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Levels of pharmaceuticals in Slovene municipal and hospital wastewaters: a preliminary study

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Pharmaceuticals in wastewater have clearly raised concern and a broad range of analytical methods has been used to assess the risk as accurately as possible. The aim of our study was to measure and compare the concentrations of atorvastatin, bisoprolol, carbamazepine, ciprofloxacin, clofibric acid, diclofenac, fluoxetine, metoprolol, and sertraline in wastewater samples taken from one municipal and one hospital wastewater treatment plant in Slovenia and to predict the potential environmental burden using the risk quotient. In both effluents only clofibric acid and fluoxetine were not detected. The measured concentrations of the remaining seven pharmaceuticals varied between the ng L^{-1} and the µg L^{-1} range. Hospital effluent showed higher concentrations, except for diclofenac and carbamazepine. However, high risk quotient was found only for ciprofloxacin and diclofenac in both municipal and hospital effluent. In conclusion, our method can provide a useful tool for systematic monitoring of pharmaceuticals commonly found in wastewater, which will enable a reliable assessment of the risks for the aquatic biota and humans. Knowing the risks will help to plan wastewater treatment and preserve our environment.

KEY WORDS: *atorvastatin; bisoprolol; carbamazepine; ciprofloxacin; clofibric acid; diclofenac, fluoxetine; hospital; LC-MS/MS; metoprolol; municipal; risk assessment; sertraline*

Pharmaceuticals in wastewater clearly pose a health risk to humans and aquatic life. First, they add to the growing problem of antimicrobial resistance caused by high and uncontrolled consumption of antibiotics and their continuous presence in the environment (1). Second, pharmaceutical residues in drinking water might disrupt the endocrine system. Endocrine disruptors may affect sperm count or cause breast and testicular cancer. One should bear in mind, however, that all evidence of direct adverse effects on human health is weak and inconclusive (2). On the other hand, the evidence of adverse effects on aquatic biota is much more convincing; long-term exposure to diclofenac impairs renal and gill function (3, 4), exposure to carbamazepine growth retardation (5), and exposure to sertraline reproductive disorders in a variety of fish species (6). Even though drug concentrations found in the environment are much lower than the therapeutic ones, some drugs, such as endocrine disruptors, can affect organisms even at extremely low concentrations. They may interfere with the endocrine system and affect vital functions such as development, reproduction, neurology, and immunity. Examples abound: intersex fish, synthesis of vitellogenin in males, feminisation, and absence of pregnancies (7, 8). Furthermore, lipophilic drugs and their metabolites tend to bioaccumulate (9).

A great many studies have introduced various monitoring methods in order to assess exposure and risk in non-target organisms as accurately as possible (9). Considering the increasing use of pharmaceuticals worldwide, their concentrations in the environment might reach toxicologically significant levels. There are two main approaches to evaluating environmental levels of a drug. The theoretical approach is based on factors such as consumption, metabolism, and excretion of these drugs. Estimating national drug consumption may be challenging, as the consumption of over-the-counter drugs is not as meticulously tracked as that of prescription drugs (10). Moreover, ingested drugs undergo biochemical reactions and their metabolites and degradation products could exhibit either the same or modified activity as the unaltered parent compound. In other words, evaluations may fail to reflect the true environmental load (11, 12). This is why it is imperative to apply the second, experimental approach, which employs sensitive analytical methods to accurately measure the concentrations of targeted compounds. For compounds present in the environment in trace concentrations an array of methods is available such as liquid or gas chromatography coupled with mass spectrometry (13-15) or enzyme-linked immunosorbent assay (ELISA) (15). Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) in combination with a sample pre-concentration step seems to be the method of choice for most analytes (16). LC-MS/MS can determine a broad range of pharmaceuticals thanks to its high

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sensitivity and selectivity in complex matrices such as wastewaters as well as better precision than with other analytical methods due to a less complicated sample preparation (e. g. no derivatisation is needed) (17).

One of the main reasons for pharmaceuticals entering bodies of water is low removal rate in wastewater treatment plants (WWTPs) (18-20). Because municipal WWTPs are considered the main route of pharmaceutical release into the aquatic environment, hospital effluents are frequently overlooked in exposure estimates. With this study we tried to address this oversight using the state-of-the art method. Our aim was (i) to measure the concentrations of eight common drugs and one metabolite from different classes that are released in Slovene wastewaters, (ii) to compare these concentrations between municipal and hospital wastewaters, and (iii) to assess environmental burden using the risk quotient.

MATERIALS AND METHODS

Our analysis targeted pharmaceuticals that are commonly used and well covered by literature data, namely atorvastatin, bisoprolol, carbamazepine, ciprofloxacin, clofibric acid, diclofenac, fluoxetine, metoprolol, and sertraline. Furthermore, their occurrence in wastewater has already been evidenced (21-24). Carbamazepine and clofibric acid were included due to their long history of use and persistence (25). Beta-blockers and NSAIDs were selected because of their widespread use (26). Diclofenac and bisoprolol are the 3rd and the 5th most prescribed drugs in Slovenia, respectively (27). In terms of ecotoxicity, carbamazepine, diclofenac, and metoprolol have been evidenced to cause growth retardation and heart abnormalities in fish embryos (28). Fluoxetine seems to cause endocrine-mediated reproduction dysfunction and developmental abnormalities, also in fish embryos (29, 30). Antibiotic residues in aquatic systems have clearly showed a potential to induce resistance in bacterial strains and become a serious threat for public health (31, 32).

