Zeitschrift:	Archives des sciences [2004-ff.]
Herausgeber:	Société de Physique et d'histoire Naturelle de Genève
Band:	65 (2012)
Heft:	1-2
Artikel:	Pharmaceuticals and their human metabolites in Lake Geneva : occurence, fate and ecotoxicological relevance
Autor:	Bonvin, Florence / Chevre, Nathalie / Rutler, Rebecca
DOI:	https://doi.org/10.5169/seals-738357

#### Nutzungsbedingungen

Die ETH-Bibliothek ist die Anbieterin der digitalisierten Zeitschriften. Sie besitzt keine Urheberrechte an den Zeitschriften und ist nicht verantwortlich für deren Inhalte. Die Rechte liegen in der Regel bei den Herausgebern beziehungsweise den externen Rechteinhabern. <u>Siehe Rechtliche Hinweise.</u>

#### **Conditions d'utilisation**

L'ETH Library est le fournisseur des revues numérisées. Elle ne détient aucun droit d'auteur sur les revues et n'est pas responsable de leur contenu. En règle générale, les droits sont détenus par les éditeurs ou les détenteurs de droits externes. <u>Voir Informations légales.</u>

#### Terms of use

The ETH Library is the provider of the digitised journals. It does not own any copyrights to the journals and is not responsible for their content. The rights usually lie with the publishers or the external rights holders. <u>See Legal notice.</u>

**Download PDF:** 30.03.2025

ETH-Bibliothek Zürich, E-Periodica, https://www.e-periodica.ch

# Pharmaceuticals and their human metabolites in Lake Geneva:

# occurrence, fate and ecotoxicological relevance

#### Florence BONVIN<sup>1</sup>, Nathalie CHEVRE<sup>2</sup>, Rebecca RUTLER<sup>3</sup> and Tamar KOHN<sup>4,\*</sup>

Ms. received the 9th August 2012, accepted 30th November 2012

#### Abstract

This study assesses the presence, fate and effects of five environmentally relevant pharmaceuticals and eight of their corresponding human metabolites in Lake Geneva. Over a 10-month period, lake samples were taken at various depths and locations in the Vidy Bay. Among the targeted metabolites, six were detected with concentrations ranging from 5 to > 100 ng  $\cdot$  L<sup>-1</sup>. The highest concentrations were detected above the wastewater treatment plant outfall, supporting the assumption that wastewater represents the main source of human metabolites to the lake. The metabolites were less recalcitrant to environmental degradation than their parent. Nevertheless, their presence in the aquatic environment may lead to an increase of the ecotoxicological risk.

**Keywords:** human metabolites, lake, ecotoxicological risk, wastewater plume, sulfamethoxazole, diclofenac, clarithromycin, carbamazepin

#### Introduction

#### Micropollutants in the aquatic environment

Over the past two decades, an increasing number of studies have documented the presence of low concentrations (ng·L<sup>-1</sup> range) of various anthropogenic organic substances, or micropollutants, in surface and groundwaters (Daughton and Ternes 1999; Heberer 2002; Kolpin et al. 1998; Kolpin et al. 2002; Schwarzenbach et al. 2006). Anthropogenic micropollutants include pharmaceuticals, personal care products, pesticides, xenoestrogens, biocides and other substances of human origin. These substances can enter the aquatic environment via runoff from

agriculture and urban landscapes, atmospheric deposition, and industrial and municipal wastewater streams. Despite the abundance of occurrence data, less is known about the fate of micropollutants once they reach the environment. Main degradation pathways in surface waters are believed to include photolytic degradation or biodegradation (Boreen et al. 2003; Kagle et al. 2009), but knowledge of the corresponding transformation rates and products is incomplete. Information is even scarcer regarding the effects that these individual substances or their mixtures exert on the ecosystem. Some studies report shifts in microbial populations (Johnson et al. 2009), feminization of fish (Sumpter 1995) or of frogs (Hayes et al. 2002). However, much remains to be understood regarding the mechanisms of action

<sup>&</sup>lt;sup>1</sup> Laboratory of Environmental Chemistry, School of Architecture, Civil and Environmental Engineering (ENAC), École Polytechnique Fédérale de Lausanne (EPFL), Station 2, 1015 Lausanne, Switzerland. E-mail: florence.bonvin@epfl.ch

<sup>&</sup>lt;sup>2</sup> Institute of Earth Sciences, Faculty of Geosciences and Environment, University of Lausanne. 1015 Lausanne, Switzerland.

E-mail: nathalie.chevre@unil.ch <sup>3</sup> Laboratory of Environmental Chemistry, School of Architecture, Civil and Environmental Engineering (ENAC), École Polytechnique Fédérale de Lausanne (EPFL), Station 2, 1015 Lausanne, Switzerland.

<sup>&</sup>lt;sup>4</sup> Laboratory of Environmental Chemistry, School of Architecture, Civil and Environmental Engineering (ENAC), École Polytechnique

<sup>Fédérale de Lausanne (EPFL), Station 2, 1015 Lausanne, Switzerland. E-mail: tamar.kohn@epfl.ch
\* Corresponding author.</sup> 

underlying these effects. As such, micropollutants in the environment have emerged as one of the preeminent research fields in water quality.

#### Micropollutants in Lake Geneva

In Europe, approximately 50 new substances are authorized for use in commercial and industrial markets every year, adding to the 100'000 of compounds already in use (Gebel et al. 2009). Through pointsource and disperse pollution sources, many of these compounds are expected to enter rivers and lakes. In Lake Geneva, a large selection of micropollutants has effectively been detected on a regular basis throughout the water column, within the framework of the International Commission for the Protection of Lake Geneva (Edder et al. 2006; Edder et al. 2007; Edder et al. 2008), as well as in other studies (Bonvin et al. 2011; Morasch et al. 2010). The range of detected compounds includes a suite of pesticides and biocides, several classes of pharmaceuticals, X-ray contrast media, corrosion inhibitors and endocrine disruptors. Pesticides mainly originated from diffuse sources, such as runoff from land. The presence of some pharmaceuticals could be related to industrial discharge into the Rhône River, the main tributary of Lake Geneva. However, most pharmaceuticals, corrosion inhibitors and selected biocides were found to be associated with wastewater discharge into the lake. Recent work has shown that during the summer stratification period of the lake, the discharged wastewater does not readily mix with the surrounding water column, but instead forms a plume of significant spatial extent below the thermocline (Bonvin et al. 2011). Within this plume, the concentrations of several micropollutants exceeded the predicted noeffect concentrations (PNEC), indicating a potential ecotoxicological risk to the environment. Thus, the practice of direct discharge of wastewater into the lake is of particular concern with respect to ecotoxicological effects of micropollutants.

