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Science of the Total Environment 442 (2013) 380–388

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data

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HIGHLIGHTS

- Regional sales relevant for monitoring and emission prediction of pharmaceuticals
- Metformin concentrations are 80 μg/L and 1 μg/L in wastewater and effluent respectively.
- 82% of metformin in wastewater recovered as guanylurea in effluent.
- Significant better removal at higher temperatures for 4 of 9 pharmaceuticals
- Significant better removal at higher HRTs for 3 of 9 pharmaceuticals

ARTICLE INFO

Article history:
Received 6 June 2012
Received in revised form 28 September 2012
Accepted 11 October 2012
Available online xxxx

Keywords:
Pharmaceuticals
Metformin
Guanylurea
Wastewater
Consumption based monitoring
Regional surface water

ABSTRACT

Local consumption data of pharmaceuticals were used to study the emission to wastewater and surface waters in two small Dutch water catchments. For nine high consumption pharmaceuticals: metformin, metoprolol, sotalol, losartan, valsartan, irbesartan, hydrochlorothiazide, diclofenac and carbamazepine, predicted emissions were compared to wastewater concentrations, removal in sewage treatment plants and recovery in regional surface water. The study shows that local consumption data can be very useful to select pharmaceuticals for monitoring and to predict wastewater concentrations. Measured influent concentrations were on average 78% with a range of 31–138% of predicted influent concentrations. Metformin is the pharmaceutical with the highest concentration in wastewater (64–98 μg/L) but it is removed with >98% in sewage treatment plants (STP). Guanylurea, a biodegradation product of metformin, was detected in STP effluents and surface waters at concentrations of 39–56 μg/L and 1.8–3.9 μg/L, respectively. The STP removal of the different pharmaceuticals varied strongly. For carbamazepine, hydrochlorothiazide and sotalol a significant better removal was found at higher temperatures and longer hydraulic retention times while for metoprolol significantly better removal was only observed at higher temperatures. Predicting environmental concentrations from regional consumption data might be an alternative to monitoring of pharmaceuticals in wastewater and surface waters.

1. Introduction

Over the last two decades, numerous articles have been published on pharmaceuticals in the environment. These studies show that a mixture of different pharmaceuticals is present in wastewaters and surface waters (Daughton and Ternes, 1999; Sacher et al., 2008; Roig, 2010). The presence of pharmaceuticals and personal care products in surface waters has given rise to concern about ecological and human health risks. Concentrations in surface waters generally fall in the ng/L to μg/L range. At these concentrations, human health effects are not expected (Bruce et al., 2010). Whether these concentrations pose a threat to the environment is difficult to assess. Acute aquatic risks are usually estimated by assessing the ratio of the predicted environmental concentration and the predicted no-effect concentration (PEC/PNEC). For a large number of pharmaceuticals, these ratios have been reviewed by Fent et al. (2006). Observed ratios were generally far below 1 indicating that there is a limited risk based on the effects of individual pharmaceuticals. However, it should be noted that eco-toxicological data are still limited (Fent et al., 2006). Especially for chronic toxicity data that consider effects of exposure during multiple life stages or even multiple generations are lacking and little is known about effects of mixtures of pharmaceuticals (Roig, 2010).

Currently, pharmaceuticals such as β-blockers, analgesics, antibiotics, lipid regulators, and X-ray contrast media have been monitored and detected in wastewaters (Kasprzyk-Hordern et al., 2007; Radjenovic et al., 2007; Miege et al., 2008; Flyborg et al., 2010; Rosal et

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0048-9697/$ – see front matter.
http://dx.doi.org/10.1016/j.scitotenv.2012.10.046

al., 2010; Hörsing et al., 2011) and surface waters (Jones et al., 2002; Kasprzyk-Hordern et al., 2007). Some pharmaceuticals have also been found in drinking water produced from surface water (Huerta-Fontela et al., 2011; Sanderson, 2011) and river bank filtrate (De Jongh et al., 2012).

Monitoring of pharmaceuticals in the environment is restricted by the available analytical techniques in laboratories. The selection of pharmaceuticals for monitoring is often based on selections of pharmaceuticals analyzed in previous studies. Consequently, some pharmaceuticals, that might be relevant based on their consumption, human excretion and passage of sewage treatment, might be omitted in current environmental monitoring studies. There are, for example, many data on the occurrence and fate of the anti-epileptic carbamazepine and the β-blockers metoprolol, sotalol and atenolol in the water cycle, while only few data exist on other widely used pharmaceuticals such as metformin (anti-diabetic), irbesartan (anti-hypertensive) and hydrochlorothiazide (diuretic). The fact that certain pharmaceuticals have not been included in water catchment-studies might result in an underestimation of the total concentrations and annual fluxes of pharmaceuticals in surface waters and can affect the risk assessment as some relevant pharmaceuticals might be ignored. Besides occurrence, human excretion and removal by wastewater treatment plants, prescription (or sales) data are suitable to select relevant pharmaceuticals for environmental monitoring (de Voogt et al., 2009) and predict environmental concentrations. Jones et al. (Richardson and Bowron, 1985; Jones et al., 2002; Siemens et al., 2008) used average consumption of the 25 most prescribed pharmaceuticals in the UK to predict environmental concentrations. Furthermore, Alder et al. (2010) predicted surface water concentrations of four β-blockers within a factor of two in the Swiss Glatt Valley from national consumption data and measured removal efficiencies in three sewage treatment plants (STPs). Discrepancies between predicted and measured concentrations were explained by higher biodegradation and photolysis in summer time when residence times of the water in the valley were 100–200 days. Additionally, Scheurer et al. (2009) described the occurrence of the widely used pharmaceutical metformin in German surface waters and reported that the high concentrations correlated well with consumption data. Finally, ter Laak et al. (2010) related the loads of pharmaceuticals in the river Rhine to the upstream consumption of these pharmaceuticals in the Rhine catchment area and could explain the loads of 15 out of the 20 most frequently detected pharmaceuticals within a factor of two.

The literature data above illustrate that, among other criteria, international or national sales data of pharmaceuticals can be valuable to select relevant pharmaceuticals for monitoring and can be used to estimate loads and average environmental concentrations in surface waters. However, as consumption of pharmaceuticals can differ on a regional basis they are less suitable to predict loads or concentrations in wastewater and regional surface water systems.

To our knowledge hardly any studies have been conducted in which regional sales of pharmaceuticals within catchments of a wastewater treatment plant have been related to measured concentrations and loads of these pharmaceuticals in the wastewater influent, effluent and the receiving surface waters. This approach can be very useful as it links the local consumption (i.e. sales) in households to concentrations in wastewater and surface water. Thereby the input of the system is better defined, allowing descriptive modeling. Furthermore, results might be useful for the prediction of loads of pharmaceuticals in other regional catchments.

In this study, local sales data of pharmaceuticals in a village and a city in the Netherlands, and information on human excretion and removal during wastewater treatment were used to select pharmaceuticals for monitoring. Besides that, guanylurea, a transformation product of metformin, was selected for monitoring because recent literature showed that this product was formed during wastewater treatment and observed in surface waters (Scheurer et al., 2009; Trautwein and Kümmerer, 2011). The pharmaceuticals with presumably the highest emissions were subsequently monitored in sewage influents, sewage effluents and receiving surface waters.