Chemicals and reagents

Standards for carbamazepine (CAR), clofibric acid (CLO), diclofenac (DIC), fluoxetine (FLU), metoprolol (MET), and sertraline (SER) were purchased from Sigma-Aldrich (Steinheim, Germany). Atorvastatin (ATOR) and bisoprolol fumarate (BIS) were purchased from Sequoia Researcher Products (Pangbourne, UK) and ciprofloxacin (CIP) from AppliChem GmbH (Darmstadt, Germany). The reagents used for standard and sample preparation including acetonitrile (ACN), acetic acid (98 %), formic acid (98-100 %), methanol (MeOH), 2-propanol (iPrOH) and potassium dihydrogen phosphate were provided by Merck (Darmstadt, Germany). Ultra-pure water was produced by a Millipore Milli-Q water purification system A10 Advantage (Millipore Corporation, Billerica, MA, USA). Solvents for LC-MS/MS analyses were LC-MS-grade acetonitrile ChromasolV® (Sigma-Aldrich, Steinheim, Germany), Milli-Q water and formic acid (98-100 %) Suprapur® (Merck, Darmstadt, Germany).

Sample collection

The samples were collected from two WWTPs in central Slovenia: one urban municipal WWTP with a capacity of 9900 population equivalents and daily flow of approximately 2200 m^3 and the other WWTP serving a medium-sized hospital with 220 beds with a capacity of 1000 population equivalents and daily flow of approximately 40 m^3. It also receives a negligible portion of wastewater from a nearby settlement. Both WWTPs employ a similar treatment: primary mechanical processing followed by biological treatment. Samples were collected both before the treatment point (influent) and after the final treatment (effluent). We obtained three litres of grab wastewater samples (influent or effluent) from each WWTP in accordance with the ISO/IEC-17025 standard. Samples were stored at 4 °C and analysed within 24 h. From the municipal WWTP influent and effluent wastewater samples were taken in March and June 2015, and from the hospital WWTP five times at regular weekly intervals in May 2015. All wastewater samples had pH around 7.

Sample extraction and analysis

Standard stock solutions of each analyte were prepared by dissolving 5 mg of accurately weighed standard in methanol to obtain the concentration of 1 mg mL^{-1}. The final concentration of the working solution of 1 mg L^{-1} was prepared by mixing the corresponding volumes of the stock solutions of each analyte and by diluting them further with ultra-pure water (standard mixture of pharmaceuticals).

To evaluate the concentration of pharmaceuticals in wastewater we employed the method of standard addition (33). The wastewater samples (250 mL) were first diluted with 250 mL of 250 mmol L^{-1} potassium dihydrogen phosphate buffer at pH 3. One aliquot of diluted sample was extracted without any additions in order to determine the original concentrations of the analytes. To other aliquots we added the appropriate amounts of the standard mixture of pharmaceuticals before extraction to obtain the final concentrations of 200, 500, 1000, and 2000 ng L^{-1}. All samples were prepared in triplicate. Analyte concentrations were calculated using the linear regression curve.

All samples were extracted using a semi-automated sample preparation system SPE-DEX (Horizon Technology, Salem, NH, USA) with HLB Horizon discs. Rough samples were sequentially treated using a pre-developed method programmed in the Envision™ platform Controller software (Horizon Technology). Of all tested rinse solvents, a mixture of ACN (50 %), MeOH (25 %), and iPrOH (25 %) provided the best results.

The obtained samples were analysed with the Agilent 1290 Infinity LC coupled to Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). One microlitre of the sample was injected onto a 100×3.0 mm, 2.7 µm Poroshell EC-C18 column (Agilent Technologies, Palo Alto, CA, USA) at 50 °C and eluted with mobile phase A (0.1 % formic acid in water) and B (ACN) using the following linear gradient (time min; % B; flow-rate mL min^{-1}): (0;5;0.35), (0.5;5;0.35), (1.1;9;0.35), (1.2;40;0.65), (1.3;50;0.65), (2.0;60;0.65), (2.5;60; 0.65). Run time was 3.2 min. After each injection, the sampling needle was washed with a washing solvent (MeOH:H_2O=80:20 v/v). For MS, a JetStream® (Agilent Technologies, Santa Clara, CA, USA) electrospray source was used. Instrument parameters were set as follows: drying gas temperature 275 °C, drying gas flow 5 L min^{-1}, nebuliser 45 psi, sheath gas temperature 320 °C, sheath gas flow 11 L min^{-1}, capillary entrance voltage 4000 V, nozzle voltage 1000 V. Quadrupoles Q_1 and Q_3 were set at a wide mass resolution (1.2 amu). Instrument control, data acquisition, and quantification were performed by MassHunter Workstation software (Agilent Technologies, Santa Clara, CA, USA). The MRM transitions and other quantification settings for analytes are presented in Table 1.

Quality control

The analytical method was validated using five calibration standards (in triplicate) in the concentration range between 0.01-10 µg L^{-1}. Process efficiency was assessed on samples of ultra-pure water and the June samples of the municipal WWTP influent and effluent wastewater spiked with the standard mixture of pharmaceuticals at different concentrations. The values were estimated by comparison of the peak area obtained from extracted sample and standard solution at the same final concentration (Eq. 1). The imprecision was expressed in terms of the relative standard deviation (RSD) of six replicates. The limit of quantification was defined as the concentration level with a signal to noise ratio of at least 10:1. The influence of the sample matrix on analyte response was determined as an absolute matrix effect (ME) according to equation 2, based on Matuszewski et al. (34). The rationale of the equation will be described in a separate publication (manuscript in preparation). Values >0 indicate ionisation enhancement and <0 ion suppression.

