suspended particles. Also for another surfactant-derived hydrophobic compounds (Linear Alkylbenzenes; LABs) it was observed that their sedimentary concentrations were one order of magnitude lower than those in suspended particles in the Sumidagawa River (Takada and Ishiwatari, 1987) and was ascribed to the dilution by soil and sand in the riverbed (Takada, 1989). In the case of APs, however, their concentrations in the sediment were in the same range as those in suspended solid, despite the dilution by soil and sand. This also can be explained by the *in-situ* transformation of AP1EO to APs in the Sumidagawa River sediment. The decreased ratio of NP1EO to NP in the sediment samples may support the facilitated anaerobic conversion of NP1EO to NP. As shown in Figure 3.31, the relative compositions of NP1EO to NP were lower in the sediment (0.4±0.4, n=17) than river water (1.5±1.6, n=44). For example, in the Sumidagawa River, NP1EO/NP ratios (weight concentration

basis) in the river waters were 1.6±1.0 (n=9), whereas the ratios in the river sediments were 0.2±0.3 (n=8). Furthermore, the range of NP1EO/NP ratios observed in the Sumidagawa River sediment (0.01-0.6, n=8) lower than those in the Tamagawa River sediment (0.2-1.3, n=5).

The difference in the sedimentary APs concentrations appears to be related with water column APs. Higher APs concentrations in water of the Sumidagawa River can be explained by resuspension and/or desorption of APs generated in the bottom sediments, if their *in-situ* formation could be operative. Also the higher APs concentration is partly due to that the Sumidagawa River receives larger amounts of wastewater from industries such as textile and paper mill that potentially discharge APs (Ministry of International Trade and Industry of Japan, 1996) than the Tamagawa River whose drainage area is mainly of residential. The seasonal trend that APs concentrations in the river water were higher in spring and summer than in autumn and winter may be related with the APs formation in the benthic environment. The seasonal trend cannot be attributable to seasonal change in water flow, because water level is normally higher in summer due to higher precipitation in the season than the other season. The seasonal trend in APs concentrations can be explained by seasonal change in microbial activities that breakdown NPnEO to NP. The water temperatures observed during spring and summer surveys (21.6-30.0 °C) were higher than that of autumn and winter surveys (9.1-18.9 °C). This temperature differences could promote degradation of the parent NPnEO to produce lower ethoxymers. The shift of EO distribution to shorter chain length in summer was already reported by Maruyama *et al.* in the same rivers (Maruyama *et al.*, 2000). Higher water temperature could also facilitate the conversion of NP1EO to NP in the benthic environment in the rivers, if the conversion process could be operative. Lower

dissolved oxygen concentrations was also observed in spring and summer in the Sumidagawa River (Bureau of Environment Tokyo Metropolitan Government, 1998), which might accelerate the conversion in the warmer season. However, the present study provided only circumstantial evidence for anaerobic conversion of NP1EO to NP in river environments. More direct evidence to support the *in-situ* production of NP in the river sediment is needed.

