Original Article

Preliminary Ecological Risk Assessment of 10 PPCPs and their Contributions to the Toxicity of Concentrated Surface Water on an Algal Species in the Middle Basin of Tama River

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ABSTRACT

Investigating the ecological risk of pharmaceuticals and personal care products (PPCPs) using recent data of the occurrence and contributions of PPCPs with potential ecological risks to ecotoxicological effects of chemicals on aquatic organisms in water environments is required. In the present study, preliminary ecological risk assessments of 10 PPCPs were conducted in the middle stream of Tama River and its tributaries using the hazard quotient. An algal growth inhibition test of concentrated river water samples using *Pseudokirchneriella subcapitata* was also conducted to evaluate the toxicity of each sample and the contributions of PPCPs to toxicity of the water sample to the algal species. The result of preliminary ecological risk assessment showed that the hazard quotients of five PPCPs were over one at some sampling locations. The relatively weak toxicities on *P. subcapitata* were observed in all the concentrated water samples. The relatively large contribution to relatively weak toxicity of the concentrated water samples on the growth of *P. subcapitata* was found for three of five PPCPs that show potential ecological risks in the middle basin of Tama River.

Keywords: bioassay, environmental risk, hazard quotient

INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) such as fragrances, stimulants, analgesics, antibiotics and hormones have been detected in aquatic environments, and they are recognized as emerging contaminants in environmental science in terms of adverse effects on aquatic organisms [1,2]. Most PPCPs could potentially have adverse effects on aquatic ecosystems due to their physiological properties [3]. The occurrence and fate of PPCPs have been reported in Japanese rivers [4–6] and treated wastewater [7,8]. Preliminary risk assessments of several PPCPs have been conducted [5,9,10]. As the occurrence of PPCPs can change temporally, ecological risks of PPCPs on aquatic organisms using recent data of the occurrence in Japanese rivers is required.

In addition to the preliminary ecological risk assessment using the hazard quotient (HQ), which is the ratio of predicted environmental concentration (PEC) to predicted no effect concentration (PNEC), investigating effects of PPCPs

with potential ecological risks on aquatic organisms in water environments is required. Ecotoxicity tests of a water sample are useful for estimating the ecological risks of chemicals contained in the water sample [11]. The toxicity of a water sample such as river water presents the toxicity of the chemicals contained in the river water sample. It is possible to estimate the contributions of PPCPs to the toxicity of a water sample by comparing the estimated toxicity of individual PPCP with the toxicity of the sample [12]. Investigating the contributions of PPCPs that present high potential ecological risks may provide important information for understanding the ecological risk of the PPCPs in Japanese rivers.

This study conducted initial ecological risk assessments of 10 PPCPs, whose ecological risks are a concern in Japanese rivers, in the middle stream of Tama River and its tributaries. In Tama River, PPCPs have been detected [4,13]. The contribution of each PPCP to the concentrated river-water toxicity to algal growth was estimated by comparing the concentrated water sample toxicity to algal growth with the

Table 1 Target compounds, CAS number, molecular mass, usage, pKa and log K_{ow} .

Compound	CAS number	Molecular mass (g/mol)	Usage	pKa	Log K_{ow} *
Azithromycin	83905-01-5	748.98	Antibiotic agent	8.74 ^{a)}	4.02
Bezafibrate	41859-67-0	361.82	Lipid lowering drug	3.6 ^{b)}	4.25
Caffeine	58-08-2	194.19	Psychoactive stimulant drug	14 ^{c)}	-0.07
Clarithromycin	81103-11-9	747.95	Antibiotic agent	8.99 ^{a)}	3.16
Crotamiton	483-63-6	203.28	Antipruritic agent	-	2.73
Ibuprofen	15687-27-1	206.28	Anti-inflammatory drug	4.41 ^{d)}	3.97
Ketoprofen	22071-15-4	254.28	Anti-inflammatory drug	4.23 ^{d)}	3.12
Levofloxacin	100986-85-4	361.37	Antibacterial agent	5.5 ^{e)}	-0.39
Sulfamethoxazole	723-46-6	253.28	Sulfonamide antibacterial agent	1.8-6.0 ^{f)}	0.89
Triclosan	3380-34-5	289.54	Antibacterial agent	8.14 ^{d)}	4.76

*log Kow values were derived from EPI Suite^{TM} ver. 4.11 [34].

a) McFarland *et al.* [35], b) Beausse [36], c) Nam *et al.* [37], d) Ozaki *et al.* [38], e) Yasojima *et al.* [39], f) Pavlović *et al.* [40]

toxicity of the PPCP on the basis of the measured concentration and chronic toxicity of PPCP to algal growth. Some reports suggest that algae are more sensitive to some PPCPs than *Daphnia* and fish species [14,15]. Therefore, an algal toxicity test was applied to investigate the toxicities of water samples and the contributions of PPCPs to the toxicity of a water sample were estimated.

