表 6. 6 物質の RP , NRP 値 (mg/L)
および回復に必要な日数 (日)

物質名	RP	NRP	回復日数
DHH	2.0	-*	3-5
ACT	10<	-	2
KTP	25	-*	1-3
PHT	5.0	10	1
EDC	60<	-	1-3
EPT	10<	-	1

*RP 記載濃度より上の濃度区で親個体全滅

D. 考察

6 物質の回復試験結果から、表 6 に回復に必要な日数を示した。この表から回復期間に移行して 1-3 日目で回復する物質が多いことが分かった。また、PHT のみ NRP が確認されたが、産仔が 1 匹も無い状態ではなく、対照区と同程度の産仔が観察されなかった。このことから回復の定義を産仔の有無にすると PHT も回復したことになると考えられた。このように回復の定義によって、または何をもってして回復とみなすかということで回復した・しないが変化するため、回復の定義を定めるためにも様々なデータパターンが必要であると考えられた。また、ACT, EDC, EPT は最高濃度区で死亡せず、産仔しない状態であった為、より高濃度で回復試験を行うと PHT のように NRP が観察されると考えられる。

E. 結論

複合試験結果では KET と LVS の組み合わせは人間では副作用を起こすことから禁忌とされているが、ミジンコに対しては相殺の作用が確認された。

回復試験では今回試験を行った医薬品 5 物質は回復することが確認されたが、PHT だけ回復しない濃度があった。また、回復の定義によって回復の有無が変わるため今後さらなる検討が必要である。

G. 成果発表

1.論文発表　なし

2.学会発表　なし

H. 知的財産権の出願・登録状況

（予定を含む）

特許取得　　なし

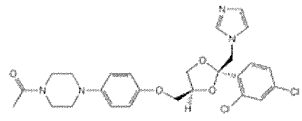
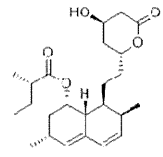
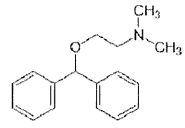
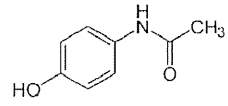
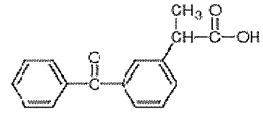
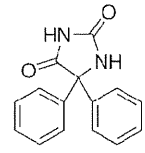
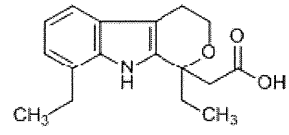
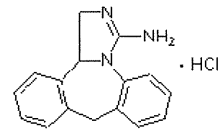
実用新案特許　　なし

その他　　なし

F. 健康危機情報

なし

表 1. 対象医薬品　8 種類

試薬名 (略称)	CAS No.	分子量	構造式	メーカー	用途
Ketoconazole (KTC)	65277-42-1	531.43		東京化成工業株式会社 (純度 98%以上)	アゾール系抗真菌薬
Lovastatin (LVS)	75330-75-5	404.54		東京化成工業株式会社 (純度 98%以上)	HMG-CoA 還元酵素阻害薬
Diphenhydramine HCl (DHH)	147-24-0	291.82		和光純薬工業株式会社 (純度 98%以上)	抗ヒスタミン薬
Acetaminophen (ACT)	103-90-2	151.16		和光純薬工業株式会社 (純度 98%以上)	解熱鎮痛薬
Ketoprofen (KTP)	22071-15-4	254.28		和光純薬工業株式会社 (純度 98%以上)	解熱鎮痛薬
Phenytoin (PHT)	57-41-0	252.27		東京化成工業株式会社 (純度 99%以上)	抗てんかん薬
Etodolac (EDC)	41340-25-4	287.35		和光純薬工業株式会社 (純度 97%以上)	解熱鎮痛薬
Epinastine HCl (EPT)	108929-04-0	285.78		東京化成工業株式会社 (純度 98%以上)	抗アレルギー薬