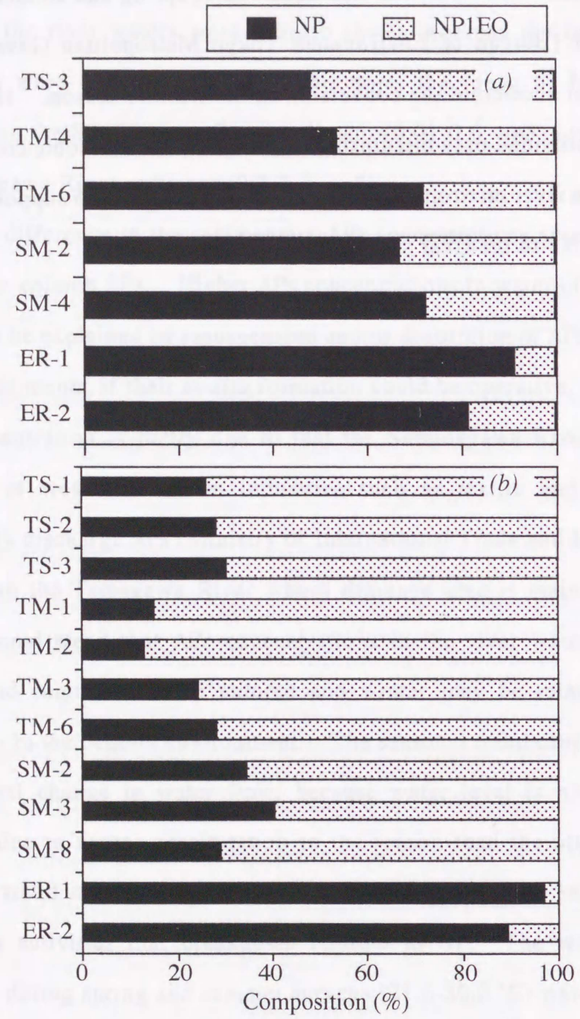


Figure 3.31 Relative composition of NP and NP1EO in the river sediment (a) and river water (b).

4. Conclusions and Future Researches

4.1 Conclusions

The analytical method for degradation products of APnEO from various environmental matrices was developed in **Section 3.1**. As shown in Table 2.1, the developed analytical method was applicable to both water samples (wastewater and river water) and solid samples (sediment, sludge and suspended particles). The detection limits of NP and NP1EC were 15 ng/L and 13 ng/L, respectively.

Concentrations and elimination and production of NP, OP, NP1EO, NP1EC and NP2EC in 5 sewage treatment plants in Tokyo were examined in **Section 3.2**. Analysis of composite samples indicated that NP, OP and NP1EO were efficiently removed from wastewater (88-97 %) while NP1EC and NP2EC were produced during sewage treatment (Table 3.7). Mass balance calculation indicated that one-quarter of nonylphenolic compounds (NPcs) were released from STPs to the aquatic environments (Figure 3.15). NPnEO (NP1EO + NP2-18EO) accounted for 90 % of the NPcs in the influent, whereas NP1EC and NP2EC were predominant (i.e., three-quarters) in the final effluent.

In **Section 3.3**, distribution and behavior of NPcs in the aquatic environment in Tokyo were demonstrated. In the river water (**3.3.1**), the ranges of concentrations were similar to those reported in the United States and Canada, but much less than extremely higher values observed in Switzerland nor in England. NP1EC and NP2EC represented majority (i.e., 80% or more) of nonylphenolic compounds in the river water, which was consistent with results reported by Ahel *et al.* (1994b).

The apparent partition coefficients normalized by organic carbon (K'_{oc}) were calculated (**3.3.2**). The estimated K'_{oc} values (log K'_{oc} = 5.3 for NP, 5.1 for OP and 4.0 for NP1EO) were in the reported range (Sekela *et al.*, 1999), and significantly higher than K_{oc} estimated from reported K_{ow}. To reveal the behavior of degradation products of APnEO in the estuary, 16 river water samples were collected from the Tamagawa Estuary (**3.3.3**). The percentage of NP in the particulate phase increased as salinity increased. This phenomenon suggests possible shifts of NP, OP, and NP1EO to the particulate phase in the estuarine water. As described in **3.3.4**, NP, OP and NP1EO were widely distributed in the surface sediments, and the possibility of formation of APs in the surface sediments were suggested.

4.2 Future Researches

Analysis of the parent compounds and other degradation products are necessary. From recent findings, significant amounts of the intermediates which had carboxylated alkyl chain and/or chlorinated or brominated benzene ring were observed. These intermediates may play important role in mass balance of APnEO and their degradation products in the STPs.

The estuarine survey suggested that NP could be removed from water column through flocculation in the estuary. This survey was conducted only once in summer, and, therefore, more observations are necessary.

Further researches focused on biological effects of APnEO and their degradation products should be examined as soon as possible. The moderate hydrophobicity and persistence of these compounds raise the possibilities of bioaccumulation and endocrine disruption in organisms.

Current monitoring programs have been focusing on NP and OP. However, the present study demonstrated that NP1EC and NP2EC represented that more than 80 percent of the degradation products of NPnEO in the wastewater effluents and the river waters. Future monitoring should cover these carboxylic metabolites. It is reported that NP1EC has almost equivalent estrogenicity to NP (Jobling and Sumpter, 1993). The concentrations of NP1EC in the river water were one order of magnitude higher than NP, implying NP1EC may be more important in terms of estrogenic activity. This also strongly suggests the need of monitoring of NP1EC together with NP and OP. There is almost no information on acute toxicity, biodegradability, and degradation pathway of NP1EC. Accumulation of these basic data through laboratory experiments is essential. Further investigations in Tokyo Bay to reveal the level of contamination of NP1EC and its mechanism are also needed.