The aim of the study was to investigate the local emission of pharmaceuticals to regional surface waters. Well defined consumption data of pharmaceuticals in a catchment of a sewage treatment plant and knowledge on the sewage treatment plant efficiencies enable one to relate consumption to loads of pharmaceuticals in sewage influent, removal by wastewater treatment, loads and concentrations in effluents, and loads and concentrations in receiving waters. The monitoring was performed in September–October and December 2010 in a sewage treatment plant with a parallel operated conventional activated sludge system (CAS) and a membrane bioreactor (MBR). This additionally allowed the comparison of removal rates of pharmaceuticals at different wastewater temperatures and with different sewage treatment technologies, varying in hydraulic retention time (HRT), sludge concentration and type of solids removal.

2. Materials and methods

2.1. Selection of pharmaceuticals

For the village of Ootmarsum (7220 inhabitants) and the city of Enschede (157,052 inhabitants) (Fig. 1) the top-50 of most sold pharmaceuticals by local pharmacies (2009) was extracted from a database of the Dutch foundation of pharmaceutical numbers (SFK). The sales data were reported in defined daily doses per year (DDD/yr). It was assumed that sales and consumption were equal. Sales of pharmaceuticals via local hospital pharmacies were not included in the data of the SFK. However, it was assumed that the contribution of hospital pharmacies to the total load was marginal since most pharmaceuticals, except some antibiotics and X-ray contrast media, are mainly acquired via generic pharmacies (Derksen et al., 2007; Ort et al., 2010a; Vergouwen et al., 2011a). The load of pharmaceuticals to the STP was calculated by use of an excretion factor and an average daily dose.

$$Consumption\ \ Enschede\ \ (g/pers.day) = \frac{(DDD/yr)_{Enschede} * (g/DDD)}{nr\ \ persons\ \ Enschede}$$

$$Load\ \ to\ \ STP\ \ Enschede\ \ (g/day) = \frac{(DDD/yr)_{Enschede} * (g/DDD) * excretion}{365}$$

Table 1 lists the nine pharmaceuticals selected for monitoring. The predicted emission was based on sales, excretion rate and STP removal rate, and analytical methods available. Initially, furosemide and omeprazol were also selected for monitoring based on their predicted emissions. However, no analytical techniques were available, so these pharmaceuticals could not be included in the current study. The high predicted consumption and emission of furosemide and omeprazol give rise to studying these pharmaceuticals in the future.

2.2. Sewage treatment plants

The sewage treatment plants of Enschede (circa 50,000 m^3/d) and Ootmarsum (circa 2500 m^3/d) both consist of a conventional activated sludge system (CAS) with biological phosphate removal and nitrogen removal via nitrification/denitrification. At STP Enschede, the wastewater passes primary clarifiers, anaerobic tank, denitrification tank, nitrification tank and secondary clarifiers. The secondary and primary sludge is digested in mesophilic sludge digesters and the digested sludge is centrifuged to remove excess of water. This 'reject water' is mixed up with the raw wastewater. Ferric chloride is added to the reject water to remove released phosphate (Fig. 2).

STP Ootmarsum consists of a combination of a MBR and a CAS system with sand filtration as post treatment. MBR and CAS with sand filtration are operated in parallel. The HRT in the MBR is 19 ± 4 h while the HRT in the CAS is three times longer (61 ± 13 h). Under dry weather

Table 1
Consumption and human excretion of the selected pharmaceuticals.

Pharmaceutical	Sales g/person/year				Human excretion (%)	Predicted emission to the STP kg/yr[e]	Ootmarsum
	Enschede	Ootmarsum	Netherlands	Europe [a]		Enschede	
Metformin	15.61	15.15	16.65	5.9–12.1	100[b]	2451	109
Metoprolol	1.76	1.96	1.63	0.04–1.0	11[c]	30	14
Sotalol	–[d]	0.17	0.12	0.06–0.28	85[b]	[d]	1.0
Valsartan	0.31	–[d]	0.33	0.1–0.15	99[b]	122	[d]
Losartan	0.30	0.16	0.28	–[d]	88[b]	42	1.0
Irbesartan	0.40	0.75	0.53	0.1–0.33	31[b]	20	1.7
Hydrochlorothiazide	0.20	0.24	0.19	0.2	100[b]	72	1.7
Carbamazepine	0.48	0.35	0.33	0.61–0.98	26[c]	9	0.3
Diclofenac	0.30	0.37	0.44	0.06–0.88	16[c]	8	0.4

[a] Data obtained from Roig (2010).
[b] Data obtained from www.fk.cvz.nl (accessed: January 2010).
[c] Data obtained from Lienert et al. (2007).
[d] No data available.
[e] Predicted emissions are calculated by multiplying the number of prescriptions with a defined daily dose (mg/d) and the human excretion.

conditions, 50% of the wastewater is treated in the MBR and the other 50% is treated in a CAS system with sand filtration. During storm rainfall circa 77% of the wastewater is treated in the CAS system since the hydraulic capacity of the MBR is limited to 150 m^3/h. After treatment the effluent of STP Ootmarsum passes a wetland with a hydraulic retention time of 4 days. Fig. 2 shows the flow diagrams of STP Enschede and STP Ootmarsum. The wastewater and effluent characteristics of both STPs are presented in Table S1 of the Supplemental Information.

2.3. Sampling

Flow proportional 24 h samples were taken from the raw wastewater and from effluent of STP Ootmarsum at September the 4th and 19th, October the 5th and December the 7th, 8th and 9th 2010. The STP Enschede was sampled at September the 1st and 9th and October the 1st. Additionally, on December the 7th, 8th and 9th grab samples were taken from effluent receiving surface water, 2.5 km downstream from the discharge of effluent of STP Ootmarsum. The total hydraulic retention time in the surface water, including the wetland from STP Ootmarsum to the point where the samples were taken was approximately four days. This allows mixing and homogenization of daily variations of loads of pharmaceuticals from the effluent (Radke et al., 2010). Therefore, grab samples were considered to represent the average concentration in the surface water which enabled to calculate loads. All samples were stored at 4 °C and processed within one week.

2.4. Analytical

Determination of all pharmaceuticals and metabolites was done using HPLC/MS–MS analysis after automated solid phase extraction (SPE) of the analytes. The target analytes were selected based on sales

