Inflow and outflow loads of 484 daily-use chemicals in wastewater treatment plants across Japan

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ABSTRACT

With the increasing number and volume of chemicals used in modern life, their adverse effects on human health and aquatic organisms have increased concerns as well. To formulate appropriate management plans, the amounts/volumes used and emitted of these chemicals must be regulated. However, no data are available on the use of most chemicals, particularly daily-use chemicals such as pharmaceuticals and personal care products (PPCPs). Herein, we tested eight activated sludge wastewater treatment plants (WWTPs) across Japan, each servicing populations of over 200,000, to investigate the emissions of 484 chemicals including 162 PPCPs. Twenty-four-hour composite samples were collected before and after the activated sludge component of treatment in each season of 2017. Targeted substances were solid-phase extracted and subsequently measured by LC-QTOF-MS-Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra Acquisition. The mean number of detected substances and their mean total concentrations in inflows (n = 32) and outflows (n = 32) were 87 and 92 and 108,517 ng L−1, respectively. Pharmaceuticals comprised 50% of the screened chemicals in the inflow. The median removal efficiency was 31.3%: 29.2% for pharmaceuticals and 20.2% for pesticides, which were similar to those in the literature. Cluster analysis showed that spatial differences among the WWTPs are larger than seasonal differences in the same WWTP. Regardless, we detected seasonal differences in the amounts of substances in the inflows: the amounts of sucralose, UV-filters, and insecticides were larger in summer than in winter, whereas those of ibuprofen and chlorpheniramine were larger in winter than in summer. The total inflow and outflow population equivalent loads estimated using wastewater volume, detected concentrations, and populations were 671 tons y−1 and 671 tons y−1, respectively. Using the data obtained in this study, we identified 13 candidates of marker substances for estimating real-time population in a sewage treatment area and 22 candidates of marker substances for sewage contamination.

Key words: municipal wastewater treatment plant; influent; effluent; daily-use chemicals; pharmaceutical and personal care product; pesticide; population equivalent load; LC-QTOF-MS-Swath

INTRODUCTION

In the pursuit of a healthy and comfortable life, the number and volume of chemical substances used worldwide have been rapidly increasing (Binetti et al., 2008; Bernhardt et al., 2017). In particular, the number and volume of both daily-use and other consumer chemicals, such as pharmaceuticals and personal care products (PPCPs) and pesticides, have been increasing in not only developed countries but also in developing countries. After using these chemicals in our daily-life, busi-
ness, and commercial activities, many of them, their metabolites, and decomposition products are introduced into the aquatic environment, where they may adversely affect aquatic organisms (Moschet et al., 2014; Eggen et al., 2014).

In Japan, environmental emissions of chemicals used in various industries are reported in the Pollutant Release and Transfer Register (PRTR, Ministry of Economy, Trade and Industry, Japan, 2001); this is used to promote voluntary improvement in the management of chemical substances, thereby reducing the volume of chemicals released into the environment. However, the numbers of chemicals included in the register are limited, and the chemicals released into the environment in volumes of less than 1 ton per year are not reported to the government. While industrial emissions are a natural focus in terms of reporting, the amount of daily-use chemicals used in homes and businesses are unknown despite many of these chemicals, including PPCPs and pesticides, being known environmental pollutants. Although the local production and imported volumes of pharmaceuticals are reported every year (Ministry of Health, Labour and Welfare, Japan, 2018), because the published data is for the whole of Japan, the amounts used in each region/prefecture are unknown. In addition, although the sales volume for pesticides used in agriculture in each prefecture are published every year (Japan Plant Protection Association, 2019), the amounts discharged from human activities other than agriculture are also unknown. Furthermore, no data are available on the amount of chemicals used in personal care products and other consumer products.

The chemicals of concern discussed above that are used domestically and in commercial and business properties are eventually discharged to the sewerage system and introduced into municipal wastewater treatment plants (WWTPs). Thus, the local emissions of such chemicals can be determined by examining their concentrations in the inflows and outflows of WWTPs. This is known as wastewater-based-epidemiology (WBE) (Lorenzo and Pico, 2019). Although the first target substances of WBE were illicit drugs (Daughton, 2012; Bade et al., 2020; O’Rourke, et al., 2020), recently it has also been applied in the case of legal substances such as metformin (Yan et al., 2020; O'Rourke, et al., 2020), and pesticides via LC-QTOF-MS (Kadokami and Ueno, 2019)). Herein, we report the survey results for the 484 polar compounds. Owing to a large number of targets and treatment plant diversity, we set the following six objectives: (1) to estimate inflow and outflow population equivalent loads (PEL, g 1,000 capita⁻¹ d⁻¹) of 484 chemicals; (2) to calculate annual loads of these substances for Japan; (3) to identify marker substances for estimating real-time treatment population; (4) to identify new marker compounds that can be used as indicators of sewage contamination; (5) to examine spatial and seasonal differences in the detected chemicals and their concentrations among sampling areas; and (6) to examine removal efficiencies of the detected chemicals. To the best of our knowledge, this study is the first comprehensive study to elucidate the amounts of chemicals discharged in the course of our daily life and the amounts in which they are emitted into the aquatic environment.