MATERIALS AND METHODS

Target PPCPs

Ten PPCPs were selected in this study as shown in **Table 1**. The PPCPs were selected based on an annual report on statistics of production by the pharmaceutical industry in 2010 [16] and previous studies [17,18]. They are mass produced or have been reported to be detected in the water environment in Japan. The 4 of 9 target PPCPs other than triclosan were included in target PPCPs of simultaneous analysis developed by Konishi *et al.* [19].

Sampling location

Tama River is a major river in Yamanashi, Kanagawa and Tokyo prefectures in Kanto region, Japan. The river is 138 km long and covers a catchment area of about 1,240 km². Effluents from sewage treatment plants are discharged into the river along the middle reaches, in which the effluents comprise up to over 50% of the river water during low-water season.

The middle basin of Tama River in Tokyo metropolitan area was selected for this study (**Fig. 1**). Surface water was collected as grab samples at three points in the middle stream

of Tama River and four of its tributaries. The location of the sampling sites is shown in **Fig. 1**. The St. 1 is located upstream of the points where effluents from two sewage treatment plants are discharged. Almost all of the river water at St. c consists of final effluent from a sewage treatment plant. The Sts. 2, 3 and d are located downstream of the points where effluents from sewage treatment plants are discharged. Murata *et al.* [20] showed that concentrations of some PPCPs in Tama River were higher in winter than in summer due to greater water flow during summer and other factors such as greater photodegradation and biodegradation. Therefore, to conduct a conservative ecological risk assessment of the 10 PPCPs in this study, sampling was performed during the daytime on January 31 and February 16, 2012.

Preparation and analytical methods for PPCPs

One liter of each sample was collected into two bottles together with EDTA and ascorbic acid, with a concentration at 1 g/L to encourage the efficiency of extraction and to prevent oxidation and metal masking, respectively. The samples were immediately transported to the laboratory under cool conditions.

The simultaneous analytical method established by Konishi *et al.* [19] was adopted for the analysis of target PPCPs other than triclosan with minor modification. One-liter sample was filtered through a glass fiber filter (GF/F, pore size: 0.7 µm, Whatman, GE Healthcare, Little Chalfont, UK) and acidified at pH 4 by hydrochloric acid. After the addition of the internal standards (azithromycin-¹³C-d₃ (TLC PharmaChem, Vaughan, Canada), bezafibrate-d₆ (CDN Isotopes, Pointe-Claire, Canada), caffeine-d₉ (CDN Isotopes), clarithromycin-

Fig. 1 Sampling location in the middle basin of Tama River. Sts. 1-3 were in the mainstream of Tama River. Sts. a-d were in tributaries. Stars indicate sewage treatment plants.

cin- ^{13}C - d_3 (TLC PharmaChem), crotamiton- d_7 (Hayashi Pure Chemical Industries, Osaka, Japan), ibuprofen- d_3 (CDN Isotopes), ketoprofen- d_4 (CDN Isotopes), levofloxacin- ^{13}C - d_3 (TLC PharmaChem) and sulfamethoxazole- d_4 (Tokyo Chemical Industry, Tokyo, Japan)), the filtrate was extracted with an Oasis HLB Plus cartridge (containing 225 mg resin, Waters, Milford, MA, USA) at the rate of 10 mL/min. Before the extraction, the cartridges were conditioned with both methanol and dilute hydrochloric acid (pH 4). After the passage of the aliquots, the cartridge was washed by dilute hydrochloric acid, dewatered by centrifugation and purged by nitrogen gas, and then eluted with 6 mL methanol. The extract was dried under a gentle stream of nitrogen gas and then redissolved in 1 mL of acetonitrile/0.1% formic acid (7:93, v/v). After the centrifugation of the extract, supernatant was analyzed by liquid chromatograph (Agilent 1100, Agilent Technology, Santa Clara, CA, USA) equipped with a tandem quadrupole mass spectrometer (API4000, AB Sciex, Framingham, MA, USA) (LC-MS/MS) in both positive-ion (azithromycin, caffeine, clarithromycin, crotamiton, ketoprofen, levofloxacin and sulfamethoxazole) and negative-ion (bezafibrate and ibuprofen) modes. In the positive-ion mode, the analyses were separated with a Hypersil GOLD column (2.1×100 mm, $3 \mu\text{m}$, Thermo Fisher Scientific, Waltham, MA, USA); acetonitrile (0.1% formic acid) and water (0.1% formic acid) were used as the mobile phase. In negative-ion mode, a Hypersil GOLD column, acetonitrile and 1 mM aqueous ammonium acetate were used. The m/z values of the precursor and product ions monitored for the analysis of target PPCPs are listed in **Table 2**. The mean recovery rate of

a surrogate for each of the nine PPCPs analyzed by LC-MS/MS from water samples ranged from 34 to 144% (**Table 2**).

