Nakada *et al.*, 2008; Zhong *et al.*, 2010; Gómez *et al.*, 2009; Cuderman and Heath, 2007; Yoon *et al.*, 2010; Sapkota *et al.*, 2007), including those that our research group measured in the areas without sewage service coverage in Tokushima, Kyoto, and Saitama, Japan (Kimura *et al.*, 2011). These MEC values are summarized and listed in Table 2.

The assessment factors of 100 and 10 were used for the risk assessment because both the acute and (sub-)chronic data are available for three aquatic organisms as recommended by the OECD (1992) using the following equations,

$$\text{PNEC (acute)} = (EC_{50} \text{ or } LC_{50})/100 \quad (1)$$

$$\text{PNEC (chronic)} = (\text{NOEC})/10 \quad (2)$$

where PNEC is the predicted no effect concentration. The MEC/PNEC (predicted no effect concentration) ratios were determined for all the monitoring data for three aquatic organisms separately for both acute and (sub-)chronic data for comparison.

RESULTS

The results of acute toxicity tests are summarized in Table 3 while the detailed toxic data (e.g. concentration–inhibition curves) are presented in the Supporting Information. As can be seen from Table 3, the algal toxicity was the strongest for TCS (EC_{50} of 5.1 $\mu g\,l^{-1}$) among the selected antimicrobial agents, followed by TCC. For the other selected antimicrobials, the EC_{50} values ranged from 7400 to 130 000 $\mu g\,l^{-1}$, and these values were at least three orders of magnitude higher than TCS. As for the EC_{50} values of *Daphnia* and fish (Table 3), the strongest toxicity was found for TCC (approximately 10 and 85 $\mu g\,l^{-1}$ for *Daphnia* and fish, respectively) and this value was 1–3 orders of magnitude lower than the values for the other compounds. Resorcinol exerted much stronger toxicity for *Daphnia* compared with algae and fish. EC_{50} values of phenoxyethanol were extremely high, at least 96 $mg\,l^{-1}$.

The results of sub-chronic toxicity tests are summarized in Table 4 while the detailed toxic data are presented in Supporting Information as with the acute data. As can be seen from Table 4, the NOEC values for TCS and TCC were relatively low and ranged from 0.53 to 30 $\mu g\,l^{-1}$, while those for the other antimicrobials ranged from 170 to 130 000 $\mu g\,l^{-1}$. The NOEC values of *p*-thymol were 2–3 orders of magnitude higher than those for TCC and/or TCS. Phenoxyethanol was the most weakly toxic among the selected antimicrobial agents, and not significantly toxic at the highest concentration for algae (NOEC of 130 000 $\mu g\,l^{-1}$) and fish (NOEC of 52 000 $\mu g\,l^{-1}$), but was slightly toxic for *Daphnia* reproduction (NOEC of 5800 $\mu g\,l^{-1}$). Again, the toxicity of resorcinol was found to be much stronger for *Daphnia* compared with algae.

The results of the preliminary ecological risk assessment for the selected antimicrobials using acute toxicity tests with equation (1) are presented in Fig. 2. As can be seen from Fig. 2, the algal MEC/PNEC ratios of TCS and TCC for several monitoring data were >1, and some of them exceeded 10 for TCS and TCC. The maximum MEC/PNEC ratio of TCC for *Daphnia* and fish was >10 and 1, respectively, while those of TCS were between 0.1 and 1. For the other three selected antimicrobial agents, all of the ratios determined for the detected concentrations were below 0.1 except for the maximum MEC/PNEC ratio of resorcinol, which was >0.1 for *Daphnia*, similar to the risk of TCS for this species.

The results of the preliminary ecological risk assessment based on the (sub-)chronic toxicity tests using equation 2 are shown in Fig. 3. Similar to the acute results (Fig. 2), the algal MEC/PNEC ratios of TCS and TCC for several monitoring data were >1, and some of them exceeded 10 for both compounds. Whereas some MEC/PNEC ratios of TCS and TCC were >0.1 for the survival (or hatching) of zebrafish larvae, those of TCC were >1 for *Ceriodaphnia* reproduction. For the other antimicrobials, the maximum MEC/PNEC value of resorcinol and phenoxyethanol for *Daphnia* was between 0.01 and 0.1, but the other MEC/PNEC values were all below 0.01. Since the results of the same algal growth inhibition tests are used for acute (EC_{50} values) and chronic (NOEC) toxicity, the acute (Table 2) and chronic (Table 3) results are within a factor of 10 for algae. For example, phenoxyethanol was not significantly toxic at the highest concentration of 130 000 $\mu g\,l^{-1}$, so that the MEC/PNEC ratio in chronic test became 10 times lower than in the acute test.

DISCUSSION

Comparing the toxicities for the three aquatic organisms, that for *Daphnia* was the strongest for triclocarban, resorcinol and *p*-thymol. The toxicity for fish was the weakest for most of the selected compounds except for triclosan, which is most strongly toxic to algae and similarly toxic for *Daphnia* and fish.

Ecotoxicity data obtained for TCS and TCC in the present study are mostly comparable to those obtained by other researchers (and organizations), except for the *Daphnia* chronic toxicity and fish chronic data. The *Daphnia* NOEC value for TCS obtained by the Ministry of Environment Japan (2009) was extremely low (0.34 $\mu g\,l^{-1}$), which may be due to the enhancement of the toxicity by the use of surfactant-like solvent. Oliveira *et al.* (2009) observed that the NOEC for the mortality of zebrafish larvae was 300 $\mu g\,l^{-1}$ in 6 days and was much lower than our values. We judged the mortality in 9 days and our results showed similar mortality in 6 days (data not shown). Comparable data are unavailable for phenoxyethanol and *p*-thymol.