Process efficiency (PE)

$$PE = 100 \times \left(\frac{\text{peak area [(spiked preextraction sample)}-\text{(blank wastewater sample)]}}{\text{peak area (standard solution)}}\right)$$

[Eq. 1]

Matrix effect (ME)

$$ME = 100 \times \left(\frac{\text{peak area [(spiked postextraction sample)}-\text{(blank wastewater sample)]}}{\text{peak area (standard solution)}} - 1\right)$$

[Eq. 2]

Risk quotient (RQ)

$$RQ = \frac{MEC}{PNEC}$$

[Eq. 3]

Environmental risk assessment

Environmental risk was assessed in terms of each compound's RQ, by calculating the ratio between measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC) according to Eq. 3. PNEC values were based on previously reported acute toxicity data for the most sensitive species (Table 4). A commonly accepted risk ranking criterion was used: RQ<0.1 means minimal risk to aquatic organisms, 0.1≤RQ<1 moderate risk, and RQ≥1 high risk (35, 36).

RESULTS AND DISCUSSION

Analytical method validation

Table 2 shows the process efficiencies, imprecision, and matrix effects, quantified in ultrapure and/or in influent and effluent wastewater. Process efficiencies were 81-106 %, 68-102 %, and 73-99 % for ultrapure, influent, and effluent wastewater, respectively. Lower recoveries in wastewater

Table 1 *Quantification settings*

Analyte	MRM (m/z)			CE (eV)	Fr (V)	P
atorvastatin	559.3	>	440.2	17	180	+
bisoprolol	326.2	>	116.1	9	144	+
carbamazepine	237.1	>	194.1	13	100	+
ciprofloxacin	332.1	>	314.1	16	134	+
clofibric acid	213.0	>	127.0	10	80	-
diclofenac	296.0	>	214.0	15	55	+
fluoxetine	310.1	>	148.1	1	100	+
metoprolol	268.2	>	116.0	12	96	+
sertraline	306.1	>	158.9	24	60	+

MRM: multiple-reaction monitoring; CE: collision energy; Fr: fragmentor; P: polarity

samples are probably related to sample complexity. Imprecision expressed as RSD (%) of six samples spiked at 0.5 μg L^{-1} in all types of water was not higher than 12 % and, not surprisingly, the most complex water sample, influent wastewater had the highest RSD.

Calibration curves were linear with determination coefficients greater than 0.999 for all compounds. The linearity using the standard addition method was also confirmed for all analytes. The matrix effects were below 2.5 %, except for ciprofloxacin. Limits of quantification (LOQ) for the investigated compounds were in the low ng L^{-1} range. Based on the validation data we concluded that the applied method was appropriate for further investigation of pharmaceuticals in wastewater.

Pharmaceutical concentrations in municipal wastewater

To the best of our knowledge, this the first study reporting pharmaceuticals in Slovene municipal and hospital wastewaters, but their concentrations in tap and river water have already been reported (37). Table 3 shows municipal wastewater influent and effluent concentrations of the selected compounds and detection frequencies. Six out of nine pharmaceuticals were detected at every sampling and one (atorvastatin) was found in 75 % of the samples. Clofibric acid, an active metabolite of the lipid-modifying drug clofibrate, and fluoxetine were not detected in any of the samples. This does not surprise, since both drugs have become quite obsolete and have been replaced by safer alternatives such as HMG CoA reductase inhibitors (statins) or new-generation selective serotonin reuptake inhibitors (sertraline, escitalopram, and paroxetine). Instead of clofibrate we found its alternative atorvastatin and instead of fluoxetine we found sertraline in both influent and effluent wastewater.

The concentrations of the remaining pharmaceuticals were mostly similar with other reports (38). Higher ciprofloxacin concentrations were reported in Italy (630 ng L^{-1}) (10) and Spain (2200 ng L^{-1}) (39). High concentrations of the nonsteroidal anti-inflammatory drug diclofenac in our study seem to confirm its ranking as the third most prescribed medicine in Slovenia (27). Another reason for its high concentration may be poor elimination efficiency via activated sludge, as reported earlier (21, 40, 41). The highest measured absolute concentration reported in literature is 11 μg L^{-1} (9), while generally, its concentrations vary from a few ng L^{-1} up to 5.5 μg L^{-1}, depending on the country and wastewater treatment technology (42-44).

The comparison between influent and effluent concentrations in our study clearly demonstrates insufficient removal rates for all monitored compounds. Another curiosity worth noting is that diclofenac effluent concentrations (that is to say, after treatment) were higher than influent concentrations in both March and June samples. This phenomenon may be closely related to diclofenac metabolism (45). Several authors suggest that

Table 2 *Validation parameters*

Analyte	Process efficiency (%)*			Imprecision (% RSD)*			Matrix effect (%)*		Determination coefficient (range 0.01-10 μg L^{-1})	LOQ (ng L^{-1})
	Ultrapure water	Influent wastewater	Effluent wastewater	Ultrapure water	Influent wastewater	Effluent wastewater	Influent wastewater	Effluent wastewater		
ATOR	84.1	80.8	81.3	4.0	10.3	4.7	1.2	1.2	0.9996	2.0
BIS	87.2	88.6	82.4	1.0	5.2	1.0	1.6	1.7	0.9996	0.2
CAR	105.9	102.1	96.8	2.0	9.0	3.6	1.6	1.8	0.9996	2.5
CIP	105.9	85.1	99.4	7.0	12.1	7.1	7.8	6.9	0.9991	5.0
CLO	89.4	89.7	92.8	5.0	4.2	1.0	1.0	1.1	0.9990	7.0
DIC	105.3	69.3	88.3	6.7	12.0	4.1	2.0	2.4	0.9993	40.0
FLU	86.6	79.5	80.2	2.0	8.2	7.0	1.1	1.2	0.9994	2.0
MET	80.5	99.2	96.6	2.0	3.8	0.9	1.2	1.3	0.9994	0.5
SER	87.6	68.2	73.2	2.0	10.4	9.7	1.2	1.2	0.9997	0.3