The method established by Nakada *et al.* [7] was adopted for the analysis of triclosan with minor modification. A one-liter sample was filtered through a glass fiber filter (GF/B, pore size: $1.0 \mu\text{m}$, Whatman). After 100 μL methanol solution containing 1 mg/L of triclosan $^{13}\text{C}_{12}$ (Cambridge Isotope Laboratories, Tewksbury, MA, USA) was added to the filtrate as a surrogate for triclosan, the filtrate was extracted with an Oasis HLB Plus cartridge (containing 225 mg resin, Waters) at the rate of 10 mL/min. Prior to the extraction, the Oasis HLB Plus cartridges were conditioned with methanol and MilliQ water. After the passage of the aliquots, the cartridge was washed with MilliQ water, dewatered by centrifugation and purged by nitrogen gas, and then eluted with 10 mL methanol. The eluent was evaporated just to dryness in a gentle stream of nitrogen gas. The residue was redissolved in 2.5 mL of hexane/dichloromethane (75:25; v/v) and then loaded onto a silica gel column (Sep-Pak Si, Waters; 690 mg silica weight) that had previously been washed with 20 mL of hexane. A further 2.5 mL of hexane/dichloromethane (75:25; v/v) was used to elute the sample. The compounds in the column were eluted with 5 mL of dichloromethane. The dichloromethane fraction in which triclosan was derivatized to acetates with pyridine and acetic anhydride. The acetates were extracted with hexane, evaporated to dryness, and redissolved in a solution of 300 μL of isooctane solution containing 100 $\mu\text{g/L}$ of *p*-terphenyl- d_{14} (Wako Pure Chemical, Osaka, Japan) as the internal standard to account for methodological analyte losses. Triclosan was quantified by

Table 2 Precursor and product ions for LC-MS/MS analysis of nine PPCPs, monitor ions for the GC-MS analysis of triclosan and mean recovery rate of surrogates from water samples.

Compound	Precursor ion (m/z)	Product ion (m/z)	Monitor ion (m/z)	Recovery rate of a surrogate from water samples (%)	
				Mean	SD
LC-MS/MS					
Azithromycin	749.6	83.2	-	38	25
Bezafibrate	360.1	274.1	-	34	5
Caffeine	195.1	138.1	-	144	11
Clarithromycin	748.6	158.3	-	57	19
Crotamiton	204.2	69.1	-	116	7
Ibuprofen	205.1	161.1	-	94	7
Ketoprofen	255.1	209.2	-	84	17
Levofloxacin	362.2	318.2	-	76	24
Sulfamethoxazole	254.1	156.1	-	40	17
GC-MS					
Triclosan	-	-	218, 288	106	9

gas chromatography employing a mass spectrometer (HP 5973 with HP 6890, Agilent Technology) (GC-MS) with an HP-5 MS (Agilent Technology) capillary column after appropriate preparation as fully described by Nakada *et al.* [7]. The *m/z* values of the monitor ions for the GC-MS analysis of triclosan are listed in **Table 2**. The mean recovery rate of the surrogate for triclosan from the water samples was 106% (**Table 2**).

The limit of detection of each pharmaceutical was defined as three times the standard deviation of five replicate analyses at the lowest concentration of a standard.

Preparation of samples for algal bioassay

Detecting the toxicity caused by micropollutants such as PPCPs in a river water sample and estimating values of the toxicity could be facilitated by conducting the algal growth inhibition test of the concentrated river water sample because bioassay in high concentrations of micropollutants could be conducted. In this study, river water samples concentrated by the solid-phase extraction (SPE) method were used for the algal growth inhibition test. Organic compounds were mainly concentrated because PPCPs constitute a group of organic compounds.

One liter of each sample was filtrated using a glass fiber filter (GF/B, Whatman), and then concentrated by the SPE method. The OASIS HLB (Waters) cartridge was washed with methanol and MilliQ water. A filtered water sample was supplied to the cartridge at a flow rate of 10 mL/min. The compounds retained on the cartridge were eluted with 10 mL

of methanol. The eluent was evaporated just to dryness in a gentle stream of nitrogen. The residue was redissolved in 100 μ L of dimethyl sulfoxide (DMSO). The SPE sample was preserved at 4°C prior to bioassay.