EXPERIMENTAL

REAGENTS AND EQUIPMENT

Chemical standards were purchased from Restek Japan (Tokyo, Japan), Kanto Chemical (Tokyo, Japan), and Hayashi Pure Chemical (Osaka, Japan). Analytical-grade pharmaceuticals were obtained from Kanto Chemical, Funakoshi (Tokyo, Japan), Tokyo Chemical Industry (Tokyo, Japan), FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), Dr. Ehrenstorfer GmbH (Augsburg, Germany), LKT laboratories (St Paul, MN, USA), Sigma-Aldrich Japan (Tokyo, Japan), Toronto Research Chemicals (North York, ON, Canada), and Santa Cruz Biotechnology (Dallas, TX, USA). LC-MS-grade methanol and pesticide-grade dichloromethane were purchased from Kanto Chemical. Stock solutions (1 mg mL⁻¹ when possible) of each substance were prepared with methanol or acetonitrile and stored at −20°C in a freezer. Multiresidue due pesticide standards obtained from reagent companies were used as the stock solution. Working mixed standard solutions were made by diluting the stock solutions with methanol. The deuterium labeled standards that were used as internal standards (IS), surrogates, and matrix substances that were used for evaluating matrix effects were purchased from Kanto Chemical, Hayashi Pure Chemical, and Sigma-Aldrich Japan. The HPLC-grade ammonium acetate (1 mol L⁻¹) used for HPLC mobile phase was obtained from FUJIFILM Wako Pure Chemical Corporation. LC-MS-grade water was obtained by purifying tap water in an Elga Purelab Chorus 1 Analytical Research (Veolia Water, Tokyo, Japan) water purification system. The solid-phase extraction (SPE) cartridges used were Oasis HLB Plus and Waters Sep-Pak Plus AC2 (Nihon Waters, Tokyo, Japan). Whatman GF/C glass fiber filters (47 mm diameter) were purchased from GE Healthcare (Tokyo, Japan). Millex-LG syringe filters (4 mm, 0.20 μm) were purchased from Merck Millipore (Darmstadt, Germany). A GL-SPE vacuum manifold system was purchased from GL Sciences (Tokyo, Japan). A Sciex ExionLC with a Sciex X500R QTOF System (AB Sciex, Tokyo, Japan) was used for chemical separation and determination. All glassware and plasticware were cleaned with detergent and water, washed in an ultrasonic cleaner, dried, and rinsed with methanol before use.

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TARGET CHEMICALS

The number of target chemicals examined herein is 484; these chemicals are categorized in Table 1 and listed in Table S1. This list includes substances with a wide range of physicochemical properties (log Pow – 1.55–8.53).

SAMPLING

Eight WWTPs representative of those found across Japan that service populations of over 200,000 and treat sewage through the conventional activated sludge process were selected (for technical specifications of the WWTPs, see Table S2). Because sewage inflows considerably vary during the day, 24-h composite samples were collected using Isco GLS composite samplers (Nikkaki Bios, Tokyo, Japan) before the first sedimentation tank (inflow) and after the final sedimentation tank (outflow) in each WWTP; these samples were collected four times in the Japanese 2017 fiscal year (April 1, 2017 to March 31, 2018). The total amount of rainfall in the four days before sampling at each WWTP in each season was below 20 mm, indicating that wastewater was not discharged without treatment from combined WWTPs. During sampling, cold gels were packed in the sampler to keep the sample cool, and after collecting, the sample was brought to a laboratory with cold gels, and the targets were extracted within the same day.

ANALYTICAL PROCEDURES

The analytical processes for the 484 target substances (Table S1) were performed according to Kadokami and Ueno (2019). The surrogate standards comprising a wide range of polarities (Table 1 and Table S1) were added to a water sample (inflow: 50 mL, outflow: 200 mL), and the mixture was filtered using a glass fiber filter. The filtrate was extracted using an Oasis HLB cartridge and a Sep-Pak AC2 cartridge. Next, the cartridges were washed with purified water and dried by nitrogen, following which the targets were eluted with methanol and dichloromethane. The suspended solids (SS) remaining on the filter paper were sonication-extracted (Ultrasonic cleaner USK-3R, AS ONE, Osaka, Japan) with methanol. After combining the eluate and the extract, the mixture was concentrated under a nitrogen stream. To the mixture thus obtained, mixed internal standards comprising a wide range of retention times and matrix standards (standards for examining the degree of the matrix effects, Table 1 and Table S1) were added and the resulting mixture was reconstituted to 500 μL with methanol. The final extract was filtered using a syringe filter prior to LC-QTOF-MS analysis.

Chemical concentrations were determined using LC-QTOF-MS (the relevant conditions are listed in Table S3). We measured samples by Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra Acquisition (SWATH); SWATH provides accurate masses of a precursor ion and product ions without the interference of co-eluted peaks, which is most suitable for simultaneously measuring numerous substances (Kadokami and Ueno, 2019). The method detection limits (MDLs, Table S1) for the substances were estimated from the concentration ratio (ratio of the first volume of a sample to the volume of a final concentrate), and the instrument detection limit (IDL) (Table S1). For 85% of the chemicals, the MDLs were below 8 and 2 ng L⁻¹ for inflow and outflow, respectively.

DATA PROCESSING AND STATISTICAL ANALYSES

Sciex OS was used for the maintenance and control of the LC-QTOF-MS instrument, sample measurement, and all mass data processing. The targets were identified using the mass accuracies of a precursor and product ions, their ratios, and retention times. Quantification of the identified target substance was performed via the internal standard method using six internal standards (Table S1).

Data processing was performed using Microsoft Excel 2016 (Microsoft Japan, Tokyo, Japan), IBM SPSS Statistics 25 (IBM Japan, Tokyo, Japan), and R ver. 3.6.3 (R Core Team, 2020). Principal component analysis (PCA) was performed using R package FactoMineR (Lê et al., 2008). Before PCA analysis, data were scaled based on their mean and standard deviation. Target concentrations below the MDLs were treated as zero.

