Occurrence of 70 pharmaceutical and personal care products in Tone River basin in Japan

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Abstract The occurrence of 70 pharmaceutical and personal care products (PPCPs) was investigated in the Tone River. The river has the largest basin in Japan, and the water is utilized not only for farming, but also as a source of water supply. One day in both January and October 2006, surface waters in the river and its tributaries and effluents from sewage treatment plants (STPs) directly discharging into the Tone River were collected, the location of which ranged over 150 km along the river. The 70 PPCPs in the samples were concentrated by solid phase cartridge and were measured by LC-MS/MS using three analytical methods. Fifty-seven PPCPs were detected in one or more samples. Bezafibrate, caffeine, carbamazepine, clarithromycin, crotamiton and sulpiride were frequently detected. Mass flow profiles of some PPCPs (e.g., crotamiton) were comparable to cumulative inhabitants in the basin, suggesting that these PPCPs could be markers of population. Total load of each PPCP into the basin from upstream, the tributaries, and the STPs were calculated. The contribution of selected PPCPs from the tributaries with lower sewerage system coverage was dominant compared to those from upstream and the STPs, suggesting the installation of sewerage systems is necessary to reduce the load of PPCPs in the Tone River basin.
Keywords Drug; mass flow profile; molecular marker; river basin

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Introduction

The occurrence of pharmaceutical and personal care products (PPCPs) in the aquatic environment has attracted interest in terms of chronic effects on human beings and aquatic organisms, as well as wide spread of resistant bacteria. Significant effects of PPCPs on aquatic organisms have not yet been clarified, except for oral contraceptive 17α-ethinylestradiol. This, however, might be due to the lack of knowledge about the toxicity of PPCPs on wildlife, and the insufficiency of monitoring data on concentrations of PPCPs in the aquatic environment.

Environmental monitoring and investigations on removal techniques for PPCPs have been conducted systematically in European countries since the late 1990s. Although the studies on PPCPs in Japan began later than those in Europe, the fate of some PPCPs in sewage treatment plants (STPs) have been demonstrated to date. However, information about environmental fates (e.g., in rivers and lakes) of PPCPs is still limited in Japan. At some rivers in Japan, points of discharge from STP and intake for water supply (followed by appropriate treatment) were often located interlaced in the same river. Therefore, appropriate management of wastewaters in sub-basins was one of the critical factors in water quality of the mainstream.

The objectives of the present study were to investigate the occurrence and fate of PPCPs in the Tone River, which receives a variety of waste; the water is utilized as water supply, for future risk assessment in terms of biological effect and for effective river basin management.

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Methods

Sampling location

Surface waters were collected at 10 points in the mainstream, at 7 confluences of major tributaries, and at 2 major distributaries of the Tone River on January 31st (winter) and October 3rd (late summer) 2006. The Tone River has the largest basin (length: 322 km, basin: 16,840 km^2, population in the basin: 12 million peoples) in Japan, and is located in the Kanto Plain (Figure 1). The river receives a variety of wastewaters from households, farmland, stockbreeders and manufactures, with and without appropriate treatment. The water of the river is utilized not only for farming and stockbreeding, but also as the source of water supply for 27 million people (corresponding to ~20% of the total population in Japan) living in the Tokyo metropolitan area, and on the periphery of Tokyo. The percentage of sewered population in municipalities along the river is 50% on average, while it is 41% in those along the tributaries.

On the same days, sewage effluents were also collected as grab samples from 9 STPs which discharge the effluent directly into the Tone River. The water flow of rivers and discharging volume were obtained from The Ministry of Land, Infrastructure and Transport Kanto Regional Development Bureau and the STPs, or were measured for two tributaries at the sampling time.

Sample preparation and analytical methods

One litre of each sample was collected into two bottles together with EDTA-Na_2 and ascorbic acid, with the concentration at 1 g/L in order to encourage the efficiency of extraction (below) and to prevent biological reduction, respectively. The collected samples were immediately transported under cool conditions. In this study, three analytical methods were adopted as below.