Algal growth inhibition test

The algal growth inhibition test, in which SPE samples were exposed to a green alga (*Pseudokirchneriella subcapitata*(NIES-35)) in 72 h, was conducted. The tests were carried out using 96-well microplates according to the method of Yamashita *et al.* [21]. For exposure of SPE samples, the dilution series was prepared by serial 1:1 dilution with culture medium up to 10 steps. The concentration factors for the SPE samples were 0.1, 0.2, 0.4, 0.8, 1.6, 3.1, 6.3, 13, 25 and 50. The volume of each sample was 240 μ L. The concentration of DMSO in each sample was 0.5%. The solvent control was prepared. Five wells for each of the dilution series and five wells for the solvent control were prepared. After the test, the no observed effect concentration (NOEC) value for each concentrated river sample was estimated. The absorbance (450 nm) of each well was measured by a microplate reader (ARVO SX-1420, Wallac, Perkin Elmer, Waltham, MA, USA). The cell density was obtained from a calibration curve of absorbance versus cell number to estimate the growth rate. The NOEC value was estimated by comparing the algal cell growth of solvent control and those of treatments with the program EcoTox-Statics Version 2.6d (The Japanese Society of Environmental Toxicology, Japan). The chronic toxicity unit (TU) values of river water samples

Table 3 Assessment factors for application to toxicity data for estimating PNEC.

Toxicity	Number of taxonomic group, in which toxicity data are available	Assessment factor
Acute	1–2	1000
	3	100
Chronic	1–2	100
	3	10

were calculated from the inverse of NOEC values of river water samples to *P. subcapitata* [22]. A large TU value of a water sample indicates a high adverse effect of the water sample on the growth of *P. subcapitata*. If the TU value of a water sample is higher than 1, it suggests that chemicals contained in the SPE sample of the water sample can have an adverse effect on the growth of *P. subcapitata* at concentrations equal to the concentrations of the chemicals in the water sample.

Preliminary ecological risk assessment

Preliminary ecological risk assessments of 10 PPCPs at each sampling location were conducted using the hazard quotient (HQ), which is the ratio of measured environmental concentration (MEC) to PNEC. The maximum measured concentration of each PPCP at each sampling location was used as the MEC of the PPCP at the sampling location. The PNECs of PPCPs for preliminary risk assessments were calculated using published data on the toxicities of three taxonomic groups of algal, crustacean and fish species together with the assessment factors according to the guideline for ecological risk assessment, as directed by the Japan Ministry of the Environment [23]. Toxicity data collected from published data were classified into two types of toxicity, acute toxicity such as EC50 or LC50 for algal growth, mortality or immobilization of crustaceans and fish mortality and chronic toxicity such as NOEC for algal growth, reproduction of crustaceans and fish growth or reproduction. The PNEC values of acute and chronic toxicities were calculated by dividing the minimum values of acute and chronic toxicity values by assessment factors, respectively. The assessment factor was determined by each type of toxicity and the number of taxonomic groups, in which toxicity values are available (**Table 3**). The lower of the two PNECs was adopted as the PNEC for initial ecological risk assessment in this study. The criteria for ecological risk in the Japan Ministry of the Environment [23] are as follows: A HQ value of less than 0.1 “needs no further survey”; between 0.1 and 1 “needs further survey”; and equal to or higher than 1 “needs detailed evaluation”.

Estimation of contributions of PPCPs to the toxicity of river water

The method by Tamura *et al.* [12] with minor modification was adopted for estimation of the contributions of PPCPs that were classified into “needs further detailed investigation” by initial ecological risk assessment to the toxicity of concentrated river water to algal growth. The contribution of each pharmaceutical was calculated by the following equation,

$$\text{Contribution (\%)} = 100 \times TU_{\text{PPCP}} / TU_{\text{river water}} \quad (1)$$

where TU_{PPCP} and $TU_{\text{river water}}$ indicate the chronic toxicity unit of the PPCP and the toxicity of concentrated river water, respectively. The TU of each PPCP was estimated by dividing the measured concentration of the PPCP in river water by the published value of the chronic toxicity (NOEC) of the PPCP to the growth of *P. subcapitata* [24]. If more than one chronic toxicity value of a PPCP to the growth of *P. subcapitata* is reported, the geometric mean of the chronic toxicity values was calculated for the TU_{PPCP} . If a single compound is responsible for the concentrated river water toxicity, the TU of the PPCP / TU of the river water ratio becomes unified.

