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Occurrence and Fate of Anti-inflammatory Drugs in Wastewater Treatment Plants in Japan

Norihide Nakada*, Koya Komori and Yutaka Suzuki

Water Environment Research Group, Public Works Research Institute
1-6 Minami-hara, Tsukuba, Ibaraki 305-8516, Japan

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The fates of anti-inflammatory drugs (e.g., ibuprofen, naproxen, mefenamic acid and ketoprofen), which are frequently detected in the discharges of wastewater treatment plants (WWTPs) and river water in Japan, were clarified in two WWTPs. The concentrations of ibuprofen, naproxen, mefenamic acid and ketoprofen were 69–1080, 179–305, 143–1580 and 160–1060 ng/L in the influent, and N.D. (< 40 ng/L), 74–166, 72–265, 64–107 ng/L in the effluent, respectively. The concentrations of the anti-inflammatory drugs analyzed were almost equal to or lower than those reported in foreign countries. High removal efficiencies of the drugs, except ibuprofen, were observed in the WWTP that has longer hydraulic retention time than that of the other WWTP. For ibuprofen, high removal efficiencies were observed in both WWTPs (84 to 98%). Disinfection by chlorination was not effective to remove the drugs surveyed. On the other hand, the effective removal of ketoprofen by ultraviolet (UV) radiation for disinfection was demonstrated, although the disinfection by-products were not identified.

1. Introduction

Recently, there has been a growing public interest in the emergence of environmental pollution of water sources by pharmaceuticals and personal care products (PPCPs).[1] Some European countries are addressing this problem through systematic research and investigation mainly on the status of PPCP pollution of drinking water sources, both tap water and ground water and on treatment technologies for removing PPCPs.[2] In Japan, there is currently no systematic investigation being conducted regarding this problem. Only a limited number of research groups are engaged in fragmentary research investigations on the level of PPCP pollution in the influent and effluent from wastewater treatment plants (WWTPs)[3–5] and river water.[5,6]

*E-mail: Nakada55@pwri.go.jp

PPCPs are physiologically active by nature. It is therefore safe to assume that they have some pharmacological actions on living systems, particularly human beings. PPCPs undergo animal and clinical testings to obtain safety certifications before they are marketed. However, such tests do not measure the potential impact of PPCPs on aquatic life when discharged into a water environment. Considering the route PPCPs take to reach aquatic life, WWTPs may facilitate their initial entry into water environments. If PPCPs are not properly treated during the sewerage treatment process, they can contaminate aquatic environments, including drinking water sources.

Considering the current level of knowledge regarding this problem, it is necessary to clarify the fates of PPCPs in WWTPs to identify appropriate treatment methods. In our research, we analyzed the presence of anti-inflammatory drugs, a class of drugs used widely among the general public, in two WWTPs to clarify the concentrations of PPCPs present in a wide variety of forms.

Our final goal was demonstrate the fate of selected anti-inflammatory drugs along the different units of municipal WWTPs. This included the understanding of the removal properties of each anti-inflammatory drug in the WWTPs.

2. Materials and Methods

2.1 *Chemicals*

Aspirin and mefenamic acid were purchased from Kanto Kagaku (Tokyo, Japan) and from Wako Chemicals (Osaka, Japan), respectively. Ketoprofen, ibuprofen, dicrofenac, naproxen, and fenoprofen were obtained from Tokyo Kasei (Tokyo, Japan).

2.2 *Study sites*

The surveys were conducted in two WWTPs in Kanagawa prefecture, Japan in July and September 2004. The details regarding population size serviced, water flow and operating conditions for the WWTPs are summarized in Table 1. The WWTPs mainly receive domestic wastewater from each treatment and wastewater from local industries. The contributions of industrial wastewaters to the total water flow are approximately 10% for WWTP-1 and 2% for WWTP-2. WWTP-1 performs wastewater treatment by conventional activated sludge treatment followed by chlorine disinfection (Fig. 1). WWTP-2 adopts the conventional activated sludge treatment, and then the secondary effluent is subjected to biofiltration followed by UV disinfection (Fig. 1).

Table 1
Population serviced, wastewater flow, and operational parameters of WWTPs.