The MEC/PNEC data obtained in this study for TCS were similar to most results reported by the other researchers and organizations. For example, Ministry of Environment Japan (2009) estimated the maximum MEC/PNEC value of TCS as 1.3 while Brausch and Rand (2011) determined the hazard quotient of TCS as approximately 18 (both are comparable to our results) and concluded that further investigation is necessary. In contrast, Lyndall *et al.* (2010) conducted probabilistic ecological risk assessment for TCS and found that 95% of surface water MEC was below the 5% possible hazardous concentration (HC_5) using the species-sensitivity distribution analysis, which suggests limited ecological risk, even though hazardous effects are possible for some species such as Monostyla/Philodina. HC_5 has been widely used to cover the 95% confidence level of the species-sensitivity distribution model and is often used for ecological risk assessment (Newman *et al.*, 2000). The MEC/PNEC value reported by TCC Consortium (2002) was 0.34 and <1, their proposed trigger level of further detailed investigation. However, the maximum MEC used in the report was 50 $ng\,l^{-1}$ and the concentration could be underestimated, especially for an urban area without sewage surface coverage (Table 2). We conservatively used the MEC for TCC as high as on the order of thousands of nanograms per liter compared with those obtained by other

Table 2. List of measured environmental concentrations (MECs) used for the ecological risk assessment

Compounds			Year	Range (ng l^{-1})	Median (ng l^{-1})	Mean (ng l^{-1})	Description of the sampling site	Reference
TCS	Switzerland	River	2001	11–74		42.5	Rivers flow into a lake (serving population of 107 000)	Lindström *et al.*(2002)
TCS	Japan	River	2008	<0.6–59.1			Nationwide survey of river water	Nakada *et al.* (2008)
TCS	USA	River	2005	60 80			1.1 and 3.8 km downstream of sewage outfall	Coogan *et al.* (2007)
TCS	Slovenia	River	2004	68			Recreational area	Cuderman and Heath (2007)
TCS	Spain	River	2007	<12–285	14	53	1 km downstream from WWTPs	Kantiani *et al.* (2008)
TCS	UK	River	2006, 2007	<5–95		48	3.5–10.5 km downstream of WWTPs	Kasprzyk-Hordern *et al.* (2008)
TCS	Japan	River	2006	11–31			Downstream of the outfall of domestic wastewater	Nishi *et al.* (2008)
TCS	China	River	2005, 2006	35–1023			Rivers receiving direct discharge of WWTP effluents	Peng *et al.* (2008)
TCS	Spain	River	2008	24–157		68	Rivers receiving urban and industrial wastewaters	Gómez *et al.* (2009)
TCS	Korea	River	2008	1–29		17	Rivers (flow rate: <90–2100 $m^3 s^{-1}$)	Yoon *et al.* (2010)
				16–82		55	Urban creeks (flow rate: <7–24 $m^3 s^{-1}$)	
TCS	China	River	2007, 2008	<4.1–26.2, 6.5–31.1, 90.2–478	11.9, 16.2, 238	13.7, 16.8, 242	Urban stream (Flow rate: 17.3–156 $m^3 s^{-1}$ 175–1120 $m^3 s^{-1}$ 3.5–5.7 $m^3 s^{-1}$)	Zhao *et al.* (2010)
TCS	Japan	River	2010, 2011	<2–177	41.3		Urban streams and rivers with sewage service coverage ranges from 0 to 100%	Kimura *et al.* (2011)
TCC	USA	River	2002, 2003	25–5600	81	985	Contaminated by raw sewage leaked from sewage pipes	Halden and Paull (2004)
TCC	USA	River	2005	80 160			1.1 and 3.8 km downstream of sewage outfall	Coogan *et al.* (2007)
TCC	USA	River	2004	0.45–45, 2–250		12 84	Upstream of a WWTP Downstream of a WWTP	Sapkota *et al.* (2007)
TCC	China	River	2007–2010	<3.9–13.9, 4.5–46.2, 68.8–338	6, 17.1, 145	7.4, 19.9, 158	Urban stream (flow rate: 17.3–156 $m^3 s^{-1}$ 175–1120 $m^3 s^{-1}$ 3.5–5.7 $m^3 s^{-1}$	Zhao *et al.* (2010)
Resorcinol	China	Lake	2008, 2009	n.d. to 53.1		8.5	Third largest freshwater lake in China	Zhong *et al.* (2010)
Resorcinol	Japan	River	2010, 2011	1.2–1150	20.8		Urban streams and rivers with sewage service coverage ranges from 0 to 100%	Kimura *et al.* (2011)
p-Thymol	Japan	River	2010, 2011	<1.1–715	95.6		Urban streams and rivers with sewage service coverage ranges from 0 to 100%	Kimura *et al.* (2011)
Phenoxyethanol	Japan	River	2010, 2011	<0.9–14000	139		Urban streams and rivers with sewage service coverage ranges from 0 to 100%	Kimura *et al.* (2011)

WWTP, Wastewater treatment plant.

Table 3. Results of the acute toxicity tests.

	Green algae (*Pseudokirchneriella ubcapitata*)	*Daphnia* (*Daphnia magna*)	Fish (*Oryzias latipes*)
($\mu g\,l^{-1}$)	72 h EC_{50}	48 h EC_{50}	96 h LC_{50}
TCS	5.1 (3.8–8.4)	180 (150–230)	210 (130–340)
TCC	29 (25–35)	10 (7.1–12)	85 (45–100)
Resorcinol	110 000 (110 000–120 000)	530 (350–740)	100 000<
p-Thymol	7400 (6800–8300)	5700 (3000–7100)	7600 (6600–8700)
Phenoxyethanol	130 000<	96 000<	123 000<

95% Confidence intervals are presented within parentheses.