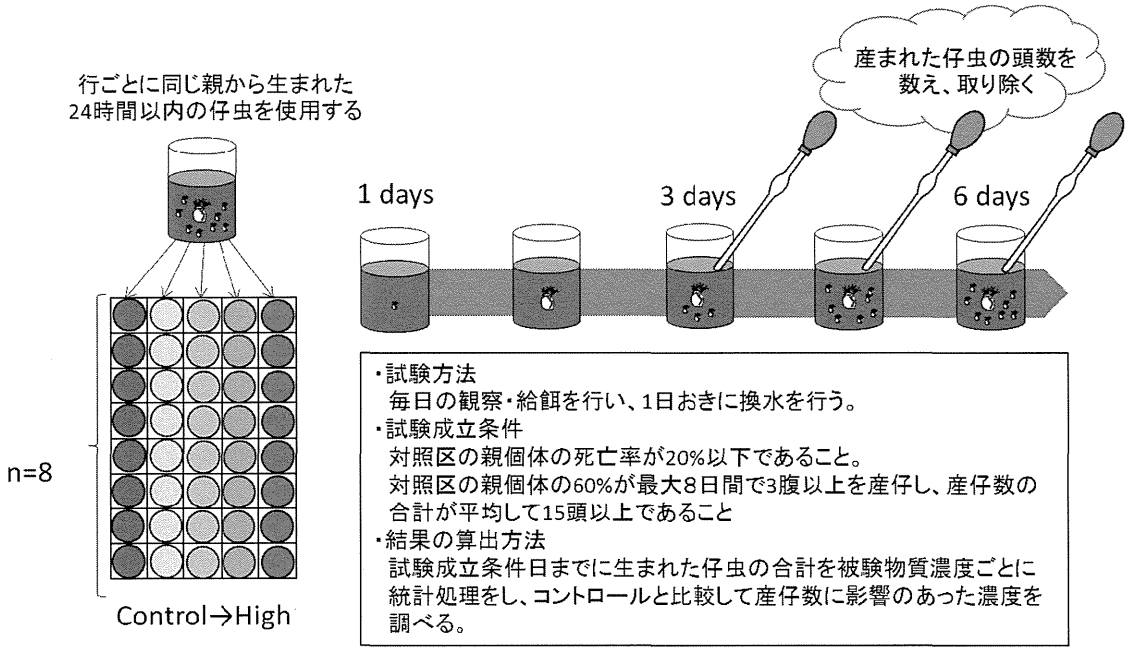


図 1B-1. 繁殖試験手順の概要

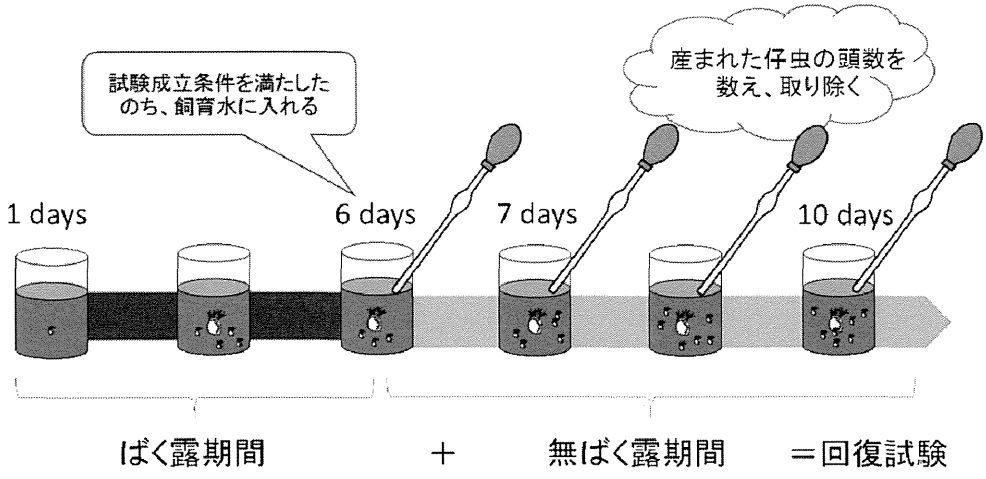


図 2B-2. 回復試験の模式図

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Suzuki, T., Kosuge, Y., Hosaka, M., Nishimura, T., Nakae, D.	Occurrence and behavior of the chiral anti-inflammatory drug naproxen in an aquatic environment	Environ. Toxicol. Chem.	33 (12)	2671-2678	2014

Ⅳ. 研究成果の刊行物・別刷

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OCCURRENCE AND BEHAVIOR OF THE CHIRAL ANTI-INFLAMMATORY DRUG NAPROXEN IN AN AQUATIC ENVIRONMENT

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Abstract: The present study reports on the occurrence and chiral behavior of the anti-inflammatory drug (S)-naproxen (NAP)—(S)-2-(6-methoxynaphthalen-2-yl)propionic acid—in an aquatic environment under both field and laboratory conditions. In influents and effluents of sewage treatment plants (STPs) in the Tama River basin (Tokyo), (S)-NAP was detected at concentrations of 0.03 $\mu g\,L^{-1}$ to 0.43 $\mu g\,L^{-1}$ and 0.01 $\mu g\,L^{-1}$ to 0.11 $\mu g\,L^{-1}$, respectively. The concentrations of a major metabolite, 6-*O*-desmethyl NAP (DM-NAP) were up to 0.47 $\mu g\,L^{-1}$ and 0.56 $\mu g\,L^{-1}$ in influents and effluents, respectively. (R)-naproxen was not detected in STP influents, although it was present in effluents, and the enantiomeric faction (= S/[S + R]) of NAP ranged from 0.88 to 0.91. Under laboratory conditions with activated sludge from STPs, rapid degradation of (S)-NAP to DM-NAP and chiral inversion of (S)-NAP to (R)-NAP were observed. During river die-away experiments, degradation and chiral inversion of NAP were extremely slow. In addition, chiral inversion of (S)-NAP to (R)-NAP was not observed during photodegradation experiments. In the river receiving STP discharge, NAP and DM-NAP concentrations reached 0.08 $\mu g\,L^{-1}$ and 0.16 $\mu g\,L^{-1}$, respectively. The enantiomeric faction of NAP in the river ranged from 0.84 to 0.98 and remained almost unchanged with the increasing contribution of rainfall to the river water. These results suggest that the absence and decrease of (R)-NAP in river waters could indicate the inflow of untreated sewage. *Environ Toxicol Chem* 2014;33:2671–2678.

Keywords: Naproxen Enantiomeric fraction Chiral inversion Sewage treatment plant

INTRODUCTION

Numerous chiral xenobiotics such as pesticides, flame retardants, and pharmaceuticals have been developed and used in various fields, resulting in pollution of the environment and biota [1–5]. Because enantiomers of chiral xenobiotics often have differing toxicities and bioactivities, it is important to assess the fate of individual enantiomers to assess the risks to human health accurately and to protect ecosystems appropriately [1,2]. Observed differences in enantiomeric ratios of chiral xenobiotics provide additional evidence for the importance and contribution of biological transformation in aquatic and terrestrial environments [6]. Enantiomeric fractions of chiral xenobiotics usually remain unchanged by dilution, adsorption, photodegradation, and abiotic degradation in natural environments [1]. Therefore, enantiomer profiles of chiral xenobiotics in environmental samples and biota have been used as diagnostic tools to trace chemical sources and chemical fates in the natural environment. Previous studies have examined chiral pesticides such as organochlorine pesticides [3], phenoxy acid herbicides [7], and phenylpyrazole insecticides [8]. In the past decade, pharmaceuticals and personal care products (PPCPs) have become a pollutant of aquatic environments [4,5]. The levels of contamination by PPCPs in the aquatic environment, such as analgesics, antiphlogistics, lipid regulators, and antidepressants, range from nanograms per liter to micrograms per liter. Their potential environmental risk is an emerging environmental issue, and the effects of PPCPs on aquatic ecosystems and human health are of concern.

Little is known about the enantiomeric compositions and fate of chiral PPCPs in the aquatic environment. The enantiomer composition of the anti-inflammatory drug ibuprofen in surface water, with the (S) form of the enantiomer being greater than the (R) form of the enantiomer, may indicate some input of untreated or insufficiently treated wastewater [9]. The enantiomeric composition of a β-blocker propranolol might be a useful indicator for leakage or overflow of sewers [10].