WWTP	Population serviced (persons)	Water flow (m^3/day)	Hydraulic retention time (h)	Sludge retention time (day)
WWTP-1	30,500	13,400	9.1	5.8
WWTP-2	70,600	21,200	12	9.3

2.3 *Sample collection and preparation*

Twenty four-hour water flow proportional composite samples were collected from the nine and seven sampling locations in each WWTP (Fig. 1). The samples of the 24-h water flow proportional composites of the return sludge from the final sedimentation tank, the return flow from the sludge dewatering process, and the drained sludge from the primary settling tank were collected particularly in WWTP-1. The aeration tank samples were collected from two locations in the WWTPs: from the mid-distance point (AT_M) and from the end of the tank (AT_E). All the samples were stored in a refrigerator or in a chest containing iced water and were immediately transported to the laboratory. Then, the samples were filtered through glass fiber filters (GF/B, pore size: 1.0 μm) within 24 h after collection. The filters were wrapped with clean aluminum foil and stored in –30°C until extraction of the drugs.

2.4 *Solid-phase extraction*

The filtered samples were analyzed for the presence of anti-inflammatory drugs by solid-phase extraction.[3] In brief, the filtrates were adjusted to pH 2.0 with 4N HCl and then subjected to solid-phase extraction using tC18 cartridge containing 900 mg of octadecyl silica gel (Waters, MA, USA). The cartridge was conditioned with 20 mL of methanol and 20 mL of water (pH 2.0). The samples were extracted at a flow rate of 10 mL/min. Next,

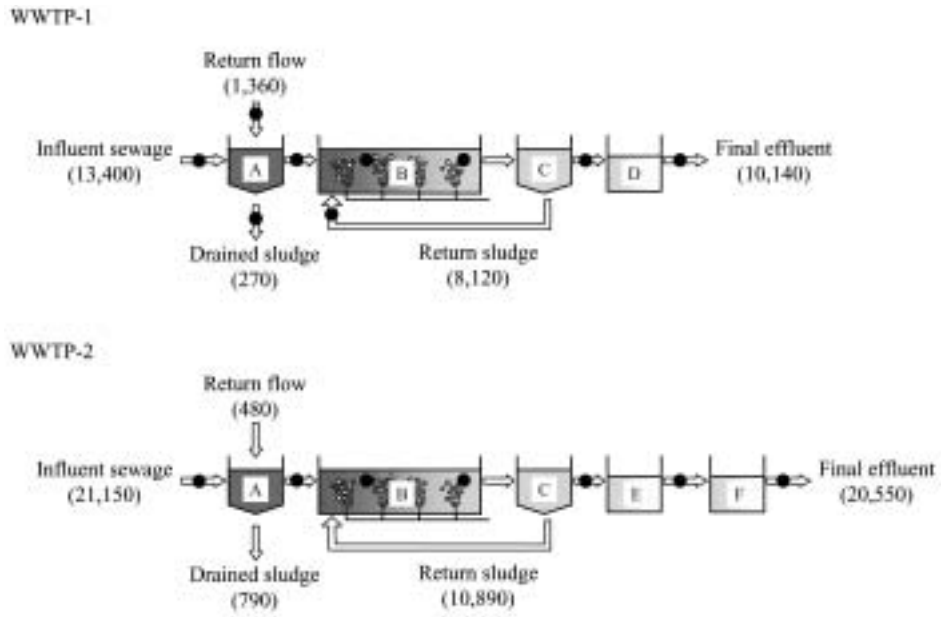

Fig. 1. Sampling location (closed circle) in municipal sewage treatment plant. A: Primary settling tank; B: Aeration tank; C: Final sedimentation tank; D: Chlorination tank; E: Biofiltration tank; F: Ultraviolet radiation tank. The primary settling tanks receive return flow from sludge dewatering process. Numbers in parentheses indicate water flow data (m^3/day) at each location.

the cartridge was dried by centrifugation (3000 rpm × 15 min) and by placing in a nitrogen stream for 15 min, and the analytes were eluted with 15 mL of methanol. The extract is defined as the dissolved-phase sample.