RESULTS AND DISCUSSION

PPCP concentration

Table 4 shows the concentrations of 10 PPCPs in water samples at three points in the middle stream of Tama River and four of its tributaries. The median concentrations of the 10 PPCPs in the mainstream were in the following order: crotamiton (670 ng/L) > clarithromycin > levofloxacin > azithromycin > caffeine > bezafibrate > sulfamethoxazole > triclosan > ibuprofen = ketoprofen (4 ng/L). The median concentrations of the 10 PPCPs in tributaries were in the following order: caffeine (355 ng/L) > crotamiton > bezafibrate > clarithromycin > triclosan > sulfamethoxazole > levofloxacin > azithromycin > ibuprofen > ketoprofen (2 ng/L). The order of median concentrations of 10 PPCPs differed between the mainstream and the tributary in the middle basin of Tama River. The highest concentrations of azithromycin, bezafi-

Table 4 Concentrations of 10 PPCPs (ng/L) and NOEC values for concentrated river water samples of algal growth inhibition test in 7 sampling locations.

Compound	LOD	Mainstream						Tributary							
		St. 1		St. 2		St. 3		St. a		St. b		St. c		St. d	
		Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16
Azithromycin	0.036	N.D.	N.D.	150	120	130	120	10	5.5	0.35	0.46	500	430	15	19
Bezafibrate	0.04	1.2	0.63	91	82	72	70	60	140	N.D.	N.D.	220	120	110	65
Caffeine	1.5	22	25	140	130	150	110	1600	1400	52	76	360	240	390	350
Clarithromycin	0.42	0.82	0.93	460	380	340	340	22	20	1.8	1.4	1200	900	100	98
Crotamiton	0.17	12	9.0	770	670	710	670	41	58	6.0	6.4	1100	1200	300	240
Ibuprofen	0.064	N.D.	N.D.	6.7	3.1	3.8	3.3	9.8	8.8	N.D.	N.D.	17	9.2	5.2	5.9
Ketoprofen	0.076	N.D.	0.54	3.6	30	2.4	18	1.1	4.3	0.17	0.64	500	400	0.19	1.9
Levofloxacin	0.71	N.D.	0.71	180	140	130	130	3.6	3.4	1.3	0.86	600	540	28	30
Sulfamethoxazole	0.11	0.89	0.93	72	49	64	56	5.9	2.3	1.3	1.4	110	100	35	31
Triclosan	0.1	2.20	2.0	54	45	46	48	15	14	2.9	3.9	177	96	27	27
Bioassay															
NOEC		≥50	≥50	3.1	3.1	6.3	6.3	13	13	≥50	≥50	3.1	3.1	13	13

N.D.: Not Detected

LOD: Limit of Detection

brate, clarithromycin, crotamiton, ibuprofen, ketoprofen, levofloxacin, sulfamethoxazole and triclosan were detected in St. c, which is located immediately downstream of a sewage treatment plant (**Table 4**). In St. c, almost all of the river water consists of effluent from the sewage treatment plant. The inflow of sewage effluent water may contribute to high concentrations of PPCPs in St. c. The highest concentrations of caffeine were observed in St. a. This tributary does not receive sewage effluent water. The inflow of untreated domestic wastewater into the tributary may cause relatively high concentration of caffeine in St. a. In the mainstream of Tama River, concentrations of PPCPs in St. 1, which is located upstream of a sewage treatment plant, were lower than those in Sts. 2 and 3. Concentrations of crotamiton in Sts. 2 and 3 were similar to those reported by Abe and Ishii [13]. High concentrations in Sts. 2 and 3 may be attributed to PPCPs contained in discharged waters and effluents from sewage treatment plants.

In this study, mean recovery rates of azithromycin, bezafibrate and sulfamethoxazole were equal to or less than 40%. Recovery rates of bezafibrate were low in all sampling sites. Compared to other sites, recovery rates of azithromycin and sulfamethoxazole were higher in Sts. 1 and b, in which domestic wastewater and effluents from sewage treatment plants were not discharged into rivers. Mean recovery rates

of caffeine and crotamiton were more than 100%. Low recovery rates of azithromycin, bezafibrate and sulfamethoxazole and high recovery rates of caffeine and crotamiton may be affected by interfering substances contained in water samples, physicochemical properties of the PPCPs and the affinity with Oasis HLB. Further study is required to develop the simultaneous analysis.

Preliminary ecological risk assessment

Preliminary ecological risks for 10 PPCPs were assessed on the basis of the PNEC and MECs in Tama River and its tributaries. **Table 5** shows the PNECs of 10 PPCPs and toxicity data to derive the PNECs. The PNECs of 9 PPCPs other than bezafibrate were determined by toxicity values to *P. subcapitata*. The PNEC of bezafibrate was determined by the toxicity of crustacean, *Ceriodaphnia dubia*.