Table 4. Results of the chronic toxicity tests

	Green Algae (*Pseudokirchneriella subcapitata*)	*Daphnia* (*Ceriodaphnia dubia*)	Fish (*Danio rerio*)
($\mu g\,l^{-1}$)	72 h NOEC	8 day NOEC	9 day NOEC
TCS	0.53	30	26
TCC	5.7	1.9	24
Resorcinol	67 000	170	—
p-Thymol	2500	1070	1500
Phenoxyethanol	130 000<	5800	52 000<

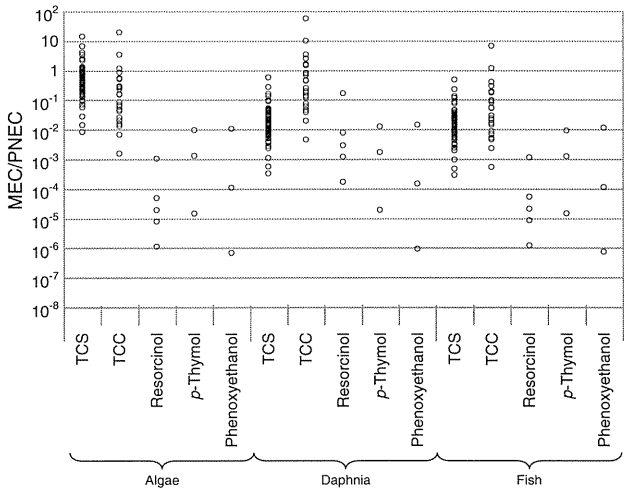

Figure 2. Results of the ecological risk assessment based on the acute toxicity data.

researchers to find the maximum MEC/PNEC ratio >10 in the present study.

According to the IPCS's Concise International Chemical Assessment Documents (WHO, 2006), the PEC/PNEC ratio of resocinol only in the receiving water of effluent from a rubber factory or hair dye treatment exceeded 1, but that for a hair

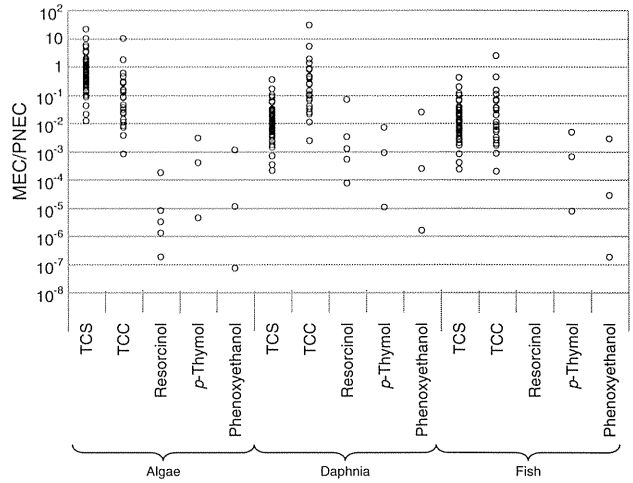

Figure 3. Results of the ecological risk assessment based on the (sub-) chronic toxicity data.

dye factory and pharmaceutical factories were <1 and the ecological risk was limited. The probability of ecological effect quotient over 0.3 was reported to be 0% using the monitoring data in Taihu Lake, China, which suggests that the ecological risk of resorcinol is limited and agrees with our results.

The maximum MEC/PNEC ratio for TCC obtained in this study was >10 using the data obtained by Halden and Paull (2004), who found the maximum concentration of TCC to be 5600 $ng\,l^{-1}$ in an urban stream. Since all sampling sites were located upstream of wastewater treatment plant inputs, leaked raw sewage might have caused the hot spot. In most of the rivers and streams, the probability of TCC concentration could not become such high concentration. The MEC/PNEC ratios >1 for TCS and TCC were in rivers with low flow rate or drought in China or the USA. These results suggest concern for potential adverse effects of TCS for algae and TCC for *Daphnia*, especially in urban areas contaminated by untreated household wastewater owing to lack of sewage service coverage, such as in Tokushima, Japan (Tamura *et al.*, 2010)

Kimura *et al.* (2011) reported maximum concentrations over 10 $\mu g\,l^{-1}$, hundreds of nanograms per liter, and on the order of 10 $ng\,l^{-1}$ for phenoxyethanol, *p*-thymol and resorcinol, respectively (Table 2). However, the maximum MEC/PNEC values of these three antimicrobials were far below 0.1, the trigger level for further investigation, which suggests no urgent risk.

Overall, the individual MEC/PNEC values of three antimicrobials – phenoxyethanol, *p*-thymol and resorcinol – are mostly <0.1 and the urgent risk for the aquatic organisms is limited, although the physiological effects other than survival, growth and reproduction, such as endocrine disruption, need to be examined in the future as well as the toxicity of mixtures of compounds. In contrast, the individual MEC/PNEC values of TCS and TCC are far above 1 for some hot spots. As presented above, TCS and TCC are frequently used together and could have additive effects owing to their similar physiological effects. We previously evaluated the ecological risk of a class of preservatives, parabens; the MEC/PNEC ratios were <0.1 and the risk for the aquatic organisms might not be a significant concern even if they are combined (Yamamoto *et al.*, 2011). However, the aquatic toxicity of TCC and TCS is 2–5 orders of magnitude stronger than that of parabens. The ecological risk of the combination of antimicrobial agents, especially TCS and TCC, for aquatic organisms is at the level of significant concern and further research is absolutely necessary.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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REFERENCES

Brausch JM, Rand GM. 2011. A review of personal care products in the aquatic environment: environmental concentrations and toxicity. *Chemosphere* **82**: 1518–1532.