2.5 *Ultrasonic extraction*

The filter samples containing suspended particles were subjected to ultrasonic extraction with the appropriate volumes of methanol (15 min) and acetone (15 min × 2), successively. After the extraction, the extracts were filtered using the glass fiber filter and the filtered extracts were termed suspended-phase samples.

2.6 *Derivatization*

Both the extracts of the dissolved- and suspended-phase samples were transferred to a 1.5-mL glass vial and concentrated to dryness under gentle stream of nitrogen. The concentrated extracts were derivatized with BF_3 in methanol (GL Science, Tokyo, Japan) for 5 h at 80°C. After the derivatization, the derivatized extracts in the vial were liquid-extracted by hexane and water three times. All hexane layers were then dewatered through a column containing anhydrous sodium sulfate.

2.7 *Purification by column chromatography*

The derivatized extracts were purified by column chromatography as described in ref.3 with a minor modification. In this study, a silica gel cartridge (690 mg, Sep-pack Si, Waters) was used for the purification. The cartridge had been washed with hexane (5 mL × 2) prior to use. The derivatized extracts were concentrated just to dryness and then pipetted onto the silica gel column. The column was eluted with 5 mL each of hexane, dichloromethane (DCM), and DCM/acetone (7:3, v/v), and then three fractions were obtained separately. The second and third fractions were analyzed by gas chromatography-mass spectrometry (GC-MS).

2.8 *Analysis by GC-MS*

Analysis of the anti-inflammatory drugs was conducted.[3] The fractions were carefully concentrated by subjecting them to a gentle flow of nitrogen and redissolved in an appropriate volume (500–3000 μL) of isooctane solution containing 50 ng/mL naphthalene-d_8, phenanthrene-d_{10} and *p*-terphenyl-d_{14} (Wako Chemicals) as an internal standard. The anti-inflammatory drugs were analyzed by GC-MS, using a Hewlett-Packard 5973 quadrupole MS fitted with an HP 6890 GC under a selected-ion monitoring condition.

3. Results and Discussion

3.1 *Analytical precision*

Table 2 shows the analytical precision of each measurement component applied to the analysis of the influent and effluent wastewaters. The recovery rates of all drugs excluding aspirin and dicrofenac were between 54% and 157%. Although some of the drugs exceeded a 100% recovery rate, a reproducibility rate of less than 17% was achieved for every drug. Aspirin and dicrofenac were excluded from the list of the anti-inflammatory drugs, as their recovery rates were extremely low in the addition-recovery test.

Table 2
Analytical precision for each anti-inflammatory drug (%).

Compound	Influent		Effluent	
	Recovery	Reproducibiliy	Recovery	Reproducibiliy
Aspirin	N.A.		N.A.	
Ibuprofen	74	17	52	10
Fenoprofen	96	1	79	8
Naproxen	70	8	58	3
Mefenamic acid	147	6	77	16
Ketoprofen	80	8	54	10
Dicrofenac	N.A.		N.A.	

N.A.: not available

3.2 *Removal efficiencies of SS, BOD and COD*

The removal efficiencies of suspended solid (SS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were 98%, 99% and 91% for WWTP-1 and 100%, 99% and 92% for WWTP-2, respectively, as reported by the operator of each plant.

3.3 *Occurrence of anti-inflammatory drugs*

The results of the analysis of the anti-inflammatory drugs, as well as the limit of quantification (LOQ) for each analyte, are shown in Table 3. All of the anti-inflammatory drugs, except for fenoprofen, were detected. The concentrations of the drugs in the influent of WWTP-2 were generally higher than those in the influent of WWTP-1. In particular, the concentrations of ibuprofen, mefenamic acid and ketoprofen in the WWTP-2 influent were 10-fold higher than those in the WWTP-1 influent. None of the drugs was detected in the suspended water sample.

Previous studies showed that the ibuprofen concentrations in the influent and effluent were 14.2–58.9 μg/L and 0.3–24.6 μg/L, respectively, in a WWTP in Canada,[7] and 2.64–5.70 μg/L and 0.91–2.1 μg/L, respectively, in a WWTP in Spain.[8] In another study, the ibuprofen concentrations in the effluent were 0.37(median)–3.4 μg/L in a WWTP in Germany.[9] The ibuprofen concentrations in our research in the influent and effluent were 69–1090 ng/L and N.D. (< 40 ng/L), respectively. The ibuprofen concentration in the influent is almost equal to or one order of magnitude lower than those in previous reports, whereas the ibuprofen concentration in the effluent is one to three orders of magnitude lower than those in previous reports.