**at the concentration of 0.5 μg L^{-1}*

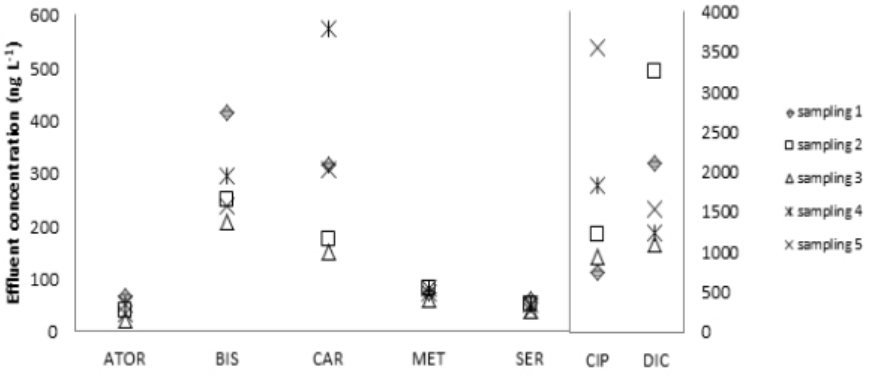

Figure 1 *Concentrations of pharmaceuticals detected in the hospital WWTP effluent wastewater over five consecutive weeks in May 2015. CIP and DIC are shown on the separate numeric scale. ATOR: atorvastatin; BIS: bisoprolol; CAR: carbamazepine; CIP: ciprofloxacin; DIC: diclofenac; MET: metoprolol; SER: sertraline*

Table 3 *Concentrations (ng L^{-1}) and frequency (%) of pharmaceuticals in the municipal WWTP samples*

Analyte	Freq. (%)	Influent concentration (ng L^{-1})		Effluent concentration (ng L^{-1})	
		March	June	March	June
ATOR	75	< LOQ	23.3	< LOQ	nd
BIS	100	130.1	77.5	216.4	36.0
CAR	100	269.6	193.8	482.4	340.5
CIP	100	135.4	165.8	101.9	< LOQ
CLO	0	nd	nd	nd	nd
DIC	100	881.1	4056.9	1155.8	4760.0
FLU	0	nd	nd	nd	nd
MET	100	12.5	18.8	67.4	25.0
SER	100	48.9	49.6	29.1	24.9

nd: not detected; <LOQ: below the limit of quantification

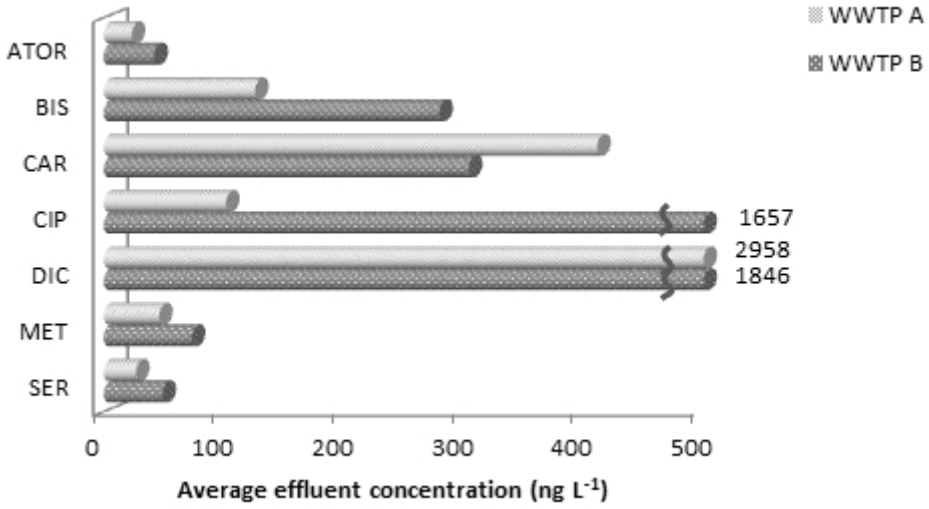

Figure 2 *Comparison between the mean concentrations of pharmaceuticals detected in municipal WWTP (A) and hospital WWTP (B) effluents. ATOR: atorvastatin; BIS: bisoprolol; CAR: carbamazepine; CIP: ciprofloxacin; DIC: diclofenac; MET: metoprolol; SER: sertraline*

glucuronide and sulphate conjugates of diclofenac may be cleaved by enzymes during wastewater treatment and convert to their parent compound (42, 46). Moreover, the results from the evaluation of the matrix effect in influent and effluent wastewater (Table 2) exclude the potential analytical error (47).

Pharmaceutical concentrations in hospital wastewater and the comparison between municipal and hospital concentrations

In order to avoid daily bias for hospital wastewater composition we measured them weekly over entire May 2015 (five samplings). Figure 1 shows their concentrations. Again, clofibric acid and fluoxetine were not detected, quite likely because of the same reason as for the municipal wastewater: these two obsolete drugs have been replaced by new ones. Concentrations of atorvastatin, metoprolol, and sertraline were roughly consistent across all five samplings, while bisoprolol and carbamazepine showed a higher variability. The highest and the most variable concentrations were observed for ciprofloxacin (0.7-3.6 μg L^{-1}) and diclofenac (1.1-3.2 μg L^{-1}), yet ciprofloxacin concentrations in our study were slightly lower than in some other hospital wastewater studies (35, 48). Diclofenac concentrations, in turn, were between the ranges reported by Oliveira et al. (0.03-0.2 μg L^{-1}) (37) and Lin and Tsai (up to 70 μg L^{-1}) (49). All in all, our findings are in line with other published hospital wastewater studies (35), and confirm differences in the loading patterns for specific pharmaceuticals, depending on several factors reported earlier (37, 50). We believe that further investigation should look into these loading patterns.

