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Occurrence and Fate of Macrolide Antibiotics in Wastewater Treatment Plants and in the Glatt Valley Watershed, Switzerland

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An analytical method was developed for determining macrolide antibiotics in treated wastewater effluents and in ambient water based on solid-phase extraction and LC/MS analysis as well as on LC/MS/MS for structural confirmation. In wastewater treatment plants (WWTPs) macrolides are only partly eliminated 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 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 WWTP Kloten-Opfikon seasonal differences revealed a load two times higher in winter than in summer. The higher abundance of erythromycin-H_2O in the effluent of WWTP Kloten-Opfikon can be explained by distinct consumption patterns due to the main international airport of Switzerland in the catchment area. In the Glatt River clarithromycin reached concentrations of up to 75 ng/L. Mass flux 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 (<20%). Investigations in the Glatt River before and after the diversion of the largest WWTP revealed an observable decrease in clarithromycin loads.

Introduction

The occurrence of pharmaceuticals as contaminants in wastewater and in the aquatic environment has attracted increased attention (*1*−*3*). A high percentage of pharmaceuticals consumed by humans are excreted unchanged via urine and feces into the sewage. In wastewater treatment plants these chemicals are only partially eliminated, and residual amounts can reach ambient surface waters or groundwater. There is a potential risk for aquatic and soil organisms associated with the presence of trace concentrations of these bioactive compounds. Studies on the fate of antibiotics in wastewater and surface water are motivated by the question whether trace concentrations in the environment may contribute to the maintenance and spread of antibiotic resistance.

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In 1997, approximately 90 t of antibiotics (including antibacterials such as fluoroquinolones and sulfonamides) were used in Switzerland; 38% in human medicine and 62% in veterinary medicine (*4*−*6*). The total amount of antibiotics used annually in human medicine is around 34 tons and has remained fairly constant since 1992. β-Lactam antibiotics, which include the penicillins and cephalosporins, represent the largest fraction of human antibiotics, accounting for approximately 18 t. They are followed by sulfonamides (5.5 t), macrolides (4.3 t), and fluoroquinolones (3.9 t). In Switzerland, private consumption of macrolides in the year 1999 amounted to 1.74 t of clarithromycin, 0.32 t of azithromycin, 0.26 t of spiramycin, 0.17 t of erythromycin, and 0.15 t of roxithromycin (*5*). Beside the private consumption, 20−40% of the total macrolide consumption is used in hospitals.

In this study, we focus on macrolide antibiotics, which are among the most important antibacterial agents used in human medicine. Macrolide antibiotics act as inhibitors of bacterial protein synthesis. Indications typically include lower and upper respiratory tract infections (bronchitis, pneumonia, sinusitis, pharyngitis) and soft-tissue infections. Macrolides are highly potent against a wide variety of gram-positive and gram-negative organisms, and they are used as penicillin substitutes. Excretion occurs primarily via bile and feces (50−67%). Urinary excretion of unchanged parent drug is 10−20% for clarithromycin, 30% for roxithromycin, 10−20% for spiramycin, 6−12% for azithromycin, and 5−10% for erythromycin (*7*). All macrolides are metabolized to a minor extent, except erythromycin, whose main metabolite is an antibacterially inactive degradation product with an apparent loss of water (erythromycin-H_2O).

The word macrolide breaks down into macro (large) and olide (lactone), as macrolides consist of a large lactone ring (Figure 1). The lactone ring is substituted with hydroxyl, alkyl, and ketone groups, and neutral or amino sugars are bound to the nucleus by substitution of hydroxyl groups. Macrolides have basic properties and the pK_as of the amino group vary between 7.1 and 9.2 (*8*). Since they are mainly positively charged at pHs of 7−8, the log K_{ow}s (octanol water coefficients), which vary between 1.5 and 4, are not very meaningful at environmental conditions.

Little is known about the environmental behavior of macrolide antibiotics. Erythromycin was found to be nonbiodegradable (*9*). Gavalchin et al. (*10*) determined a half-life time ($t_{1/2}$) of 11.5 days in soil for the biotransformation of erythromycin. Loke et al. (*11*) reported a $t_{1/2}$ of less than 2 days for tylosin in manure, but they could not determine whether the elimination was due to sorption or abiotic or biotic transformation. Later, the same group published data on aerobic degradation and found half-lives of 9.5−40 days for tylosin with initial lag phases of 31−40 days in simulated surface water (*12*). Tylosin was considered to be moderately persistent in surface water systems. Tylosin was found to sorb to soil, correlated to soil clay content with a K_D of 8−128 mL/g and a K_{oc} of 553−7988 mL/g (*13*).