#### Pharmaceuticals and their human metabolites

Among the different micropollutant classes detected in Lake Geneva and worldwide, pharmaceuticals are of exceptional interest. Because they are designed to fulfill a specific biological function, they are prone to exert undesired biological effects in the environment. In particular antibiotics have been a priority, as they may not only exert ecotoxicologial effects, but may also promote antibiotic resistance in the environment (Kummerer 2009).

An important route of pharmaceuticals into the environment is the ingestion by humans and subsequent excretion into the sewage system (Ternes and Joss 2006). After intake by humans, pharmaceuticals are metabolized to various extents. Thus, a variable fraction of the active pharmaceutical, along with its metabolites, is released via urine and feces into raw wastewater. Due to incomplete removal during wastewater treatment (Oulton et al. 2010), many pharmaceuticals are ultimately discharged into receiving waters. Therefore, centralized sewage treatment plants represent an important point source for pharmaceuticals and their metabolites into the aquatic environment.

The presence of metabolites in the environment has received little attention to date. Nevertheless, available data show that metabolites are present in wastewater effluent in concentrations comparable to or higher than their parent compound (Diaz-Cruz et al. 2008; Gobel et al. 2004; Hummel et al. 2006; la Farre et al. 2008; Miao et al. 2005; Perez and Barcelo 2008a; Stulten et al. 2008). Leclercq et al. found 2-5 times higher concentrations of the main metabolite of carbamazepine (10,11-dihydro-10,11-trans-dihydroxycarbamazepine) in wastewater of three different treatment plants, in addition to other metabolites of this antiepileptic drug (Leclercq et al. 2009). Hilton reported high concentrations of N4-acetylsulfamethoxazole, a metabolite of sulfamethoxazole, in treated sewage (Hilton and Thomas 2003). Human metabolites were not only detected in wastewaters, but also in the receiving environment, though data remain scarce. Trace concentrations of human metabolites were found in streams receiving wastewater effluent, in drinking water (the main metabolite of carbamazepine) and even in bottled water (Diaz-Cruz et al. 2008; Hummel et al. 2006).

Human metabolites of pharmaceuticals are generally more polar and less biologically active than their parent substance, though some metabolites are known to retain biological function (Debska et al. 2004; Trovo et al. 2009). Furthermore, human metabolites have been found to back-transform to the parent substance via both biotic (Radke et al. 2009) and abiotic processes (Bonvin et al. 2012). Human metabolites could thus present a source of pharmaceuticals to the environment. Finally, despite their increased polarity, some human metabolites have been found to be more persistent to typical environmental degradation processes, such as photolysis, compared to their parent substance (Bonvin et al. 2012; Boxall et al. 2004). Combined, these arguments make a compelling case that human metabolites should be included in the study of presence, fate and effects of micropollutants.

The goal of this study was to determine the presence of a selection of human metabolites in Lake Geneva, to assess their fate with respect to degradation over time and space, and to evaluate their contribution to the overall ecotoxicological risk. We have focused our work on the Vidy Bay, as this area was found to

exhibit high micropollutant concentrations, likely due to the discharge of effluent wastewater from the Vidy treatment plant. In the present study, we focus on the human metabolites of a selection of pharmaceuticals. Specifically, we investigated human metabolites of three antibiotics (clarithromycin, norfloxacin and sulfamethoxazole), one analgesic (diclofenac) and an anti-epileptic (carbamazepine). The spatial temporal distribution of the parent compounds was already examined in a previous study; the first four were found to originate primarily from wastewater effluent, and were previously reported in concentrations close to or beyond the PNEC (Bonvin et al. 2011). Conversely, carbamazepine, though also present in wastewater effluent, mainly enters the lake from industrial inputs into the Rhône River.

#### Materials and Methods

#### Site description and sampling strategy

The study area, Vidy Bay, lies on the northern shore of Lake Geneva, and represents 0.3% of the lake's total volume (Chevre et al. 2011). Aside from lake currents, 100'000 m<sup>3</sup> of wastewater effluent are discharged per day into Vidy Bay from Lausanne's wastewater treatment plant (WWTP). The WWTP outfall is located 700 m from shore at -30 m depth. During rain events the WWTP capacity is rapidly exceeded, and untreated wastewater is released directly into the lake (Pote et al. 2009). Other sources of water to the Bay are the Chamberonne River and the Flon stormwater outlet. Monthly water samples were collected at various depths and locations in Vidy Bay between April 2010 and January 2011. The central sampling location ("WWTP outfall") was above the discharge point of the effluent wastewater. The two other sampling sites were located ca. 1.5 km upstream ("REF up") and downstream ("REF down") of the discharge point (Fig. 1). They will be considered together and referred to as reference sites or REF. Between 5 and 9 depths were sampled at each site. Samples were taken from the R/V "La Licorne" (Forel Institut, Geneva) equipped with a crane and a rosetta water sampler (1018 Rosette Sampling System, General Oceanics Inc.). The rosetta consisted of 11 Niskin bottles (1.7 L), coupled to a CTD device (OCEAN SEVEN 316Plus CTD, IDRONAUT Srl) which were externally powered via a sea cable and yielded instantaneous information on temperature and electrical conductivity. Temperature and electrical conductivity were processed with REDAS-5 Release 5.40 (IDRONAUT Srl).