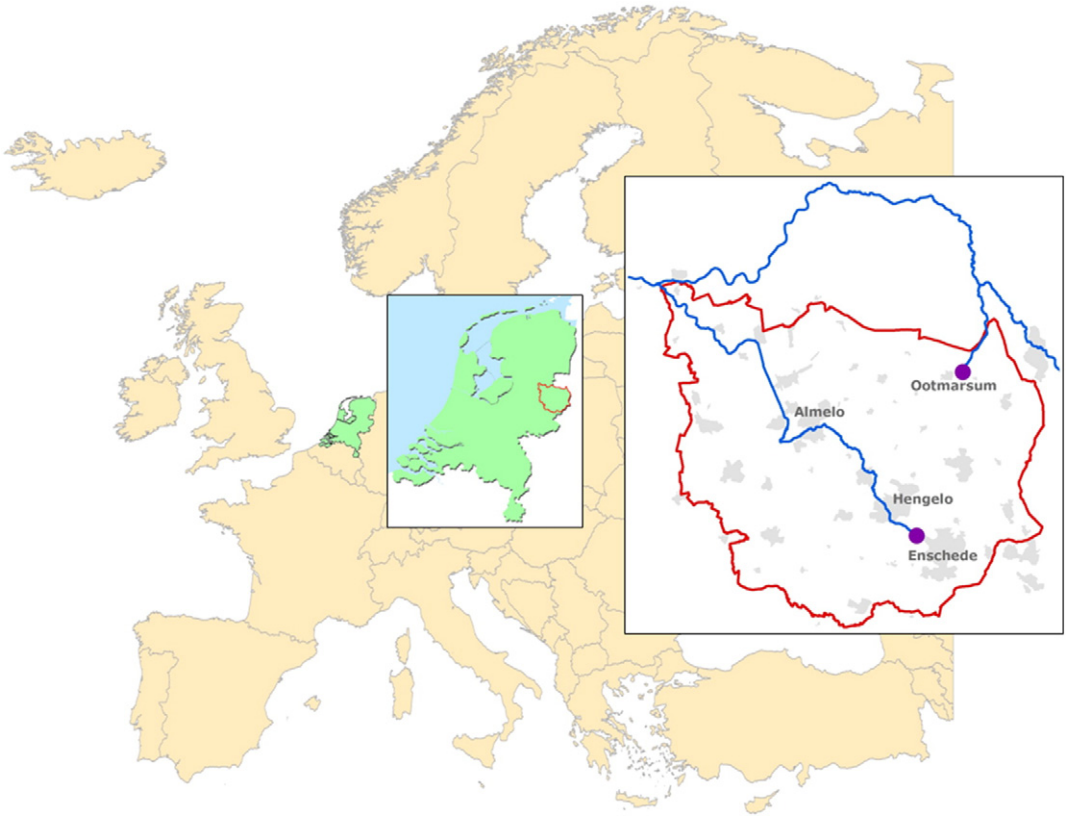

Fig. 1. Sampling locations.

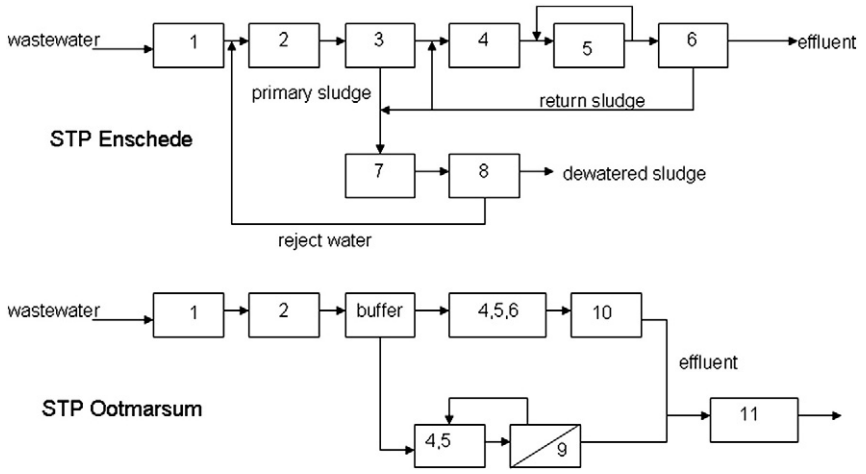

Fig. 2. Simplified flow scheme of STP Enschede and STP Ootmarsum. Legend: 1) grid removal, 2) sand trap, 3) primary clarifiers, 4) selector + anaerobic tank, 5) denitrification and nitrification, 6) secondary clarifiers, 7) sludge digestion, 8) sludge dewatering, 9) UF membranes, 10) sand filtration, 11) and wetland passage.

and consumption data. Several existing methods were applied to tackle the analysis of the chemically diverse set of target compounds. Prior to analysis, wastewater samples were diluted with tap water that was free of pharmaceuticals to reduce matrix effects.

Carbamazepine, diclofenac, irbesartan, losartan and valsartan were analyzed following a method that is described in detail in Sacher et al. (2008). The water samples were adjusted to a pH of 3 by addition of hydrochloric acid. Then carbamazepine-d10, diclofenac-d4, valsartan-d3, irbesartan-d3 and ibuprofen-d3 were added as internal standards. Solid-phase extraction was done on plastic cartridges filled with 200 mg of Bakerbond SDB 1 material (Mallinckrodt Baker, Deventer, The Netherlands). The SPE material was dried for 60 min in a gentle stream of nitrogen and elution was done with 10 mL acetone. The solvent was evaporated to dryness in a stream of nitrogen and the dry residue was reconstituted with 50 μL methanol and 50 μL HPLC grade water. An aliquot was injected into the HPLC/MS–MS system (HPLC HP 1100 from Agilent Technologies, Waldbronn, Germany and API 2000 mass spectrometer from AB Sciex, Langen, Germany). For HPLC separation a Luna C18 column (250 mm × 2 mm, 5 μm particle size) from Phenomenex (Aschaffenburg, Germany) was used. Injection volume was 12.5 μL and flow rate of the eluent was 0.2 mL/min. Gradient elution was applied with a 20 mM ammonium formate solution in MilliQ water and with a 20 mM ammonium formate solution in a 2:1 (v:v) mixture of acetonitrile and methanol. An electrospray interface was used and MS detection was done in the positive ionization mode with an ionization voltage of +5500 V. For MS–MS detection the MRM (multi reaction monitoring) mode was used.

Hydrochlorothiazide was analyzed with a similar method as the one previously described. The samples were adjusted to pH 3 and hydrochlorothiazide-^{13}C-d2 was added as internal standard. For pre-concentration of the analyte 200 mg SPE material Strata X from Phenomenex was used and elution was done with 5 mL methanol and subsequently with 1 mL acetone. The solvents were evaporated to dryness and reconstituted with 20 μL methanol and 80 μL HPLC grade water. An aliquot was injected into the HPLC/MS–MS system (HPLC 1200 SL from Agilent Technologies and API 4000 mass spectrometer from AB Sciex). HPLC separation was done on a Gemini column (250 mm × 2 mm, 5 μm) from Phenomenex. Injection volume was 20 μL and flow rate of the eluent was adjusted to 0.3 mL/min. For gradient elution a 20 M ammonium formate solution in MilliQ water and a 2 mM ammonium formate solution in a 2:1 (v:v) mixture of acetonitrile and methanol were used. MS detection of hydrochlorothiazide was done in the negative ionization mode with an ionization voltage of −4500 V. MS–MS detection was again done in the MRM mode.

For analysis of metoprolol and sotalol, the samples were adjusted to a pH of 7 (if necessary) and spiked with clenbuterol-d9 and sotalol-d6 as internal standards. Solid-phase extraction was done on Bond Elut PPL material (200 mg) from Agilent Technologies. Again, the eluate was evaporated to dryness and reconstituted with 50 μL methanol and 50 μL of a 95:5 (v:v) mixture of a 20 mM aqueous ammonium acetate solution and acetonitrile. An aliquot was injected into the HPLC/MS–MS system (HPLC HP 1100 from Agilent Technologies and API 2000 mass spectrometer from AB Sciex). Separation of the analytes was achieved on a Nucleosil column (250 mm × 2 mm, 3 μm) from Bischoff Chromatography (Leonberg, Germany). Injection volume was 12.5 μL and flow rate of the eluent was 0.2 mL/min. Gradient elution was applied with a 20 mM ammonium formate solution in MilliQ water and with a 20 mM ammonium formate solution in a 2:1 (v:v) mixture of acetonitrile and methanol. An electrospray interface was used and MS detection was done in the positive ionization mode with an ionization voltage of +5500 V. For MS–MS detection the MRM mode was used.