Hirsch et al. (*14*) first investigated the occurrence of several representatives from the main groups of antibiotics in wastewater treatment plant effluents and in river water. They describe the analysis of various water samples for 18 antibiotic substances from the antibiotic classes of macrolides, sulfonamides, penicillins, and tetracyclines. They observed the frequent occurrence of erythromycin-H_2O, roxithromycin, and sulfamethoxazole with concentrations of up to 6 μg/L

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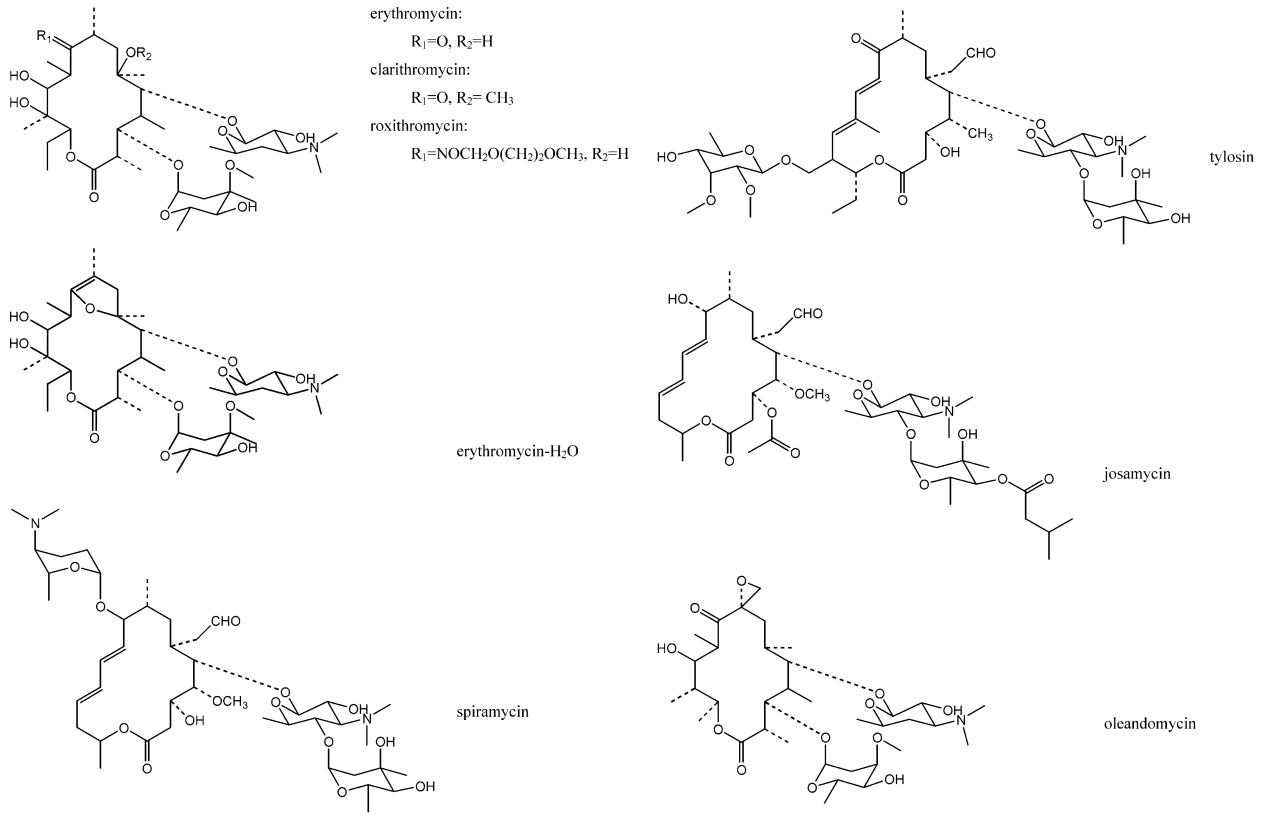

FIGURE 1. Chemical structure of the analyzed macrolide antibiotics.

in the effluent of wastewater treatment plants (WWTPs). Neither tetracyclines nor penicillins could be detected at concentration levels above 50 and 20 ng/L, respectively. Penicillins are not very likely to occur in the aquatic environment because of their chemically unstable β-lactam ring, which is readily susceptible to hydrolytic cleavage. Sacher et al. (*15*) analyzed 105 groundwater wells in Baden-Wuerttemberg, Germany. Among 60 pharmaceuticals, they found erythromycin-H_2O and sulfamethoxazole, which were the only antibiotics out of eight compounds detected in at least three groundwater samples. Recently, a study was published which shows the occurrence of 95 organic wastewater contaminants including pharmaceuticals in 139 streams across the United States (*3*). Among 31 antibiotics from the groups of tetracyclines, macrolides, sulfonamides, and fluoroquinolones, erythromycin-H_2O and sulfamethoxazole were found in concentrations of up to 1.7 and 1.9 μg/L, respectively. In previous publications, we have reported on the occurrence of macrolide antibiotics in different surface waters and WWTP effluents and demonstrated different input pathways of veterinary and human antibiotics (*16*, *17*).

The objective of this study was to conduct a detailed investigation on the environmental behavior of macrolide antibiotics. This included the development of an analytical method for analyzing environmental samples and the assessment of wastewater treatment. The analytical method by Hirsch et al. (*18*), which uses LC/MS/MS for analysis, was adapted for measuring environmental samples with LC/MS. Regional and seasonal studies of WWTP effluents were performed for three WWTPs in Switzerland. Additionally, the occurrence and fate of macrolide antibiotics in the Glatt Valley Watershed was studied. The same watershed was investigated for the fluoroquinolone antibiotics ciprofloxacin and norfloxacin (*19*), and therefore the behavior and the mass fluxes of the two antibiotic groups could be compared. In addition, the impact of the elimination of a point source could be studied, because the largest WWTP that was discharging into the Glatt River, the WWTP Zurich-Glatt, was decommissioned and the wastewater was diverted to the WWTP Zurich-Werdhoelzli in another watershed.