(S)-naproxen (NAP)—2-(6-methoxynaphthalen-2-yl) propionic acid—is a member of the 2-aryl-propanoic acid series of nonsteroidal anti-inflammatory drugs that has potent inhibitory effects on prostaglandin E2 synthesis [11]. It is commonly used to treat pain, fever, inflammation, rheumatoid arthritis, psoriatic arthritis, and gout [12]. Naproxen has an asymmetric carbon atom and 2 enantiomers, the (R) form and the (S) form, as shown in Figure 1. In practice, the profens are generally administered as a racemic mixture; however, NAP is administered only in the (S) form, because the (R) isomer has the effect of increasing the burden on renal clearance [13] and is also substantially less potent than the (S) form [14]. The principal metabolic pathways of (S)-NAP in animals and humans are demethylation of 6-methoxy group to convert to 6-*O*-desmethyl desmethyl-NAP (DM-NAP) in the phase I reaction by microsomal CYP2C9, and glucuronide and sulfate conjugation in the phase II reaction, as shown in Figure 1 [15]. Chiral inversion of (S)-NAP to (R)-NAP was not observed in rabbits [16] or female Sprague-Dawley rats [17], although the conversion of (R)-NAP to (S)-NAP occurred [16–17]. Its physicochemical property, with a disassociation constant of 4.2 to 4.9 [18,19], suggests high mobility in the

All Supplemental Data may be found in the online version of this article.
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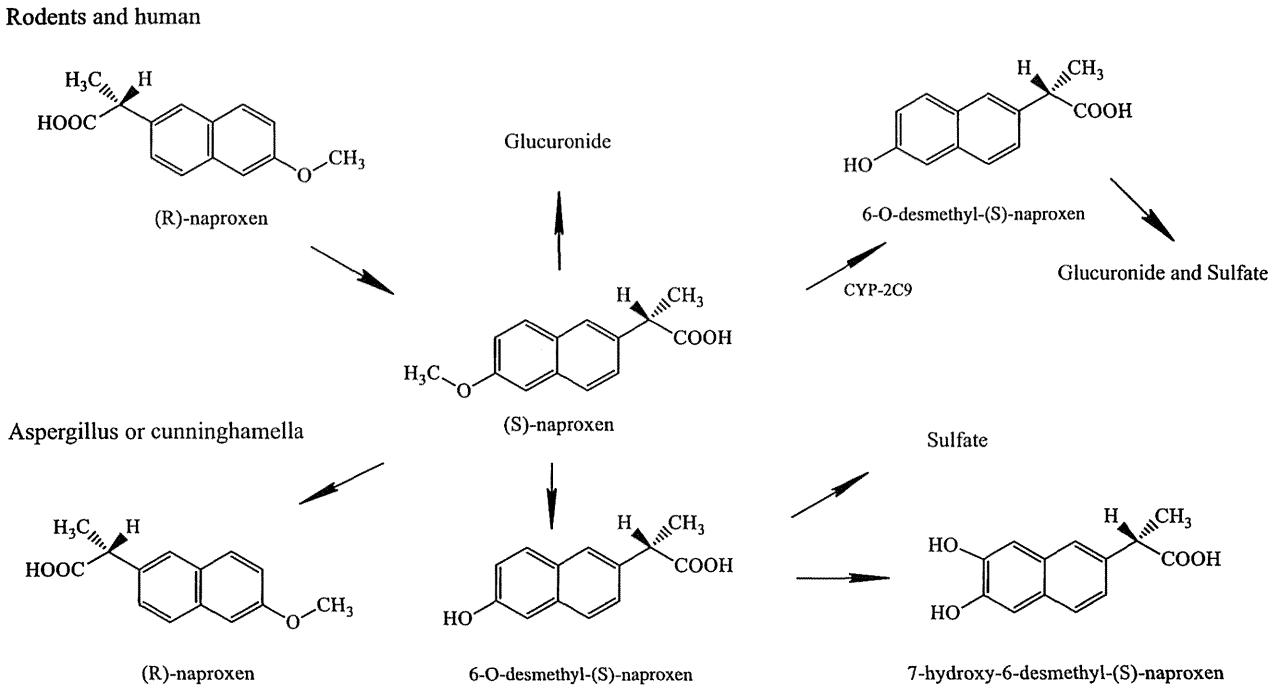

Figure 1. Metabolic pathways of (S)-naproxen in mammals and bacteria.

natural aquatic environment. In previous aquatic monitoring studies, NAP has been observed in urban river water [20–22], in influents and effluents of sewage treatment plants (STPs) in the European Union [23] and Japan [24], and in drinking water sources in the United States [25] at concentrations from several nanograms per liter to several micrograms per liter in combination with other PPCPs. No known risks associated with exposure of aquatic organisms or humans to low concentrations of (S)-NAP and (R)-NAP have been identified. Regarding the degradation of NAP in an aquatic environment, the major metabolite of NAP is DM-NAP in aerobic degradation experiments with activated sludge [26]. A fungal strain, *Aspergillus niger* ATCC, has also metabolized (S)-NAP to DM-NAP and to 7-hydroxy-DM-NAP [27]. *Cunninghamella* species transformed NAP to DM-NAP and then to a sulfate conjugate of DM-NAP [28]. To our knowledge, however, no studies have investigated the possibility of the chiral inversion of NAP in STPs and the aquatic environment.

To clarify chiral behavior of NAP in the aquatic environment, we examined the 2 enantiomers of NAP and its major metabolite DM-NAP in the influent and effluent of STPs located in the Tama River system and in the Tama River, which flows through Tokyo, Japan. Additional culture experiments were performed with activated sludge and river water under laboratory conditions to simulate the biotransformation of NAP in water from the STPs and the river.

MATERIALS AND METHODS

Chemicals

We purchased (Rac)-NAP, (R)-NAP, and (S)-NAP from Wako Pure Chemicals. (Rac)-6-*O*-Desmethyl NAP (DM-NAP) was synthesized from (Rac)-NAP by demethylation with boron bromide and purified by recrystallization in dichloromethane. Identifying DM-NAP was performed by gas chromatography–mass spectrometry (GC/MS) and liquid chromatography–mass spectrometry (LC/MS; see Supplemental Data).