Metformin and guanylurea were analyzed by a method described in detail by Scheurer et al. (2009). Briefly, this method consists of a pre-concentration of the analytes from the water samples at neutral pH onto Strata X-CW material from Phenomenex. Metformin-d6 was used as internal standard and elution of the analytes from the SPE material was done with 5 mL of a methanol/acetonitrile mixture (20:80, v:v) containing 2% formic acid. After elution the solvent was evaporated to dryness in a stream of nitrogen and the dry residue was reconstituted in a mixture of 50% HPLC grade water and 50% acetonitrile. Then 10 μL was injected into the HPLC tandem–MS system (1200 HPLC system from Agilent Technologies and 4000 Q-Trap Triple–Quadrupole mass spectrometer from AB Sciex with an electrospray interface operated in positive ionization mode). HPLC separation took place on a ZIC-HILIC column (150 × 2.1 mm, 3.5 μm) from Merck SeQuant AB (Umeå, Sweden). Gradient elution was applied with an aqueous 10 mM ammonium formate solution set to pH 3 with concentrated formic acid and with acetonitrile. Flow rate was 0.35 mL/min. MS–MS detection was again done in the MRM mode.

Isotope-labeled standards were used whenever available. If no labeled standard was available similar isotope-labeled compounds were used to correct for extraction recoveries and analytical variations (e.g. ionization effects). The labeled compound that showed the most similar 'analytical behavior' to the target compound was used as internal standard. For carbamazepine, diclofenac, valsartan, irbesartan, sotalol and metformin deuterated analogs were used as internal standard. For hydrochlorothiazide a ^{13}C labeled and deuterated internal standard was used. For metoprolol, isotope-labeled clenbuterol was used as internal standard, for guanylurea metformin-d6 was used,

while for losartan valsartan-d3 was used. For all methods calibrations were made for the overall procedure from tap water. Validation parameters characterizing the performance of the analytical methods used are summarized in Table S8 in the Supplemental Information. Validation was done in surface water to account for the matrix effects occurring in the samples analyzed in this study (surface water and diluted wastewater). The validation data prove that the methods are well suited for the application in the monitoring campaign. Analytical uncertainties are in the range of 20 to 30% for all compounds under investigation.

3. Results and discussion

3.1. Consumption and emission

Table 1 shows the consumption data of pharmaceuticals for the city of Enschede and the village of Ootmarsum, extracted from the database of the SFK. The emission is predicted from the consumption of the patients living in the zip code area that was covered by the respective STPs.

Various discrepancies can exist between the predicted consumption and the actual consumption. First, differences between predicted and observed concentrations in the influents can be attributed to incomplete use of prescribed and sold pharmaceuticals (Ruhoy and Daughton, 2008; Musson and Townsend, 2009). This so called medication compliance for cardiovascular pharmaceuticals has been estimated to be 71% (Claxton et al., 2001). Secondly, sales data do not account for pharmaceuticals obtained from other sources due to consumption of pharmaceuticals obtained from other regions or illegal use. Finally, temporal (seasonal) trends in consumption of pharmaceuticals such as antibiotics, anti viral drugs and analgesics can lead to corresponding trends in emissions and loads in surface waters (Singer et al., 2008; ter Laak et al., 2010). Together, these discrepancies can bias predicted emissions resulting in differences between predicted and observed loads of pharmaceuticals in the STPs or surface waters.

It can be observed that the predicted emission of metformin is very high compared to other pharmaceuticals. This is due to its large share of users (>3.5% of the Dutch population), its high daily dose of 2000 mg/d and 100% excretion by humans.

Table 1 and Table S2 of the Supplemental Information show only marginal differences in consumption of pharmaceuticals between Enschede and Ootmarsum or between the Dutch average and these locations. Consumption patterns of the studied region were generally rather similar to the average Dutch consumption, despite the observation from a national survey on pharmaceutical consumption in the Netherlands of Van den Berg Jeths and Van Batenburg-Eddes (2003), which showed that there can be large regional variations in the consumption of pharmaceuticals. If the regional and Dutch consumption data are compared to European averages, larger differences are observed. The consumption of carbamazepine, ketoprofen and bezafibrate is considerably lower than elsewhere in Europe, while the consumption of furosemide, valsartan, irbesartan, metoprolol and metformin largely exceeds the average European consumption.

Five of the six pharmaceuticals with the highest predicted emissions show consumptions that exceed European averages (Table 1). Moreover, the consumption of pharmaceuticals is likely to increase in the coming decades due to aging populations (van der Aa et al., 2011).

3.2. Measurements in wastewater and surface water

Based on the consumption data, metformin, valsartan, losartan, irbesartan, metoprolol, sotalol, carbamazepine and diclofenac were monitored in raw wastewater, effluent and receiving surface water. Table 2 presents predicted influent concentrations in raw wastewater (i.e. the calculated daily emission divided by average wastewater flow on the sampling days), average measured influent and effluent concentrations and average removal efficiency calculated from these (see Supplemental Information) at the STPs. Due to varying influent concentrations and wastewater flows the STPs are not at steady state, and removal efficiencies can change from day to day. Furthermore the HRT exceeded one day. Therefore, the concentrations and related removal efficiencies in Table 2 are based on average mass flows of influent and effluent (mg/d) and average wastewater flows (m^3/d) over the sampling period (Ort et al., 2010b). Additionally, it should be noted that the analytical uncertainty is estimated to be 20–30% so minor differences between influents and effluents or between predicted and measured values can be attributed to analytical uncertainties. The standard deviation of the influent and effluent concentrations represents the variation observed between the individual 24 h composite samples. The individual data are presented in the Supplemental Information, Table S3–S7.

The predicted concentrations in the influents are generally slightly higher than the measured concentrations. Measured concentrations were on average 78% with a range of 31–138% of predicted concentrations. Loads of sotalol for Enschede and valsartan for Ootmarsum could not be predicted as regional consumption data were not available. This shows that the consumption based prediction is rather accurate. Differences between predicted and observed concentrations in the influents can be attributed to incomplete use of sold pharmaceuticals, additional consumption from hospital pharmacies not included in this study (Vergouwen et al., 2011b), transformation of pharmaceuticals during sewer passage, temporal variations in pharmaceutical consumption (ter Laak et al., 2010) and analytical uncertainties (Ort et al., 2010b). A more detailed discussion on the predicted concentrations, measured concentrations and removal during sewage treatment for the individual pharmaceuticals is given below.

3.3. STP removal of pharmaceuticals

Table 3 presents removal efficiencies at STP Ootmarsum at different temperatures and HRTs. It can be observed that for some pharmaceuticals the removal efficiency increases with increasing temperature and HRT. The results of the individual pharmaceuticals are discussed below.