Konishi *et al.* (2006) established a broad-spectrum analytical method for 57 PPCPs. The method was adapted for the first survey, with minor modification (method A). In brief, one litre sample filtrated through a glass fiber filter (pore size: 1 μm) was acidified to pH 2 with HCl, and then the sample was divided into four 200 mL aliquots. A series of standard solution mixture of target PPCPs was injected into three aliquots with a common ratio of 10. For some analytes which has corresponding isotope-labeled standard, surrogate solution was injected into all aliquots of each sample. The four aliquots were extracted with Oasis HLB cartridge (Waters, containing 500 mg resin in 6 mL syringe) which was previously

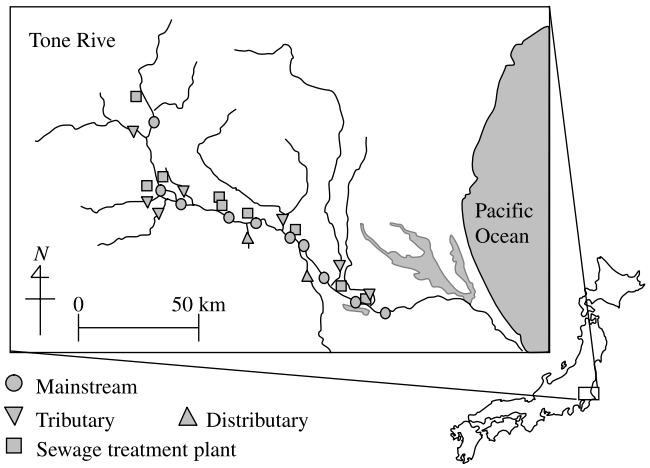

Figure 1 Sampling location in the Tone River basin

conditioned with both methanol and dilute hydrochloric acid. After passage of the aliquots, the cartridge was washed by dilute hydrochloric acid and dewatered by centrifugation and passing of nitrogen gas, and then eluted with methanol. The extract was dried under a gentle stream of nitrogen gas and then redissolved with 5% aqueous acetonitrile (0.1% formic acid). The extract in aqueous acetonitrile was analyzed by liquid chromatograph (LC: Agilent 1,100) equipped with tandem quadrupole mass spectrometer (MS/MS: thermo Quantum Discovery Max), in both positive-ion (57 PPCPs) and negative-ion (13 PPCPs) mode. In the positive-ion mode, the analytes were separated with a Thermo Hypersil GOLD column (2.1 × 150 mm, 5μm); 5% methanol in water (0.1% formic acid) and acetonitrile (0.1% formic acid) were used as mobile-phase solvents. In the negative-ion mode, Zorbax Eclipse XDB-C18 column (2.1 × 150 mm, 3.5 μm), 1 mM aqueous ammonium and acetonitrile were used. The mobile phase gradient pattern and monitor ions were optimized by flow injection analysis of both single and mixture of target PPCPs. Appropriate analytical data from one of the spiked samples was used for quantifying (i.e., recovery correction) the analytes in the unspiked sample, or corrected by recovery of corresponding surrogate.

For the second survey, method A was adapted with minor modification (method B). One litre sample was divided into five 200-mL aliquots for the river surface samples, and into five 50-mL aliquots for the STP effluent samples. The aliquots were filtrated, acidified, and then a series of standard solution mixture of target PPCPs was injected into four aliquots with a common ratio of 5. The five aliquots were extracted using the same procedure in method A and then subjected to LC-MS/MS (Agilent 1,100 equipped with Applied Biosystems API 4,000) analysis. Thermo Hypersil Advance column (2.1 × 150 mm, 5μm) was used in both positive- and negative-ion mode. The mobile phase gradient pattern and monitor ions were also optimized by using both single and mixture of target PPCPs. These two methods (A and B) were verified by analysis of the same samples (surface water and effluents) collected in the second survey.

For clarithromycin, azithromycin and levofloxacin, the previously reported method (Yasojima *et al*., 2005) was adopted for the same samples in the two surveys (method C).

Recoveries of the target PPCPs were previously confirmed thorough the analysis of STP effluents, the averaged recovery was 102 ± 21% ($n = 3$) for method A, and 104 ± 31% ($n = 4$) for method B. Limits of quantification (LOQ) ranged between sub and hundreds ng/L (Table 1) and depended on the analyte, analytical methods and the sample matrix,.