Sampling location and collection of water samples

River water samples were collected from the Tama River basin in Tokyo, Japan, from January 2004 to March 2005 (Figure 2). Water samples were stored in 1-L amber glass bottles, which had been cleaned with 50 mL of acetone. Flow rate data at the Tamagawara Bridge sampling point were obtained from the Keihin Office of River, the Ministry of Land, Infrastructure, and Transport, Tokyo. Composites samples of influent and effluent (all 24-h flow proportionally collected) from the 6 STPs located near the Tama River system were collected from October 2004 to March 2005 into amber glass bottles that had been washed with acetone. Table 1 lists the influent and effluent flow rates and the populations served by the 6 STPs.

Sample preparation and clean up

A 500-mL sample of river water or STP effluent was acidified to pH 3 or 4 with formic acid and passed though tandem solid-phase extraction (SPE) cartridges. The first was a Sep-Pak PS-2 Plus (300 mg/80 μm; Waters) and the second was an OASIS HLB Plus (225 mg/60 μm; Waters). The SPE cartridges had been washed by 5 mL of acetonitrile (CH_3CN; Wako Pure Chemical) and then 5 mL of water at a flow rate of 20 mL/min. In the case of the STP influent (500 mL) samples, solids were separated by glass filter (45 mm inner diameter, pore size 0.45 μm; Millipore) prior to the SPE. The filter was first sonicated for 5 min in 5 mL of methanol, then the methanol solution was added to the filtrate, and finally the sample was subjected to the SPE. The 2 cartridges were dried with passing air for 30 min. The analytes were eluted from the tandem SPE cartridges by the back-flush method using 5 mL of CH_3CN. The CH_3CN solution eluted from the SPE cartridges was divided into 2 portions and then dried under a stream of nitrogen at 40 °C. For GC/MS analysis, the extract was dissolved in 250 μL of dichloromethane, and then NAP and DM-NAP were trimethylsilylated by 50 μL of N, O-bistrifluoroacetamide prior to being injected into the

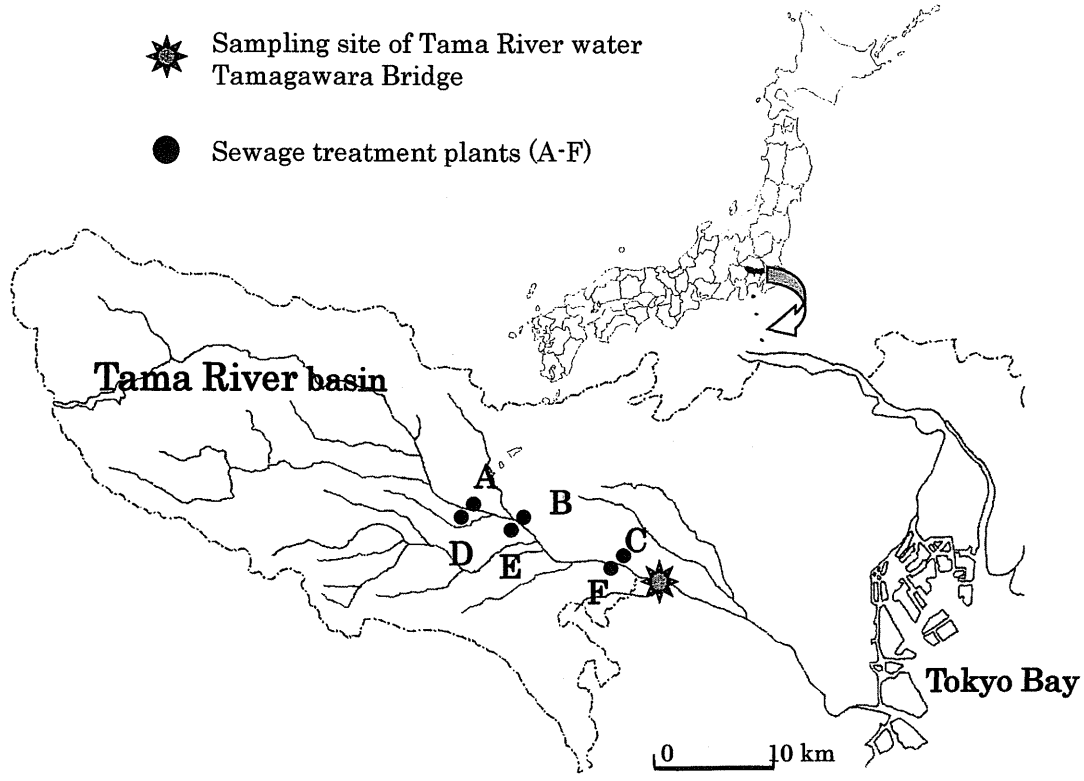

Figure 2. Sampling locations of sewage treatment plants and river water in Tama River basin. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

GC/MS system. The analytes were ascertained by internal standard methods using fluoranthene-d_{10} as an internal standard. For liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, the extract was dissolved in 250 μL of 0.1% formic acid:CH_3CN (10:90, v/v). The extracts of STP influent and effluent were cleaned by reverse-phase high-performance liquid chromatography (HPLC) under the following conditions: column, Lichrosorb RP-18, 10 mm inner diameter × 250 mm (Cica Merck); column temperature, 40 °C; mobile phase, 0.1% formic acid:CH_3CN (30:70, v/v); flow rate, 5 mL/min; ultraviolet detector, 260 nm. The fraction containing NAP was obtained at retention times from 3.7 min to 4.4 min under these HPLC conditions. The fraction was dried under a stream of nitrogen at 40 °C and dissolved in 0.1% formic acid:CH_3CN (10:90, v/v).

For accuracy and reproducibility of the GC/MS and LC-MS/MS methods, the recoveries of NAP and DM-NAP were more than 90% from river water and were 100% to 110% from STP influent and effluent, respectively. The relative standard deviations of the analytes were lower than 20% at the spiked concentrations of 100 ng L^{-1}.

