Prof. Dr. Walter Giger
EAWAG, Swiss Federal Institute of Aquatic Science and Technology
Ueberlandstrasse 133
8600 Dübendorf
giger@eawag.ch

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Dr. Alfredo Alder
EAWAG, Division Chemical Pollutants, Dübendorf
alder@eawag.ch

Dr. Hans-Peter Kohler
EAWAG, Division Microbiology and Ecotoxicology, Dübendorf
kohler@eawag.ch

Occurrence of human-use antibiotics and antibiotic resistance in the aquatic environment (HUMABRA)

Objectives Antibiotics used in human medicine are found at trace concentrations in municipal wastewaters, and residual amounts reach ambient waters. Aim of the project is to study sources and fate of antibiotics in the aquatic environment.

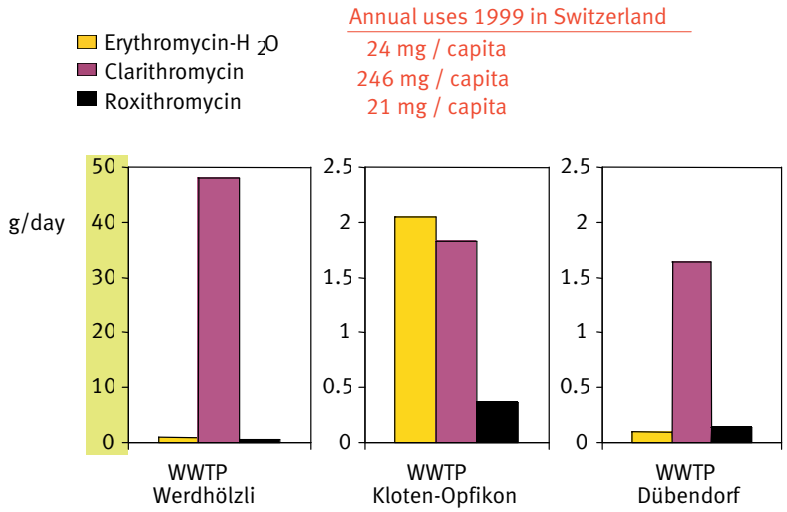
Conclusions New analytical methods allowing qualitative determination of the ß-lactam antibiotics and quantitative determination of trace concentrations of the antibiotics fluoroquinolones, macrolides and sulfonamides in wastewater, sewage sludge and ambient water were developed. For these methods, crucially important were efficient enrichment techniques (solid-phase extraction and accelerated solvent extraction) and liquid chromatography directly coupled to electro spray ionisation mass spectrometry.
While representatives from the classes of fluoroquinolones, macrolides and sulfonamides could be quantitatively measured in hospital wastewaters, in municipal wastewaters before and after mechanical-biological wastewater treatment as well as in rivers, the highly important ß-lactams could only be detected semi-quantitatively in hospital wastewaters. The concentrations found in wastewater correlated reasonably well with the known use data, and in-depth knowledge about the behaviour of the concentrations during wastewater treatment could be established.
Ciprofloxacin, norfloxacin, clarithromycin, azithromycin, trimethoprim, sulfamethoxazole and sulfapyridine were ubiquitously found in the lower ng/L range in Swiss rivers and sulfamethoxazole also occurred in groundwaters, which contain high amounts of bank filtrates.

Main results and findings

Validation of analytical methods Analytical methods were developed for the determination of sulfonamide and macrolide antimicrobials in aqueous and solid phases. For the extraction of activated and digested sewage sludge samples, accelerated solvent extraction was used. For diluted sludge extracts and water samples, solid-phase extraction and reversed-phase liquid chromatography were applied. Finally, for the detection of the selected compounds, tandem mass spectrometry with electrospray positive ionization in the multiple reaction mode was used. The limits of quantification ranged between 3–214 ng/L in raw influent, 1–23 ng/L in secondary and tertiary effluent. For activated sludge samples the limits of quantification varied between 3–41 µg/kg dry weights. Recoveries were generally above 80%, and the combined measurement uncertainty ranged between 2.4 and 16% for aqueous samples.