#### Choice of substances

Previous research in Vidy Bay retained a selection of 27 pharmaceuticals as priority pollutants for this area based on consumption loads, the degree of human metabolism, removal in the WWTP, previous detection in the Vidy bay, and – if known – ecotoxicological relevance (Bonvin et al. 2011, Perazzolo et al. 2010). A subset of these chemicals, namely carbamazepine (CBZ), clarithromycin (CLA), diclofenac (DCF), norfloxacin (NOR) and sulfamethoxazole (SMX), were chosen herein for analysis of their human metabo-



Fig. 1. Situation and map of the Vidy Bay showing the sampling locations WWTP outfall (Swiss coordinates: 534'672/151'540), REF-up (Swiss coordinates: 536'000/151'000) and REF down (Swiss coordinates: 533'048/150'920). Coordinates are in Swiss Grid system with datum CH1903.

lites. Deciding factors included their ecotoxicological impact in Vidy Bay, the commercial availability of their metabolites, and previous evidence for their presence in wastewater or surface waters in literature. The targeted metabolites included: trans-10,11dihydro-10,11-dihydroxy carbamazepine (DiOH-CBZ), carbamazepine 10,11-epoxide (epoxy-CBZ), 10,11-dihydro-10-hydroxy carbamazepine glucuronide (DiOH-CBZ-glu), N-desmethyl clarithromycin (desm-CLA), 4'-hydroxy diclofenac (4-OH-DCF), Nacetyl norfloxacin (acetyl-NOR), sulfmethoxazole  $\beta$ -D glucuronide (SMX-glu) and N-acetyl sulfamethoxazole (acetyl-SMX). The structures of the selected micropollutants and their corresponding human metabolites are shown in Fig. 2.

#### Chemicals and reagents

Most human metabolites were purchased from Toronto Research Chemicals (Canada). N-acetyl sulfamethoxazole was obtained from CHEMOS GmbH (Germany), and carbamazepine 10,11-epoxide from Sigma Aldrich (Switzerland). Other chemicals and reagents used in the analytical procedure have been listed previously (Morasch et al. 2010).

Precursor ion [m/z]	Fragment ions [m/z]	Cone [V]	Collision [V]	Retention time [min]	LOQ <sup>a</sup> [ng·L <sup>-1</sup> ]	Extraction efficiency	PNEC <sup>b</sup> [ng·L <sup>-1</sup> ]
253.27	167.15	22	36	3.8	<1	0.3 ± 0.08	2500
P(B)rule	179.7	22	22				
271.33	210.18	22	14	3.5	<1	1.8 ± 0.23	2500
	236.18	22	12				
206.22	65.01	34	36	3.1	<1	1.39 ± 0.19	28
230.22	134.12	34	28				
212 22	230.14	20	36	6.1	1.1	1.12 ± 0.19	100
512.22	266.15	20	12				
362.43	231.15	58	38	4.6	<1	0.7 ± 0.08	40100
	274.24	54	34				
430.3	156.08	26	30	2.1	1	0.99 ± 0.11	28
	254.16	26	14				
431.29	194.18	24	34	3.3	19	n.d.	2500
	237.14	24	12				
734.8	83.14	30	54	6.3	<1	0.62 ± 0.04	60
	144.18	30	24				
	Precursor         253.27         271.33         296.22         312.22         362.43         430.3         431.29         734.8	Precursor ion [m/z]Fagment ions [m/z]253.27 253.27167.15 210.18 236.18271.33 236.18236.18 230.14296.22 296.22 312.22 362.43 362.43 362.43 362.43 231.15 	Precursor (on [n/c])Fragment (on [n/c])Schei (n/c)253.27167.1522271.33210.1822271.33236.1822296.2265.0134296.24230.1424312.22230.1420312.22230.1420362.43231.1558362.43231.1558362.43254.1620430.34254.1620431.29194.1824734.883.1430	Precursor ion (m/c)Fragment ions (n/c)Collision (N)253.27137.152.23.2179.72.22.22.2271.332.10.182.214.271.332.36.182.21.2296.2265.013.43.6296.222.30.143.42.8312.222.30.143.43.6362.432.30.145.83.8362.432.31.155.83.8362.432.54.165.43.4430.32.54.162.63.4431.29194.182.43.4734.83.143.02.4	Precursor ion (n/z)Fragment ons (n/z)Collision ivel (n/z)Retention ime (n/z)253.27167.152.23.63.8219.27179.772.22.23.8271.33210.182.21.43.8236.182.21.43.73.1296.2265.013.43.63.1236.183.43.63.13.1312.2265.013.43.63.1312.23230.143.43.63.1362.43230.145.83.83.1362.43231.155.83.83.6362.43254.165.43.43.1343.432.53.63.13.1343.432.43.43.33.3373.483.63.13.33.3373.483.33.53.43.3373.483.02.43.43.3	Precursor ion [nv]Fagment ion [nv]Cone ivRetention ive [nu]LOQ a ive [nu]253.27167.152.23.63.8-1179.72.22.23.8-1179.72.22.23.8-1210.182.21.4-3.5-123.182.21.4-3.5-1236.182.21.2-3.5-1236.182.21.2-3.1-1236.183.43.6-3.1-1134.123.42.8-3.1-1312.22230.143.42.03.6-1312.23230.142.03.6-6.11.1362.43230.145.83.8-4.6-1362.432.75.83.8-4.6-1430.3156.083.63.0-2.11431.29194.182.43.4-3.3-1734.83.8.143.05.4-4.6-4.6734.83.143.05.4-4.6-4.6	Precursor ion [nv]Fragment ons [nv]Coll inpact (nopact)Retention inpact (nopact)Extraction efficiency253.27167.1522363.8.33.9.33.9.33.9.33.9.33.9

<sup>a</sup>LOQ: limit of quantification; <sup>b</sup>PNEC (predicted no effect concentration). PNEC of the corresponding parent compound was used for human metabolite PNEC (see text for details).

#### Sample manipulation

1 L water samples were collected in Niskin bottles and transferred into amber glass bottles, immediately acidified to pH 2 with concentrated HCl to inhibit biological activity, and transported to the laboratory within 4 hours of sampling, where they were directly filtered through >1µm glass fiber filters (Whatman). Before further treatment, all filtrates were spiked with a set of deuterated standards (final concentration 120 ng·L<sup>-1</sup>) to account for losses of the parent compound during extraction, as described previously (Morasch et al. 2010). As deuterated standards of the metabolites were not available for spiking at the time of sampling, their losses during extraction were determined in experiments using spiked lake water, as described in the following paragraph. All glassware used for samples was immersed for 24h in a Contrad bath, machine-washed and finally rinsed with methanol and sample water before use

#### Analytical method

The concentrations of the parent compounds in lake water samples were analyzed following a previously developed analytical method involving solid-phase

> extraction (SPE) and ultra-performance liquid chromatography coupled to a tandem mass spectrometer (UPLC-MS/MS) (Bonvin et al. 2011, Morasch et al. 2010). Additionally, an analytical method for the detection of human metabolites was developed. Briefly, the target compounds were extracted on hand-assembled 6mL cartridges using an automated SPE system, eluted and evaporated, under a flux of nitrogen, at 40°C to 200 µL and finally stored at -20°C until UPLC-MS/MS analysis. Analytical details regarding the determination of the parent compound concentration have been described previously (Bonvin et al. 2011). For analysis of targeted metabolites, the stored extracts were diluted 1:6 with UPLC eluent (A) and analyzed twice by UPLC-MS/MS. Reported values represent the average of the two separate measurements. Compounds were

Table 1. Details of analytical method for MS/MS analysis of targeted human metabolites and PNEC used in risk assessment.