Coogan MA, Edziyie RE, La Point TW, Venables BJ. 2007. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater treatment plant receiving stream. *Chemosphere* **67**: 1911–1918.

Cuderman P, Heath E. 2007. Determination of UV filters and antimicrobial agents in environmental water samples. *Anal. Bioanal. Chem.* **387**: 1343–1350.

Dussault B, Balakrishnan VK, Sverko E, Solomon KR, Sibley PK. 2008. Toxicity of human pharmaceuticals and personal care products to benthic invertebrates. *Environ. Toxicol. Chem.* **27**(2): 425–432.

Environment Canada. 2007. Second Edition Biological Test Method: Test of Reproduction and Survival Using the Cladoceran Ceriodaphnia dubia. EPS 1/RM/21.

Gómez MJ, Gómez-Ramos MM, Agüera A, Mezcua M, Herrera S, Fernández-Alba AR. 2009. A new gas chromatography/mass spectrometry method for the simultaneous analysis of target and non-target organic contaminants in waters. *J. Chromatogr. A* **1216**: 4071–4082.

Halden RU, Paull DH. 2004. Analysis of triclocarban in aquatic samples by liquid chromatography electrospray ionization mass spectrometry. *Environ. Sci. Technol.* **38**: 4849–4855.

Ishibashi H, Matsumura N, Hirano M, Matsuoka M, Shiratsuchi H, Ishibashi Y, Takao Y, Arizono K. 2004. Effects of triclosan on the early life stages and reproduction of medaka *Oryzias latipes* and induction of hepatic vitellogenin. *Aquat. Toxicol.* **67**: 167–179.

Kantiani L, Farre M, Asperger D, Rubio F, Gonzalez S, Lopezdealda M, Petrovic M, Shelver W, Barcelo D. 2008. Triclosan and methyl-triclosan monitoring study in the northeast of Spain using a magnetic particle enzyme immunoassay and confirmatory analysis by gas chromatography–mass spectrometry. *J. Hydrol.* **361**(1–2): 1–9.

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Res.* **42**: 3498–3518.

Kimura K, Kameda K, Yamamoto H, Nakada N, Masunaga S. 2011. Abstracts for *20th Symposium on Environmental Chemistry*; 178–180 (in Japanese).

Lindström A, Buerge IJ, Poiger T, Bergqvist PA, Muller MD, Buser HR. 2002. Occurrence and environmental behavior of the bactericide triclosan in and its methyl derivative in surface waters and in wastewater. *Environ. Sci. Technol.* **36**: 2322–2329.

Lyndall J, Fuchsman P, Bock M, Barber T, Lauren D, Leigh K, Perruchon E, Capdeviella M. 2010. Probabilistic risk evaluation for triclosan in surface water, sediments, and aquatic biota tissues. *Integr. Environ. Assess. Manag.* **6**(3): 419–440.

McAvoy DC, Schatowitz B, Jacob M, Hauk A, Eckhoff WS. 2002. Measurement of triclosan in wastewater treatment systems. *Environ. Toxicol. Chem.* **21**: 1323–1329.

Ministry of Environment Japan. 2009. Environmental Risk Assessment of Chemical Pollutants. 7, Initial Ecological Risk assessment of Chemical Substance. [3] 5-Chloro-2-(2,4-dichlorophenoxy)phenol.

Nakada N, Tanishima T, Shinohara H, Kiri K, Takada H. 2006. Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. *Water Res.* **40**: 3297–3303.

Nakada N, Kiri K, Shinohara H, Harada A, Kuroda K, Takizawa S, Takada H. 2008. Evaluation of pharmaceuticals and personal care products as water-soluble molecular markers of sewage. *Environ. Sci. Technol.* **42** (17): 6347–6353.

Newman MC, Ownby DR, Mézin LCA, Powell DC, Christensen TRL, Lerberg SB, Anderson B. 2000. Applying species-sensitivity distributions in ecological risk assessment: assumptions of distribution type and sufficient numbers of species. *Environ. Toxicol. Chem.* **19**: 508–515.

Nishi I, Kawakami T, Onodera S. 2008. Monitoring of triclosan in the surface water of the Tone Canal, Japan. *Bull. Environ. Contam. Toxicol.* **80**: 163–166.

Nishihara T, Korai H. 2005. Development and Prospect of Biocontrol Agents. CMC.

OECD. 1992. Report on the OECD Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment. Environment Monograph No. 59. Available from: http://www.oecd.org/dataoecd/30/48/34528236.pdf (accessed 17 March 2012).

OECD. 1992. Fish, acute toxicity test. OECD guidelines for testing of chemicals no. 203.

OECD. 1992. Fish, early-life stage toxicity test. OECD guidelines for testing of chemicals no. 210.

OECD. 1998. Fish, short-term toxicity test on embryo and sac-fry stages. OECD guidelines for testing of chemicals no. 212.

OECD. 2002. *Manuals for Investigation of HPV chemicals*, Chapter 4, Initial assessment of data. Available from: http://www.oecd.org/dataoecd/6/14/2483645.pdf (accessed 29 March 2012).

OECD. 2004. *Daphnia* sp., acute immobilization test and reproduction test. OECD guidelines for testing of chemicals no. 202.

OECD. 2006. Alga, growth test. OECD guidelines for testing of chemicals no. 201.

OECD. 2008. *Daphnia magna* reproduction test. OECD guidelines for testing of chemicals no. 211.

Okuda T, Kobayashi Y, Nagao R, Yamashita N, Tanaka H, Tanaka S, Matsukawa H, Tanabe K. 2008. Removal efficiency of 66 pharmaceuticals during wastewater treatment process in Japan. *Water Sci. Technol.* **57**(1): 65–71.