Results and discussion

Occurrence of PPCPs in the Tone River basin

Fifty-seven out of 75 PPCPs were detected in one or more samples (Table 1). As the sampling points located on the lower reaches, the number of detection and detected concentrations were high. Among the two surveys, the detection and the concentrations of individual PPCPs were higher in the winter survey than those in the summer survey for the river and STP effluent samples. The water flows in the mainstream in the summer survey were 1.3 to 3.8 times (average: 2.7) longer than those in the winter survey; therefore the pollutant concentration at the summer survey might have been diluted.

Concentrations of the PPCPs were generally high in the following order: the effluents, surface water in the tributaries, and surface water in the mainstream. Azithromycin (macrolide), bezafibrate (lipid regulator), carbamazepine (antiepileptic), clarithromycin (macrolide), crotamiton (antipruritic), diethyltoluamide (insect repellent), diltiazem (vasodilator), disopyramide (antiarrhythmic), levofroxacin (quinolone), nalidixic acid (quinolone), pirenzepine (anti-ulcer agent) and sulpiride (gastric mucosal protective and anti-psychotic drug) were frequently detected in mainstream, tributaries, and effluents (Table 1).

Table 1 Limits of quantification (LOQ) and concentrations of target PPCPs in the Tone River basin

Compound	LOQ	Mainstream (n = 20)			Tributary (n = 14)			STP effluent (n = 18)		
		no. > LOQ	Median	Max	no. > LOQ	Median	Max	no. > LOQ	Median	max
			(ng/L)			(ng/L)			(ng/L)	
acetaminophen	6–40	13	22	52	7	18	110	1	263	263
amitriptyline	0.5–3	0	< LOQ		0	< LOQ		15	7.6	14
atenolol	1–30	8	3.8	46	4	11	39	18	291	930
azithromycin	0.8–10	20	8.0	70	14	6.5	16	18	165	441
bezafibrate	2–40	17	16	77	10	35	170	18	425	1,500
caffeine	50–270	18	264	2,100	13	420	1,500	4	565	3,500
carbamazepine	1–3	16	4.5	12	11	5.6	15	18	54	86
chloramphenicol	0.3–50	0	< LOQ		0	< LOQ		5	74	140
clarithromycin	0.5–2	20	12	38	14	13	60	18	568	726
clofibric acid	1–10	9	4.1	7.0	7	4.2	21	12	14	110
crotamiton	6–30	20	37	160	12	45	210	18	900	1,900
DEET	10–40	12	18	36	9	16	26	17	69	191
diclofenac	1–200	4	1.7	3.3	2	2.6	3.3	9	50	220
diltiazem	0.1–2	11	0.4	2.2	6	0.7	3.6	18	43	90
dipyridamole	40–420	0	< LOQ		0	< LOQ		4	200	460
disopyramide	0.3–1	20	5.9	19	13	8.9	37	18	297	710
ethenzamide	0.6–30	8	2.0	2.8	5	1.4	1.5	15	23	47
furosemide	6–50	2	9.1	9.1	1	21	21	18	320	940
griseofulvin	2–30	5	3.2	4.4	3	3.2	6.6	3	11	29
haloperidol	0.1–4	1	0.4	0.4	2	0.1	0.2	8	2.2	4.5
ibuprofen	30	0	< LOQ		0	< LOQ		6	41	77
ifenprodil	1 –4	0	< LOQ		0	< LOQ		15	15	140
indomethacin	5–40	1	16	16	3	6.3	8.7	17	140	251
isopropylantipyrine	1–5	6	2.0	3.1	2	2.6	3.1	5	10	12
josamycin	0.2–0.7	2	0.3	0.4	1	0.3	0.3	7	2.6	6.1
ketoprofen	20–100	1	24	24	0	< LOQ		15	266	820
levofloxacin	10–20	10	17	29	7	16	32	18	336	990
mefenamic acid	4–950	0	< LOQ		0	< LOQ		7	30	62
metoclopramide	1–4	0	< LOQ		0	< LOQ		17	24	76
metoprolol	2–40	0	< LOQ		0	< LOQ		4	21	23

Table 1 (*continued*)