Wastewater treatment plants (WWTPs) Macrolides are only partly eliminated in wastewater treatment plants (WWTPs) and can therefore reach the aquatic environment. In treated effluents from three WWTPs in Switzerland, clarithromycin, roxithromycin and erythromycin-H_2O, the main degradation product of erythromycin, were found. The following figure shows the daily loads of macrolides in (g/day) in the treated effluents of three WWTPs, Zurich-Werdhoelzli, Kloten-Opfikon and Duebendorf (see Figure 1):

Figure 1
Daily loads of macrolides in (g/day) in the treated effluents of three wastewater treatment plants, Zurich-Werdhoelzli, Kloten-Opfikon and Duebendorf



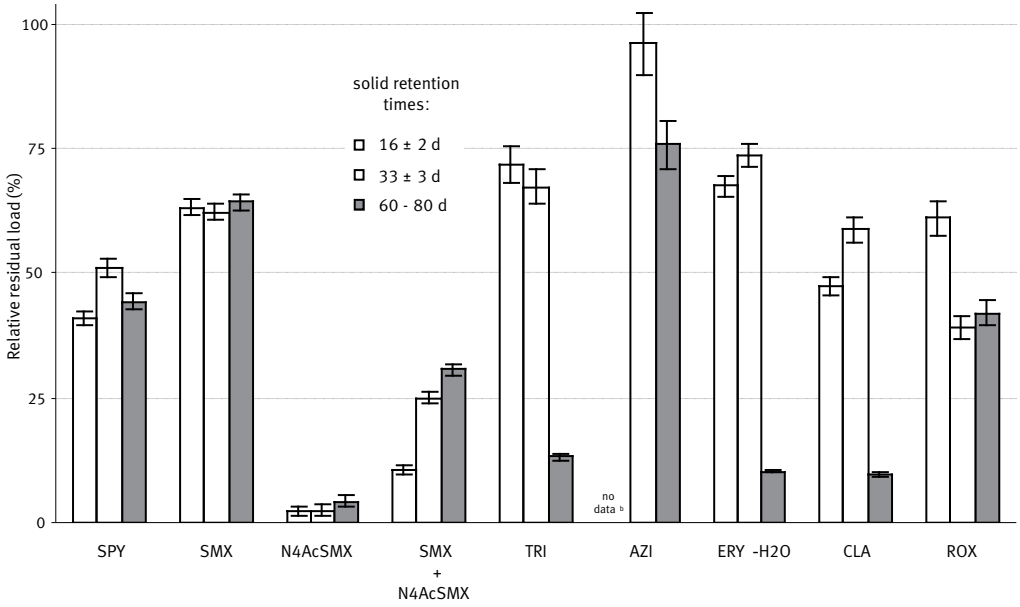
In particular, it is worthy of notice that:
- the most abundant, clarithromycin, reflects the consumption pattern of macrolide antibiotics. Summer concentrations of clarithromycin varied between 57 and 330 ng/L in treated WWTP effluents;
- in the Kloten-Opfikon WWTP seasonal differences revealed a load two times higher in winter than in summer. The higher abundance of erythromycin-H_2O in the effluent of the Kloten-Opfikon WWTP can be explained by distinct consumption patterns due to the main international airport of Switzerland in the catchment area.

Field studies on the behavior and fate of macrolide and sulfonamide antimicrobials, including trimethoprim, were performed in selected WWTPs using activated sludge, a fixed-bed reactor or a membrane bioreactor, operated at three different solid retention times, as secondary treatment step. Moreover, the ozonation of wastewater effluents was investigated as a tool to further reduce the loads entering the aquatic environment. Futhermore, mass balances were performed, including sorption to sewage sludge, to assess the elimination of sulfonamide and macrolide antimicrobials in wastewater treatment. The results can be summarised as follows:
- In accordance with consumption data, clarithromycin and sulfamethoxazole were the most predominant macrolide and sulfonamide, respectively, found in Swiss wastewater.
- For sulfamethoxazole, it proved to be crucial to include the amount present as the main human metabolite, N4-acetylsulfamethoxazole.
- No significant elimination was observed for all investigated compounds in primary treatment. During secondary treatment, the elimination observed depended on the treatment technology investigated.
- Overall sorption to secondary sludge is of minor importance for all compounds investigated.