The comparison between the municipal and hospital wastewater shows that hospital effluents contained pharmaceuticals in 1.5 to 24 times higher concentrations, except for diclofenac and carbamazepine (Figure 2). The greatest difference concerns the antibiotic ciprofloxacin, which is not surprising, due to far more intense use of antibiotics among hospitalised patients. In contrast, diclofenac concentrations were 1.6 times higher in municipal wastewater effluents. One of the reasons could be that the nonsteroidal anti-inflammatory drug diclofenac is not as common among hospitalised patients as in outpatients and the ageing population with musculoskeletal disorders.

In terms of environmental threat one should bear in mind that effluent concentrations were measured directly at the outlet and that wastewater gets significantly diluted before it reaches surface waters. Therefore, we expect that even the highest concentrations measured in our study should drop below the predicted critical downstream concentrations reported by Fick et al. (51). This does not mean that we should neglect the potential environmental threat of ciprofloxacin, diclofenac, or carbamazepine.

Environmental risk assessment

Our findings do not provide grounds for a precise risk assessment due to the low number of samples and the nature of grab sampling. However, they may give a general idea of the risk. We calculated the risk quotient for the lowest and highest measured concentrations and expressed it as a range. To do that we used previously reported PNECs for the most sensitive species (52-57) and combined them with the highest MECs to establish the worst-case scenario. Table 4 shows high risk for only two compounds: ciprofloxacin and diclofenac. Both belong to therapeutic classes (antibiotics and NSAIDs, respectively) categorised as environmental hazards (58). When the risk quotient is higher than 1 (as is the case with these two drugs) the threat to exposed organisms is serious. Permanent presence of antibiotics in the environment leads to antimicrobial resistance (36). Even the WWTP sludge that retains about 70 % of the influent concentration is known to be used as a fertiliser and can seriously affect the human food chain (59). Global studies have demonstrated that almost all bacterial species have become resistant to fluoroquinolone. *Neisseria gonorrhoeae* has rapidly developed fluoroquinolone resistance, which is now as high as 100 % in Asia and 10-30 % in Europe and North America (1). While ciprofloxacin poses a risk in terms of long-term exposure, diclofenac's toxicity is both acute and chronic in *Daphnia*. Long term exposure, in turn, leads to renal and gill function impairment in several fish species (3, 4). Furthermore, diclofenac residues have virtually destroyed the Pakistani vulture population (*Gyps bengalensis)* due to renal failure and visceral gout, as it fed on dead domestic livestock treated with diclofenac (60).

The risk quotient for carbamazepine and sertraline ranged between 0.1 and 1 in our study, which implies moderate environmental risk. Exposure to carbamazepine in an early life stage can slow down growth in *Danio rerio* (61). Sertraline as one of the most active selective serotonin re-uptake inhibitors may affect the nervous and/or hormonal systems of non-target organisms, such as the ones reported in *Ceriodaphnia dubia* (6). While our findings should not raise immediate concern if we look separately at the levels of each drug, the mixture of these pharmaceuticals may pose a far greater risk than either compound individually (52, 61).

CONCLUSION

This leads us to the shortcomings of our research. In addition to suboptimal sampling method and the modest number of samples mentioned above, it lacks experimental toxicology data about the exposed organisms and information about downstream (diluted) concentrations measured in surface water. Even so, our findings do confirm that antibiotics and NSAIDs are the most hazardous drug classes regardless of the source of wastewater, as reported

Table 4 *PNEC (for the most sensitive species) and the range of risk quotients for the selected pharmaceuticals*

Compound	PNEC (ng L^{-1})	Species assayed	RQ (municipal WWTP)	RQ (hospital WWTP)
atorvastatin	160	*(P. subcapitata) (53)*	0.0	0.1-0.4
bisoprolol	na	-	-	-
carbamazepine	2500	*(C. dubia) (54)*	0.1-0.2	0.1-0.2
ciprofloxacin	50	*(M. aeroginosa) (55)*	0.7-2.0	14.7-71.2
clofibric acid	64	*(C. dubia) (54)*	no risk	no risk
diclofenac	100	*(C. dubia) (54)*	11.6-55.6	11.0-32.5
fluoxetine	50	*(P. subcapitata) (56)*	no risk	no risk
metoprolol	7900	*(D. subspicatus) (52)*	0.0	0.0
sertraline	90	*(C. dubia) (57)*	0.3-0.3	0.4-0.7

PNEC: predicted no-effect concentration; na: not available in literature; RQ: risk quotient; WWTP: wastewater treatment plant

earlier (35). Our method can serve as a preliminary tool for monitoring pharmaceutical compounds in wastewater, as it allows expanding from a small to a large number of compounds. We believe that with a substantial number of samples this method can provide enough information for a relevant and reliable risk assessment.

However, in light of these findings, we believe that wastewater treatment should include new technologies of hazardous compound removal. For instance, diclofenac is significantly better eliminated by oxidative treatment with ozone or hydrogen peroxide (62), and replacing activated sludge with attached-growth biomass has also shown promising effects (40).