Oliveira R, Domingues I, Koppe Grisolia C, Soares AMVM. 2009. Effects of triclosan on zebrafish early-life stages and adults. *Environ. Sci. Pollut. Res. Int.* **16**(6): 679–688.

Orvos DR, Versteeg DJ, Inauen J, Capdevielle M, Rothenstein A, Cunningham V. 2002. Aquatic toxicity of triclosan. *Environ. Toxicol. Chem.* **21**: 1338–1349.

Peng XZ, Yu YJ, Tang CM, Tan JH, Huang QX, Wang ZD. 2008. Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China. *Sci. Total Environ.* **397**: 158–166.

Perencevich EN, Wong MT, Harris AD. 2001. National and regional assessment of the antibacterial soap market: a step toward determining the impact of prevalent antibacterial soaps. Am. *J. Infect. Control.* **29**: 281–283.

Sapkota A, Heidler J, Halden RU. 2007. Detection of triclocarban and two co-contaminating chlorocarbanilides in US aquatic environments using isotope dilution liquid chromatography tandem mass spectrometry. *Environ. Res.* **103**(1): 21–29.

Tamura I, Nitta K, Hirata Y, Yamamoto A, Sekizawa J, Yamamoto H. 2010. Fate and transport of pollutants originated from household effluent in unsewered area and construction of a simple numerical model to predict their aquatic concentrations. *J. Environ. Chem.* **20**: 339–349 (in Japanese with English abstract).

Tatarazako N, Ishibashi H, Teshima K, Kishi K, Arizono K. 2004. Effects of triclosan on arious aquatic organsims. *Environ. Sci.* **11**: 133–140.

TCC Consortium. 2002. High Production Volume (HPV) Chemical Challenge Program Data Availability and Screening Level Assessment for Triclocarban. CAS no. 101-20-2. Report No. 201-14186A; 1–35.

US EPA. 2002. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, 4th edn. EPA-821-R-02-013.

US EPA. 2011. Ecotox Database. Available from: http://www.epa.gov/ecotox. (accessed 22 July 2012).

WHO. 2006. Concise International Chemical Assessment Document 71 Resorcinol. Available from: http://www.who.int/ipcs/publications/cicad/cicad71.pdf (accessed 17 March 2012).

Yamamoto H, Watanabe M, Hirata Y, Nakamura Y, Nakamura Y, Kitani C, Sekizawa J, Uchida M, Nakamura H, Kagami Y, Koshio M, Hirai N, Tatarazako N. 2007. Preliminary ecological risk assessment of butylparaben and benzylparaben – 1. Removal efficiency in wastewater treatment, acute/chronic toxicity for aquatic organisms, and effects on medaka gene expression. *Environ. Sci.* **14**(suppl.): 73–87.

Yamamoto H, Tamura I, Hirata Y, Kato J, Kagota K, Katsuki S, Yamamoto A, Kagami Y, Tatarazako N. 2011. Aquatic toxicity and ecological risk assessment of seven parabens. *Sci. Total Environ.* **410–411**: 102–111.

Yoon Y, Ryu J, Oh J, Choi BG, Snyder SA. 2010. Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). *Sci. Total Environ.* **408**: 636–643.

Yoshioka Y. 2001. Ecotox-Statics ver.2.6d. *Japan J. Environ. Toxicol.* **4**(2): 113. Available from: http://www.intio.or.jp/jset/ecotox.htm (accessed 28 March 2012).

Zhao JL, Ying GG, Liu YS, Chen F, Yang JF, Wang L. 2010. Occurrence and risks of triclosan and triclocarban in the Pearl River system, South China: from source to the receiving environment. *J. Hazard. Mater.* **179**: 215–222.

Zhong W, Wang D, Xu X, Luo Q, Wang B, Shan X, Wang Z. 2010. Screening level ecological risk assessment for phenols in surface water of the Taihu Lake. *Chemosphere* **80**(9): 998–1005.

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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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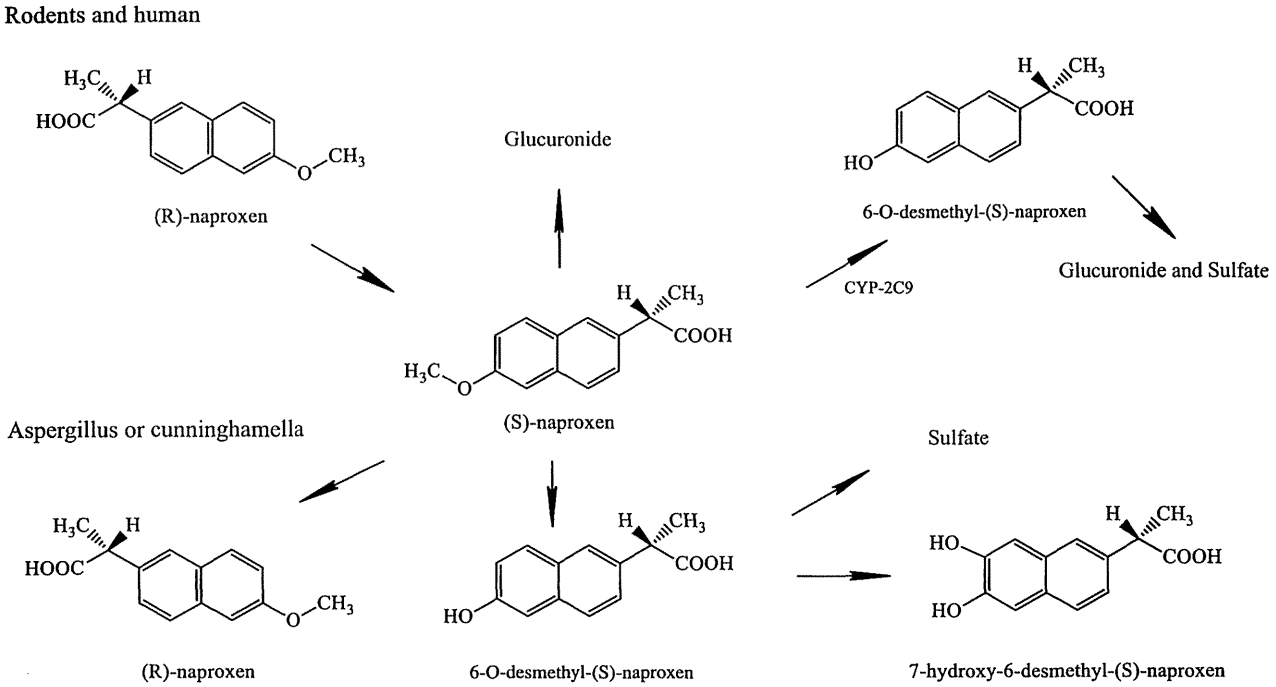