3.4. Metoprolol and sotalol

The measured influent concentrations of the two β-blockers metoprolol and sotalol of STP Ootmarsum are 31 ± 7% higher than predicted wastewater concentrations based on regional consumption data. These marginal differences could be explained by analytical uncertainties, minor changes in consumption between 2009 and 2010 and additional consumption from hospital pharmacies.

The β-blockers are only partly removed in the STP of Ootmarsum and Enschede. The performance of the CAS system of Enschede and STP Ootmarsum reveals no significant differences (Table 2). The removal rate of metoprolol appeared slightly higher than that of sotalol in both STPs. Typically, the opposite is reported in literature (Vieno et al., 2007; Roig, 2010). However, Roig mentions that the collected data lack STPs with hydraulic retention times >25 h and sludge retention times >20 d. Both STPs in the current study have a hydraulic retention time >25 h and sludge retention times >20 d. Additionally, Maurer et al. (2007) showed that removal of β-blockers by sorption to activated sludge is negligible and that degradation rate constants of metoprolol (0.58 L/d/g_{COD}) were twice as high as for sotalol (0.29 L/d/g_{COD}). This corresponds to the observed higher removal rate of metoprolol.

The STP of Ootmarsum, where a conventional activated sludge system and a Membrane Bioreactor are operated in parallel, was sampled in September–October and December (Fig. 2). The removal in September–October was significantly higher than in December for both β-blockers (Table 3). This illustrates that the removal is more effective at higher temperatures, which is probably due to higher biological activity. Additionally, a significantly better removal by the MBR was observed for metoprolol, while the removal of sotalol was more effective in the conventional

Table 2
Predicted and measured concentrations in wastewater influent and effluent of STPs Enschede and Ootmarsum and recovered amount in surface water. Standard deviations are given in brackets.

	Ootmarsum (n = 7)					Enschede (n = 3)			
	Predicted influent	Influent	Effluent	Removal[a]	Recovery surface water[b]	Predicted influent	Influent	Effluent	Removal
	μg/L	μg/L	μg/L		%	μg/L	μg/L	μg/L	
Carbamazepine	0.72	0.22 (0.08)	0.20 (0.04)	10% (19%)	–[c]	1.09	0.56 (0.15)	0.54 (0.32)	3% (34%)
Diclofenac	0.47	0.34 (0.24)[d]	0.20 (0.05)	41% (20%)	131%	0.43	0.25 (0.04)	0.31 (0.15)	−9% (36%)
Guanylurea	–[e]	[c]	48.01 (24.95)		87%	–[e]	–[f]	–[f]	–[e]
Hydrochlorothiazide	1.90	1.65 (0.64)	1.27 (0.26)	23% (23%)	71%	1.79	1.46 (0.45)	1.01 (0.43)	31% (9%)
Irbesartan	1.88	1.55 (0.57)	1.46 (0.26)	6% (46%)	98%	1.09	0.62 (0.23)	0.88 (0.41)	−42% (114%)
Losartan	1.11	0.50 (0.17)	0.06 (0.03)	88% (7%)	–[c]	2.34	0.79 (0.17)	0.09 (0.04)	89% (8%)
Metformin	122.01	73.73 (9.45)	1.82 (0.63)	98% (1%)	187%	141.38	84.41 (13.61)	1.22 (0.50)	99% (1%)
Metoprolol	1.74	2.40 (0.64)	1.39 (0.26)	42% (16%)	93%	1.70	2.24 (1.18)	1.60 (0.72)	29% (25%)
Sotalol	1.37	1.70 (0.42)	1.29 (0.19)	24% (23%)	89%	1.05[g]	1.06 (0.38)	0.88 (0.47)	17% (19%)
Valsartan	2.63[g]	1.93 (0.59)	0.21 (0.13)	89% (7%)	–[c]	2.71	2.93 (0.50)	0.14 (0.07)[h]	95% (2%)

[a] The calculation of average removal and standard deviations is explained in the supplemental information.
[b] Recovery of pharmaceuticals in surface water in December compared to effluent load (i.e. (flow∗conc. surface water)/(flow∗conc. effluent)), see Table S7 of the Supplemental Information for individual data.
[c] Not detected.
[d] n = 6.
[e] Cannot be calculated.
[f] Not measured.
[g] No individual consumption data available, calculated from average Dutch consumption.
[h] n = 2.

activated sludge treatment. This difference is difficult to interpret. The mixed liquor suspended solids (g/L) in the MBR is 2.4 times higher while the hydraulic retention time is 3.2 times shorter than in the CAS system. It is unlikely that the higher removal of metoprolol in the MBR system can be attributed to sorption to the higher suspended solids load, as sorption coefficient of metoprolol to sewage sludge is too low to allow significant removal, even in the MBR system (Maurer et al., 2007). Possibly, the removal of sotalol is improved by longer hydraulic retention that promotes a microbial community that is more effective in degrading sotalol, while the microbial community in the MBR is more suitable for metoprolol removal. More detailed studies on the (microbial) removal efficiency of the parallel MBR and CAS treatment in Ootmarsum are necessary to generate conclusive results.

The concentrations of the β-blockers in the effluents of the two Dutch STPs are higher than the mean European effluent concentrations (metoprolol (0–0.8 μg/L), sotalol (0.18–0.87 μg/L)). This can be explained by the regional and national Dutch consumption of metoprolol and sotalol that both exceed the European average (see Table 1 and Table S2 of the Supplemental Information). The recoveries of metoprolol and sotalol in surface waters (residence time of water ~4 days) were 93% and 89% of the calculated emission, respectively. This illustrates that removal due to biodegradation and sorption in surface waters is low. However, sampling of surface water and effluent was done at the same time so the recoveries could not be determined exactly.

Table 3
Removal of pharmaceuticals at STP Ootmarsum at different wastewater temperatures and hydraulic retention times.

	Temperature		HRT	
	Dec 2010	Sept, Oct 2010	MBR	CAS
	8 °C	17 °C	HRT = 19 h	HRT = 61 h
Carbamazepine	−8%[a]	24%[a]	−2%[b]	13%[b]
Diclofenac	30%	44%	39%	44%
Hydrochlorothiazide	3%[a]	43%[a]	9%[a]	31%[a]
Irbesartan	17%	−40%	−10%	−5%
Losartan	85%	91%	93%	82%
Metformin	97%	98%	98%	97%
Metoprolol	33%[b]	54%[b]	50%[b]	34%[b]
Sotalol	9%[a]	40%[a]	18%[b]	27%[b]
Valsartan	85%	94%	93%	85%

[a] Significant differences in removal with temperature or HRT ($p<0.01$).
[b] Significant differences in removal with temperature or HRT ($p<0.05$).

3.5. Diclofenac

The concentrations of diclofenac observed in the influents are 72% and 58% of the predicted average concentrations for Ootmarsum and Enschede and the removal of diclofenac is −9% and 41% in the STPs of Ootmarsum and in Enschede, respectively. The removal efficiency did not change significantly with temperature or hydraulic retention time. The poor removal efficiency of diclofenac observed in both treatment systems is in line with literature data (Roig, 2010). The effluent concentrations of diclofenac are within the range of mean environmental concentrations reported in Europe: 0.03–1.8 μg/L. This corresponds to the regional consumption of diclofenac, which is similar to the average European consumption (Table 1). The recovery of diclofenac in surface water was 131% of the calculated emission. This shows that diclofenac is persistent in the aquatic environment.