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REFERENCES

1. Dalhoff A. Global fluoroquinolone resistance epidemiology and implictions for clinical use. Interdiscip Perspect Infect Dis 2012;97:62-73. doi: 10.1155/2012/976273
2. Fawell J, Nieuwenhuijsen MJ. Contaminants in drinking water: Environmental pollution and health. Br Med Bull 2003;68:199-208. doi: 10.1093/bmb/ldg027
3. Schwaiger J, Ferling H, Mallow U, Wintermayr H, Negele RD. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac. Part I: histopathological alterations and bioaccumulation in rainbow trout. Aquat Toxicol 2004;68:141-50. doi: 10.1016/j.aquatox.2004.03.014
4. Triebskorn R, Casper H, Scheil V, Schwaiger J. Ultrastructural effects of pharmaceuticals (carbamazepine, clofibric acid, metoprolol, diclofenac) in rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). Anal Bioanal Chem 2007;387:1405-16. doi: 10.1007/s00216-006-1033-x
5. Ferrari B, Paxéus N, Lo Giudice R, Pollio A, Garric J. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicol Environ Saf 2003;55:359-70. doi: 10.1016/S0147-6513(02)00082-9
6. Fent K, Weston AA, Caminada D. Ecotoxicology of human pharmaceuticals. Aquat Toxicol 2006;76:122-59. doi: 10.1016/j.aquatox.2005.09.009
7. Söffker M, Tyler CR. Endocrine disrupting chemicals and sexual behaviors in fish - a critical review on effects and possible consequences. Crit Rev Toxicol 2012;42:653-68. doi: 10.3109/10408444.2012.692114
8. Sária MP, Santos MM, Castro LF, Vieira NM, Monteiro NM. Estrogenic chemical effects are independent from the degree of sex role reversal in pipefish. J Hazard Mater 2013;263:746-53. doi: 10.1016/j.jhazmat.2013.10.043
9. Verlicchi P, Al Aukidy M, Zambello E. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment - A review. Sci Total Environ 2012;429:123-55. doi: 10.1016/j.scitotenv.2012.04.028
10. Verlicchi P, Al Aukidy M, Jelic A, Petrović M, Barceló D. Comparison of measured and predicted concentrations of selected pharmaceuticals in wastewater and surface water: a case study of a catchment area in the Po Valley (Italy). Sci Total Environ 2014;470-471:844-54. doi: 10.1016/j.scitotenv.2013.10.026
11. Celiz MD, Tso J, Aga DS. Pharmaceutical metabolites in the environment: analytical challenges and ecological risks. Environ Toxicol Chem 2009;28:2473-84. doi: 10.1897/09-173.1
12. Evgenidou EN, Konstantinou IK, Lambropoulou DA. Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: a review. Sci Total Environ 2015;505:905-26. doi: 10.1016/j.scitotenv.2014.10.021
13. Castiglioni S, Bagnati R, Calamari D, Fanelli R, Zuccato E. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. J Chromatogr A 2005;1092:206-15. doi: 10.1016/j.chroma.2005.07.012
14. Robles-Molina J, Gilbert-López B, García-Reyes JF, Molina-Díaz A. Monitoring of selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain. Sci Total Environ 2014;479-480:247-57. doi: 10.1016/j.scitotenv.2014.01.121