Table 1. Occurrence of naproxen (NAP) and 6-*O*-desmethyl-naproxen (DM-NAP) in influent and effluent of sewage treatment plants (STPs) located at the Tama River basin in Tokyo

			Influent					Effluent				
				NAP					NAP			
STP[a]	Population served	Date	Flow m^3/day	μg/L	g/day	EF[b]	DM-NAP μg/L	Flow m^3/day	μg/L	g/day	EF	DM-NAP μg/L
A	453 232	Jan-2005	145 360	0.43	63	1.00	0.35	120 500	0.05	6	0.91	0.34
		Mar-2005	155 740	0.25	39	1.00	0.47	130 720	0.08	11	0.88	0.54
B	138 024	Jan-2005	44 310	0.04	2	1.00	0.11	44 290	0.02	1	0.91	0.11
		Mar-2005	37 270	0.10	4	1.00	0.13	40 180	0.04	1	0.90	0.28
C	471 527	Jan-2005	178 440	0.11	19	1.00	0.19	183 330	0.09	16	0.90	0.34
		Mar-2005	161 450	0.12	19	1.00	0.19	160 910	0.11	17	0.91	0.56
D	279 028	Jan-2005	72 250	0.03	2	—	—	72 200	0.01	1	—	—
		Mar-2005	73 660	0.05	4	—	—	73 610	0.02	1	—	—
E	224 516	Jan-2005	63 400	0.08	5	—	—	63 360	0.02	1	—	—
		Mar-2005	63 730	0.19	12	—	—	63 700	0.06	4	—	—
F	339 400	Jan-2005	96 210	0.03	3	—	—	96 140	0.03	3	—	—
		Mar-2005	97 590	0.07	6	—	—	97 510	<0.01	0	—	—

[a]Treatment process: filtration, primary sedimentation, biological reaction (activated sludge), secondary sedimentation, and chlorine contact.
[b]Enantiomeric fraction (EF) = (S)-NAP/[(S)-NAP + (R)-NAP].

Degradation of NAP with activated sludge and river water

To simulate the biotransformation of NAP in STPs, 1000 mL of STP influent was transferred into a 2000-mL amber flask, to which we added 10 g (wet wt) of activated sludge obtained from the same STP. Then, (S)-NAP (100 mg L^{-1} in acetone) was added into the flask at the final concentration of 10 μg L^{-1}. The flask was incubated at 20 °C in the dark under aerobic conditions by aeration with 200 mL/min of ambient air, which was first passed through an activated-carbon column. An aliquot (100 mL) of the water samples was taken at incubation times of 0 h, 2 h, 4 h, 8 h, and 24 h and was then centrifuged at 3000 rpm for 10 min. The supernatant was extracted by the SPE as described in *Sample preparation and clean up* and was then made up to 1 mL. The analytes were measured by GC/MS after trimethylsilyl (TMS) derivatization and by LC-MS/MS.

For the river die-away experiment, 1000 mL of Tama River water was transferred into an amber flask. Either (S)-NAP or (R)-NAP (100 mg L^{-1} in acetone) was added to the flask at the final concentration of 10 μg L^{-1}. The flask was incubated in the dark at 20 °C under aerobic conditions by bubbling with 50 mL/min of ambient air, which was first passed through an active-carbon column. An aliquot (100 mL) of the water sample was taken at incubation times of 0 d, 1 d, 3 d, 5 d, 7 d, 14 d, and 30 d and then centrifuged at 3000 rpm for 10 min. The supernatant was analyzed in a similar manner as the experiment with activated sludge.

To check the biodegradability in the 2 degradation systems, benzoic acid was used as a reference compound, as recommended by the Organisation for Economic Co-operation and Development [29], and was fortified at a concentration of 10 μg L^{-1}. Some PPCPs were also observed at concentrations of nanograms per liter in the influent and river water samples. Sterile controls of both degradation systems were prepared by autoclaving the medium at 121 °C for 15 min, after which the test substances were spiked and incubated in the same way as nonsterile samples.

Photodegradation of NAP in river water

(S)-naproxen was dissolved in the Tama River water at a concentration of 10 μg L^{-1}. The solution (50 mL) was put into a Petri dish made of quartz (6 cm inner diameter, depth 3 cm), and the water surface was irradiated with an XC-100BSS solar lamp (30 W/m^2; 300 nm < λ < 700 nm; Solax). The temperature of the solution was kept at 17 °C by an electric cooler unit. An aliquot (100 μL) of the water samples was taken at incubation times of 0 h, 1 h, 3 h, 5 h, 7 h, and 22 h and was analyzed by LC-MS/MS.

Analysis of NAP and DM-NAP by GC/MS

Analytical conditions for the TMS derivatives of NAP and DM-NAP were as follows: GC model, HP-5890 Series II (Agilent); injector temperature, 220 °C; column head pressure, 80 kPa (constant pressure mode); carrier gas, helium (99.999% Tomoe Shokai); auto-injector, HP-7673 (Hewlett-Packard); sample size, 2 μL (splitless injection, purge on time for 1 min; glass wool was not inserted into the splitless insert); analytical column, HP5-MS, 0.25 mm inner diameter × 30 m; and film thickness, 0.25 μm (J&W Scientific). The GC oven temperature was programmed as follows: held at 50 °C for 1 min; increased from 50 °C to 200 °C at 10 °C/min; and increased from 200 °C to 300 °C at 6 °C/min. The MS conditions were set as follows: Automass II mass spectrometer (JEOL); ionization potential, 70 eV; ionization current, 300 μA; ion source temperature, 220 °C; and temperature of transfer line between GC and MS, 250 °C. The TMS derivatives of the analytes were identified and quantified by single ion monitoring using the monitor ions at *m/z* 185 and 302 for NAP and *m/z* 243 and 360 for DM-NAP. The complete separation of NAP and DM-NAP could be performed, and the GC/MS chromatograms showed no interference under these chromatographic conditions (Supplemental Data, Figure S1). The limits of quantification (signal-to-noise ratio of 10) for NAP and DM-NAP were 1 μg L^{-1}. A calibration curve was acquired with a determination coefficient of $R^2 = 0.998$ to 0.999 at the concentrations of NAP from 1 μg L^{-1} to 100 μg L^{-1}.