Table 1 Summary of target substances, detected substances and annual Japan-wide loads, ton y⁻¹

| Class                   | Number of targeta | Number of detected substances | Annual Japan-wide loads |
|------------------------|-------------------|-------------------------------|-------------------------|
|                        |                   | Inflow | Outflow | Inflow | Outflow |
| Pesticide              | 296               | 47     | 56      | 9.7    | 8.1     |
| Pharmaceutical         | 156               | 82     | 88      | 1033.7 | 294.2   |
| Personal care product  | 6                 | 5      | 5       | 199.8  | 72.2    |
| Consumer product       | 12                | 9      | 5       | 554.0  | 246.6   |
| Industrial chemical    | 10                | 4      | 5       | 52.6   | 28.0    |
| Others                 | 4                 | 3      | 2       | 228.7  | 21.9    |
| Total                  | 484               | 150    | 161     | 2078.6 | 671.0   |

|                  |                   |       |         |       |         |
| Internal standard | 6                  |       |         |       |         |
| Surrogateb       | 4                  |       |         |       |         |
| Matrix substancec| 5                  |       |         |       |         |

a = including metabolites; b = substances for evaluating analysis; c = substances for evaluating matrix effects
**ESTIMATION OF INFLOW AND OUTFLOW POPULATION EQUIVALENT LOADS (G 1,000 CAPITA−1 D−1) IN WWTPS AND TOTAL ANNUAL JAPAN-WIDE INFLOW AND OUTFLOW LOADS**

Inflow (outflow) PELs of the detected substances in Japan were calculated via two methods. The first method estimated the PELs using the following equation.

Inflow (outflow) PEL (g 1,000 capita−1 d−1) = Concentration in inflow (outflow) water (ng L−1) x Volume of inflow (outflow) water (m3 d−1) / (Serving population of the surveyed WWTP x 1,000) — (Eq. 1)

After calculating the inflow (outflow) PELs of each WWTP, mean inflow (outflow) PELs are obtained as inflow (outflow) PELs across Japan.

The second method calculated inflow (outflow) PELs using Eq. 2 because the annual volume of the inflow (outflow) water across Japan (15,094 x 106 m3 y−1) and the serving population of all WWTPs in Japan (100.306 x 106) can be obtained from Statistics of Sewage in 2017 (Japan Sewage Works Association, 2019).

Inflow (outflow) PELs across Japan (g 1,000 capita−1 d−1) = Mean concentration in inflow (outflow) water (ng L−1) of the surveyed WWTPs x Annual volume of inflow (outflow) water across Japan (m3 y−1) / (Serving population of all WWTPs in Japan x 365 x 1,000) — (Eq. 2)

Total annual Japan-wide inflow loads (ton y−1) of each substance were calculated as the product of the inflow PELs of each substance by population in Japan (127.388 x 106 in 2017, Japan Sewage Works Association, 2019) + Serving population of all WWTPs in Japan (100.306 x 106) can be obtained from Statistics of Sewage in 2017 (Japan Sewage Works Association, 2019). In contrast, the total annual Japan-wide outflow loads (ton y−1) of each substance were calculated using the following equation (Eq. 3). In Eq. 3, we assumed that removal efficiencies of the combined septic tank system are identical to those of WWTPs (Japan Sewage Works Association, 2019). In the case of the single septic tank, only black water is treated, and gray water is directly discharged into the aquatic environment; therefore, we assumed that removal efficiencies of only pharmaceuticals that are in black water are identical to those of WWTPs.

Total annual Japan-wide outflow loads (ton y−1) of each substance were calculated as the product of the outflow PELs of each substance by population in Japan (11.530 x 106 in 2017, Japan Sewage Works Association, 2019)) x 365 / 1 x 109 — (Eq. 3)

**QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)**

The analytical methods described above were validated using a performance-based approach that included an analysis of procedural blanks and duplicate samples. In addition, we examined recoveries of the surrogates spiked into the samples and the detected amounts of matrix standards spiked into the final samples together with the internal standards (Kadokami and Ueno, 2019).

**RESULTS AND DISCUSSION**

**RESULTS OF QA/QC**

Recoveries of the surrogates and matrix standards are listed in Table S4. In Japan, the official recommendation is that surrogate recovery is acceptable if it is in the range of 50%–120% in samples (Ministry of the Environment, Japan, 2012). Mean recoveries of almost all of the surrogates in blanks and inflow and outflow samples satisfied this criterion. However, recoveries of the surrogates and the matrix standards in inflow and outflow samples were slightly lower than those in blanks, which may be due to matrix effects (Kadokami and Ueno, 2019).

The number of duplicated analyses was 16, of which 14 were inflow samples and two were outflow samples. Most (78%) of the detected substances satisfied the Japan guidelines that state that the difference between two samples is less than 30% of their average even though 15 out of 16 samples were inflow samples containing substantial organic matter (Table S5).

**OVERVIEW OF SURVEY RESULTS OF THE 484 TARGET COMPOUNDS**

The mean number of the detected substances and their mean total concentrations in inflows and outflows were 87 and 92 and 108,517 and 31,537 ng L−1, respectively (Table 2). The main reason for the number of substances detected in outflow samples being larger than that detected in inflow samples are the lower MDLs of outflow samples due to their larger sample volume (200 mL compared to 50 mL). The sum concentration of pharmaceuticals in inflow samples constituted half the total concentration, and the sum of the concentrations of personal care and consumer products constituted 36% of the total even

| Class | Mean number of detected substances | Concentration of inflow, ng L−1 | Concentration of outflow, ng L−1 |
|-------|-----------------------------------|-------------------------------|---------------------------------|
|       | Inflow | Outflow | Min | Max | Mean | RSD, % | Min | Max | Mean | RSD, % |
| Pesticide | 10.3 ± 3.9 | 16.9 ± 5.1 | 101.6 | 1303.3 | 508.5 | 57.9 | 140.2 | 970.9 | 411.6 | 41.4 |
| Pharmaceutical | 59.4 ± 3.7 | 64.2 ± 4.2 | 29242.3 | 79367.0 | 53963.6 | 21.3 | 7469.8 | 2522.1 | 13537.9 | 31.0 |
| Personal care product & Consumer product | 11.7 ± 1.7 | 6.8 ± 1.4 | 25057.7 | 75799.0 | 39357.6 | 33.5 | 8522.6 | 22792.4 | 14370.0 | 26.0 |
| Industrial chemical | 2.4 ± 0.5 | 2.8 ± 0.7 | 917.5 | 8899.8 | 2744.9 | 71.2 | 320.1 | 4568.3 | 1333.9 | 65.4 |
| Others | 2.9 ± 0.3 | 1.0 ± 0.3 | 2706.2 | 26130.9 | 11942.1 | 46.3 | 0 | 255.9 | 64.3 | 101.3 |
| Total | 86.8 ± 7.1 | 91.7 ± 7.8 | 74416.8 | 136349.9 | 108516.8 | 16.2 | 19529.3 | 46510.6 | 31537.6 | 22.0 |