The naproxen concentrations in our research were 179–305 ng/L in the influent and 74–166 ng/L in the effluent. In a WWTP in Spain, the naproxen concentrations were 1.79–4.6 μg/L in the influent and 0.8–1.85 μg/L in the effluent,[8] whereas in WWTPs in Germany, the concentrations were 0.3(median)–0.52 μg/L for naproxen in the effluent.[9] Our concentration data are approximately a factor of 10 lower for the influent and between a factor of 10 and 1000 lower in the effluent than those reported in the literature.

In a Canadian WWTP survey, it was reported that the ketoprofen concentration in the influent was 5.7 μg/L.[7] The ketoprofen concentrations in the effluents of two different

Table 3
Fates of pharmaceuticals along wastewater treatment processes and limits of quantification for each anti-inflammatory drug[a].

Plant ID	Sample[b]	Ibuprofen (ng/L)	Fenoprofen (ng/L)	Mefenamic acid (ng/L)	Ketoprofen (ng/L)	Naproxen (ng/L)
WWTP-1	Influent	69	N.D.	143	160	179
	Primary effluent	383	N.D.	N.D.	322	253
	AT_M	69	N.D.	60	173	177
	AT_E	N.D.	N.D.	62	166	146
	Secondary effluent	N.D.	N.D.	288	90	179
	Final effluent	N.D.	N.D.	265	107	166
	Return flow	505	N.D.	N.D.	N.D.	239
	Drained sludge	367	N.D.	149	149	246
	Return sludge	N.D.	N.D.	60	170	112
WWTP-2	Influent	1090	N.D.	1580	1060	305
	Primary effluent	998	N.D.	1650	911	241
	AT_M	405	N.D.	187	595	197
	AT_E	74	N.D.	157	536	149
	Secondary effluent	69	N.D.	104	409	55
	Biofiltration effluent	52	N.D.	233	334	67
	Final effluent	N.D.	N.D.	72	64	74
Limit of Quantification		40	20	50	20	10

[a]N.D.: not detected; [b]activated sludge in the aeration tank collected from the mid-distance point (AT_M) and from the end of the tank (AT_E).

WWTPs were 0.013 μg/L in a Canadian WWTP[10] and 0.20(median)–0.38 μg/L in a German WWTP.[9] These concentrations are almost the same as those obtained in our study, that is, 160–1060 ng/L in the influent and 64–107 ng/L in the effluent.

Although there are no useful data for comparing the concentrations of mefenamic acid, the concentrations of the anti-inflammatory drugs analyzed in our research for the WWTP influent and effluent were generally lower than those reported in foreign countries. These findings are contrary to those reported by Yasojima *et al.*[4] who found that the concentrations of the antibiotics levofloxacin and clarithromycin in secondary treated water in Japan are higher than those in foreign countries.

3.4 *Fate of anti-inflammatory drugs*

The flux of each anti-inflammatory drug was calculated from its concentration and wastewater flow rate at the sampling points for WWTP-1(Fig. 2). As shown in Fig. 2(a), the flux of ibuprofen significantly increased in the primary settling tank, and was efficiently removed in the aeration tank. Naproxen was also found to have a high flux in the primary settling tank, but no drastic reduction in its flux was observed in the aeration tank or final sedimentation tank. A slight reduction in the flux of naproxen was observed in the