Figure 1. Metabolic pathways of (S)-naproxen in mammals and bacteria.

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

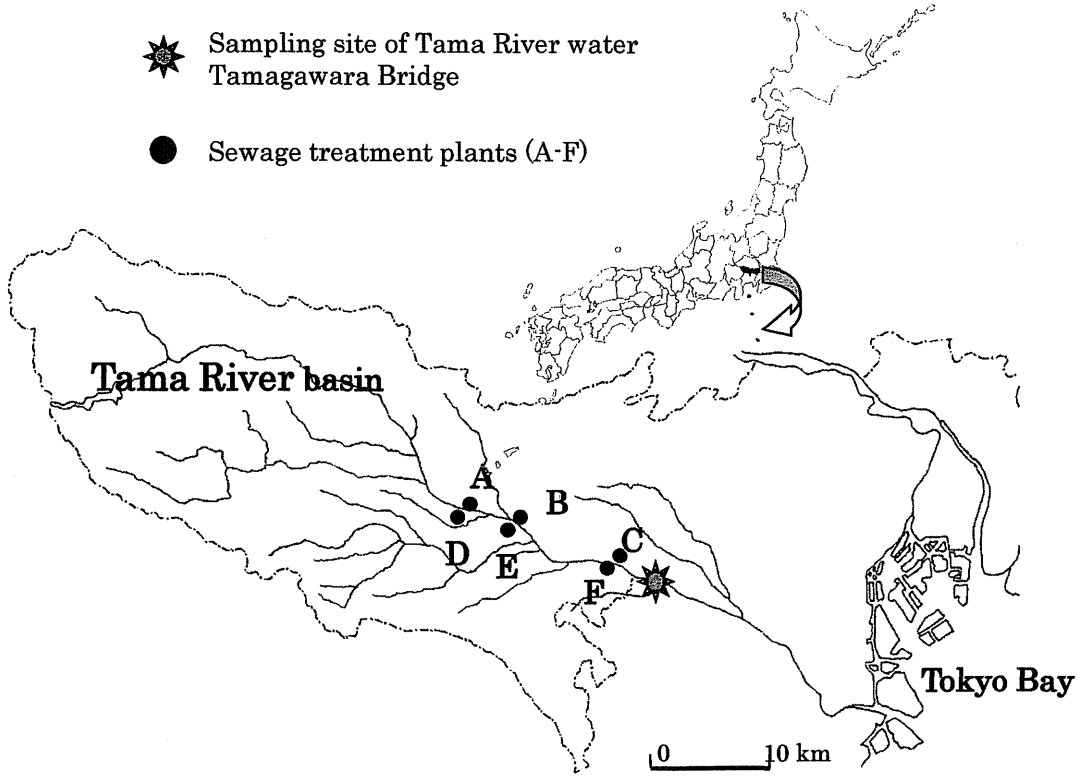

Figure 2. Sampling locations of sewage treatment plants and river water in Tama River basin. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