Fig. 2. Structures of the targeted pharmaceuticals (blue box) and their selected human metabolites.

separated on a Waters ACQUITY UPLC HSS T3 1.8µm column. The mobile phases were (A) 95% H<sub>2</sub>O; 5% MeOH; 1% Formic acid; 5mM Ammonium formate and (B) 95% MeOH; 5% H<sub>2</sub>O; 1% Formic acid; 5mM Ammonium formate. The first minute the portion of A decreased from 90 to 60%, followed by a linear gradient for 7 min to 5% A. Thereafter the initial conditions were restored and held for 3 minutes. The injection volume was 10 µl, and the flow rate 0.3 mL·min<sup>-1</sup>. UPLC-MS/MS parameters for our selection of human metabolites are listed in Table 1; a positive ionization potential (ESI-mode) was applied for all compounds. A set of 10 to 13 standards with concentrations between 1 and 700  $\mu$ g·L<sup>-1</sup> were analyzed in duplicate along with the samples. The analytical limit of quantification (LOQ; Table 1) was defined as the concentration of the lowest standard with a signal-to-noise ratio >10. Human metabolite concentrations in the sample concentrates were calculated based on calibration curves using at least 6 calibration points closest to the sample concentration. Correlation coefficients for the calibration curves were typically > 0.990. Extraction recoveries (Table 1) and repeatability were determined by a method reproducibility test, in which 6 replicates of lake water spiked with all metabolites to 50 ng·L<sup>-1</sup> were processed by the entire sample workup procedure. The concentrations in the original lake water samples were determined taking into account the extraction efficiencies determined by this test. The reproducibility of the entire method was high, resulting in a relative standard deviation of less than 25% of the calculated concentration.

#### Risk assessment

For a single compound i, the risk for aquatic organisms is normally evaluated by comparing the measured environmental concentration (MEC) in the aquatic system under consideration to either the predicted no-effect concentration (PNEC; [European-Commission 2003]) or the hazardous concentration (HC; [Backhaus et al. 2003b]). In this study, we calculated the risk based on the PNEC, as not enough data was available for the compounds under consideration to determine a HC.

The ratio of  $MEC_i$  to  $PNEC_i$  (RQ<sub>i</sub>, Eq. 1) must be smaller than one to ensure that a given chemical ipresents an acceptable risk to the environment.

$$RQ_{i} = \frac{MEC_{i}}{PNEC_{i}} < 1$$
 (1)

For mixtures of compounds exhibiting a similar mode of action, the mixture effect can be predicted by the concept of concentration addition (CA; [Backhaus et al. 2003a]). As no ecotoxicological information was available for the metabolites, we assumed that the mode of action of a metabolite is similar to that of its parent compound, and therefore defined the PNEC of metabolites as equivalent to the PNEC of the parent compound. This assumption will be discussed in the Results and Discussion section. The risk quotient for mixtures ( $RQ_m$ ) of a parent compound and its metabolites thus corresponds to the sum of the individual  $RQ_i$  (Eq. 2). The  $RQ_m$  must remain smaller than one to ensure that a mixture of chemicals presents an acceptable risk to the environment:

$$RQ_{m} = \sum_{i=1}^{n} RQ_{i} = \sum_{i=1}^{n} \frac{MEC_{i}}{PNEC_{i}} < 1$$
 (2)

PNECs of the individual parent compounds (and hence of their metabolites; Table 1) that were used to calculate RQ have been determined in a previous study (Bonvin et al. 2011 and references therein).

#### Results and discussion

#### Occurrence of human metabolites in Lake Geneva

Over 140 lake water samples were analyzed for the presence of five pharmaceuticals along with a selection of their human metabolites. The frequency of detection and the observed concentrations varied with time and compound. Two metabolites, namely CBZ-glucuronide and N-acetyl NOR, were never detected over the entire sampling period. SMX-glucuronide and 4-OH-DCF showed a relatively low overall frequency of detection of 9 and 21% respectively. These compounds were mainly detected at the WWTP outfall sampling site. Higher frequencies of detection were observed for DiOH-CBZ, desm-CLA, acetyl-SMX and epoxy-CBZ, which were detected in 96, 89, 50 and 43% of all samples, respectively. Some difficulties were encountered with the detection of epoxy-CBZ which was unstable during analysis; thus the reported detection frequencies and concentrations for this compound may underestimate the actual occurrence.

Generally, the detected concentrations of human metabolites were in the same range or below those of their parent compound (Fig. 3). The absolute range of concentrations was compound specific: CBZ and its metabolite epoxy-CBZ showed the highest concentrations with a median of 20 ng·L<sup>-1</sup>. The concentrations of all the other compounds and metabolites were generally below 10 ng·L<sup>-1</sup>. In a few samples, namely those obtained in close vicinity to the wastewater outfall, significantly higher concentrations of up to > 100 ng L<sup>-1</sup> were detected.

Though numerous human metabolites have been identified for CBZ, the major metabolic route involves transformation to epoxy-CBZ, which is subsequently



Fig. 3. Range of concentrations detected at all sampling sites over the entire sampling period of the parent compounds (shaded box) and their corresponding human metabolites. The frequency of detection (f) is given for each compound. Boxes represent 25th percentile, median and 75th percentile. The whiskers are determined by the 5th and 95th percentiles. Stars show the mean value.

hydrated to DiOH-CBZ. In addition, DiOH-CBZ can be conjugated with a glucuronide. Therefore DiOH-CBZ and the corresponding glucuronide are the main metabolites of CBZ (Bellucci et al. 1987). Several studies have effectively detected DiOH-CBZ in influent and effluent wastewater samples, as well as in surface waters in concentrations up to three times those of the parent compound CBZ (Leclercq et al. 2009; Miao and Metcalfe 2003; Miao et al. 2005). Conversely, the present study found significantly lower concentrations of DiOH-CBZ than its parent. This was attributed to the industrial sources of CBZ to Lake Geneva (Bonvin et al. 2011), which maintain relatively high and stable concentrations of the parent compound throughout the water column. Accordingly, significant differences of detected concentrations were observed between the WWTP outfall and the reference point only for the human metabolites and not CBZ. The other major metabolite, CBZglucuronide has never been reported to date in environmental samples, however there is evidence for cleavage of the glucuronide acid moiety in wastewater treatment plants (Leclercq et al. 2009 and references therein). The CBZ-glucuronide standard was highly unstable in aqueous and organic solutions; hence its absence from the lake water samples is not surprising. Concentrations of clarithromycin were relatively low (<5 ng L<sup>-1</sup>) and corresponding metabolite concentrations were significantly lower. Though the desmethylated metabolite of clarithromycin was detected in many samples, the detected concentrations were frequently below the limit of quantification. To our knowledge, this is the first report of the occurrence of a human metabolite of clarithromycin in wastewater or surface water samples.