3.6. Carbamazepine

The measured influent concentrations of carbamazepine in Ootmarsum and Enschede were respectively a factor 3 and 2 lower than the predicted influent concentrations. It should be mentioned that the excretion of carbamazepine was set at 26% While Lienert et al. (2007) reports 2% excretion of unmetabolized carbamazepine via urine and 24% excretion of an unknown mixture of metabolites and parent compound via feces. The excretion of unchanged carbamazepine is therefore unknown and does not allow prediction of the influent concentration on the basis of consumption and average excretion.

Carbamazepine is one of the most studied pharmaceuticals and is known to be very persistent. 43 of 48 records collected by Roig (2010) showed removal efficiencies below 20%. Our data are in line with the literature data reported, with average removal efficiencies of 10% and 3% in Ootmarsum and Enschede respectively. Even though removal was marginal, detailed studies of the STP Ootmarsum show a significantly more effective removal in September–October than in December (Table 3). Furthermore, the conventional activated sludge system appeared to be significantly more effective than the MBR system (Table 3). This suggests that the longer residence time of the activated sludge in the conventional system enables higher biodegradation. Despite the marginal removal of carbamazepine, effluent concentrations are relatively low compared to other European countries (0.2–0.5 μg/L versus 0.2–1.2 μg/L). This is in line with the consumption per capita that is approximately half of the European average (Table 1). Due to the rather low concentrations

in the effluent and high dilution in receiving waters, carbamazepine was not detected in the receiving surface water. Thus the recovery in surface water could not be determined.

3.7. Losartan, irbesartan and valsartan

The predicted concentration of losartan exceeds the measured concentrations by a factor of 2 to 3. This deviation might be attributed to removal in the sewer system before entering the STP as the high removal rate during treatment suggests that this compound is readily biodegradable. Furthermore, cardiovascular medication is known to have relatively low medication compliance (Ruhoy and Daughton, 2008; Musson and Townsend, 2009), which means that less pharmaceutical is actually consumed than sold. Together, these factors might explain the overestimation of influent concentrations.

Irbesartan shows a poor removal rate (−42% to 6%), while losartan and valsartan are removed to a larger extent (88–97%) in the STP.

The removal efficiency of losartan, irbesartan and valsartan did not change significantly with sludge temperature or hydraulic retention time. Very few literature data are available on STP removal of losartan and valsartan. However, Batt et al. (2008) reported 62% removal of losartan and Kasprzyk-Hordern et al. (2008) reported 84% removal of valsartan in a CAS system and 44% valsartan removal in a trickling filter. The observed removal rates in our study are higher (88–97%). The difference between the trickling filter and CAS system observed by Kasprzyk-Hordern suggests that solids and hydraulic retention time are both relevant for the removal of valsartan and possibly also for losartan.

To our knowledge there is no literature data available on the removal of irbesartan in STPs. However, the very low adsorption coefficients to secondary sludge for irbesartan according to Hörsing et al. (2011) ($K_f = 5.3 * 10^{-4}$ L/g) suggest that irbesartan is practically not removed by sorptive processes in activated sludge. Huerta-Fontela et al. (2011) show that 19% of irbesartan even passes advanced treatment techniques, such as chlorination, sand filtration, ozonation and granulated activated carbon filtration, applied for drinking water production. The poor removal rate of irbesartan (−42% to 6%), shows that sewage treatment techniques are not effective in removing this compound from wastewater. The recovery of irbesartan in surface water was 98% which illustrates that irbesartan is also persistent in surface water. Losartan and valsartan could not be detected in surface water, so their recoveries in surface water could not be determined.

For irbesartan, its poor removal efficiency in STPs, its resistance to advanced oxidation and adsorption by activated carbon and the limited literature data on occurrence and environmental risks suggest to further study the fate and risks of this pharmaceutical in the water cycle.

3.8. Hydrochlorothiazide

The predicted influent concentrations of hydrochlorothiazide in Ootmarsum and Enschede of 1.9 and 1.8 μg/L are respectively 15% and 22% higher than the measured influent concentrations. These marginal differences could be explained by analytical uncertainties, and incomplete use of sold medication or minor changes in consumption between 2009 and 2010.

The STPs of Ootmarsum and Enschede removed on average 23% and 31% of the diuretic drug hydrochlorothiazide, respectively. Observed removal rates fall into the broad range reported in the literature that spans from 0 to 77% (Castiglioni et al., 2004; Radjenovic et al., 2007). Detailed studies of the STP Ootmarsum show a significantly more effective removal in September–October (43 ± 17%) while the removal in December was negligible (3 ± 17%) (Table 3). Furthermore, the conventional activated sludge system appeared to be significantly more effective with an average removal of 31 ± 25% than the MBR system with a removal of only 9 ± 26% (Table 3). These observations are in line with the literature. Radjenovic et al. (2007) reported 0% removal of hydrochlorothiazide in a STP with effluent NH_4–N concentrations ranging from 7 to 43 mg/L, while Rosal et al. reported 53% removal in a CAS-system with lower effluent NH_4–N levels of 8.5 mg/L and presumably better nitrification (Rosal et al., 2010). Nitrification capacity is mainly related to the hydraulic and solid retention times and oxygen concentration in an STP. Together, this suggests that higher temperatures and longer hydraulic retention times in the conventional system enable higher biodegradation of hydrochlorothiazide. The recovery of hydrochlorothiazide in surface water was 71%. Possibly some of the hydrochlorothiazide was degraded in surface water during 4 days retention time. However, sampling of surface water and effluent was done at the same time so the recoveries could not be determined exactly.

3.9. Metformin and guanylurea

Metformin is an anti-diabetic drug that is widely used in Europe (OECD, 2009). It is probably the pharmaceutical with the highest emission to the environment on a mass basis (Scheurer et al., 2009). The average concentration of metformin in raw wastewater of Enschede and Ootmarsum was 79 ± 12 μg/L. This is similar to observations from literature that ranged from 57 to 129 μg/L (Scheurer et al., 2009; Trautwein and Kümmerer, 2011; Scheurer et al., 2012). These concentrations exceed concentrations of the other pharmaceuticals by roughly 2 orders of magnitude. Furthermore around 60% of the consumed metformin was recovered in the STP influent. The marginal overestimation of the metformin concentrations might be attributed to known low compliance of medication for the alimentary tract (Ruhoy and Daughton, 2008) and analytical uncertainties. The major fraction of metformin is removed during wastewater treatment (97.8 ± 1.0%) in the STPs, which is in line with the literature (Scheurer et al., 2009; Trautwein and Kümmerer, 2011). No significant difference in removal was observed between the STPs of Enschede and Ootmarsum and the removal efficiency did not change significantly with temperature or hydraulic retention time.

Furthermore, guanylurea, a biodegradation product of metformin, was detected at an average concentration of 48 μg/L in the effluent of STP Ootmarsum. Trautwein and Kümmerer (2011) reported the formation of guanylurea in laboratory biodegradation studies and their occurrence in STP effluents. However, concentrations in these effluents were more than one order of magnitude lower than those observed in the current study, even though influent concentrations of metformin were similar. On the other hand Scheurer et al. (2012) reported STP effluent concentrations between 18 and 99 μg/L which are similar to concentrations observed in the present study.