For secondary wastewater treatment, different treatment technologies were investigated concerning the elimination of the selected compounds. Similar results were obtained in two conventional activated sludge systems and a fixed-bed reactor. In particular:

- While no significant removal was observed for trimethoprim, sulfamethoxazole, including the amount present as N4-acetyl-sulfamethoxazole, was eliminated by about 60%. Approximately 80% of total sulfamethoxazole load was eliminated in the membrane bioreactor, independently of the solid retention time.
- For the macrolides, the results varied between sampling campaigns, with an elimination of up to 55% in one single case.
- In the membrane bioreactor, macrolides and trimethoprim were generally eliminated by 25–50% at a solid retention time of 16 and 33 days. For these compounds, significantly higher elimination, of up to 90%, was observed at a solid retention time of about 77 days. Figure 2 shows the relative residual loads of sulfonamides, macrolides and trimethoprim in a membrane bioreactor operated at different solid retention times. High solid retention time and correlated low substrate loading seem to have an influence on the diversity of the microbial population and, consequently, on the multitude of degradation pathways being expressed.

Figure 2
Relative residual loads of sulfonamides, macrolides and trimethoprim in a membrane bioreactor operated at different solid retention times



- Elimination in tertiary treatment was observed only for macrolides and trimethoprim in one of the sand filters investigated and seemed to be related to oxygen limitations.
- Ozonation proved to be efficient for the removal of sulfonamide and macrolide antimicrobials from wastewater effluents. An ozone dose of 2 mg/L resulted in a reduction of the respective loads by over 90%, interdependently of the amount of suspended solids present.

Occurrence and fate of macrolide antibiotics in the Glatt River watershed In the Glatt River clarithromycin reached concentrations of up to 75 ng/L. Load determinations in treated effluents and in river water in the Glatt Valley watershed showed that elimination of clarithromycin along the river stretch of 36 km is insignificant. Investigations in the Glatt River before and after the diversion of the largest, WWTP, revealed an observable decrease in clarithromycin loads. Figure 3 shows clarithromycin loads in the Glatt Valley watershed in winter 2001.

Figure 3
Clarithromycin loads in the Glatt Valley watershed in winter 2001 in g/day. The inputs of four WWTP effluents were measured (bars on dark points representing the WWTPs) and the input of the Bassersdorf WWTP was estimated. Samples from three stations along the Glatt River were analyzed (bars at Greifensee outlet, Oberglatt and Rheinsfelden). In Oberglatt, the actual input was calculated and is shown in the figure as a calculated sum.

Hospital wastewaters in Switzerland Analyses of hospital wastewaters were performed from the start of HUMABRA in the Cantons of Zurich and Ticino. The concentrations of antibiotics contained in wastewaters of University Hospital Zurich are summarized in Figure 4A. It should be noted that for the ß-lactams only semi quantitative results could be obtained, while the other antibiotics were measured by fully validated analytical methods. An attempt was made to obtain annual mass flow data (see Figure 4B).

Figure 4A
Antibiotics in wastewater of University Hospital Zurich: Concentrations µg/L