a = Standard deviation; b = Relative standard deviation
though in this case, only 18 substances were involved. In addition, the ratio of personal care and consumer products increased to 46% of the total concentration in outflow samples, indicating their resistance to physical removal and/or degradation in the treatment process. Over 10 pesticides were detected in both types of samples and their concentrations in outflow samples were higher than those in inflow samples.

CONCENTRATIONS OF THE TARGET SUBSTANCES IN INFLOW AND OUTFLOW SAMPLES

Mean detected numbers of pharmaceuticals, personal care and consumer products, and pesticides in inflows were 59 out of 156 substances, 12 out of 18 substances, and 10 out of 296 substances, respectively (Table S6); their mean concentrations were 53,964 (49.7% of the total), 39,358 (36.3% of the total), and 509 (0.5% of the total) ng L$^{-1}$, respectively (Table 2). Pharmaceuticals and personal care and consumer products constituted 86% of the total concentration. Post activated sludge treatment, although the number of the detected substances did not change substantially, the total concentration of chemicals was reduced by 71% (Table 2). The 30 substances of highest concentration in inflows and outflows are listed in Tables 3 and 4, respectively. The detected compounds and their concentrations are consistent with those of previous reports (Miege et al., 2009; Ratola et al., 2012; Loos et al., 2013; Luo et al., 2014; Tsui et al., 2014; Petrie et al., 2015; Kot-Wasik et al., 2016; Yang et al., 2017; Paiga et al., 2019).

ESTIMATION OF INFLOW AND OUTFLOW POPULATION EQUIVALENT LOADS (G 1,000 CAPITA$^{-1}$ D$^{-1}$) IN WWTPS AND TOTAL ANNUAL JAPAN-WIDE INFLOW AND OUTFLOW LOADS

Marx et al. (2015) suggested that analytical data from 20–40 WWTP influent samples per year are needed to reliably estimate annual input load of micropollutants to the WWTP. In this study, to obtain reliable inflow (outflow) PELs in Japan, we analyzed 32 inflow and 32 outflow samples a year at eight WWTPs. The mean inflow (outflow) PELs obtained from Eq. 1 agreed with the mean PELs obtained using Eq. 2 (Table S7); the ratios of PELs obtained from Eq. 1 and Eq. 2 for inflow

| No. | Compound                  | Summer | Autumn | Winter | Spring | Year-around |
|-----|---------------------------|--------|--------|--------|--------|-------------|
| 1   | Metformin                 | 16,830 | 18,545 | 22,485 | 22,335 | 31,567      |
| 2   | Theophylline              | 12,192 | 12,023 | 10,863 | 10,902 | 26,158      |
| 3   | Theobromine               | 6,909  | 11,578 | 15,603 | 10,875 | 25,557      |
| 4   | Sucralose                 | 13,596 | 10,260 | 8,410  | 10,017 | 9,330       |
| 5   | Distyrylbiphenyl disulfonate (FB351) | 7,221 | 9,663 | 11,829 | 8,607 | 9,330 |
| 6   | N,N-Dimethyldodecylamine N-oxide | 3,092 | 6,563 | 14,760 | 8,580 | 8,248 |
| 7   | Fluorescent brightener 71 (FB71) | 4,525 | 8,224 | 5,589  | 5,646 | 5,996 |
| 8   | Salicylamide              | 2,697  | 4,050  | 4,861  | 3,330  | 3,923       |
| 9   | 1H-Benzotriazole          | 3,786  | 2,274  | 1,331  | 2,149  | 2,385       |
| 10  | Bezafibrate               | 1,969  | 2,287  | 2,230  | 2,588  | 2,186       |
| 11  | 4-Hydroxybenzoic acid-methyl ester | 1,761 | 2,074 | 2,800  | 1,506  | 2,035       |
| 12  | Acetaminophen             | 1,197  | 1,849  | 1,830  | 1,603  | 1,620       |
| 13  | Diphenhydramine           | 1,277  | 1,228  | 2,390  | 1,270  | 1,541       |
| 14  | Sulpiride                 | 1,515  | 1,100  | 1,351  | 1,700  | 1,432       |
| 15  | Cotinine                  | 1,264  | 1,318  | 1,431  | 1,468  | 1,370       |
| 16  | Ibuprofen                 | 776    | 1,161  | 2,066  | 1,031  | 1,256       |
| 17  | Clarithromycin            | 562    | 1,281  | 1,659  | 1,167  | 1,167       |
| 18  | Levofloxacin/Ofloxacin    | 983    | 984    | 1,187  | 1,268  | 1,106       |
| 19  | Ethylhexyl methoxyccinnamate | 1,070 | 542   | 834    | 907    | 838         |
| 20  | Lidocaine base            | 525    | 672    | 1,328  | 732    | 1,917       |
| 21  | Ketoprofen                | 627    | 630    | 467    | 669    | 1,194       |
| 22  | Ampicillin trihydrate    | 533    | 718    | 575    | 536    | 1,379       |
| 23  | Azithromycin              | 337    | 468    | 926    | 542    | 1,786       |
| 24  | Taurodeoxycholic acid     | 596    | 400    | 667    | 535    | 1,263       |
| 25  | Epinastine                | 204    | 234    | 402    | 670    | 1,383       |
| 26  | Etodolac                  | 856    | 117    | 301    | 144    | 1,632       |
| 27  | Furosemide                | 243    | 381    | 490    | 272    | 899         |
| 28  | 4-&5-Methyl-1H-benzotriazole | 358  | 274    | 359    | 356    | 1,015       |
| 29  | Disopyramide              | 503    | 217    | 378    | 241    | 2,579       |
| 30  | Cimetidine                | 281    | 310    | 312    | 432    | 632         |