15. Deng A, Himmelsbach M, Zhu Q-Z, Frey S, Sengl M, Buchberger W, Niessner R, Knopp D. Residue analysis of the pharmaceutical diclofenac in different water types using ELISA and GC−MS. Environ Sci Technol 2003;37:3422-9. doi: 10.1021/es0341945
16. Gros M, Petrović M, Barceló D. Multi-residue analytical methods using LC-tandem MS for the determination of pharmaceuticals in environmental and wastewater samples: a review. Anal Bioanal Chem 2006;386:941-52. doi: 10.1007/s00216-006-0586-z
17. Fatta D, Achilleos A, Nikolaou A, Meriç S. Analytical methods for tracing pharmaceutical residues in water and wastewater. TrAC Trends Anal Chem 2007;26:515-33. doi: 10.1016/j.trac.2007.02.001
18. Garcia-Segura S, Keller J, Brillas E, Radjenovic J. Removal of organic contaminants from secondary effluent by anodic oxidation with a boron-doped diamond anode as tertiary treatment. J Hazard Mater 2015;283:551-7. doi: 10.1016/j.jhazmat.2014.10.003
19. Ribeiro AR, Nunes OC, Pereira MFR, Silva AMT. An overview on the advanced oxidation processes applied for the treatment of water pollutants defined in the recently launched Directive 2013/39/EU. Environ Int 2015;75:33-51. doi: 10.1016/j.envint.2014.10.027
20. Santos LH, Araújo AN, Fachini A, Pena A, Delerue-Matos C, Montenegro MC. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J Hazard Mater 2010;175:45-95. doi: 10.1016/j.jhazmat.2009.10.100
21. Bendz D, Paxeus NA, Ginn TR, Loge FJ. Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Hoje River in Sweden. J Hazard Mater 2005;122:195-204. doi: 10.1016/j.jhazmat.2005.03.012
22. Gros M, Petrović M, Barceló D. Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching. Anal Chem 2009;81:898-912. doi: 10.1021/ac801358e
23. Bort R, Ponsoda X, Carrasco E, Gómez-Lechón MJ, Castell JV. Comparative metabolism of the nonsteroidal antiinflammatory drug, aceclofenac, in the rat, monkey, and human. Drug Metab Dispos 1996;24:969-75. PMID: 8886606
24. Rosal R, Rodríguez A, Perdigón-Melón JA, Petre A, García-Calvo E, Gómez MJ, Agüera A, Fernández-Alba AR. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Res 2010;44:578-88. doi: 10.1016/j.watres.2009.07.004
25. Huerta-Fontela M, Galceran MT, Ventura F. Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. Water Res 2011;45:1432-42. doi: 10.1016/j.watres.2010.10.036
26. Gu Q, Dillon CF, Burt VL. Prescription drug use continues to increase: U.S. prescription drug data for 2007-2008. NCHS Data Brief 2010;42:1-8. PMID: 20854747
27. National Institute of Public Health of Slovenia. Poraba ambulatno predpisanih zdravil v Sloveniji v letu 2013 [Consumption of outpatient prescribed medicines in Slovenia in 2013, in Slovene] [displayed 31 May 2016]. Available at http://www.nijz.si/sites/www.nijz.si/files/publikacije-datoteke/poraba_ambulantno_predpisanih_zdravil_2013.pdf
28. van den Brandhof EJ, Montforts M. Fish embryo toxicity of carbamazepine, diclofenac and metoprolol. Ecotoxicol Environ Saf 2010;73:1862-6. doi: 10.1016/j.ecoenv.2010.08.031
29. Brooks BW. Fish on Prozac (and Zoloft): Ten years later. Aquatic Toxicol 2014;151:61-7. doi: 10.1016/j.aquatox.2014.01.007
30. Stewart AM, Grossman L, Nguyen M, Maximino C, Rosemberg DB, Echevarria DJ, Kalueff AV. Aquatic toxicology of fluoxetine: Understanding the knowns and the unknowns. Aquatic Toxicol 2014;156:269-73. doi: 10.1016/j.aquatox.2014.08.014
31. Halling-Sørensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lützhøft HC, Jørgensen SE. Occurrence, fate and effects of pharmaceutical substances in the environment - A review. Chemosphere 1998;36:357-93. doi: 10.1016/S0045-6535(97)00354-8
32. Hirsch R, Ternes T, Haberer K, Kratz K-L. Occurrence of antibiotics in the aquatic environment. Sci Total Environ 1999;225:109-18. doi: 10.1016/S0048-9697(98)00337-4
33. Bader M. A systematic approach to standard addition methods in instrumental analysis. J Chem Educ 1980;57:703. doi: 10.1021/ed057p703
34. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 2003;75:3019-30. doi: 10.1021/ac020361s
35. Verlicchi P, Al Aukidy M, Galletti A, Petrovic M, Barceló D. Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. Sci Total Environ 2012;430:109-18. doi: 10.1016/j.scitotenv.2012.04.055
36. Hernando MD, Mezcua M, Fernández-Alba AR, Barceló D. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 2006;69:334-42. doi: 10.1016/j.talanta.2005.09.037
37. Kosjek T, Heath E, Krbavčič A. Determination of non-steroidal anti-inflammatory drug (NSAIDs) residues in water samples. Environ Int 2005;31:679-85. doi: 10.1016/j.envint.2004.12.001
38. Oliveira TS, Murphy M, Mendola N, Wong V, Carlson D, Waring L. Characterization of Pharmaceuticals and Personal Care products in hospital effluent and waste water influent/effluent by direct-injection LC-MS-MS. Sci Total Environ 2015;518-519:459-78. doi: 10.1016/j.scitotenv.2015.02.104
39. Gracia-Lor E, Sancho JV, Hernández F. Multi-class determination of around 50 pharmaceuticals, including 26 antibiotics, in environmental and wastewater samples by ultra-high performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 2011;1218:2264-75. doi: 10.1016/j.chroma.2011.02.026
40. Zupanc M, Kosjek T, Petkovšek M, Dular M, Kompare B, Širok B, Blažeka Ž, Heath E. Removal of pharmaceuticals from wastewater by biological processes, hydrodynamic cavitation and UV treatment. Ultrason Sonochem 2013;20:1104-12. doi: 10.1016/j.ultsonch.2012.12.003
41. Kosjek T, Heath E, Kompare B. Removal of pharmaceutical residues in a pilot wastewater treatment plant. Anal Bioanal Chem 2007;387:1379-87. doi: 10.1007/s00216-006-0969-1
42. Lacey C, Basha S, Morrissey A, Tobin JM. Occurrence of pharmaceutical compounds in wastewater process streams