Compound	LOQ	Mainstream (n = 20)			Tributary (n = 14)			STP effluent (n = 18)		
		no. > LOQ	Median (ng/L)	Max	no. > LOQ	Median (ng/L)	Max	no. > LOQ	Median (ng/L)	max
nalidixic acid	2–80	8	3.2	3.9	3	3.8	9.4	12	112	345
phenytoin	2–10	3	1.9	2.3	2	1.9	2.1	8	36	54
pirenzepine	0.2–30	9	0.7	1.1	5	0.9	1.6	15	31	100
primidone	11–50	0	< LOQ		0	< LOQ		5	76	180
propranolol	9–30	0	< LOQ		0	< LOQ		6	15	23
sulfadimethoxine	0.3–4	5	1.6	3.4	4	1.1	1.7	4	7.2	10
sulfamethoxazole	6–60	2	6.7	7.2	3	71	160	10	40	76
sulfamonomethoxine	3–20	7	3.9	20	2	77	130	0	< LOQ	
sulpiride	0.6–4	20	30	110	14	27	170	18	1,200	2,100
theophylline	10–40	5	13	50	6	42	76	17	71	930
trimethoprim	6–30	0	< LOQ		0	< LOQ		8	28	56
verapamil	0.1–4	1	0.4	0.4	0	< LOQ		15	5.2	11

Antipyrine, chlorpromazine, danofloxacin, mepirizole, naproxen, norfloxacin, pentxifylline, 2-quinoxainecarboxylic acid, salbutamol, scopolamine, sotalol, sulfadimizine, terbutaline, and tetracycline were rarely detected, while amoxicillin, benzylpenicillin, carbazochrome, chlortetracycline, clenbuterol, cyclophosphamide, dextromethorphan, diclazuril, diphenidol, fenoprofen, fulfenamic acid, gemfibrozil, imipramine, oleandmycin, oxytetracycline, prednisolone, promethazine, thiamphenicol, tilmicosin, tolbutamide, and tolperisone were not detected.

Furosemide (diuretic), indomethacin (anti-inflammatory), ketoprofen (anti-inflammatory) and theophylline (bronchodilator) were also frequently detected in the effluent samples at concentration of hundreds ng/L, but not or rarely detected in the river water samples. These results might be caused by biodegradation and photodegradation (especially for ketoprofen as Lin *et al*. (2005) demonstrated), or by high LOQ.

Among sulfonamide analyzed, sulfamethoxazole was occasionally detected both in river and effluent samples, while sulfamonomethoxine was only detected in river samples, especially in tributaries. Sulfamonomethoxine is used as veterinary drug in Japan, therefore detection of this drug in the tributaries implies the influence of livestock practices in the river basin.

Mass flow of PPCPs in the Tone River basin

To demonstrate the fate of PPCPs in the Tone River basin, mass flow profiles of selected PPCPs detected regularly in the mainstream were calculated. The mass flows were calculated by multiplying concentrations in the mainstream by flow rate at each sampling point (Figure 2 (a)). For caffeine, mass flows in both surveys were comparable, except for one point in the winter survey (Figure 2 (b)). At 130 km distance from the Tone estuary, the largest tributary in the basin joins the Tone River. The relatively higher value was also observed for clarithromycin (Figure 2 (c)) and azithromycin. For crotamiton (Figure 2 (d)), bezafibrate, disopyramide and sulpiride, the mass flows closely parallel each other in the surveys. The profiles were also comparable to cumulative inhabitants (Figure 2 (e)) at each sampling point. The inhabitants were calculated from the census of population (FY 1995) in 109 frames (based on the watershed) in the Tone River basin, and if water is taken out of the river, the corresponding population was excluded from the cumulative population. Relationships of mass flows of crotamiton, bezafibrate, disopyramide and sulpiride with the cumulative inhabitants were examined. As a result, crotamiton showed highest correlation in both relatively low flow period ($R^2 = 0.95$) and high flow period ($R^2 = 0.91$), suggesting the effectiveness of crotamiton as a molecular marker of population in a river basin.