Fig. 3 relates consumption of metformin and guanylurea to recovery in raw wastewater, effluent and surface water in Ootmarsum. 82 ± 52% of the degraded metformin can be recovered as guanylurea after the

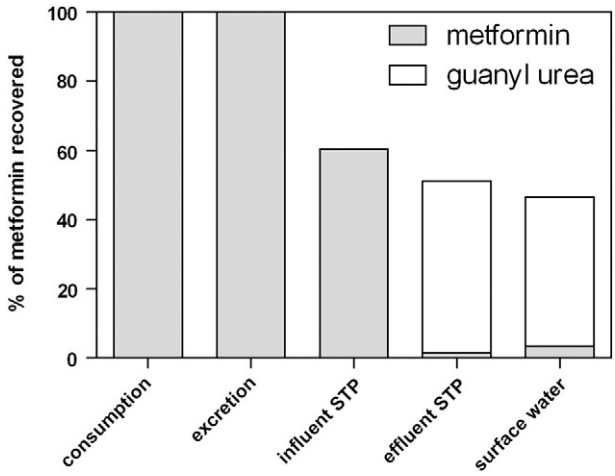

Fig. 3. Fate of metformin from consumption to surface water in Ootmarsum.

activated sludge taking into account the molecular weight ratio of guanylurea and metformin. Furthermore metformin and guanylurea were monitored in receiving surface waters. 87% of the effluent load of guanylurea could be recovered in the surface water showing that guanylurea is recalcitrant in surface water. The recovery of metformin in surface water exceeded 100% by nearly a factor two (187%). The apparent increase of the effluent load of metformin from effluent to surface water can probably not be attributed to grab sampling of the surface water, as potential daily variations of concentrations of metformin are averaged by one day retention in the treatment system and four days retention in the wetland system and surface water. However, biological degradation efficiency in CAS and MBR systems can vary in time (Tchobanoglous et al., 2003). This might explain that the recovery in surface water exceeded 100%, as the concentrations in the surface water originate from emissions with a different ratio of metformin and guanylurea four days earlier.

The number of users of metformin in the Netherlands increased with 34% from 2006 to 2010 (GIP, health insurance college, www.gipdatabank.nl, accessed April 2012). The consumption of metformin is expected to grow as the number of people which suffer from diabetes increases in the future (van der Aa et al., 2011). The high and increasing consumption of metformin and the formation of the recalcitrant guanylurea advocates further research on the behavior and possible ecotoxicological and human health risks of metformin and guanylurea in the water cycle.

4. General discussion and outlook

In the current study, the applied selection of pharmaceuticals for monitoring was based on regional consumption data and their related emission to the sewer system on the basis of daily dose and human excretion rate. It has been shown before that emissions of pharmaceuticals could be estimated on the basis of national consumption data (Richardson and Bowron, 1985; Siemens et al., 2008). However, regional consumption data might provide more accurate predictions of influent concentrations when large discrepancies between regional and national consumption of pharmaceuticals exist. This was for example observed for sotalol and irbesartan (see Table S2 of the Supplemental Information). The regional consumption of sotalol and irbesartan in Ootmarsum exceeded national consumption with 40%. In this case the predictions based on regional consumption were more accurate than predictions based on national sales data would have been.

For most studied pharmaceuticals in this study however, we found low differences between national and regional consumption. National consumption data can thus be a first tier in selecting relevant pharmaceuticals and sampling locations in monitoring programs but prediction of influent concentrations, based on regional consumption, can be more accurate when large discrepancies between regional and national consumption of pharmaceuticals exist.

It should be noted that discrepancies between sales data and actual consumption can exist due to delays between sales and actual use of medication and temporal (seasonal) variations in pharmaceutical consumption. This can bias consumption based predictions of influent concentrations and emissions into the environment. In this study annual regional sales data of 2009 were compared to monitoring data in 2010. Consumption patterns might have shifted to some extent, however, this difference is expected to be marginal for the selected pharmaceuticals. Furthermore, incomplete medication compliance might have biased our predictions. The magnitude of medication compliance is largely unknown, however, Claxton et al. (2001) reported rather low medication compliance of β-blockers of 71%. Biases due to variations in seasonal consumption and delay between sales and actual use are expected to be less relevant since patients use all studied pharmaceuticals except diclofenac on a daily basis.

Regional sales data, human excretion rates, and STP removal rates can also be applied to predict emissions into the aquatic environment. The removal of the pharmaceuticals in the studied STPs was generally comparable to literature data, so average removal rates, reported in literature might be applicable to predict STP effluent and surface water concentrations. However, the variation observed for the removal of certain compounds reported in the literature, as well as significantly different removal rates at different temperatures and hydraulic retention times in this study illustrate that emissions do vary within STPs and between STPs. Removal efficiencies for carbamazepine, hydrochlorothiazide and sotalol increased with wastewater temperature and hydraulic retention time (Table 3). When the effects of temperature and hydraulic retention time on removal of pharmaceuticals in STPs are taken into account, a more accurate prediction of effluent and surface water concentrations is possible.

The recovery of most pharmaceuticals in surface water after 4 days retention was high (71–187%) which suggests that the pharmaceuticals are persistent in the aqueous environment. However, the recovery in surface water was studied in December at low temperatures and is not representing the average situation. Finally, a remarkable result of this study was that circa 50% of the consumed metformin could be recovered as guanylurea in surface water.

It has to be emphasized that sales data do not give any information on possible ecotoxicological or human health risks of pharmaceuticals. So in addition to the consumption based assessment of the emissions and occurrence in the aqueous environment, toxicological evaluation is necessary to evaluate potential (environmental) risks.

5. Conclusions

- The current study illustrates that national consumption data of pharmaceuticals can be very helpful for the selection of relevant pharmaceuticals for environmental monitoring.
- Prediction of surface water concentrations can be improved when regional consumption data are used in combination with accurate data of STPs, i.e. removal rates, conditions and treatment techniques applied.
- Carbamazepine, hydrochlorothiazide, metoprolol and sotalol were significantly better removed at higher wastewater temperatures.
- Carbamazepine, hydrochlorothiazide and sotalol were significantly better removed by conventional activated sludge systems with longer hydraulic retention times while metoprolol was significantly better removed in a membrane bioreactor with a short hydraulic retention time.
- Metformin is readily biodegradable in activated sludge but is nevertheless detected at high concentrations in effluent and surface waters due to its high influent load. The high recovery of the biodegradation product guanylurea illustrates that monitoring stable degradation products can be relevant.
- Highest effluent loads of pharmaceuticals to the environment are expected at low sludge temperatures. Therefore a worst case study of emissions of pharmaceuticals into the environment should be carried out in winter.

Acknowledgements

This work was funded by the water board Regge and Dinkel and Agentschap NL. Supervision on data interpretation and literature research was given within the joint research program of the Dutch drinking water companies (BTO). We thank local pharmacists for providing consumption data and human excretion rates and Pim de Voogt for constructive comments to the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2012.10.046.

References

Alder AC, Schaffner C, Majewsky M, Klasmeier J, Fenner K. Fate of β-blocker human pharmaceuticals in surface water: comparison of measured and simulated concentrations in the Glatt Valley Watershed, Switzerland. Water Res 2010;44:936–48.