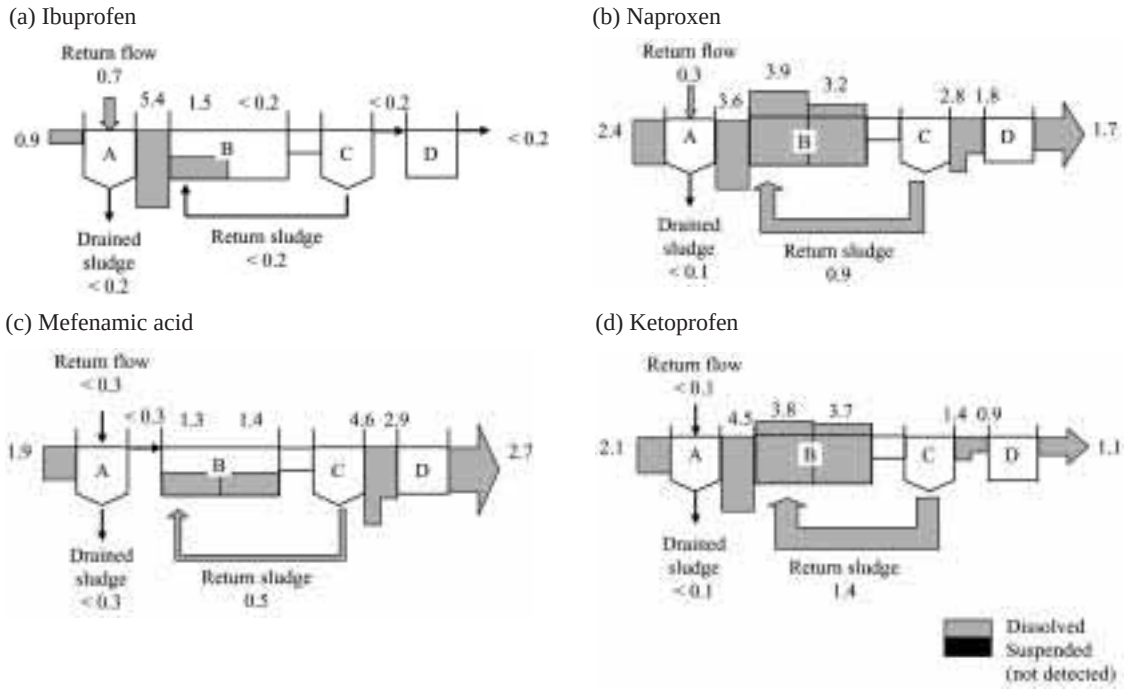

Fig. 2. Mass balances (g/d) of ibuprofen (a), naproxen (b), mefenamic acid (c), and ketoprofen (d) subjected to physicochemical treatments (A and C), biological treatment (B), and chlorination (D) in WWTP-1. A: Primary settling tank; B: Aeration tank; C: Final sedimentation tank; D: Chlorination tank.

chlorination tank (Fig. 2(b)). Because approximately one-third of the effluent from the final sedimentation tank is reused in WWTP-1, it seems that the flux is reduced as a result of the chlorination. The mefenamic acid concentration was considerably decreased after passing through the primary settling tank (Fig. 2(c)), but it increased again in the final sedimentation tank, which is somewhat difficult to explain. The ketoprofen concentration exhibited the same pattern as that of naproxen (Fig. 2(d)). The anti-inflammatory drugs surveyed in our study have a carboxy group that dissociates depending on the pH of the samples. The fates of the drugs, particularly ibuprofen, in the primary settling tank in WWTP-1 (Fig. 2) were not the same as those in WWTP-2 (described later), although pH changes through the primary settling tank in the two plants were not significant (7.3 to 7.2 in WWTP-1 and 7.7 to 7.5 in WWTP-2). The low concentration of the drugs in the plant influent of WWTP-1 might be caused by analytical problems.

Although the primary sludge, return flow and return sludge were not sampled and analyzed in WWTP-2, the flux of each anti-inflammatory drug in the plant was also calculated (Fig. 3). In WWTP-2, the fluxes of the anti-inflammatory drugs in the return flow, drained sludge and return sludge seemed insignificant in terms of the mass balance, because