Table 3 The 30 highest concentration substances in inflows, ng L$^{-1}$
Inflow and outflow loads of 484 chemicals

The sums of all inflow (outflow) PELs obtained using Eq. 1 and Eq. 2 were also in good agreement: 43.8 and 44.7 (13.2 and 13.0) g 1,000 capita−1 d −1 , respectively. Thus, we used the PELs obtained by Eq. 2 for calculating the total annual Japan-wide inflow (outflow) loads (ton y−1 ).

Several papers have reported inflow and/or outflow PELs of PPCPs in Italy (Castiglioni et al., 2006), Switzerland (Hollender et al., 2009), Greece (Papageorgiou et al., 2016), India (Subedi et al., 2015), Korea (Subedi et al., 2014), and China (Heeb et al., 2012; Qi et al., 2015; Yan et al., 2019). Although inflow PELs of most pharmaceuticals are generally similar to the PELs obtained herein, inflow PEL of sucralose, an artificial sweetener, in Japan was much higher than that in India (Table 5), indicating that amounts of food additives used may differ among countries. Inflow PELs of some of the pharmaceuticals have been reported to be markedly different even within the same country because of differences in the economic and social factors (Yan et al., 2019), e.g., significant spatial variations (5.54 ± 4.28 mg d −1 capta −1) in the consumption of metformin in north-east China owing to differences in the per capita disposable income of urban residents. In that context, the spatial variations (8.16 ± 2.62 mg d −1 capta −1) in the consumption of metformin in Japan suggested by this study’s data were less than half of those observed in the case of north east China. Additionally, 39.9% mean spatial variation was detected in the consumption of 47 substances detected in all samples. This relatively low spatial variation may indicate economic and social uniformity in Japan.

In combined sewer systems during/after heavy rain events, most inflows to WWTPs bypass the system and are discharged into the aquatic environment without any treatment (Phillips et al., 2012; Launay et al., 2016). Herein, we did not assume overflow owing to a lack of data. Therefore, actual outflow loads released into the aquatic environment are likely to be larger than the estimated loads of the total annual Japan-wide inflow load (2,079 tons y−1) and outflow load (671 tons y−1) (Tables 1 and S8). Fig. 1 and 2 show the 30 largest inflow and outflow load substances, respectively. The annual Japan-wide inflow (outflow) loads of pharmaceuticals reached nearly half of the total load and considering their biological activities, they

| No. | Compound                          | Summer Mean | Autumn Mean | Winter Mean | Spring Mean | Det freq. % | Min | Max | Mean |
|-----|-----------------------------------|-------------|-------------|-------------|-------------|-------------|-----|-----|------|
| 1   | Sucralose                         | 11,317      | 8,910       | 7,813       | 8,650       | 100         | 4,787| 16,480| 9,172|
| 2   | Metformin                         | 4,773       | 5,921       | 6,749       | 5,873       | 100         | 1,838| 13,719| 5,829|
| 3   | Distyrylbiphenyl disulfonate (FB351) | 3,257      | 3,408       | 2,504       | 3,091       | 100         | 603  | 6,244 | 3,065|
| 4   | Fluorescent brightener 71 (FB71)  | 1,666       | 1,872       | 1,423       | 1,589       | 100         | 314  | 5,500 | 1,637|
| 5   | Sulpiride                         | 1,172       | 1,098       | 1,081       | 1,257       | 100         | 590  | 2,033 | 1,152|
| 6   | 1H-Benzotriazolamide              | 1,524       | 938         | 524         | 852         | 100         | 113  | 3,680 | 960  |
| 7   | Clarithromycin                    | 356         | 814         | 1,166       | 765         | 100         | 220  | 1,551 | 775  |
| 8   | Diphenhydramine                   | 620         | 609         | 963         | 776         | 100         | 287  | 1,412 | 742  |
| 9   | Guanyurea                         | 393         | 1,131       | 483         | 739         | 84          | 0    | 2,584 | 687  |
| 10  | Lidocaine base                    | 442         | 517         | 750         | 632         | 100         | 359  | 855  | 585  |
| 11  | Levofloxacin/Olofoxacin           | 369         | 381         | 425         | 491         | 100         | 54   | 1,016 | 416  |
| 12  | Azithromycin                      | 284         | 337         | 547         | 468         | 100         | 111  | 1,100 | 409  |
| 13  | Disopyramide                      | 696         | 257         | 368         | 244         | 100         | 92   | 4,319 | 391  |
| 14  | Epinastine                        | 221         | 240         | 350         | 656         | 100         | 139  | 1,301 | 367  |
| 15  | Ampicillin trihydrate             | 318         | 480         | 340         | 312         | 94          | 0    | 1,068 | 363  |
| 16  | Furosemide                        | 215         | 308         | 462         | 267         | 100         | 35   | 824  | 313  |
| 17  | Sulfapyridine                     | 285         | 266         | 270         | 344         | 100         | 79   | 755  | 291  |
| 18  | N,N-Dimethylldodecylamine N-oxide | 455         | 201         | 146         | 325         | 63          | 0    | 2,963 | 282  |
| 19  | 4- & 5-Methyl-1H-benzotriazolide  | 278         | 284         | 284         | 247         | 100         | 99   | 803  | 274  |
| 20  | Bezafibrate                       | 172         | 267         | 245         | 347         | 100         | 15   | 1,267 | 258  |
| 21  | Ketoprofen                        | 202         | 234         | 229         | 252         | 97          | 0    | 683  | 229  |
| 22  | Atenolol                          | 192         | 199         | 207         | 237         | 100         | 121  | 344  | 209  |
| 23  | Hydrochlorothiazide               | 150         | 193         | 172         | 187         | 100         | 93   | 329  | 175  |
| 24  | Cimetidine                        | 137         | 161         | 166         | 203         | 78          | 0    | 426  | 167  |
| 25  | Amitriptyline                     | 146         | 138         | 158         | 161         | 100         | 48   | 295  | 151  |
| 26  | Diclofenac                        | 143         | 157         | 118         | 174         | 100         | 56   | 280  | 148  |
| 27  | 4-Hydroxybenzoic acid-methyl ester| 520         | 6           | 0           | 0           | 22          | 0    | 1,179 | 131  |
| 28  | Carbendazim                       | 128         | 118         | 104         | 116         | 100         | 21   | 234  | 117  |
| 29  | Sulfasalazine                     | 88          | 131         | 125         | 114         | 100         | 15   | 283  | 114  |
| 30  | Roxithromycin                     | 65          | 105         | 143         | 141         | 100         | 30   | 341  | 114  |