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

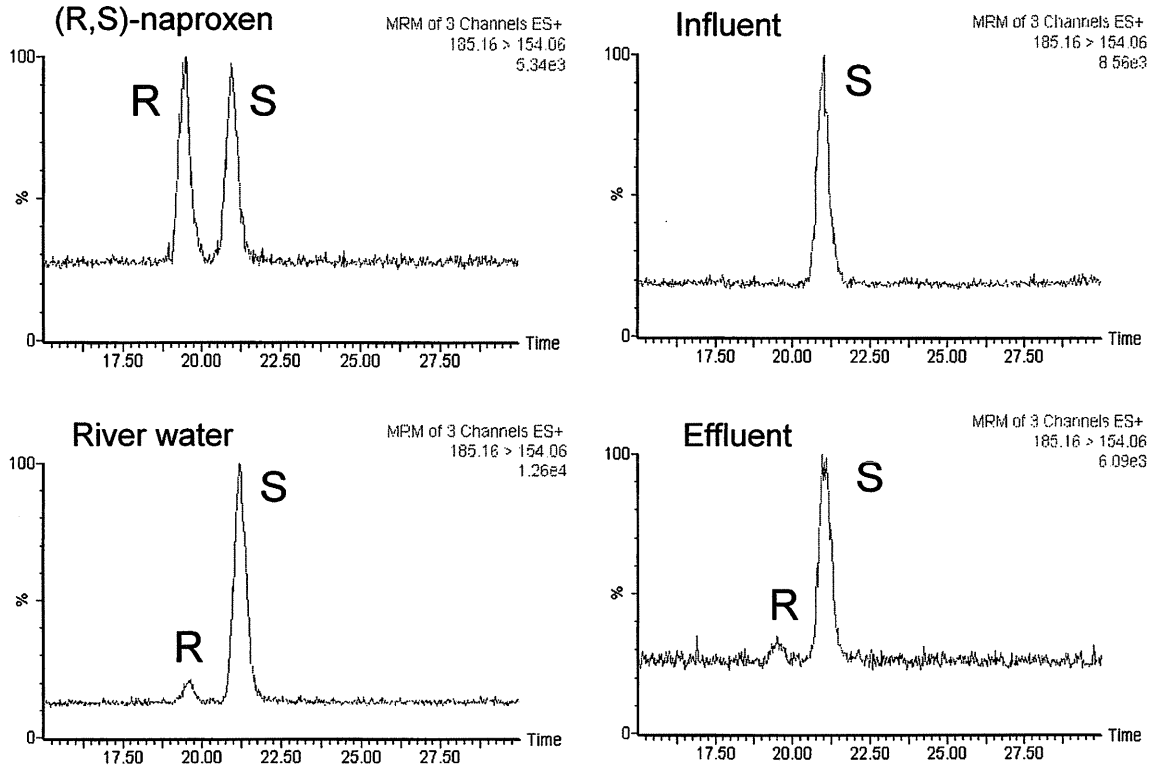

Figure 3. Liquid chromatography–mass spectrometry chromatograms of naproxen in water samples with chiral separation column. S = (S)-naproxen; R = (R)-naproxen. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

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

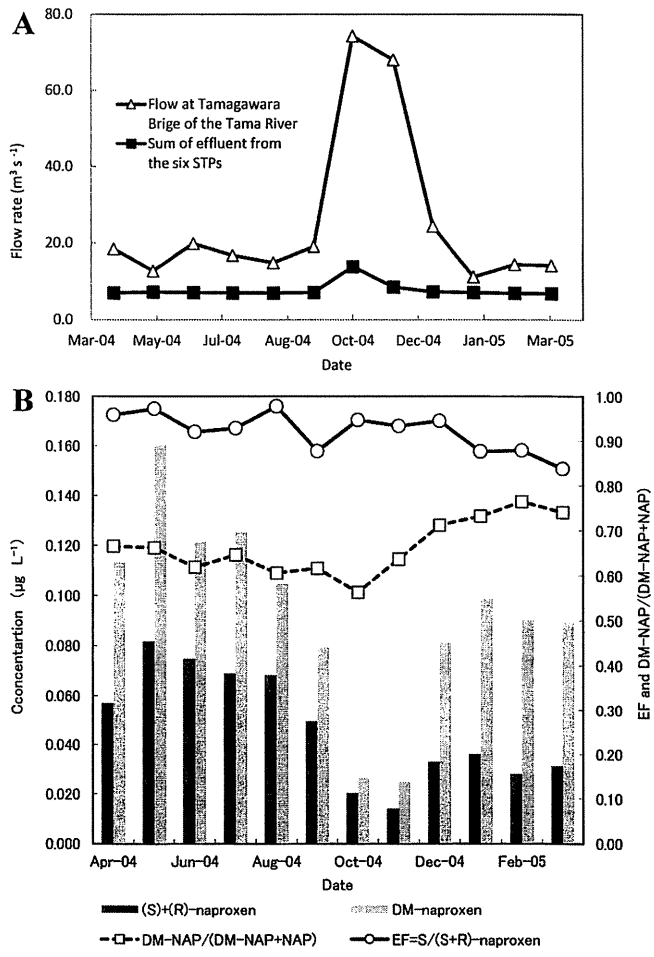

Figure 4. Seasonal changes of naproxen, 6-*O*-desmethyl-naproxen (DM-NAP), and enantiomaric fraction (EF) of naproxen at the Tamagawara Bridge in the Tama River. (**A**) Flows of river and the sum of effluent of the 6 sewage treatment plants (STPs); (**B**) concentration of naproxen and DM-NAP, and the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