Up to 50% of ingested diclofenac is excreted as the hydroxylated metabolite 4-OH-DCF and its corresponding glucuronide, while less than 10%remains unaltered (Stierlin et al. 1979). Thus the presence of the major metabolites in untreated sewage is to be expected. Indeed, various studies have found 4-OH-DCF in both influent and effluent wastewater, with only partial removal (<50%) via various wastewater treatment processes (Perez and Barcelo 2008b; Stulten et al. 2008). The concentrations were sometimes as elevated as those of the parent compound in effluent samples. Averaged over all samples (Fig. 3), the present study found similar concentration ranges of 4-OH-DCF and its parent in the Vidy Bay. More

detailed analysis of individual sample concentrations, however, show generally higher concentrations of the metabolite above the WWTP outfall, whereas the inverse is observed at the reference sites.

Approximately 14% of ingested sulfamethoxazole is excreted unchanged; the remaining fraction is metabolized mainly to acetyl-SMX (47%) and SMXglucuronide (14%) (Vanderven et al. 1995). Acetyl-SMX has been detected in wastewater effluent (Gobel et al. 2004) as well as surface waters at concentrations comparable to its parent compound (Ashton et al. 2004). For SMX-glucuronide, in contrast, this is the first evidence of its occurrence in surface waters. Its low frequency of detection (15%) was expected given the unstable nature of the glucuronide bond, which can be microbially cleaved (Radke et al. 2009). The overall concentrations of acetyl-SMX detected in this study were significantly lower than those of SMX. As for DCF, however, a sample-by-sample analysis revealed that in samples taken above the WWTP outfall, the metabolite concentration was significantly higher than that of the parent compound.

#### Peak metabolite concentrations in wastewater plume

The presence of a wastewater plume originating from the direct discharge of treated (and during high rain events, also untreated) wastewater into the Vidy Bay was reported in a recent study (Bonvin et al. 2011).



Fig. 4. Depth profiles of relative concentrations of the targeted human metabolites (full lines) and their corresponding parent compound (dashed lines) for a) April, b) August, c) October and d) December 2010. Temp: temperature; EC: electrical conductivity.

Locally high concentrations of pharmaceuticals were observed above the WWTP outfall site. The plume depth followed the thermocline during thermal lake stratification, which moved to lower depths over the course of the warm seasons. Additionally, a strong linear correlation between electrical conductivity and concentrations of wastewater-derived micropollutants was identified. In absence of thermal stratification, from November to January, no plume was observed.

The presence of human metabolites of pharmaceuticals in wastewater has been well-established (Gobel et al. 2004; Leclercq et al. 2009; Perez and Barcelo 2008b); hence the latter should logically show the plume feature. Indeed, notably higher concentrations of our targeted human metabolites were observed in vicinity to the WWTP outfall in April (Fig. 4a) and August (Fig. 4b). These high concentrations coincided with an elevated conductivity, which is indicative of wastewater. A slight plume feature could still be observed at 26 m depth in October (Fig. 4c); whereas homogenous concentrations are established in December (Fig. 4d) when complete mixing of the water column was restored.

In general, the relative peak concentrations of the metabolites were more pronounced than their associated precursor. This could either be due to faster degradation of the metabolite with regard to its parent, or to high background concentrations of the parent, which may diminish the peak amplitude. For example, carbamazepine, which enters the lake with the Rhône River, showed little to no peak feature in the wastewater plume. Conversely, its human metabolite, DiOH-CBZ, showed evident concentration peaks above the WWTP outfall, in accordance with the hypothesis that its main source is wastewater.

The unstable nature of SMX-glucuronide mentioned earlier was confirmed by a low detection frequency in the water column. However, it was detected in the plume in April and August, as well as in other months (not shown), displaying a strong plume feature.



Fig. 5. Temporal and spatial variation of detected concentrations of DiOH-CBZ (left), 4-OH-DCF (middle) and acetyl-SMX (right). The solid line shows the median concentration and shaded area the 25-75% quantile range.

## Spatial and temporal variation of metabolite concentrations in Vidy Bay

During thermal stratification, the locally high concentrations of human metabolites associated with the wastewater plume led to notably elevated median concentrations at the WWTP outfall site (Fig. 5). Additionally, the 25-75% quantile ranges were large due to this plume feature. Conversely, after mixing of the wastewater with the surrounding water mass and travel to the reference sites, the water column concentrations of the targeted human metabolites were lower and more homogeneous. These homogeneous conditions, depicted by a narrow 25-75% quantile range in Fig. 5, were also observed during the cold months (November to January) at the WWTP outfall site. This can be explained by enhanced mixing throughout the water column, as temperature profiles straighten during the winter.

Generally, metabolite concentration trends at the reference sites closely tracked those of the wastewater plume. Among the metabolites considered, only acetyl-SMX exhibited noticeable concentration differences at the reference sites between the warm and cold seasons, consistent with the higher concentrations in the plume during the same period. Notably, however, all metabolites appeared to decrease in concentration during the month of August.

#### Degradation within the Vidy Bay

After discharge from the WWTP, and upon dispersion of the wastewater plume, wastewater-derived micropollutant concentrations decrease with distance from the WWTP outlet due to dilution with surrounding water masses. In addition, environmental degradation processes, such as biodegradation or photolysis, can decrease micropollutant concentrations in the water column. The substances studied herein have previously been reported to undergo various extents of degradation in the Vidy Bay (Bonvin et al. 2011; Morasch et al. 2010): The concentrations of CLA and DCF were found to readily diminish, whereas SMX was more recalcitrant. As CBZ's main source was not the WWTP, no apparent decrease in concentration was found throughout the Vidy Bay. Furthermore, CBZ is known to be particularly recalcitrant to environmental degradation (Tixier et al. 2003).