Batt AL, Kostich MS, Lazorchak JM. Analysis of ecologically relevant pharmaceuticals in wastewater and surface water using selective solid-phase extraction and UPLC–MS/MS. Anal Chem 2008;80:5021–30.

Bruce GM, Pleus RC, Snyder SA. Toxicological relevance of pharmaceuticals in drinking water. Environ Sci Technol 2010;44:5619–26.

Castiglioni S, Fanelli R, Calamari D, Bagnati R, Zuccato E. Methodological approaches for studying pharmaceuticals in the environment by comparing predicted and measured concentrations in River Po, Italy. Regul Toxicol Pharmacol 2004;39:25–32.

Claxton AJ, Cramer J, Pierce C. A systematic review of the associations between dose regimens and medication compliance. Clin Ther 2001. [23, NO. 8].

Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 1999;107:907–38.

De Jongh CM, Kooij PJ, De Voogt P, Ter Laak TL. Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water. Sci Total Environ 2012;427–428:70–7.

de Voogt P, Janex-Habibi M-L, Sacher F, Puijker LM, Mons M. Development of a common priority list of pharmaceuticals relevant for the water cycle. Water Sci Technol 2009;59:39–46.

Derksen JGM, Roorda JH, Swart D. Verg(h)ulde pillen. Utrecht, the Netherlands: STOWA; 2007. p. 58.

Fent K, Weston AA, Caminada D. Ecotoxicology of human pharmaceuticals. Aquat Toxicol 2006;76:122–59.

Flyborg L, Bjorlenius B, Persson KM. Can treated municipal wastewater be reused after ozonation and nanofiltration? Results from a pilot study of pharmaceutical removal in Henriksdal WWTP, Sweden. Water Sci Technol 2010;61:1113–20.

Hörsing M, Ledin A, Grabic R, Fick J, Tysklind M, Jansen JLC, et al. Determination of sorption of seventy-five pharmaceuticals in sewage sludge. Water Res 2011;45:4470–82.

Huerta-Fontela M, Galceran MT, Ventura F. Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. Water Res 2011;45:1432–42.

Jones OAH, Voulvoulis N, Lester JN. Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. Water Res 2002;36:5013–22.

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. J Chromatogr A 2007;1161:132–45.

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res 2008;42:3498–518.

Lienert J, Gudel K, Escher BI. Screening method for ecotoxicological hazard assessment of 42 pharmaceuticals considering human metabolism and excretory routes. Environ Sci Technol 2007;41:4471–8.

Maurer M, Escher BI, Richle P, Schaffner C, Alder AC. Elimination of β-blockers in sewage treatment plants. Water Res 2007;41:1614–22.

Miege C, Choubert JM, Ribeiro L, Eusebe M, Coquery M. Removal efficiency of pharmaceuticals and personal care products with varying wastewater treatment processes and operating conditions—conception of a database and first results. Water Sci Technol 2008;57:49–56.

Musson SE, Townsend TG. Pharmaceutical compound content of municipal solid waste. J Hazard Mater 2009;162:730–5.

OECD. The 2007 OECD list of high production volume chemicals. Brussels, Belgium: Organisation for Economic Co-operation and Development; 2009. p. 104.

Ort C, Lawrence MG, Reungoat J, Eaglesham G, Carter S, Keller J. Determining the fraction of pharmaceuticals residues in wastewater originating from a hospital. Water Res 2010a;44:605–15.

Ort C, Lawrence MG, Rieckermann Jr, Joss A. Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review. Environ Sci Technol 2010b;44:6024–35.

Radjenovic J, Petrovic M, Barcelo D. Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. Anal Bioanal Chem 2007;387:1365–77.

Radke M, Ulrich H, Wurm C, Kunkel U. Dynamics and attenuation of acidic pharmaceuticals along a river stretch. Environ Sci Technol 2010;44:2968–74.

Richardson, Bowron. J Pharm Pharmacol 1985;37(1):1-12.Zuccato, et al. Lancet 1985: 355-1789.Calamari. ES&T 1985;37:1241.

Roig B. Pharmaceuticals in the environment—current knowledge and need assessment to reduce presence and impact. London, UK: IWA; 2010. p. 198.

Rosal R, Rodriguez A, Perdigon-Melon JA, Petre A, Garcia-Calvo E, Gomez MJ, et al. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Res 2010;44:578–88.

Ruhoy IS, Daughton CG. Beyond the medicine cabinet: an analysis of where and why medications accumulate. Environ Int 2008;34:1157–69.

Sacher F, Ehmann M, Gabriel S, Graf C, Brauch HJ. Pharmaceutical residues in the river Rhine—results of a one-decade monitoring programme. J Environ Monit 2008;10:664–70.

Sanderson H. Presence and risk assessment of pharmaceuticals in surface water and drinking water. Water Sci Technol 2011;63:2143–8.

Scheurer M, Sacher F, Brauch H-J. Occurrence of the antidiabetic drug metformin in sewage and surface waters in Germany. J Environ Monit 2009;11:1608–13.

Scheurer M, Michel A, Brauch H-J, Ruck W, Sacher F. Occurrence and fate of the antidiabetic drug metformin and its metabolite guanylurea in the environment and during drinking water treatment. Water Res 2012;46:4790–802.

Siemens J, Huschek G, Siebe C, Kaupenjohann M. Concentrations and mobility of human pharmaceuticals in the world's largest wastewater irrigation system, Mexico City-Mezquital Valley. Water Res 2008;42(8–9):2124–34.

Singer AC, Howard BM, Johnson AC, Knowles CJ, Jackman S, Accinelli C, et al. Meeting report: risk assessment of Tamiflu use under pandemic conditions. Environ Health Perspect 2008;116:1563–7.

Tchobanoglous G, Burton FL, Stencel DH. Wastewater engineering: treatment and reuse. McGraw-Hill; 2003.

ter Laak TL, Van der Aa M, Stoks P, Houtman C, van Wezel AP. Relating environmental concentrations of pharmaceuticals to consumption: a mass balance approach for the river Rhine. Environ Int 2010;36:403–9.

Trautwein C, Kümmerer K. Incomplete aerobic degradation of the antidiabetic drug Metformin and identification of the bacterial dead-end transformation product Guanylurea. Chemosphere 2011;85:765–73.

Van den Berg Jeths A, Van Batenburg-Eddes T. The Netherlands swallows differently. Regional variations in cost and usage of medicines. Pharm Weekbl 2003;138:840–4.

van der Aa NGFM, Kommer GJ, van Montfoort JE, Versteegh JFM. Demographic projections of future pharmaceutical consumption in the Netherlands. Water Sci Technol 2011:825–32.

Vergouwen L, Hehenkamp M, van Veldhuizen H, Breedveld R. Gebiedsstudie geneesmiddelen in de provincie Utrecht. H2O; 2011b. p. 23–5.

Vergouwen AA, Pieters BJ, Kools S. Inventarisatie van emissie van geneesmiddelen uit zorginstellingen. Amersfoort, The Netherlands: ZORG, Deel C. STOWA; 2011a.

Vieno N, Tuhkanen T, Kronberg L. Elimination of pharmaceuticals in sewage treatment plants in Finland. Water Res 2007;41:1001–12.