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Appendix

Appendix 1-1. Sampling data in June 6, 2000.

	Time	Tw(°C)	pH	EC(mS/cm)	Salinity	depth (m)
Edogawa River						
Shingyotoku	9:20	23.4	8.5	34.3	20.4	6.2
Tozaisen	9:30	23.8	8.79	34.9	21.7	3
Ichikawaohashi	9:40	23.2	8.75	40.5	24.4	3.3
Shiohamabashi	9:50	23.2	7.96	40.8	24.6	6.2
Kyu-Edogawa River						
Maihama	10:30	23.3	8.23	9.76	5.2	3.4
Sakongawa	10:45	23.1	8.07	8.42	4.6	3.6
Urayasu	11:00	23.3	7.84	4.77	2.7	5.7
Imaibashi	11:25	23.3	8	2.18	1.4	3.6
Arakawa River						
Heriport	13:30	23.8	7.5	16.95	9.4	2.9
Wanganbashi	13:20	23.7	7.45	14.29	7.8	4
Kasaibashi	12:55	24.2	7.37	12.42	6.7	5
Shinfunakoshi	12:45	24	7.54	6.18	3.4	6.8

Appendix 1-2. Sampling data in July 13, 2000.

	Time	Ta(°C)	Tw(°C)	pH	EC(μS/cm)	Salinity
Tamagawa River						
Inagi-o-hashi	9:40	28.8	23.3	8.07	251	
Kamigawara-seki	10:15	31.0	23.9	7.86	259	
Chofu-seki	11:25	32.6	27.4	7.95	263	
Daishi-bashi	12:25	34.0	27.5	7.57	10400	5.6
Tsurumigawa River						
Tsurumi-o-hashi	13:00	32.4	27.8	7.72	17100	9.3
Morinaga-bashi	13:45	31.6	27.3	7.28	11500	5.7
Nippa-bashi	14:30	30.1	26.7	7.59	414	
Chiyo-bashi	16:00	30.2	28.1	8.14	394	

Appendix 1-3. Sampling data from Tamagawa Estuary survey in August 3, 2000.

	Time	Ta(°C)	Tw(°C)	pH	EC(mS/cm)	Salinity
No. 1	9:35	34.7	28.4	7.40	0.419	0.4
No. 2	9:55	36.7	28.2	7.36	0.505	0.5
No. 3	10:00	34.8	28.4	7.26	0.802	0.7
No. 4	10:12	35.7	28.6	7.31	1.98	1.4
No. 5	10:30	33.8	29.2	7.44	3.61	2.3
No. 6	11:10	33.0	29.4	7.49	8.07	4.2
No. 7	11:25	33.0	29.9	7.60	8.78	5.1
No. 8	11:40	32.8	30.2	7.63	13.7	8.1
No. 9	11:55	32.9	30.2	7.69	13.8	8.3
No. 10	12:10	32.8	29.8	7.74	20.1	12.1
No. 11	12:30	32.8	29.8	7.81	21.8	13.1
No. 12	12:40	31.3	29.8	7.82	23.0	14.4
No. 13	12:50	31.1	29.5	7.99	29.0	19.0
No. 14	13:05	29.3	28.3	8.37	40.3	26.7
No. 15	13:15	29.6	28.9	7.99	29.0	18.5
No. 16	13:20	13.4	28.4	8.04	31.2	19.9

Appendix 1-4. Sampling data in August 18, 2000.