Compared to their parent compounds, human metabolites may be more susceptible to environmental degradation processes. For example, in a laboratory batch reactor, 4-OH-DCF was found more susceptible to biodegradation than its parent (Perez and Barcelo 2008b). However, incomplete removal of this compound during wastewater treatment (Perez and Barcelo 2008b; Stulten et al. 2008) indicates that biodegradation proceeds at a slow rate. Furthermore, both acetyl-SMX and SMX-glucuronide were reported to undergo biodegradation back to SMX during wastewater treatment (Gobel et al. 2005) and in sediment tests (Radke et al. 2009). Finally, SMX-glucuronide has recently been found to be more photolabile than SMX, whereas acetyl-SMX was slightly more photostable (Bonvin et al. 2012).

To assess if human metabolites undergo environmental degradation in the Vidy Bay, and to determine the relative extent of degradation compared to the parent substance, we calculated the ratio of metabolite/parent concentration close to the wastewater outfall, as well as at the reference points located 1.5 km up- or downstream (Fig. 6). Both metabolites and parents are equally affected by dilution; consequently, the difference in ratio between the wastewater discharge and the reference indicates the relative propensity of the metabolite to undergo environmental degradation during passage through the Vidy Bay. This analysis could only be conducted for those substances for which both parent and metabolites concentrations were detected at a high frequency.

As can be seen in Fig. 6, the metabolite/parent ratio was generally greater for samples taken near the



Fig. 6. Seasonal and spatial (WWTP-plume vs reference sites) variation of the metabolite/parent compound ratio.

wastewater outlet than in the corresponding reference samples. The difference was most drastic in April, where the wastewater sample captured a particularly pronounced plume feature, and thus consisted of poorly diluted wastewater (Fig. 4).

Correspondingly, metabolites were present at concentrations up to 4.5-fold greater than their parent compound, consistent with the notion that pharmaceuticals are mainly excreted into sewage in metabolized form. In both April and August, the metabolite/parent ratio dropped off steeply between the plume and the reference points, indicating that metabolites underwent more rapid environmental degradation than their parent compounds. During the months of October and December, the difference in ratio was less pronounced. This could be due to enhanced mixing of the discharged wastewater with the surrounding water column during the colder months (Fig. 4). Furthermore, both biodegradation and photolysis processes are less efficient in fall and winter. Compared to dilution, the degradation of metabolites may thus play a smaller role during the cold period.

Overall, it can be stated that the metabolites considered herein are less recalcitrant in the environment than their parent compounds. As it is not fully clarified which products are formed during environmental degradation, however, it cannot be concluded that metabolites therefore are of lesser ecotoxicological concern. In particular for those metabolites which can be back-transformed to their parent compound, environmental degradation may lead to an increase in ecotoxicological risk.

#### Ecotoxicological risk assessment

Given the presence of metabolites in Lake Geneva, the important question arises whether these compounds are of ecotoxicologial relevance. Explicitly, our final goal was to determine if metabolites make a significant contribution to the ecotoxicological risk presented by the parent compounds. As was



Fig. 7. Time course of mixture risk quotient  $(RQ_m)$  for the sum of pharmaceuticals carbamazepine, clarithromycin, diclofenac and sulfamethoxazole and their associated metabolites for concentrations detected in the plume (left) and average water column concentrations detected at REF down (right). RQ > 1 (red line) indicates a potential ecotoxicological risk.

determined previously, DCF and SMX both presented an ecotoxicological risk (i.e., RQ > 1) in several plume samples (Bonvin et al. 2011). In contrast, the detected concentrations of CLA and CBZ were always below the corresponding PNEC.

Even if metabolites are taken into account, a negligible risk is estimated for all mixtures of parent compound and metabolites at the reference point. At the plume, however, a risk is highlighted for DCF and metabolites in June and for SMX and metabolites in April and June. Taking into account metabolites leads to both a more frequent excess of the RQ of 1, as well as to higher overall RQs. For SMX, the RQ reaches a value of 3.3 in June, compared to 2.4 taking into account only the parent compound, suggesting that this pharmaceutical may be of environmental concern. Similarly, for DCF in June, the RQ only exceeded 1 if metabolites were considered, whereas it amounted to 0.56 for the parent alone. Similarly, other studies have reported that the combination of several xenoestrogens led to ecotoxicological effects, even if each individual compound was present at no effect concentrations (Silva et al. 2002). This highlights the importance of taking into account not only individual substances, but classes of compounds with the same mechanisms of action. To our knowledge, no ecotoxicological information is available to date on human metabolites of pharmaceuticals; therefore it is not possible to determine accurate PNECs. In this study, we therefore assumed the PNEC of the metabolites to be similar to those of the parent compound. This assumption is supported by the study of Boxall et al. (Boxall et al. 2004), who showed that 81% of the degradates are less or similarly toxic as the parent compound. However, one has to note that 29% of the metabolites are more toxic that the parent compound. We can therefore not exclude that we underestimated the toxicity of any given metabolite.

Furthermore, by applying the concentration addition model we assumed that the metabolites have the same mode of action as the parent compound. To our knowledge, this was never evaluated in environmental organisms. From a pharmacological point of view, i.e. in humans, this assumption is correct for many, but not all metabolites. For example, the mode of action of 4-hydroxy-tamoxifen is similar to its parent compound, the anti-cancer drug tamoxifen (Dahmane et al. 2010). In contrast, another metabolite of tamoxifen, endoxifen, has a different mode of action (Dahmane et al. 2010). Furthermore, even in humans, the mode of action of pharmaceuticals and metabolites are not always well understood. The CA approach applied in this study is therefore used as a worst case scenario, as proposed by Backhaus et al. (Backhaus et al. 2003a), when the mode of action of the chemicals in a mixture are not known.