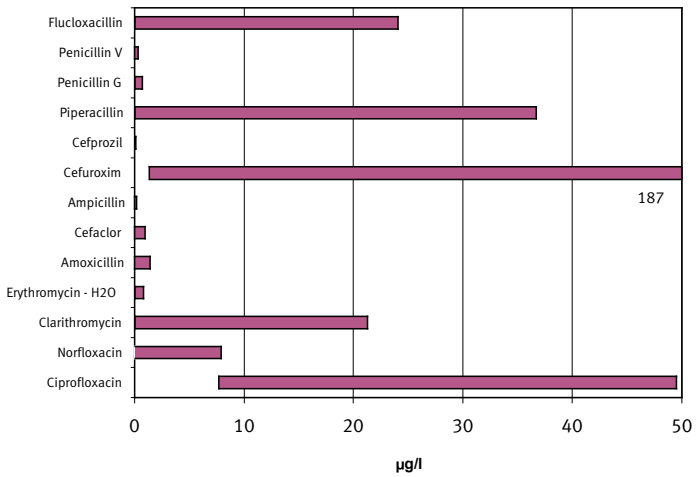
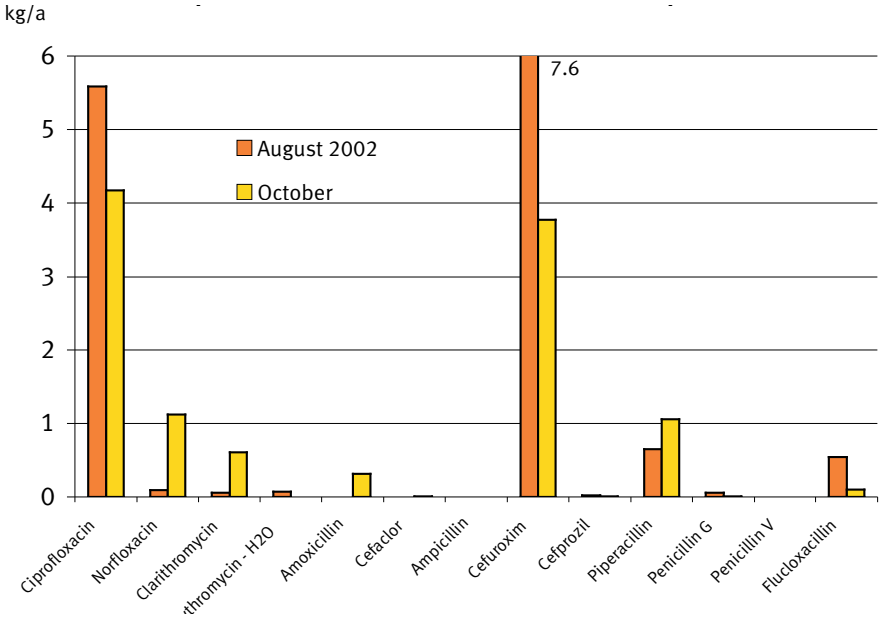


Figure 4B
Antibiotics in wastewater of University Hospital Zurich: Annual mass flows (loads) kg/J



Wastewaters of the long-term care facility (Pflegeheim) Mattenhof in Zurich In August 2005, 4 grab samples of the wastewater of the long-term care facility (Pflegeheim) Mattenhof in Zurich were analyzed for fluoroquinolone, macrolide and sulfonamide antibiotics. Concentrations 10–50 times lower than the corresponding values in the wastewaters of University Hospital Zurich were found. The annual mass flows are estimated to be less than 10% of those in hospital wastewaters.

Antibiotics in wastewaters in Southeast Asia: Vietnam Another objective was to compare use patterns and occurrences of human-use antibiotics in wastewaters in Southeast Asia (Hanoi, Vietnam). This project was the first investigation that provided information on the occurrence of residual human-use antibiotics in the aquatic environment in Vietnam. The two fluoroquinolones (FQs) ciprofloxacin and norfloxacin are consumed in large amounts in Vietnam. Because hospital wastewaters represent a significant source of antibiotics, FQs were determined in samples from untreated wastewater effluents of five hospitals in Hanoi. The results can be summarised as follows:

- The concentration of the FQs in aqueous grab samples from the hospital wastewater effluents varied from 1 to 15 µg/L (see Table 1).
- The FQs levels in Hanoi were generally in the same order of magnitude as in Switzerland.
- In the Huu Nghi hospital the wastewater is treated in small treatment facility before it is discharged into the receiving surface water, the To Lich River.
- Grab samples were taken every 3 hours and combined to a 24 h composite sample.
- The removal of the analysed FQs from the water stream during wastewater treatment was between 80 and 87%, presumably mainly through sorption to particulates. These elimination rates are in agreement with the values reported in the literature.

Table 1
Antibiotic concentrations and loads in Vietnamese hospitals.

Hospital	Concentration [b] (µg/L)		Load [c] (g/d)	
	NOR	CIP	NOR	CIP
Thanh Nhana [a]	15.2 ± 0.3	7.0 ± 0.1	4.6	2.1
Viet Duc [a]	3.4 ±0.4	10.9 ± 0.8	3.4	10.9
Hanoi K [a]	nd	1.2 ± 0.2		0.4
Central Obstetric [a]	13.6 ± 0.3	2.1± 0.1	4.6	0.7
Hanoi Obstetric [a]	nd	1.1 ± 0.1		0.3
Huu Nghi				
raw wastewater	6.8 ± 1.1	33.3 ± 7.7		
treated wastewater	1.4 ± 0.2	4.3 ± 0.9	0.5	1.2

[a] untreated wastewater; [b] average ± STD;
[c] = average concentration x average wastewater volume; nd: not detected
CIP: ciprofloxacin NOR: norfloxacin

The To Lich River, with a total length of 13.5 km, runs from north to south of Hanoi and is in reality one of the four main wastewater channels of Hanoi.