1. Kallenborn R, Huhnerfuss H. 2001. *Chiral Environmental Pollutants*. Springer-Verlag, Berlin, Germany.
2. Garrison AW. 2006. Probing the enantioselectivity of chiral pesticides. *Environ Sci Technol* 40:16–23.
3. Janak K, Covac A, Voorspoels S, Becher G. 2005. Hexabromocyclododecane in marine species from Western Scheldt Estuary: Diastereoisomer- and enantiomer-specific accumulation. *Environ Sci Technol* 39:1987–1994.
4. Daughton C, Ternes TA. 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environ Health Perspect* 107:907–938.
5. Kolpin DW, Furlong ET, Meyer MT, Thruman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environ Sci Technol* 36:1202–1211.
6. Renner R. 1996. Chiral compounds show promise as environmental tracers. *Environ Sci Technol* 30:16A–17A.
7. Zipper C, Suter MJF, Haderein SB, Gruhl M, Kohler HPE. 1998. Changes in the enantiomeric ratio of (R)- to (S)-mecoprop indicate in situ biodegradation of this chiral herbicide in a polluted aquifer. *Environ Sci Technol* 32:2070–2076.
8. Jones WJ, Mazur CS, Kenneke JF, Garrison AW. 2007. Enantioselective microbial transformation of the phenylpyrazole insecticide Fipronil in anoxic sediments. *Environ Sci Technol* 41:8301–8307.
9. Buser HR, Poiger T, Muller MD. 1999. Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface water and in wastewater. *Environ Sci Technol* 33:2529–2535.
10. Fono L, Sedlak D. 2005. Use of the chiral pharmaceutical propranolol to identify sewage discharge into surface waters. *Environ Sci Technol* 39:9244–9252.
11. Tomlinson RV, Ringold HJ, Qureshi MC, Forchielli E. 1972. Relationship between inhibition of prostaglandin synthesis and drug efficacy: Support for the current theory on mode of action of aspirin-like drugs. *Biochem Biophys Res Commun* 46:552–559.
12. Hutt AJ, Caldwell J. 1983. The metabolic chiral inversion of 2-arylpropanoic acids – A novel route with pharmacological consequences. *J Pharm Pharmacol* 35:693–704.
13. Strong M. 1999. FDA policy and regulation of stereoisomers: Paradigm shift and the future of safer, more effective drugs. *Food Drug Law J* 54:463–487.
14. Harrison IT, Lewis B, Nelson P, Rooks W, Roszkowski A, Tomolonis A, Fried JH. 1970. Nonsteroidal antiinflammatory agents. I. 6-Substituted 2-naphthylacetc acid. *J Med Chem* 13:203–205.
15. Sugawara Y, Fujiwara M, Miura Y, Hayashida K, Takahashi T. 1978. Studies of the fate of NAP. II. Metabolic fate in various animals and man. *Chem Pharm Bull* 26:3312–3321.
16. Goto J, Goto N, Nambara T. 1982. Separation and determination of NAP enantiomers in serum by high-performance liquid chromatography. *J Chromatogr* 239:559–564.
17. Anderson JV, Hansen SH. 1992. Simultaneous determination of (R)- and (S)-NAP and (R)- and (S)-6-*O*-desmethylNAP by high-performance liquid chromatography on a chiral-AGP column. *J Chromatogr* 577:362–365.
18. Packer JL, Wener JJ, Latch DE, McNeil K, Arnold WA. 2003. Photochemical fate of pharmaceuticals in the environment: NAP, diclofenac, clofibric acid, and ibuprofen. *Aquat Sci* 65:342–351.
19. Jones OAH, Voulvoulis N, Lester JN. 2002. Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. *Water Res* 36:5013–5022.
20. Vieno NM, Harkki H, Tuhkanen T, Kronberg L. 2007. Occurrence of pharmaceuticals in river water and their elimination in a pilot-scale drinking water treatment plant. *Environ Sci Technol* 41:5077–5084.
21. Zhang S, Zhang Q, Darisaw S, Ehie O, Wang G. 2007. Simultaneous quantification of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pharmaceuticals and personal care products (PPCPs) in Mississippi river water, in New Orleans, Louisiana, USA. *Chemosphere* 66:1057–1069.
22. Tixier C, Singer HP, Oellers S, Muller SR. 2003. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen and NAP in surface waters. *Environ Sci Technol* 37:1061–1068.
23. Ternes TA. 1998. Occurrence of drugs in German sewage treatment plants and rivers. *Water Res* 32:3245–3260.
24. Nakada N, Tanishima T, Shinohara H, Kiri K, Takada H. 2006. Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. *Water Res* 40:3297–3303.
25. Mark JB, Rebecca A, Trenholm BJ, Vanderford JC, Holady DS, Shane AS. 2009. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. *Environ Sci Technol* 43:597–603.
26. Quintana JB, Weiss S, Reemtsma T. 2005. Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. *Water Res* 39:2654–2664.
27. He A, Rosazza JPN. 2003. Microbial transformations of S-NAP by *Aspergillus niger* ATCC 9142. *Pharmazie* 58:420–422.
28. Da-Fang Z, Lu S, Lei L, Hai-Hua H. 2003. Microbial transformation of NAP by Cunninghamella species. *Acta Pharmacol Sin* 24:442–447.
29. Organisation for Economic Development and Co-Operation. 1992. Test No. 301: Ready biodegradability—CO_2 in sealed vessels (headspace test). *OECD Guidelines for the Testing of Chemicals*. Paris France.
30. Kimura K, Hara H, Watanabe Y. 2007. Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors. *Environ Sci Technol* 41:3708–3714.
31. Nakada N, Komori K, Suzuki Y. 2005. Occurrence and fate of anti-inflammatory drugs in wastewater treatment plants in Japan. *Environ Sci* 12:359–369.
32. Carballa M, Omil F, Lema JM, Llompart M, Garcia-Jares C, Rodriguez I, Gomez M, Ternes T. 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Res* 38:2918–2926.
33. Qurie M, Khamis M, Malek F, Nlr S, BufoJihad Abbadi SA, Scrano SA, Karaman R. 2014. Stability and removal of naproxen and its metabolite by advanced membrane wastewater treatment plant and micelle-Clay complex. *Clean Soil Air Water* 42:594–600.
34. Vieno NM, Tuhkanen T, Kronberg L. 2005. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. *Environ Sci Technol* 39:8220–8226.
35. Lindqvist N, Tuhkanen T, Kronberg L. 2005. Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. *Water Res* 39:2219–2228.

36. Bosca F, Marin ML, Miranda MA. 2001. Photoreactivity of the nonsteroidal anti-inflammatory 2-arylpropipnic acids with photosensitizing side effects. *Photochem Photobiol* 74:637–655.
37. Lin AYC, Reinhard M. 2005. Photodegradation of common environmental pharmaceuticals and estrogens in river water. *Environ Toxicol Chem* 24:1303–1309.
38. Felis E, Marciocha D, Surmacz-Gorska J, Miksch K. 2007. Photochemical degradation of NAP in the aquatic environment. *Water Sci Technol* 55:281–286.
39. Nakada N, Kiri K, Shinohara H, Harada A, Kuroda K, Takizawa S, Takada H. 2008. Evaluation of pharmaceuticals and personal care products as water-soluble molecular markers of sewage. *Environ Sci Technol* 42:6347–6353.