The HQ values of 10 PPCPs at each sampling location are shown in **Table 6**. The HQ values of azithromycin, clarithromycin and triclosan were greater than one in Sts. 2, 3, a, c and d. Our results were similar to those of Komori *et al.* [5], in which the MEC/PNEC value of clarithromycin was 9.0, indicating the high potential environmental risk of clarithromycin to aquatic organisms in Japanese rivers. The HQ value of levofloxacin exceeded one in Sts. 2, 3 and c. The HQ value of ketoprofen was greater than one in St. c. Results indicate

Table 5 Toxicity data and PNEC values of 10 PPCPs.

Compound	Toxicity	Taxon of minimum toxicity value	Species name	Endpoint	Effect Measurement	Test duration (day)	Minimum toxicity value (µg/L)	Number of taxonomic groups*	Assessment factor	PNEC (µg/L)**	Reference
Azithromycin	Acute	Alga	<i>Pseudokirchneriella subcapitata</i>	EC50	Growth	4	19	2	1000	0.019	[41]
	Chronic	Alga	<i>P. subcapitata</i>	NOEC	Growth	4	52	1	100	0.52	[41]
Bezafibrate	Acute	Alga / Crustacean	<i>P. subcapitata</i> / <i>Daphnia magna</i>	EC50	Growth / Immobilization	4 / 2	10000***	2	1000	10	[41]
	Chronic	Crustacean	<i>Ceriodaphnia dubia</i>	NOEC	Reproduction	7	23	3	10	2.3	[42]
Caffeine	Acute	Crustacean	<i>D. magna</i>	EC50	Immobilization	2	10000	3	100	100	[41]
	Chronic	Alga	<i>P. subcapitata</i>	NOEC	Growth	4	520	1	100	5.2	[41]
Clarithromycin	Acute	Alga	<i>P. subcapitata</i>	EC50	Growth	3	2	3	100	0.02	[43]
	Chronic	Alga / Crustacean	<i>P. subcapitata</i> / <i>D. magna</i>	NOEC	Growth / Reproduction	4 / 21	3.1***	2	100	0.031	[21]
Crotamiton	Acute	Alga	<i>P. subcapitata</i>	EC50	Growth	4	3500	2	1000	3.5	[41]
	Chronic	Alga	<i>P. subcapitata</i>	NOEC	Growth	4	2100	1	100	21	[41]
Ibuprofen	Acute	Fish	<i>Pimephales promelas</i>	LC50	Mortality	28	680	3	100	6.8	[44]
Ketoprofen	Chronic	Alga	<i>P. subcapitata</i>	NOEC	Growth	3	10	3	10	1	[45]
	Acute	Alga	<i>P. subcapitata</i>	EC50	Growth	4	330	2	1000	0.33	[46]
Levofloxacin	Chronic	Alga	<i>P. subcapitata</i>	NOEC	Growth	4	16	1	100	0.16	[46]
	Acute	Alga	<i>P. subcapitata</i>	EC50	Growth	5	7.9	3	100	0.079	[47]
Sulfamethoxazole	Chronic	Crustacean	<i>D. magna</i>	NOEC	Reproduction	21	31	3	10	3.1	[21]
	Acute	Alga	<i>P. subcapitata</i>	EC50	Growth	4	280	3	100	2.8	[46]
Triclosan	Chronic	Alga	<i>P. subcapitata</i>	NOEC	Growth	4	160	2	100	1.6	[46]
	Acute	Alga	<i>P. subcapitata</i>	EC50	Growth	3	0.53	3	100	0.0053	[48]
	Chronic	Alga	<i>P. subcapitata</i>	NOEC	Growth	3	0.2	3	10	0.02	[48]

* Number of taxonomic groups of which toxicity values are available.

** Bold value was used for initial ecological risk assessment.

*** Toxicity values to two species are the same.

Table 6 Hazard quotient (HQ) values of 10 PPCPs at sampling locations.