in Dublin, Ireland. Environ Monit Assess 2012;184:1049-62. doi: 10.1007/s10661-011-2020-z
43. Vieno N, Sillanpää M. Fate of diclofenac in municipal wastewater treatment plant - A review. Environ Int 2014;69:28-39. doi: 10.1016/j.envint.2014.03.021
44. Andreozzi R, Raffaele M, Nicklas P. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere 2003;50:1319-30. doi: 10.1016/S0045-6535(02)00769-5
45. Tang W. The metabolism of diclofenac-enzymology and toxicology perspectives. Curr Drug Metab 2003;4:319-29. doi: 10.2174/1389200033489398
46. Aceña J, Heuett N, Gardinali P, Pérez S. Suspect screening of pharmaceuticals and related bioactive compounds, their metabolites and their transformation products in the aquatic environment, biota and humans using LC-HR-MS techniques. Comprehen Anal Chem. doi: 10.1016/bs.coac.2016.02.011
47. Caban M, Migowska N, Stepnowski P, Kwiatkowski M, Kumirska J. Matrix effects and recovery calculations in analyses of pharmaceuticals based on the determination of β-blockers and β-agonists in environmental samples. J Chromatogr A 2012;1258:117-27. doi: 10.1016/j.chroma.2012.08.029
48. Santos LHMLM, Gros M, Rodriguez-Mozaz S, Delerue-Matos C, Pena A, Barceló D, Montenegro MCBSM. Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: Identification of ecologically relevant pharmaceuticals. Sci Total Environ 2013;461-462:302-16. doi:10.1016/j.scitotenv.2013.04.077
49. Lin AY-C, Tsai Y-T. Occurrence of pharmaceuticals in Taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities. Sci Total Environ 2009;407:3793-802. doi: 10.1016/j.scitotenv.2009.03.009
50. Beier S, Cramer C, Koster S, Mauer C, Palmowski L, Schröder HF, Pinnekamp J. Full scale membrane bioreactor treatment of hospital wastewater as forerunner for hot-spot wastewater treatment solutions in high density urban areas. Water Sci Technol 2011;63:66-71. doi: 10.2166/wst.2011.010
51. Fick J, Lindberg RH, Tysklind M, Larsson DG. Predicted critical environmental concentrations for 500 pharmaceuticals. Regul Toxicol Pharmacol 2010;58:516-23. doi: 10.1016/j.yrtph.2010.08.025
52. Cleuvers M. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol Lett 2003;142:185-94. doi: 10.1016/S0378-4274(03)00068-7
53. William E. Acree J, Grubbs LM, Abraham MH. Prediction of toxicity, sensory responses and biological responses with the Abraham Model. Chapter 12. In: Acree W, editor. Toxicity and drug testing. InTech 2012. p. 261-70.
54. Ferrari B, Mons R, Vollat B, Fraysse B, Paxeus N, Lo Giudice R, Pollio A, Garric J. Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environ Toxicol Chem 2004;23:1344-54. doi: 10.1897/03-246
55. Halling-Sorensen B, Lutzhoft HC, Andersen HR, Ingerslev F. Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. J Antimicrob Chemother 2000;46(Suppl 1):53-8; discussion 63-5. PMID: 11051625
56. Boillot C. Évaluation des risques écotoxicologiques liées aux rejets d'effluents hospitaliers dans les milieux aquatiques. Contribution à l'amélioration de la phase «caractérisation des effets» [Ecotoxicological risk assessment related to hospital wastewater discharge into aquatic environments. Contribution to the improvement of the "characterization of effects" phase, in French]. INSA de Lyon, 2008.
57. Henry TB, Kwon JW, Armbrust KL, Black MC. Acute and chronic toxicity of five selective serotonin reuptake inhibitors in *Ceriodaphnia dubia*. Environ Toxicol Chem 2004;23:2229-33. doi: 10.1897/03-278
58. The European Commission. A watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council [displayed 31 May 2016]. Available at http://eur-lex.europa.eu/eli/dec_impl/2015/495/oj.
59. Lindberg RH, Björklund K, Rendahl P, Johansson MI, Tysklind M, Andersson BAV. Environmental risk assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. Water Res 2007;41:613-9. doi: 10.1016/j.watres.2006.11.014
60. Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad HL, Ahmed S, Chaudhry MJ, Arshad M, Mahmood S, Ali A, Khan AA. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature 2004;427:630-3. doi: 10.1038/nature02317
61. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. Environ Sci Technol 2002;36:1202-11.
62. Zwiener C, Frimmel FH. Oxidative treatment of pharmaceuticals in water. Water Res 2000;34:1881-5. doi: 10.1016/S0043-1354(99)00338-3

Koncentracije zdravilnih učinkovin v slovenskih komunalnih in bolnišničnih odpadnih vodah: preliminarna raziskava

Pojavljanje ostankov zdravilnih učinkovin v odpadnih vodah postaja vedno bolj aktualna tematika in posledično se širi nabor analiznih metod, ki omogočajo natančno ugotavljanje njihove prisotnosti in služijo kot orodje za napovedovanje tveganja teh onesnažil v vodnem okolju. Namen naše raziskave je bil kvantitativno ovrednotiti prisotnost izbranih zdravilnih učinkovin (atorvastatin, bisoprolol, ciprofloksacin, diklofenak, fluoksetin karbamazepin, klofibrinska kislina, metoprolol in sertralin) na iztoku ene komunalne in ene bolnišnične čistilne naprave. Na osnovi meritev koncentracij smo z uporabo količnika tveganja ocenili okoljsko breme vključenih spojin. Ugotovili smo prisotnost sedmih zdravilnih učinkovin, medtem ko klofibrinske kisline in fluoksetina nismo zaznali v nobenem vzorcu. Izmerjene koncentracije so bile v širokem koncentracijskem območju (od ng L^{-1} do µg L^{-1}), praviloma višje v bolnišnični odpadni vodi, z izjemo diklofenaka in karbamazepina. Izračunan količnik tveganja nakazuje na visoko tveganje za ciprofloksacin in diklofenak v vseh analiziranih vzorcih odpadnih voda. Raziskava je pokazala, da je razvita metoda primerno orodje za nadaljnje študije, ki bodo na podlagi sistematičnega spremljanja teh novodobnih onesnažil v odpadnih vodah omogočile zanesljivejšo oceno tveganja za izpostavljene vodne organizme in tudi za zdravje ljudi. Poznavanje teh tveganj bo prispevalo k načrtovanju ustrezne tehnologije čiščenja odpadnih voda in posledično k ohranjanju čistega in zdravega okolja.

KLJUČNE BESEDE*: atorvastatin; bisoprolol; bolnišnična čistilna naprava; ciprofloksacin; diklofenak; fluoksetin; karbamazepin; klofibrinska kislina; komunalna čistilna naprava; LC-MS/MS; metoprolol; ocena tveganja; sertralin*