Table 4 The 30 highest concentration substances in outflows, ng L−1
indicated a possibility of effects on the aquatic ecosystem. Emission amounts of pesticides, which also have strong bioactive activities, were 8.1 tons y\(^{-1}\) discharged into the aquatic environment through human activities excluding agricultural activities.

### COMPARISON BETWEEN ANNUAL JAPAN-WIDE INFLOW LOADS TO POLLUTANT RELEASE AND TRANSFER REGISTER DATA AND SALES DATA

In Japan, the annual amounts of 462 PRTR substances that had been transferred to WWTPs in the whole country are reported (Ministry of Economy, Trade and Industry, Japan, 2019). However, PRTR substances in consumer products are not eligible for the PRTR law. Majority of the non-pesticide substances targeted herein are not designated as PRTR substances. Consequently, inflow loads obtained in this study were much higher than those published by the PRTR law. In contrast, the Ministry of Health, Labour and Welfare, Japan publishes annual information on the volume of pharmaceuticals sold in Japan (Ministry of Health, Labour and Welfare, Japan, 2019). However, as the absorption, metabolism, and excretion of pharmaceuticals are not well known (Marx et al., 2015), assuming that the pharmaceuticals used will be discharged to WWTPs after use in either their active or metabolized form is reasonable. However, grasping the exact amounts of substances discharged from our daily-life and business activities to WWTPs and the aquatic environment using the existing system including PRTR is difficult. Moreover, the target screening analysis is highly useful in estimating the amount of substances such as daily-use chemicals including PPCPs discharged to WWTPs and the aquatic environment.

### EXAMINATION OF SPATIAL AND SEASONAL DIFFERENCES IN THE SUBSTANCES DETECTED IN INFLOWS

The eight sampling WWTPs are located from the southwest to the north of Japan (1,500 km long), and the climatic...
Inflow and outflow loads of 484 chemicals

Conditions they experience are very different from each other (average temperature in 2017: 7.1°C to 16.8°C) both within and between seasons. Consequently, spatial and seasonal differences in the detected substances and in their emission amounts are to be expected. In that context, the cluster analysis (CLA) revealed that the same WWTPs were classified in the same group even in different seasons (Fig. 3), indicating that spatial differences in chemical usage are more important than seasonal differences. This result was confirmed through PCA analysis; the score plot of PC1 and PC2 classified WWTPs into eight groups corresponding to individual WWTPs (Fig. 4–1). The score plots of PC2 and PC3 classified WWTPs into four groups corresponding to four seasons, with winter and summer being clearly separated (Fig. 4–2). The cause for the larger spatial differences than seasonal differences is the larger contribution rate of PC1 (20.5%) compared to PC3 (9.1%). The reason may be that most of the detected substances are PPCPs, and as such the type and volumes used differ between...
regions, but do not vary much throughout a year in the same area. This is consistent with Choi et al. (2019) and Yan et al. (2019) who reported that chemicals in wastewater reflect the social, demographic, and economic properties of the respective populations. However, seasonal differences existed within the WWTP groups. For example, emission loads of 21 out of 88 substances with detection frequencies >50% in summer and winter were significantly lower in summer than in winter; in particular, erythromycin (antibiotic) was detected only in winter. Only three substances, etodolac, sucralose, and 1H-benzotriazole, were discharged in significantly larger amounts in summer compared to winter. These results show that some substances have seasonality owing to climate conditions and infectious disease epidemics (Tsui et al., 2014; Sun et al., 2016).

Inflow loads are directly related to chemical concentrations in influent water, which subsequently change in accordance with changes in consumption and influent water volume. The latter is not constant, showing daily and seasonal temperature variations. For example, in northern Kyushu, the influent volume in summer (June to September) is ~35% larger than that in winter (December to February) (Kitakyushu City Water and Sewer Bureau, 2019). To clarify seasonal change in chemical concentrations, we compared concentrations in inflows between the four seasons. In that context, total chemical concentrations in summer, autumn, and spring were 74.5%, 85.2%, and 86.0%, respectively, of winter concentrations, reflect-

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**Fig. 3** Dendrogram of all the influent samples by cluster analysis using the 86 substances with above 50% of detection frequency Ward method and squared Euclidean distance.
Inflow and outflow loads of 484 chemicals

Fig. 4–1 Score plot of PC1 and PC2 by principle component analysis using the 86 substances with above 50% of detection frequency