Analysis of NAP enantiomers by LC-MS/MS

The 2 enantiomers of NAP were measured under the following conditions: LC model, 2690 Separation Module (Waters); solvents, 0.1% formic acid:CH_3CN (50:50, v/v); flow rate, 0.2 mL/min; column, CHIRALPAK AD-RH, 4.6 mm × 15 cm (Daicel Chemical Industry); column temperature, 35 °C; MS model, Quattro Ultima PT tandem quadrupole mass spectrometer (Micromass; Waters); ion source temperature, 120 °C; desolvation temperature, 300 °C; mode, positive electron spray ionization; capillary voltage, 3 kV; cone voltage, 150 V; collision energy, 15 eV; precursor ion, *m/z* 185; and product ion, *m/z* 154 and 170. The 2 enantiomers of NAP were separated completely (Figure 3). The limits of quantification of (S)-NAP and (R)-NAP were 100 ng L^{-1} under these LC-MS/MS conditions. A calibration curve of (S)-NAP and (R)-NAP was acquired, having a determination coefficient $R^2 = 0.998$ to 0.999, respectively, at concentrations ranging from 10 μg L^{-1} to 100 μg L^{-1}.

RESULTS AND DISCUSSION

Occurrence of NAP and DM-NAP in STPs

In STP influent and effluent, (S)-NAP was detected at concentrations of 0.03 μg L^{-1} to 0.43 μg L^{-1} and 0.01 μg L^{-1} to 0.11 μg L^{-1}, respectively (Table 1). The removal rate of (S)-NAP for the 6 STPs was 50 ± 14%. Other profens, such as ibuprofen and ketoprofen, were present in the STP influent and were removed at efficiencies of 97% and 50%, respectively, across the 6 STPs. The biological reactors at the STPs use a hydraulic retention time of 6 h to 8 h, and total treatment time was 11 h to 12 h. Previous studies have observed 50% to 65% removal of NAP in STPs [24,30,31]. Sewage treatment plants were reported to remove NAP and ibuprofen via biological treatment but not via a sedimentation process because of the compounds' acidic structures [32]. Sewage treatment plant influent and effluent showed DM-NAP concentrations of 0.11 μg L^{-1} to 0.47 μg L^{-1} and 0.11 μg L^{-1} to 0.56 μg L^{-1}, respectively (Table 1). This suggests that DM-NAP concentration increased during the STP treatment process due to biodegradation. In activated sludge, (S)-NAP underwent biodegradation within 3 d, giving DM-NAP [33]. Naproxen and DM-NAP are excreted mainly as glucuronide and sulfate conjugates in mammals such as rats, rabbits, and humans [15]. Therefore, the increase of DM-NAP during biological treatment at the STPs might be due to biodegradation of NAP and the cleavage of their conjugates.

Chiral analysis indicated that (R)-NAP was not detected in STP influent but occurred in effluent (Figure 3 and Table 1). The enantiomeric fraction in STP effluent ranged from 0.88 to 0.91. Prior studies have reported that chiral inversion of (S)-NAP to (R)-NAP was not observed in rats and rabbits [15,21]. It was reported that residual ibuprofen in STP effluent showed lower

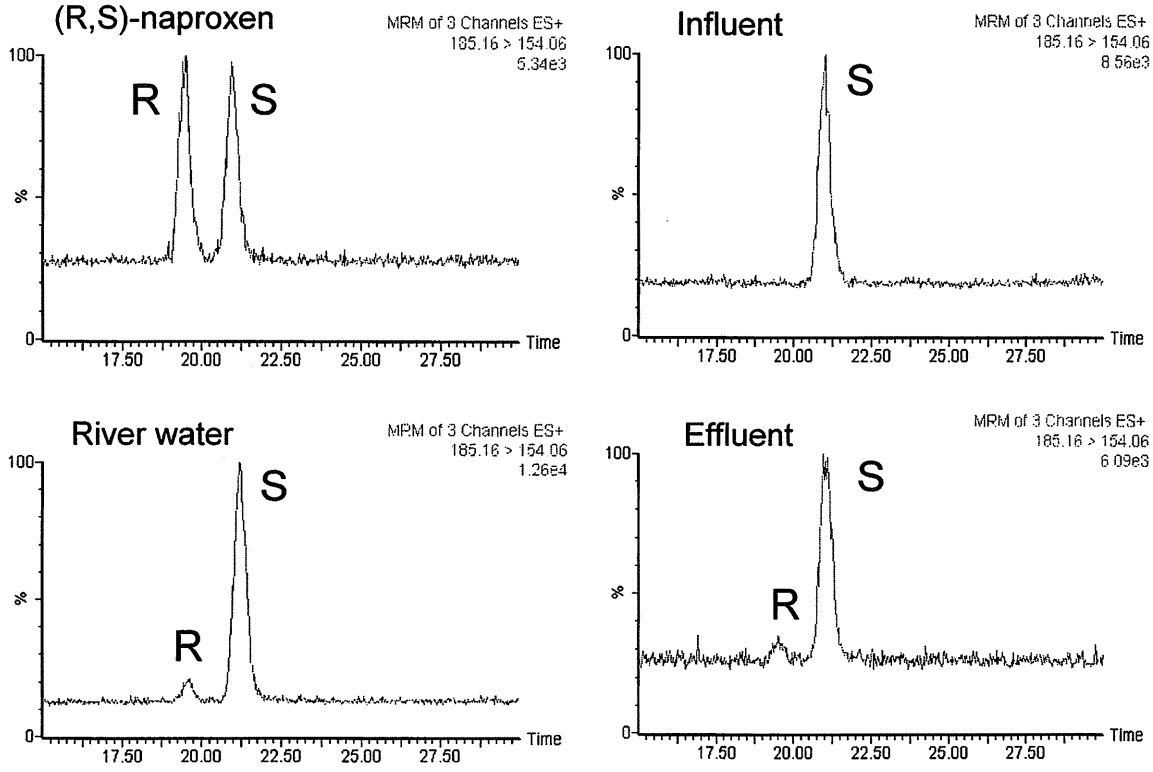

Figure 3. Liquid chromatography–mass spectrometry chromatograms of naproxen in water samples with chiral separation column. S = (S)-naproxen; R = (R)-naproxen. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

enantiomeric excess of the (S) form than the residual ibuprofen in the influents [2]. For the chiral stability of NAP in water, chiral inversion of (S)-NAP to (R)-NAP and (R)-NAP to (S)-NAP in the purified water did not occurred after 21 d at 20 °C in the dark. From these results, chiral inversion of (S)-NAP to (R)-NAP occurred during the treatment process of the STPs.