Furthermore, we propose a novel indicator. In contrast to the low biodegradability of crotamiton during sewage treatment and regular detection (Nakada *et al*., 2006), almost perfect removal efficiencies of caffeine in STPs have been reported (Thomas *et al*., 2005). Because caffeine is susceptible to biodegradation, the ratio of caffeine to crotamiton in treated wastewater is smaller than that in untreated wastewater. In fact, the ratio in STP effluents analyzed in this study were 0.8 ± 1.8 ($n = 11$, including data for caffeine which were detected but below the LOQ). The ratio in tributaries and in mainstream samples were 8.3 ± 5.5 ($n = 12$) and 6.9 ± 3.0 ($n = 18$), suggesting wastewater with insufficient treatment flow into the mainstream via tributaries. Further research will be carried out as our future work.

Source distribution of PPCPs in the Tone River basin

To demonstrate source distribution of PPCPs in the Tone River basin, total load of each PPCP from upstream, the tributaries and the STPs in the basin were calculated by multiplication of the concentrations and water flows. As for the contribution of selected PPCPs detected with a frequency over 60% in the mainstream samples, the tributaries were dominant compared to upstream and the STPs, suggesting that sewerage systems in the tributaries are insufficient. For caffeine, the total load was estimated approximately 6 kg/day in the both surveys (Figure 3 (a)). Caffeine is contained in beverages, as well as in some drugs as stimulants. Because of global consumption of caffeine and its biodegradability in STPs, the dominant load from tributaries without seasonal variation was reasonable. The total load for bezafibrate and crotamiton showed seasonal variation especially from STPs (Figure 3 (a)). Removal efficiencies of bezafibrate in winter (median: 15%) and in summer (87%)

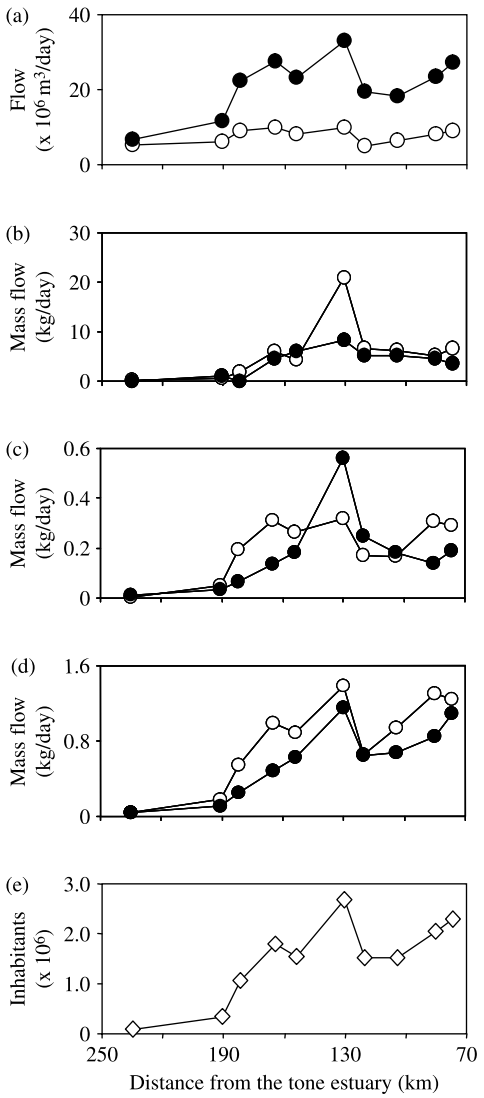

Figure 2 Flow (a) at each sampling point in the mainstream, mass flux profiles of caffeine (b), clarithromycin (c) and crotamiton (d), and cumulative inhabitants in the basin (e). The open circles indicate data from the first survey (winter), while the closed circles are from the second survey (summer)

in Italian STPs were reported (Castiglion *et al.*, 2006), which is consistent with the load of bezafibrate from STPs observed in the present study. On the other hand, stability of crotamiton without seasonal variation in STP was reported (Nakada *et al.*, 2006). Therefore, the total load of crotamiton from STPs implies the consumption of personal products containing this compound increase during the winter period. In an area without sewerage systems, onsite wastewater treatment tank is the dominant treatment equipment in Japan. Because the onsite wastewater treatment tank has probably the same or lower efficiency than STP due to high loading rate and the fact that it leaves gray water untreated, the load of PPCPs which has stability during treatment processes, from tributaries were heavier than that from STPs. As discussed above, possible factors contributing to the load and its distribution of PPCPs from each source were governed by its stability during treatment processes and seasonal variation in consumption.