	ID	station	Time	Ta(°C)	Tw(°C)	pH	EC(mS/cm)	Salinity
1	st 5	Shimo	9:00	27.0	25.1	7.35	0.323	
2	st 8	ShinArakawa	9:20	25.9	24.8	7.30	0.200	
3	st 12	Denu	10:45	26.0	28	6.68	0.679	
4	st 11	Takumi	11:15	26.3	27.5	7.00	0.389	
5	st 4	Shirahige	12:00	27.8	25.7	7.14	0.316	
6	st 7	Kine (Ara)	12:30	29.9	25.8	7.34	0.229	
7	st 10	Kine (Aya)	12:30	29.9	26.7	7.43	0.332	
8	st 3	Azuma	13:20	29.0	26.0	7.06	0.326	
9	st 2	Ryogoku	13:50	31.9	26.2	7.13	0.434	
10	st 1	Kachidoki	14:30	29.7	26.4	7.00	11.09	6.0
11	st 6	Kasai (A)	15:45	29.6	25.6	7.32	0.784	
12	st 9	Kasai (N)	15:50	29.6	27.6	7.27	0.566	
13	st 13	Urayasu	16:20	28.4	26.3	7.48	0.372	
14	st 14	Imai	17:00	28.6	26.1	7.45	0.269	
15	st 15	ShinImai	17:25	27.3	26.3	7.53	0.322	
16	st 16	ShinGyotoku	18:00	-	27.7	8.14	38.5	23.1
17	st 17	Ichikawa	19:20	-	25.5	7.51	0.222	

Appendix 1-5. Sampling data in August 30, 2000.

	ID	station	Time	Ta(°C)	Tw(°C)	pH	EC(mS/cm)	Salinity
1	st 5	Shimo	9:15	31.6	27.9	7.38	0.544	
2	st 8	ShinArakawa	9:25	31.9	28.8	7.35	0.424	
3	st 12	Denu	10:30	31.9	30.5	6.96	0.900	
4	st 11	Takumi	10:40	32.0	29.3	7.33	0.486	
5	st 4	Shirahige	11:30	32.4	28.8	7.42	0.690	0.6
6	st 3	Azuma	11:50	34.9	29.6	7.40	1.088	0.8
7	st 2	Ryogoku	12:30	33.7	29.0	7.38	2.73	1.6
8	st 1	Kachidoki	13:00	35.4	29.5	7.39	14.98	8.1
9	st 6	Kasai (A)	13:30	33.5	30.1	7.52	5.24	2.9
10	st 9	Kasai (N)	13:45	34.4	30.4	7.68	2.06	1.3
11	st 13	Urayasu	14:10	33.9	30.3	7.69	3.55	2.0
12	st 16	ShinGyotoku	14:30	35.4	30.5	7.95	27.2	15.7

Appendix 1-6. Sampling data for discharge from STP-2

	Date	Time	Ta(°C)	Tw(°C)	pH	EC(μS/cm)
2000	11-Oct	11:45		24.0	6.70	394
	28-Aug		30.3	26.9	7.52	423
	25-Jul	10:00	26.8	26.2	6.82	511
	30-Jun	9:50	29.3	24.9	7.09	446
	30-May	9:30	27.1	24.2	6.98	462
	24-Apr	10:30	16.0	20.0	6.83	450
1999	15-Nov	9:30		22.2	6.60	440
	10-Jun	9:30	24.0	24.6	6.58	475
	21-May	9:00	26.3	22.5	6.82	472
1998	4-Sep		28.8	24.4	6.93	401
	1-Jul	10:00	29.0	24.1		450
1997	22-Dec		6.3	18.8	6.81	546

Appendix 2. Site correspondance

Sumidagawa River	**ES&T**	**This Thesis**
Shimobashi	SR-9701	SM-1
Shirahigebashi	SR-9702	SM-2
Adzumabashi		SM-3
Kuramaebashi	SR-9703	SM-4
Kiyosubashi	SR-9704	SM-5
Eitaibashi	SR-9705	SM-6
Tsukudaohashi	SR-9706	SM-7
Kachidokibashi	SR-9707	SM-8
Harumibashi	SR-9708	SM-9
Rainbow Bridge	SR-9709	SM-10
Tokyo Port Tunnel	SR-9710	SM-11

Tmagawa River	**ES&T**	**This Thesis**
Inagiohashi		TM-1
Kamigawara Dam	TR-9701	TM-2
Chofu Dam	TR-9702	TM-3
Tamagawaohashi	TR-9703	TM-4
Rokugobashi	TR-9704	TM-5
Daishibashi	TR-9705	TM-6
Haneda	TR-9706	TM-7
Kawasaki Futo	TR-9707	TM-8

Sewage Treatment Plants	**ES&T**	**This Thesis**
Kitatamaichigo	STP-1	STW-1
Shibaura	STP-2	STW-2
Shingashi	STP-3	STW-3
Odai	STP-4	STW-4
Mikawajima	STP-5	STW-5

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