Degradation of NAP with activated sludge obtained from STPs

Laboratory data from the incubation of (S)-NAP in STP influent mixed with activated sludge are shown in Table 2. Degradation of (S)-NAP did not occur in the sterilized sample. On the other hand, little or no dissipation of (S)-NAP was observed during initial incubation, followed by rapid dissipation of (S)-NAP to less than 1% after 24 h of incubation for a nonsterile sample. The degradation rate of (S)-NAP followed a pseudo–zero order kinetics with a rate constant 0.408 h^{-1} ($R^2 = 0.971$) and 14-h half-life in these conditions. As a principal metabolite of NAP, DM-NAP appeared after 8 h incubation. It was also a major metabolite of (S)-NAP in previous laboratory experiments with activated sludge and showed low persistence [26]. In the present study, the amount of DM-NAP after 24 h incubation was approximately 30% of the parent compound. This suggests that DM-NAP might degrade further to lower molecular weight metabolites. A minor metabolite of (S)-NAP by *Aspergillus niger* ATCC 9142 was 7-hydroxy-DM-NAP, which appeared gradually along with decreasing DM-NAP [27]. Under the present study's conditions, benzoic acid fortified as a reference compound degraded more rapidly than (S)-NAP, and the other profens contained in the STP influent, such as ibuprofen and ketoprofen, showed the same dissipation rates as (S)-NAP. The incomplete removal of the profens usually observed in the STPs might be due to the shorter hydraulic retention time of 6 h to 8 h.

The enantiomeric fraction of NAP decreased gradually with incubation time (Table 2), reaching 0.91 after 24 h. Chiral inversion of (S)-NAP to (R)-NAP occurred during biodegradation, and the dissipation rate of (S)-NAP was faster than that of (R)-NAP. These results suggest that microorganisms in activated STP sludge could perform the chiral inversion of (S)-NAP to (R)-NAP.

Degradation of NAP in river water

The results from the river die-away experiments using (S)-NAP and (R)-NAP are shown in Table 3. No degradation of the 2 enantiomers of NAP was observed in the sterile control samples up to 30 d incubation. Conversely, (S)-NAP and (R)-NAP degraded under nonsterile conditions, with the degradation rate of the (S) enantiomer being faster than that of the (R) enantiomer. The degradation rate of (S)-NAP and (R)-NAP followed pseudo–zero order kinetics with a rate constant 0.141 h^{-1} ($R^2 = 0.965$) and 0.049 h^{-1} ($R^2 = 0.857$) in these conditions, respectively. The

Table 2. Degradation of (S)-NAP with activated sludge of the sewage treatment plant located in the Tama River basin

		Incubation time (h)				
Compound	Unit	0	2	4	8	24
(S)-NAP	μg L^{-1}	10.00	9.90	8.80	8.50	0.50
(R)-NAP	μg L^{-1}	ND	0.10	0.10	0.30	0.05
EF[a]	—	1.00	0.99	0.99	0.97	0.91
DM-NAP	μg L^{-1}	0.20	0.15	0.10	0.05	2.80
Benzoic acid[b]	μg L^{-1}	10.00	0.50	0.40	0.40	0.30

[a]Enantiomeric fraction (EF) = (S)-NAP/[(S)-NAP + (R)-NAP]; when the concentration of (S)-Nap or (R)-NAP was ND, (S) or (R) = 0.
[b]Reference compound.
(S)-NAP = (S)-naproxen; (R)-NAP = (R)-naproxen; DM-NAP = 6-*O*-desmethyl-naproxen; ND = less than 0.05 μgL^{-1}.

Table 3. River die-away experiment of (S)-naproxen and (R)-naproxen with the Tama River water

		(S)-NAP						(R)-NAP					
		Incubation time (d)						Incubation time (d)					
Compound	Unit	0	2	4	8	16	30	0	2	4	8	16	30
(S)-NAP	μg L^{-1}	10.00	10.00	9.50	9.00	8.50	5.70	ND	ND	ND	ND	0.20	0.20
(R)-NAP	μg L^{-1}	ND	ND	ND	ND	0.05	0.05	10.00	10.00	9.50	9.10	9.00	8.50
EF[a]	—	1.00	1.00	1.00	1.00	0.99	0.99	0.00	0.00	0.00	0.00	0.02	0.02
DM-NAP	μg L^{-1}	ND	ND	ND	ND	ND	0.07	ND	ND	ND	ND	ND	0.05
Benzoic acid[b]	μg L^{-1}	10.00	0.40	0.30	0.30	0.40	0.10	10.00	0.60	0.40	0.40	0.30	0.10

[a]Enantiomeric fraction (EF) = (S)-NAP/[(S)-NAP + (R)-NAP]; when the concentration of (S)-Nap or (R)-NAP was ND, (S) or (R) = 0.
[b]Reference compound.
(S)-NAP = (S)-naproxen; (R)-NAP = (R)-naproxen; DM-NAP = 6-*O*-desmethyl-naproxen; ND = less than 0.05 μg L^{-1}.

half-lives of (S)-NAP and (R)-NAP were determined to be 37 d and 99 d, respectively. Their degradation rates in the river water were very slow compared with those by activated sludge; the degradation of the benzoic acid reference compound was also slower than in the scenario with activated sludge. As for the chiral inversion of NAP under these incubation conditions, the (S) enantiomer to (R) enantiomer was 0.1 μg L^{-1} at 30 d incubation, whereas the (R) enantiomer to the (S) enantiomer was 0.2 μg L^{-1}. The major metabolite DM-NAP was not observed in up to 30 d incubation, which suggests that any residual represents less than 1% of the initial concentration.