Compound	Mainstream						Tributary							
	St. 1		St. 2		St. 3		St. a		St. b		St. c		St. d	
	Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16	Jan. 31	Feb. 16
Azithromycin	-*	-	7.9**	6.3	6.8	6.3	0.5	0.3	0.02	0.02	26	23	0.8	1.0
Bezafibrate	<0.01	<0.01	0.04	0.04	0.03	0.03	0.03	0.06	-	-	0.10	0.05	0.05	0.03
Caffeine	<0.01	<0.01	0.03	0.03	0.03	0.02	0.3	0.3	0.01	0.01	0.07	0.05	0.08	0.07
Clarithromycin	0.04	0.05	23	19	17	17	1.1	1.0	0.1	0.1	60	45	5.0	4.9
Crotamiton	<0.01	<0.01	0.22	0.19	0.20	0.19	0.01	0.02	<0.01	<0.01	0.31	0.34	0.09	0.07
Ibuprofen	-	-	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	-	-	0.02	<0.01	<0.01	<0.01
Ketoprofen	-	<0.01	0.02	0.19	0.02	0.11	0.01	0.03	<0.01	<0.01	3.1	2.5	0.00	0.01
Levofloxacin	-	0.01	2.3	1.8	1.6	1.6	0.05	0.04	0.02	0.01	7.6	6.8	0.35	0.38
Sulfamethoxazole	<0.01	<0.01	0.05	0.03	0.04	0.04	<0.01	<0.01	<0.01	<0.01	0.07	0.06	0.02	0.02
Triclosan	0.4	0.4	10	8.5	8.7	9.0	2.8	2.6	0.5	0.7	33	18	5.0	5.1

* Hazard quotient was not calculated because the compound was not detected.

** Bold values are greater than one.

that effects of the 5 PPCPs on aquatic organisms and water quality monitoring are necessary for further evaluation in the middle stream of Tama River and its tributaries. High potential ecological risks of azithromycin, clarithromycin and triclosan in rivers outside of Japan have been reported. For example, Valcárcel *et al.* [25] reported that median HQ values of azithromycin and clarithromycin in Spanish rivers were 27.6 and 5.11, respectively. In addition, Stasinakis *et al.* [26] reported that the HQ value of triclosan for algae in a Greek river was 70.

Algal growth inhibition test

Table 4 shows the NOEC values of concentrated river water samples for algal growth. The chronic TU values of the concentrated river water samples for algal growth are shown in Fig. 2. The chronic TU value differed in sampling locations and ranged from 0.02 to 0.32. The maximum TU value was observed in Sts. 2 and c. The results indicate that chemicals contained in the SPE sample of the water sample cannot have an adverse effect on the growth of *P. subcapitata* at concentrations equal to the concentrations of the chemicals in the water sample because the TU value of any of the samples was lower than 1.

Contributions of PPCPs to the toxicity of river water

Table 7 shows the NOEC values of azithromycin, clarithromycin, ketoprofen, levofloxacin and triclosan for the growth of *P. subcapitata*. There was a relatively large difference between two NOEC values of ketoprofen. Ketoprofen is a photolabile chemical, and the photoproducts may be toxic [27]. The differ-

Fig. 2 Results of algal growth inhibition tests of concentrated river water samples. Chronic toxicity unit was derived from the inverse of NOEC value of a river water sample.

ences in test conditions such as the light intensity between the two laboratories might cause the difference between the two NOEC values of ketoprofen. The NOEC value of triclosan was relatively lower in the flask test based on OECD TG 201 than in the microplate test. The NOEC values of the 5 PPCPs were in the following order: triclosan, azithromycin, clarithromycin, ketoprofen and levofloxacin.

The estimated chronic TU values of 5 PPCPs to the growth of *P. subcapitata* in Sts. 2, 3 and c, in which TU values of concentrated river water were relatively high, were shown in Fig. 3. All estimated TU values of 5 PPCPs were lower than 1. The results indicate that 5 PPCPs in the water sample may have no adverse effect on the growth of *P. subcapitata* at the concentrations of the PPCPs in the water sample.

The contributions of 5 PPCPs to the toxicities of the

Table 7 NOEC values of 5 PPCPs to the growth of *Pseudokirchneriella subcapitata*.

Compound	Geometric Mean ($\mu\text{g/L}$)	Data ($\mu\text{g/L}$)	Type*	Reference
Azithromycin	7.0	4.2	M	[49]
		5.2	M	[41]
		16	M	[46]
Clarithromycin	10.1	3.1	M	[21]
		5.2	M	[41]
		10	M	[49]
		16	M	[46]
		<40	F	[47]
Ketoprofen	126	16	M	[46]
		1000	M	[41]
Levofloxacin	370	260	M	[41]
		310	M	[21]
		630	M	[46]
Triclosan	1.3	0.2	F	[47]
		0.5	F	[10]
		1	F	[50]
		1	M	[49]
		6.3	M	[46]
		8.3	M	[41]

* F and M indicate algal growth inhibition test using flask and microplate, respectively.

concentrated river water samples to *P. subcapitata* in Sts. 2, 3 and c are shown in **Fig. 4**. The contributions of azithromycin, clarithromycin and triclosan to the toxicities of the concentrated river water samples were relatively higher than those of ketoprofen and levofloxacin. The contributions of azithromycin, clarithromycin and triclosan in Sts. 2 and 3 on Jan. 31 and Feb. 16 and St. c on Jan. 31 were in the following decreasing order: triclosan, clarithromycin and azithromycin. In St. c on Feb. 16, the contribution of clarithromycin was higher than that of triclosan. The results indicate that PPCPs contained in effluent from sewage treatment plants may contribute to the toxicity of concentrated river water to algal growth.