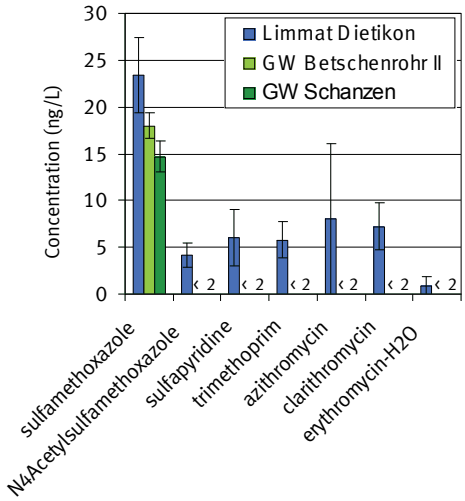
- The wastewater discharge into the To Lich is around 140–150,000 m^3/d, of mostly untreated wastewater from residential areas, hospitals, industries and agriculture located along the river.
- Aqueous grab samples were collected at different locations, and the FQs concentrations varied from 0.2 to 0.5 µg/L.
- Ciprofloxacin and norfloxacin were also determined in sediments from the To Lich, and the concentrations ranged from 0.5 to 2.1 mg/kg dry matter.
- Concentrations in To Lich are 3–7 higher than wastewater effluents in Switzerland. Concentrations in To Lich sediment are in the same order of magnitude as in sewage sludge in Switzerland.

Antibiotics in groundwater of the Limmat Valley Pollutants like antibiotics might reach groundwater by infiltration of polluted surface water or from defect wastewater pipes. To investigate these input pathways, a project was started in collaboration with AWEL (Amt für Abfall, Wasser, Energie und Luft, Kanton Zurich). Fate studies were performed in the Limmat Valley watershed to study inputs from the municipal wastewater treatment plant (WWTP) of Zurich-Werdhölzli, to evaluate the fate of selected antibiotics in the Limmat River as well as to assess their behaviour during bank filtration into groundwater (Blüm, 2005). Eleven macrolide and sulfonamide antibiotics were analyzed with LC/MS/MS according to a validated analytical method (Göbel, 2004). All of them were found in the effluent of the WWTP, where they are only partly eliminated (Göbel, 2005). The results can be summarised as follows:

- An unusually high concentration of 1100 ng/L of the macrolide antibiotic azithromycin was found in the treated sewage effluent, which could be possibly attributed to an increased contribution of hospital wastewaters.
- The azithromycin loads were substantially eliminated (70%) in the Limmat River within a river stretch of 7 km.
- The loads of the other studied trace contaminants were not affected.
- Due to dilution, concentrations in the river were around twenty times lower than in the effluent of the WWTP (Figure 5).
- Only sulfamethoxazole could be detected in measurable concentrations (11–19 ng/L) in the groundwater wells.
- In the Limmat River concentrations of sulfamethoxazole were only slightly higher (17–29 ng/L) than in the groundwater.

– It is known that in the investigated Schanzen groundwater well more than 80% of the groundwater is directly infiltrated water from the Limmat River. The source of the contamination of the groundwater can therefore be attributed to direct infiltration of river water and not to other sources like leaking water pipes. By comparing groundwater concentration to the average concentration in the Limmat River it can be inferred that up to 30% of sulfamethoxazole was eliminated in the infiltrated sediment. All other investigated antibiotics were eliminated to concentrations below detection limits during the passage through the river sediment.
– The concentrations of sulfamethoxazole observed in the groundwater, which is pumped for drinking water usage, are very low, and a negative influence on human health can be excluded based on current knowledge. However, these findings raise the question of whether this contamination can be accepted or should be avoided following the precautionary principle.

Figure 5
Concentrations of sulfonamide and macrolide antibiotics in the Limmat River at Dietikon and in two groundwater wells (GW), Betschenrohr II and Schanzen. For details, see Blüm et al. (2005)



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