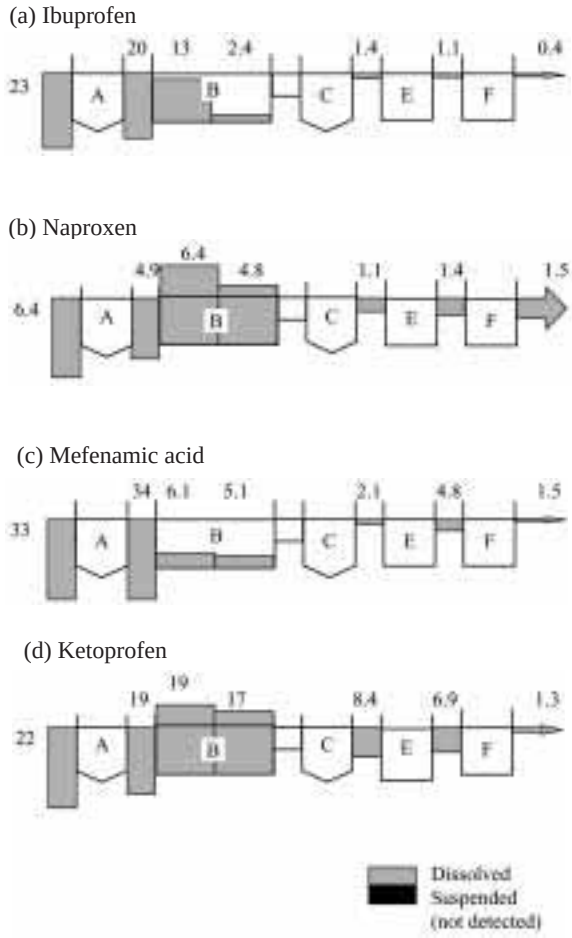

Fig. 3. Mass balances (g/d) of ibuprofen (a), naproxen (b), mefenamic acid (c), and ketoprofen (d) subjected to physicochemical treatments (A and C), biological treatments (B and E), and UV radiation for disinfection (F) in WWTP-2.
A: Primary settling tank; B: Aeration tank; C: Final sedimentation tank; E: Biofiltration tank; F: UV disinfection tank.

large increases in the flux of each drug were not observed in the primary settling tank and in the aeration tank of WWTP-2 (Fig. 3). The effective removal of the anti-inflammatory drugs was expected in the UV radiation processing stage (discussed below), even though the biofiltration processing stage seemed not effective in removing the anti-inflammatory drugs detected (Fig. 3).

3.5 *Removal efficiencies of anti-inflammatory drugs*

Figure 4 shows the overall removal efficiency and the removal efficiency for each anti-inflammatory drug in each treatment process for the two WWTPs investigated in our research. In general, WWTP-2 had a better removal efficiency for each anti-inflammatory drug. As previously discussed, ibuprofen, naproxen and ketoprofen all showed a characteristic increase in concentration in the primary settling tank, resulting into their low removal efficiencies in WWTP-1. The ibuprofen and naproxen concentrations were previously reported to have increased in the primary treatment process (that is, in the pretreatment and sedimentation tanks), which was presumed to be caused by their acidic structures.[8] According to Metcalfe *et al*.,[7] the removal efficiencies of ibuprofen and naproxen can be correlated with hydraulic retention time (HRT) but not with sludge retention time (SRT). When the HRT was 12 h or longer, the removal efficiencies of these substances were estimated to be more than 90%. The HRTs of the WWTPs investigated in this study were 9.1 h for WWTP-1 and 12 h for WWTP-2 (Table 1), which are consistent with the HRTs of previously mentioned reports.[7] The high removal efficiencies of the anti-inflammatory drugs in the aeration tank of WWTP-2 are believed to be promoted by the long HRT;