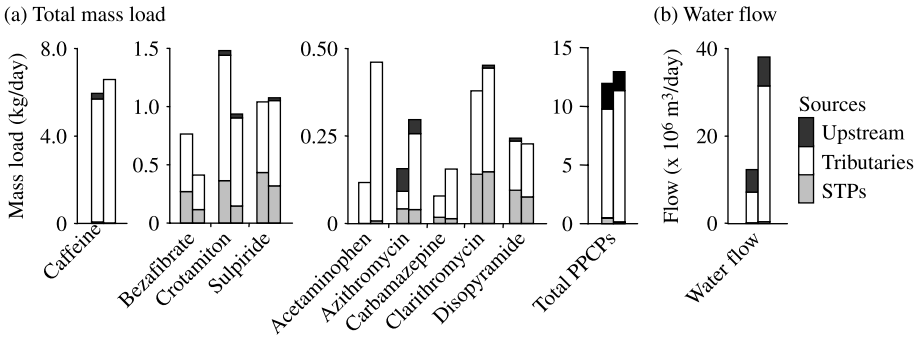

Figure 3 Source distributions of individual and total PPCPs analyzed in the Tone River basin (a) and water flow (b) from upstream, tributaries and STPs. The left bar for each indicates data from the first (winter) survey, while the right bar is from the second (summer) survey

The total load of PPCPs analyzed was also calculated from the sum of the loads of 70 PPCPs in the first survey and 75 in the second survey (Figure 3 (a)). The total load of PPCPs into the Tone River basin was estimated at approximately 12 kg/day (Figure 3 (a)), and half was calculated to be drawn off for many uses (data not shown), including as the source of water supply. The major part of the total load was estimated to come from the tributaries. Further investigations on effective treatment and its condition for persistent PPCPs are required, as well as seasonal surveys on STP.

Conclusion

To demonstrate the occurrence of PPCPs in a river basin in Japan, basin-wide surveys were conducted in two seasons using three broad-spectrum analytical methods for PPCPs in the Tone River basin. Fifty-seven in 75 PPCPs were detected in one or more samples, depending on utilities and physicochemical properties during treatment processes or transportation of the PPCPs. Mass flow profiles of PPCPs calculated in the mainstream indicate that the load increases with descent down the river. In the results of mass balance analysis in the basin, the contribution of PPCPs from tributaries were dominant relative to those from upstream and the STPs surveyed, suggesting the spread of sewerage systems is necessary to reduce the load of PPCP in the Tone River basin.

References

Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D. and Zuccato, E. (2006). Removal of pharmaceuticals in sewage treatment plants in Italy. *Environ. Sci. Technol.*, **40**(1), 357–363.

Konishi, C., Houwa, I., Nakada, N., Komori, K., Suzuki, Y. and Tanaka, H. (2006). Development of simultaneous analysis of pharmaceuticals in aqueous samples using LC-MS/MS. *Environ. Eng. Res.*, **43**, 73–82 (in Japanese with abstract in English).

Lin, A.Y. and Reinhard, M. (2005). Photodegradation of common environmental pharmaceuticals and estrogens in river water. *Environ. Toxicol. Chem.*, **24**(6), 1303–1309.

Nakada, N., Tanishima, T., Shinohara, H., Kiri, K. and Takada, H. (2006). Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. *Water Res.*, **40**(17), 3297–3303.

Thomas, P.M. and Foster, G.D. (2005). Tracking acidic pharmaceuticals, caffeine, and triclosan through the wastewater treatment process. *Environ. Toxicol. Chem.*, **24**(1), 25–30.

Yasojima, M., Nakada, N., Komori, K., Suzuki, Y. and Tanaka, H. (2006). Occurrence of levofloxacin, clarithromycin and azithromycin in wastewater treatment plant in Japan. *Water Sci. Technol.*, **53**(11), 227–233.