Fig. 4–2 Score plot of PC2 and PC3 by principle component analysis using the 86 substances with above 50% of detection frequency
ing differences in ambient temperatures and inflow volume (Table S9). However, when focusing on each chemical, several different seasonal changes were detected. Cotinine (metabolite of nicotine), testosterone (androgen), and metformin (antidiabetic drug), which would not be expected to have seasonal changes in usage, showed the same seasonal changes as those observed in the sum of concentrations. Concentrations of succralse (artificial sweetener), 1H-benzotriazole (corrosion inhibitor), benzophenone-3 (UV filter), fungicides, and herbicides were lower in winter than those in other seasons, indicating the lower consumption amounts in the colder season. Interestingly, seasonal changes among drugs classified as non-steroidal anti-inflammatory drugs (NSAIDs) were different from each other. Ibuprofen and acetaminophen exhibited the highest concentration in the colder seasons, indicating the higher prevalence of mild fevers in the season of cold and flu. In contrast, concentrations of ketoprofen and indomethacin were lowest in winter because these are used for after-exercise troubles.

**IDENTIFYING INDICATOR SUBSTANCES FOR ESTIMATING DE FACTO POPULATION SERVED BY A SEWAGE TREATMENT PLANT AT A SAMPLING TIME**

Population is one of the most important statistical data to know a society. Population is usually defined as de jure population by a census. However different from residence areas, de facto population in city centers, particularly in shopping and business areas, largely varies between the daytime and the nighttime, weekdays and weekend, and seasons; this is because numerous people enter and exit the city center for work, study, shopping, and sightseeing. Census cannot fully account for these temporal fluctuations. Therefore, the de facto population estimation methods based on amounts of chemicals present in sewage have been reported as WBE (Daughton, 2012). To accurately estimate the de facto population, chemicals that do not exhibit temporal and spatial changes are needed; thus, several substances were recommended as markers for WBE. These are classified into two groups: endogenous substances such as creatinine and coprostanol (Daughton, 2012) and exogenous substances such as caffeine, nicotine (Chen et al., 2014; Senta et al., 2015), and PPCPs (O’Brien et al., 2014; Gracia-Lor et al., 2017). In that context, 13 substances were selected owing to their low relative standard deviations (< 40%) in inflow loads (Table 6). However, de facto population estimation using a single marker can increase error because their PELs are different in locations and/or seasons; in contrast, estimation using multiple markers including substances measured via GC-MS may be appropriate to accurately estimate the de facto population.

**IDENTIFYING MARKER COMPOUND CANDIDATES FOR SEWAGE CONTAMINATION**

Domestic wastewater is one of the major water pollution sources for surface water and groundwater. Groundwater pollution occurs even in reticulated sewage treatment systems owing to the leakage of wastewater from sewer pipes (Musolff et al., 2008). Identifying the source of groundwater pollution is more difficult compared to identifying that of surface water; hence, molecular markers that are characteristic of domestic wastewater are required. Molecular markers should satisfy the following three conditions: they must exist only in sewage, at high concentration, and be resistant to decomposition. In that context, we identified 22 candidates that were detected in all the outflows at above 100 ng L\(^{-1}\) (Table 7). Of these, succralse has been reported as a good marker of domestic wastewater (Oppenheimer et al., 2011), but 16 were pharmaceuticals that, to the best of our knowledge, have not been used previously as molecular markers for sewage contamination. Because their used amounts are different among locations including countries, the more are the markers, the better is the detection of domestic wastewater pollution. Namely, if one or more of these substances are detected in the environmental waters, it is suspected that the water is polluted with domestic wastewater.

**EXAMINATION OF REMOVAL EFFICIENCIES OF THE DETECTED CHEMICALS**

The activated sludge method was used to treat the eight WWTPs surveyed herein (Table S2). Although the activated

| No. | Compound            | Inflow PEL, g 1,000 capita\(^{-1}\) d\(^{-1}\) | RSD\(^{a}\), % |
|-----|---------------------|-----------------------------------------------|----------------|
| 1   | Cotinine            | 0.565                                         | 26.5           |
| 2   | Acetaminophen       | 0.668                                         | 28.1           |
| 3   | Levofloxacin/Ofloxacin | 0.456                                   | 34.0           |
| 4   | Testosterone        | 0.0623                                        | 34.9           |
| 5   | Sucralse            | 4.36                                          | 36.2           |
| 6   | Sulpiride           | 0.590                                         | 36.4           |
| 7   | Theophylline        | 4.74                                          | 37.0           |
| 8   | Chlorpheniramine maleate | 0.0498                                 | 37.1           |
| 9   | Sulfamethoxazole    | 0.0547                                        | 37.5           |
| 10  | Atenolol            | 0.137                                         | 38.1           |
| 11  | Clarithromycin      | 0.481                                         | 38.4           |
| 12  | Candesartan         | 0.0758                                        | 39.3           |
| 13  | Metformin           | 8.27                                          | 39.4           |

\(a\) – Relative standard deviation
Table 7 Candidates of marker substance for sewage contamination