Occurrence of NAP and DM-NAP in river water

Naproxen and DM-NAP in the Tama River were measured at the Tamagawara Bridge (Figure 2), which is downstream from the discharge points of the 6 STPs; however, NAP and DM-NAP were not detected in the river water taken from the site upstream from the 6 STPs. Naproxen was observed at concentrations from 0.01 μg L^{-1} to 0.08 μg L^{-1} (Figure 4). The concentrations of the major metabolite DM-NAP ranged from 0.025 μg L^{-1} to 0.160 μg L^{-1}. This concentration is higher than that of NAP, and the ratios of DM-NAP/(NAP + DM-NAP) ranged from 0.56 to 0.76. The concentrations of NAP and DM-NAP also decreased from September to November. This phenomenon was also observed for the other PPCPs. On the other hand, the enantiomeric fraction of NAP ranged from 0.84 to 0.98 and did not change drastically despite variations in river flow (Figure 4). The sum of effluent of the 6 STPs located in the Tama River basin was lower than the sum of treatment capacity of the STPs, 12 m^3/sec, from September to November in 2004. The decrease of NAP and DM-NAP and the slight change of enantiomeric fraction indicate that the effluents from the STPs were diluted by the surface water as a result of high precipitation.

Prior studies have suggested that biodegradation and absorption are possible mechanisms to eliminate PPCPs in the aquatic environment. A study of rivers in Finland receiving STP effluent found that elimination of NAP and other profens had not yet occurred [34]. The lower level of NAP at the site downstream from the effluent discharge point can be attributed mainly to dilution and adsorption to particles and sedimentation [35]. In another previous study, decarboxylation of NAP seemed to be the only photodegradation process [36]. Under irradiation with a xenon arc lamp (765 W/m^2; 290 nm < λ < 700 nm), the half-life of NAP ranged from 1 h to 2.5 h [37]. (S)-naproxen was degraded readily with an ultraviolet-reactor and was eliminated within 5 min [38]. As another potential mechanism, dissipation of (S)-NAP in the Tama River was due mainly to photodegradation [39]. It was reported that the half-life of NAP was 42 min in river water, and its first photodegradation product was 1-(6-methyoxy-2-naphthyl)ethanol [18]. These previous studies contain no observations on the chiral inversion of (S)-NAP to (R)-NAP. In the present study of photodegradation of NAP in river water under laboratory conditions, the half-life of (S)-NAP was calculated as 3.79 h by first-order kinetics (Supplemental

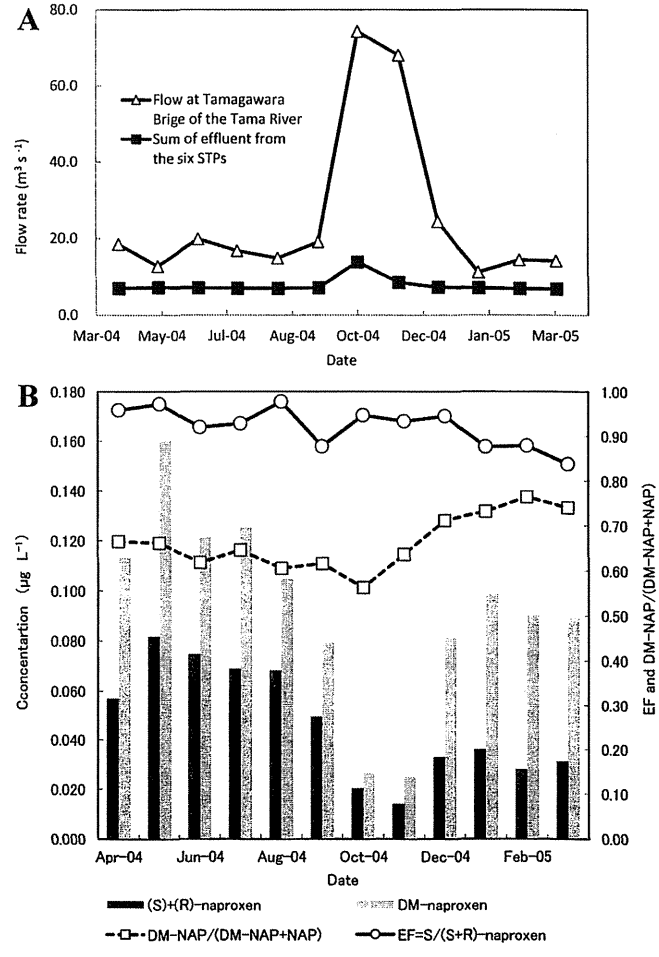

Figure 4. Seasonal changes of naproxen, 6-*O*-desmethyl-naproxen (DM-NAP), and enantiomaric fraction (EF) of naproxen at the Tamagawara Bridge in the Tama River. (**A**) Flows of river and the sum of effluent of the 6 sewage treatment plants (STPs); (**B**) concentration of naproxen and DM-NAP, and the enantiomeric faction (EF) of naproxen and the ratio of DM-NAP to DM-NAP plus NAP.

Data, Figure S2). Although some unidentified photodegradation products were observed with the disappearance of (S)-NAP, the inversion of (S)-NAP to (R)-NAP was not observed during the irradiation period.

The average flow rate of downstream sites of the Tama River basin is approximately 0.5 m sec^{-1}. The distance from the first point of effluent discharge from STPs to Tokyo Bay is approximately 30 km. Therefore, the traveling time of (S)-NAP and (R)-NAP and DM-NAP in the Tama River basin is shorter than 1 d. The contribution of chiral inversion of NAP and the biodegradation of NAP to DM-NAP in the STPs located in the Tama River system was predominant compared with the same processes in the river.

CONCLUSIONS

The present study revealed 4 findings. First, (R)-NAP occurred in the effluents but was not detected in the influents of the STPs located in the Tama River system. Second, under the laboratory degradation conditions with activated sludge, inversion of (S)-NAP to (R)-NAP was observed within 24 h. Third, in the river die-away experiment, the inversion rate and the concentrations of (S)-NAP to (R)-NAP were much less than those of the STPs. Fourth, chiral inversion of (S)-NAP to (R)-NAP was not observed during the photodegradation experiment. Therefore, (R)-NAP in river water might indicate the inflow of STP effluent if the drug is used around the river basin.

SUPPLEMENTAL DATA

Figures S1–S2. (107 KB DOC).

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