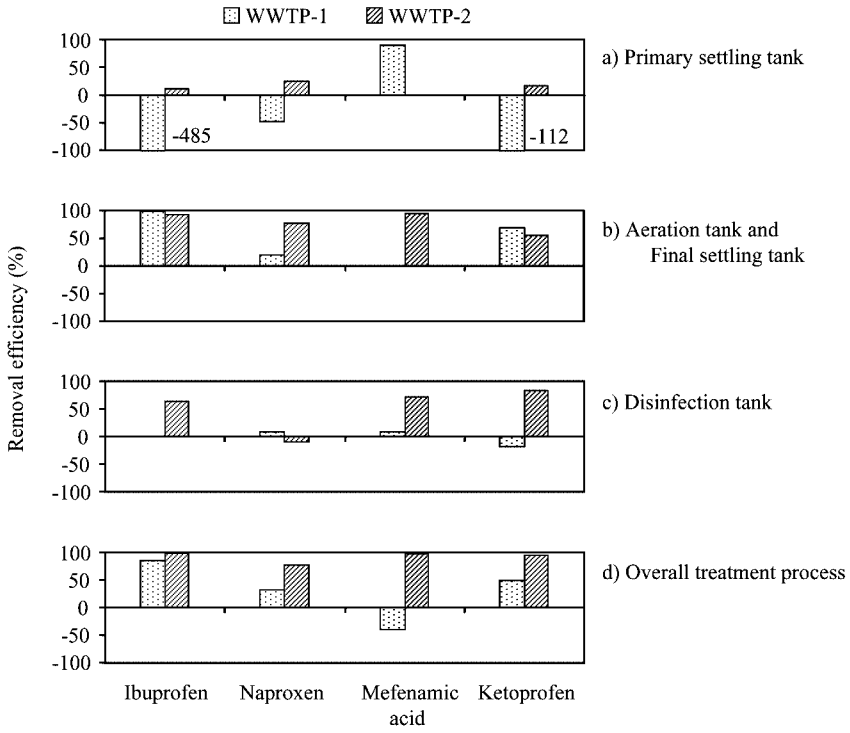

Fig. 4. Removal efficiencies of anti-inflammatory drugs subjected to primary treatment (a), secondary treatment and sedimentation (b), disinfection (c), and overall treatment process (d).

however, this hypothesis requires additional research for confirmation.

In the case of WWTP-2, in which UV radiation is used in the disinfection process, the removal efficiencies of ibuprofen, mefenamic acid and ketoprofen are high (Fig. 3(c)). Given this, the fates of the anti-inflammatory drugs particularly in the UV radiation processing stage were investigated. First, spot sampling was conducted on the influent and effluent surrounding the UV radiation process in WWTP-2, and the anti-inflammatory drugs in the samples were analyzed (as an additional survey). The results indicated that the removal efficiencies of naproxen, mefenamic acid and ketoprofen by UV radiation were 10%, –5% and 90%, respectively. No ibuprofen was detected in either the influent or the effluent after the UV radiation. An extensive follow-up survey was carried out in another WWTP (WWTP-3) where UV radiation is also performed. The results indicated a high removal efficiency (83%) of ketoprofen in WWTP-3, as was the case in WWTP-2 (Fig. 5). These results suggest that UV radiation is effective in removing ketoprofen. Tixier *et al.* have recently suggested the photodecomposition of ketoprofen in the environment.[11]

4. Conclusion

The fates of anti-inflammatory drugs which are frequently detected in the discharges of WWTPs and river water in Japan were investigated in two WWTPs. From our results, we make the following conclusions:

1. The concentrations of ibuprofen, naproxen, mefenamic acid and ketoprofen were almost equal to or lower than those reported in foreign countries.
2. In a WWTP having a long HRT, high removal efficiencies of the anti-inflammatory drugs, except ibuprofen, were observed, particularly in the aeration tank.
3. High removal efficiencies of the anti-inflammatory drugs were not observed in the disinfection by chlorination.
4. The effective removal of ketoprofen by UV radiation as a disinfection method was demonstrated, although the disinfection by-products had not been demonstrated.

The biological impacts of other drugs and their presence in WWTPs and in the environment must be further clarified in future studies.

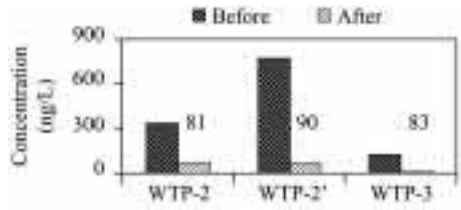

Fig. 5. Changes in ketoprofen concentration and removal efficiency when subjected to disinfection processes. Numbers indicate the removal efficiencies (%) of ketoprofen by UV radiation treatment processes (WTP-2, 2' and 3). An additional survey was conducted for the UV irradiation treatment process in the WWTP-2 (WWTP-2'). An extensive follow-up survey was also carried out in another WWTP (WWTP-3). See text for details.

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