#### Conclusion

Over a period of 10-months, the concentrations of five environmentally relevant pharmaceuticals and eight of their human metabolites were monitored at several locations and depths in Lake Geneva's Vidy Bay. Among the targeted metabolites, six were detected with variable frequency in the lake water samples. The highest concentrations were detected above the WWTP outfall, supporting the assumption that wastewater effluent represents the main source of human metabolites to the lake. The concentration profiles at the WWTP discharge location displayed a plume-like feature corresponding to the wastewater plume. On average, the detected concentrations of human metabolites were similar to, or lower than, their associated parent compound. Moreover, the metabolites targeted in this study were found to be less recalcitrant to typical environmental degradation processes than their parent. Nevertheless, their presence in the aquatic environment may still lead to an increase of the ecotoxicological risk. Assuming a worst-case scenario, in which pharmaceuticals and their metabolites present a similar ecotoxicological impact, we found a critical increase of the environmental risk for two compounds with their metabolites, namely SMX and DCF. The actual PNECs of human metabolite are still to be determined for a more precise evaluation of the environmental risk. However, the precautionary principle and the results of the present study underline the importance of including human metabolites of pharmaceuticals in environmental studies.

#### Acknowledgements

Thanks to Hugues Fournier for the map of Vidy Bay. The research was funded by the Swiss National Science Foundation (Project no. PDFMP2-123028/1) and is part of an interdisciplinary project (www.leman21.ch).

### **Bibliographie**

- ASHTON D, HILTON M, THOMAS KV. 2004. Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. Science of the Total Environment, 333: 167-184.
- BACKHAUS T, ALTENBURGER R, ARRHENIUS A ET AL. 2003a The BEAM-project: prediction and assessment of mixture toxicities in the aquatic environment. Continental Shelf Research, 23: 1757-1769.
- **BELLUCCI G, BERTI G, CHIAPPE C, LIPPI A, MARIONI F.** 1987. The metabolism of carbamezepine in humans steric course of the enzymatic hydrolysis of the 10,11-epoxide. Journal of Medicinal Chemistry, 30: 768-773.
- BONVIN F, RUTLER R, CHEVRE N, HALDER J, KOHN T. 2011. Spatial and Temporal Presence of a Wastewater-Derived Micropollutant Plume in Lake Geneva. Environmental Science & Technology, 45: 4702-4709.
- BONVIN F, OMLIN J, RUTLER R, SCHWEIZER WB, ALAIMO PJ, STRATHMANN T, MCNEILL K, KOHN T. 2012. Direct photolysis of human metabolites of the antibiotic sulfamethoxazole: Evidence for abiotic back-transformation. Environmental Science & Technology, Accepted. DOI: 10.1021/es303777k
- **BOREEN AL, ARNOLD WA, McNeill K,** 2003. Photodegradation of pharmaceuticals in the aquatic environment: A review. Aquatic Sciences, 65: 320-341.
- BOXALL ABA, SINCLAIR C, FENNER, KOLPIN D, MAUND SJ. 2004. When synthetic chemicals degrade in the environment. Environmental Science and Technology, 38: 369A-375A.
- CHÈVRE N, GUIGNARD C, ROSSI L, PFEIFER HR, BADER HP, SCHEIDEGGER R. 2011. Substance flow analysis as a tool for urban water management. Water Science and Technology, 63: 1341-1348.
- DAHMANE E, MERCIER T, ZANOLARI B CRUCHON S, GUIGNARD N, BUCLIN T, LEYVRAZ S, ZAMAN K, CSAJKA C, DECOSTERD LA. 2010. An ultraperformance liquid chromatography-tandem MS assay for tamoxifenmetabolites profiling in plasma: First evidence of 4 -hydroxylated metabolites in breast cancer patients. Journal of Chromatography B, 878: 3402-3414.
- **DAUGHTON CG, TERNES TA.** 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environmental Health Perspectives, 107: 907-938.
- **DEBSKA J, KOT-WASIK A, NAMIESNIK J.** 2004. Fate and analysis of pharmaceutical residues in the aquatic environment. Critical Reviews in Analytical Chemistry, 34: 51-67.
- DIAZ-CRUZ MS, GARCIA-GALAN MJ, BARCELO D. 2008. Highly sensitive simultaneous determination of sulfonamide antibiotics and one metabolite in environmental waters by liquid chromatography-quadrupole linear ion trap-mass spectrometry. Journal of Chromatography A, 1193: 50-59.
- **EDDER P, ORTELLI D, RAMSEIER S.** 2006. Métaux et micropolluants organiques dans les eaux du Léman. Rapport de la Commission Internationale pour la Protection des Eaux du Léman contre la Pollution, Campagne 2005. Lausanne, Switzerland, p. 65-87.
- **EDDER P, ORTELLI D, RAMSEIER S, CHÈVRE N.** 2007. Métaux et micropolluants organiques dans les eaux du Léman. Rapport de la Commission Internationale pour la Protection des Eaux du Léman contre la Pollution, Campagne 2006. Lausanne, Switzerland, p. 59-81.
- **EDDER P, ORTELLI D, KLEIN A, RAMSEIER S.** 2008. Métaux et micropolluants organiques dans les eaux du Léman. Rapport de la Commission Internationale pour la Protection des Eaux du Léman contre la Pollution, Campagne 2007. Lausanne, Switzerland, p. 57-84.
- **EUROPEANCOMMISSION** 2003. Technical guidance document on risk assessment. TGD Part II.; Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission (EC): Ispra, Italy.
- **GEBEL T, LECHTENBERG-AUFFARTH E, GUHE C.** 2009. About hazard and risk assessment: Regulatory approaches in assessing safety in European Union chemicals legislation. Reproductive Toxicology, 28: 188-195.
- GOBEL A, MCARDELL CS, SUTER MJF, GIGER W. 2004. Trace determination of macrolide and sulfonamide antimicrobials, a human sulfonamide metabolite, and trimethoprim in wastewater using liquid chromatography coupled to electrospray tandem mass spectrometry. Analytical Chemistry, 76: 4756-4764.
- **GOBEL A, THOMSEN A, MCARDELL CS, Joss A, GIGER W.** 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environmental Science & Technology, 39: 3981-3989.
- HAYES T, HASTON K, TSUI M, HOANG A, HAEFFELE C, VONK A. 2002. Feminization of male frogs in the wild. Nature, 419: 895-896.
- **HEBERER T.** 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicology Letters, 131: 5-17.
- **HILTON MJ, THOMAS KV.** 2003. Determination of selected human pharmaceutical compounds in effluent and surface water samples by high-performance liquid chromatography-electrospray tandem mass spectrometry. Journal of Chromatography A, 1015: 129-141.
- **HUMMEL D, LOFFLER D, FINK G, TERNES TA.** 2006. Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass spectrometry. *Environmental Science & Technology*, 40: 7321-7328.
- **JOHNSON DR, CZECHOWSKA K, CHEVRE N, VAN DER MEER JR.** 2009. Toxicity of triclosan, penconazole and metalaxyl on *Caulobacter crescentus* and a freshwater microbial community as assessed by flow cytometry. Environmental Microbiology, 11: 1682-1691.
- KAGLE J, PORTER AW, MURDOCH RW, RIVERA-CANCEL G, HAY AG. 2009. Biodegradation of Pharmaceutical and Personal Care Products. In: Laskin AI (ed.), Advances in Applied Microbiology, Elsevier Academic Press Inc: San Diego, Vol. 67, p. 65-108.
- **KOLPIN DW, THURMAN EM, LINHART SM.** 1998. The environmental occurrence of herbicides: The importance of degradates in ground water. Archives of Environmental Contamination and Toxicology, 35: 385-390.
- KOLPIN DW, FURLONG ET, MEYER MT, THURMAN EM, ZAUGG SD, BARBER LB, BUXTON HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environmental Science & Technology, 36: 1202-1211.