| No. | Compound                                      | Mean concentration, ng L⁻¹       | Removable efficiency, % |
|-----|-----------------------------------------------|----------------------------------|-------------------------|
|     |                                               | Influent | Effluent |                           |
| 1   | Sucralose                                     | 10,571   | 9,172    | 8.6                      |
| 2   | Metformin                                     | 20,049   | 5,829    | 66.7                     |
| 3   | Distyrylbiphenyl disulfonate (FB351)          | 9,330    | 3,065    | 64.5                     |
| 4   | Fluorescent brightener 71 (FB71)              | 5,996    | 1,637    | 68.9                     |
| 5   | Sulpiride                                     | 1,432    | 1,152    | 15.5                     |
| 6   | Clarithromycin                                | 1,167    | 775      | 31.3                     |
| 7   | Diphenhydramine                               | 1,541    | 742      | 39.9                     |
| 8   | Lidocaine base                                | 814      | 585      | 19.5                     |
| 9   | Levofloxacin/Olofoxacin                       | 1,106    | 416      | 62.8                     |
| 10  | Azithromycin                                  | 568      | 409      | 21.4                     |
| 11  | Disopyramide                                  | 335      | 391      | 10.5                     |
| 12  | Epinastine                                    | 378      | 367      | 7.0                      |
| 13  | Furosemide                                    | 346      | 313      | 6.2                      |
| 14  | Sulfapyridine                                 | 310      | 291      | 4.6                      |
| 15  | Beazafibrate                                  | 2,186    | 258      | 87.6                     |
| 16  | Atenolol                                      | 333      | 209      | 32.6                     |
| 17  | Amantadine                                    | 135      | 151      | 20.5                     |
| 18  | Diclofenac                                    | 165      | 148      | 3.0                      |
| 19  | Sulfasalazine                                 | 185      | 114      | 26.4                     |
| 20  | Roxithromycin                                 | 110      | 114      | 21.2                     |
| 21  | Chlorpheniramine maleate                      | 121      | 107      | 6.0                      |
| 22  | Indomethacine                                  | 143      | 104      | 18.1                     |

Fig. 5 Boxplots of removal efficiencies of chemicals detected in all the inflow and outflow samples (32 each)
The lines within the box mark the median, boundaries indicate the 25th and 75th percentiles, error bars indicate the maximum and the minimum, and circles and asterisks are outliers.

Sludge method, a biological treatment, is the most used treatment system because of its high removal efficiency of natural organic matter and nutrients discharged from domestic sources, its removal efficiencies in the case of heavy metals and a part of artificial organic substances are lower than those of natural organic substances (e.g., Luo et al., 2014; Komesli et al., 2015; Petrie et al., 2015; Wang and Wang, 2016; Thiebault et al., 2017; Yang et al., 2017). Some micropollutants, including
heavy metals and pesticides in wastewater discharged from facilities to the sewerage system, are therefore regulated in Japan. In contrast, pharmaceuticals and most pesticides targeted herein are not regulated. Because these substances have biological activities, investigating their removal efficiencies in addition to their concentrations in WWTP effluent is useful for conserving the aquatic ecosystem.

In this study, 137 substances were detected in both the inflow and outflow at the same WWTP (Table S10, Fig. 5). Of these 137 substances, 12% substances were removed at the same level as natural products. The median removal efficiency was 31.3%: 29.2% for pharmaceuticals and 20.2% for pesticides, indicating that the activated sludge method cannot fully cope with artificial chemicals. Some chemicals with very low removal efficiencies may be regenerated by the decomposition of their conjugates during water treatment (Calisto and Esteves, 2009; Blair et al., 2015). Others, such as guanlyurea, a decomposition product of metformin produced during activated sludge treatment (Scheurer et al., 2012; Tisler and Zwiener, 2019), are detected at high concentrations only in the outflow in water. The median of removal rates of 44 polar pesticides was 20.2%, and 19 pesticides had negative removal rates, particularly thiamethoxam, tebuconazole, and tebuthiuron (Table S10). This is consistent with the work of Kock-Schulmeyer et al. (2013). Possible explanations for these negative removal rates are, among many others (e.g., sampling time, sample volume, and method biases), the deconjugation of metabolites and/or transformation products, hydrolysis, and desorption from particulate matter during wastewater treatment. Therefore, although most polar pesticides are designed to be readily decomposed in the environment, many are not decomposed and/or adsorbed on sludge through the conventional activated sludge method. Overall, our data suggests that, while activated sludge treatment is very good at removing organic matter, other treatment methods need to be added, e.g., ozonation (Eggen et al., 2014; Luo et al., 2014; Wang and Wang, 2016; Zhang et al., 2017; Allinson et al., 2018) to remove multiple chemicals of concern from the WWTP effluent.

CONCLUSION

To estimate the amount of chemicals used in our daily-life and business activities that are discharged by Japanese society, we examined 484 chemicals in inflows and outflows of eight WWTPs across Japan for a year. The mean number of the detected substances and their mean total concentrations in inflows and outflows were 87 and 92 and 108,517 and 31,537 ng detected substances and their mean total concentrations in inflows; Table S3, LC-QTOF-MS conditions; Table S4, Recovery of surrogates and matrix substances; Table S5, Results of duplicated analyses; Table S6, Concentration (ng L\(^{-1}\)) and detected frequency (%); Table S7, Comparison of inflow and outflow population equivalents (g 1,000 capita\(^{-1}\) d\(^{-1}\)) calculated using Eq. 1 and Eq. 2; Table S8, Annual inflow and outflow loads (ton y\(^{-1}\), 95% confidential interval) across Japan; Table S9, Seasonal change in concentrations of inflows; Table S10, Removal efficiency (%) of substances detected in both samples of the same WWTP. This material is available on the Website at https://doi.org/10.5985/emcr.20200002.

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SUPPLEMENTARY MATERIAL

Table S1, List of target compounds; Table S2, Specifications of the surveyed sewage treatment plants; Table S3, LC-QTOF-MS conditions; Table S4, Recovery of surrogates and matrix substances; Table S5, Results of duplicated analyses; Table S6, Concentration (ng L\(^{-1}\)) and detected frequency (%); Table S7, Comparison of inflow and outflow population equivalents (g 1,000 capita\(^{-1}\) d\(^{-1}\)) calculated using Eq. 1 and Eq. 2; Table S8, Annual inflow and outflow loads (ton y\(^{-1}\), 95% confidential interval) across Japan; Table S9, Seasonal change in concentrations of inflows; Table S10, Removal efficiency (%) of substances detected in both samples of the same WWTP.

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