While river samples in Sts. 2 and c had the highest toxicity value, the contributions of azithromycin, clarithromycin and triclosan in St. c were higher than those in St. 2 at two surveys. In St. 2, other chemicals may contribute to the toxicity of the concentrated river water.

The contributions of azithromycin, clarithromycin and triclosan were equal to or more than 20% in St. c (**Fig. 4**). Our results show that in St. c, azithromycin, clarithromycin and triclosan contributed to the toxicity of the concentrated water sample ranging from 20 to 54% (**Fig. 4**) and the TU value of the water sample to the growth of *P. subcapitata* was lower

than 1 (**Fig. 2**). The results indicate relatively large contributions of PPCPs to relatively weak toxicity of the concentrated water samples on the growth of *P. subcapitata* in the middle basin of Tama River.

The toxicity of a river water sample was estimated by conducting an algal growth inhibition test of the SPE sample derived from the river water sample. Some chemicals such as volatile chemicals, metals and inorganic chemicals could not be trapped by the SPE cartridges. These chemicals might attenuate or enhance the toxicity of river water samples. Therefore, the contributions of PPCPs to the toxicities of water samples to the growth of *P. subcapitata* might be overestimated or underestimated in this study. In addition, chemicals such as DOM can interfere with the recovery from the SPE cartridge. Conducting whole effluent toxicity tests of river water samples is required.

If the algal species that live in the middle basin of Tama River are more tolerant to PPCPs than *P. subcapitata*, no algal species may be harmed in the basin by PPCPs. However, as interspecific and intraspecific variations in the sensitivity to a chemical is frequently observed [28,29], algal species more sensitive to PPCPs than *P. subcapitata* can inhabit the middle basin of Tama River. For example, blue-green algal species are more sensitive than green algal species to

Fig. 3 Estimated chronic toxicity units of 5 PPCPs in Sts. 2, 3 and c. The chronic toxicity unit of a PPCP in a sampling site was calculated by dividing the measured concentration of the PPCP in the sampling site by the published value of the chronic toxicity of the PPCP to the growth of *P. subcapitata*.

antibiotics [30,31]. Investigating variations in sensitivities to PPCPs among algal species and the effects of PPCPs on the algal community may help us to examine the effects of PPCPs on algal species in the middle basin of Tama River.

In this study, contributions of PPCPs to the toxicities of the concentrated river water samples to the growth of *P. subcapitata* were presented using NOEC values. Values of NOEC are discrete and highly dependent on the statistical analysis and the variance of the control [32]. Further study may be required to investigate the contributions of PPCPs to the toxicities of river water samples to the growth of *P. subcapitata* without use of NOEC values and compare them with results in this study.

CONCLUSIONS

Preliminary ecological risk assessments of 10 PPCPs in the middle basin of Tama River suggest that azithromycin, clarithromycin, ketoprofen, levofloxacin and triclosan require detailed-level ecological risk assessment. Algal growth inhibition tests of water samples show that there were no adverse effects on an algal species in all water samples. The contributions of 5 PPCPs that had potential ecological risks to the toxicity of each concentrated water sample to an algal species were estimated. Results indicate that the contributions of three PPCPs (azithromycin, clarithromycin and triclosan) were higher than the other selected PPCPs. Relatively large contributions of the PPCPs to relatively low toxicity of the concentrated water samples to the growth of the spe-

Fig. 4 Contributions of 5 PPCPs to toxicities of concentrated river water samples in St. 2, St. 3 and St. c to the growth of *Pseudokirchneriella subcapitata*.

cies were found in the middle basin of Tama River. Further studies on variations in sensitivities to PPCPs among algal species and the effects of PPCPs on the algal community are needed to understand the effects of PPCPs on algal species in the middle basin of Tama River. In addition, concentrations of PPCPs in river water and effluents discharged from sewage treatment plants can vary in season and during the day [27,33]. In further studies, seasonal and diurnal variations in the concentrations of PPCPs in river water are required for estimating the ecological risks of PPCPs.

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