- KUMMERER K. 2009. Antibiotics in the aquatic environment A review Part II. Chemosphere, 75: 435-441.
- LA FARRÉ M, PÉREZ S, KANTIANI L, BARCELÓ D. 2008. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. Trac-Trends in Analytical Chemistry, 27: 991-1007.
- LECLERCQ M, MATHIEU O, GOMEZ E, CASELLAS C, FENET H, HILLAIRE-BUYS D. 2009. Presence and Fate of Carbamazepine, Oxcarbazepine, and Seven of Their Metabolites at Wastewater Treatment Plants. Archives of Environmental Contamination and Toxicology, 56: 408-415.
- MIAO X-S, METCALFE CD. 2003. Determination of Carbamazepine and Its Metabolites in Aqueous Samples Using Liquid Chromatography—Electrospray Tandem Mass Spectrometry. Analytical Chemistry, 75: 3731-3738.
- MIAO XS, YANG JJ, METCALFE CD. 2005, Carbamazepine and its metabolites in wastewater and in biosolids in a municipal wastewater treatment plant. Environmental Science & Technology, 39: 7469-7475.
- MORASCH B, BONVIN F, REISER H, GRANDJEAN D, DE ALENCASTRO LF, PERAZZOLO C, CHEVRE N, KOHN T. 2010. Occurence and fate of micropollutants in the Vidy Bay of Lake Geneva, Switzerland. Part II: Micropollutant removal between wastwater and raw drinking water. Environmental Toxicology and Chemistry, 29: 1658-1668.
- **OULTON RL, KOHN T, CWIERTNY DM.** 2010. Pharmaceuticals and personal care products in effluent matrices: A survey of transformation and removal during wastewater treatment and implications for wastewater management. Journal of Environmental Monitoring, 12: 1956-1978.
- PERAZZOLO C, MORASCH B, KOHN T, MAGNET A, THONNEY D, CHÈVRE N. 2010. Occurence and fate of micropollutants in the Vidy Bay of Lake Geneva, Switzerland. Part I: Priority list for environmental risk assessment of pharmaceuticals. Environmental Toxicology and Chemistry, 29: 1649-1657.
- PEREZ S, BARCELO D. 2008a. Applications of LC-MS to quantitation and evaluation of the environmental fate of chiral drugs and their metabolites. Trac-Trends in Analytical Chemistry, 27: 836-846.
- PEREZ S, BARCELO D. 2008b. First Evidence for Occurrence of Hydroxylated Human Metabolites of Diclofenac and Aceclofenac in Wastewater Using QqLIT-MS and QqTOF-MS. Analytical Chemistry, 80: 8135-8145.
- **POTE J, HALLER L, KOTTELAT R, SASTRE V, ARPAGAUS P, WILDI W.** 2009. Persistence and growth of faecal culturable bacterial indicators in water column and sediments of Vidy Bay, Lake Geneva, Switzerland. Journal of Environmental Sciences-China, 21: 62-69.
- **RADKE M, LAUWIGI C, HEINKELE G, MURDTER TE, LETZEL M.** 2009. Fate of the Antibiotic Sulfamethoxazole and Its Two Major Human Metabolites in a Water Sediment Test. Environmental Science & Technology, 43: 3135-3141.
- Schwarzenbach RP, Escher BI, Fenner K, B. Hofstetter TB, Johnson CA, von Gunten U, Wehrli B. 2006. The challenge of micropollutants in aquatic systems. Science, 313: 1072-1077.
- **SILVA E, RAJAPAKSE N, KORTENKAMP A.** 2002. Something from «nothing» eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environmental Science and Technology, 36: 1751-1756.
- **STIERLIN H, FAIGLE JW, SALLMANN A, KÜNG W, RICHTER WJ, KRIEMLER HP, ALT KO, WINKLER T.** 1979. Biotransformation of diclofenac sodium (voltaren) in animals and in man. 1. Isolation and identification of principal metabolites. Xenobiotica, 9: 601-610.
- **STULTEN D, ZUHLKE S, LAMSHOFT M, SPITELLER M.** 2008. Occurrence of diclofenac and selected metabolites in sewage effluents. Science of the Total Environment, 405: 310-316.
- **SUMPTER JP.** 1995. Feminized responses in fish to environmental estrogens. Toxicology Letters, 82-83: 737-742.
- **TERNES TA, Joss A.** 2006. Human pharmaceuticals, hormones and fragrances-the challenge of micropollutants in urban water management. IWA Publishing, London.
- **TIXIER C, SINGER HP, OELLERS S, MÜLLER SR.** 2003. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. Environmental Science & Technology, 37: 1061-1068.
- **TROVÓ AG, NOGUEIRA RF, AGÜERA A, FERNANDEZ-ALBA AR, SIRTORI C, MALATO S.** 2009. Degradation of sulfamethoxazole in water by solar photo-Fenton. Chemical and toxicological evaluation. Water Research, 43: 3922-3931.
- VAN DER VEN AJ, VREE TB, VAN EWIJK-BENEKEN KOLMER EW, KOOPMANS PP, VAN DER MEER JW. 1995. Urinary recovery and kinetics of sulfamethoxazole and its metabolites in HIV-seropositive patients and healthy-volunteers after a single oral dose of sulfamethoxazole. British Journal of Clinical Pharmacology, 39: 621-625.