Experimental Section

Reagents. The macrolide antibiotics erythromycin, roxithromycin, spiramycin, oleandomycin, and tylosin were purchased from Sigma-Aldrich Co. (Buchs, Switzerland). Josamycin was bought from Fluka Chemicals (Buchs, Switzerland). Clarithromycin was kindly supplied by Abbott GmbH (Wiesbaden, Germany). Erythromycin-H_2O was obtained by adjusting the pH of an erythromycin solution to 3.0 using 3 M H_2SO_4. After 4 h of stirring at room temperature complete conversion to erythromycin-H_2O was achieved. The completeness of the reaction was verified by checking for leftover erythromycin and possible metabolites using mass spectrometry. All solvents (HPLC grade) were obtained from Scharlau Chemie S. A. (Barcelona, Spain). Ammonium acetate (NH_4Ac) and acetic acid (HAc) were purchased from Merck (Darmstadt, Germany).

Sample Collection. Treated effluent of the WWTPs Kloten-Opfikon, Zurich-Werdhoelzli, and Duebendorf were sampled in July 2000, the effluents of the WWTPs Kloten-Opfikon and Zurich-Glatt were sampled in February 2001, and the effluent of WWTP Faellanden was sampled in September 2002. The WWTPs are equipped with primary and secondary clarifiers followed by sand filtration without any post disinfection. In all cases, 24 h flow proportional composite samples were collected and analyzed during one week. Samples were collected in glass bottles and kept at 4 °C until extraction, which was performed less than 2 days later. At three sampling stations on the Glatt River weekly composite samples were collected. At the locations Greifensee outlet and Oberglatt, samples were taken on a time-proportional (every 20 min)

basis; at the sampling station Rheinsfelden the sampling was flow proportional. Flow data were supplied by the Cantonal Office for Waste, Water, Energy, and Air of Zurich (AWEL). During five succeeding weeks in winter 2001 (calendar weeks 4 to 8), samples were taken from the Glatt River. At this time, WWTP Zurich-Glatt was still discharging its treated wastewater completely into the Glatt River. Analogous sampling campaigns were repeated in summer 2001 (weeks 24 to 27), when 80% of the wastewater from WWTP Zurich-Glatt was diverted from the Glatt River to the Limmat River, and in winter 2002 (weeks 4 to 7), when 100% was diverted.

Sample Preparation. The method of Hirsch et al. (*18*) based on LC/MS/MS for analyzing environmental samples was adapted for the application of LC/MS. A 1-L aliquot of sample was filtered through 0.45-μm glass fiber filters (Whatman Int. Ltd., England), and the pH was adjusted to pH 7 using 3 M H_2SO_4 or NaOH. Solid-phase extraction was performed on 3-mL extraction cartridges filled with 100 mg of LiChrolute EN and 250 mg of LiChrolute RP-18 (Merck, Darmstadt, Germany). The cartridges were conditioned with 3×2 mL of *n*-hexane, 3×2 mL of methanol, and 6×2 mL of water (pH 3.0). The samples were extracted at a flow rate of 5−20 mL/min. The cartridges were dried in a nitrogen stream for 60 min, and the analytes were eluted 5× with 1 mL of methanol. The eluent was collected in conical reaction vials (6 mL, Supelco, Bellefonte, CA) and the volume was reduced to 50−200 μL with a gentle flow of nitrogen at room temperature. The extracts were then transferred to 1-mL volumetric flasks and brought to volume with 20 mM phosphate buffer at pH 6.

Liquid Chromatography. HPLC analyses were performed on a liquid chromatograph (Hewlett-Packard series 1100, Waldbronn, Germany) equipped with a vacuum solvent degassing unit (DG4 from Henggeler Analytic Instruments, Riehen, Switzerland), a binary high-pressure gradient pump, an automatic sample injector, and a column thermostat. Separation was accomplished with a 125×2 mm Nucleosil 100-5 C_{18} HD end-capped column (Macherey-Nagel, Dueren, Germany) equipped with an 8×2 mm precolumn that also contained the same sorbent. The column was maintained at 30 °C at a flow rate of 0.15 mL/min. Solvent A was 10 mM NH_4Ac buffer at pH 6 and acetonitrile (90:10), solvent B was acetonitrile. The gradient started at 20% B, was brought to 30% B in 10 min, to 50% in the next 10 min, and to 100% in another 18 min. The eluent was kept at 100% B for 5 min. For reequilibration, the gradient was brought down to 20% B in 10 min and kept there for 8 min. Sample volumes of 50 μL were injected.

Mass Spectrometry. A Platform LC mass spectrometer with an electrospray interface was used (Micromass, Manchester, UK). Nitrogen was used for drying as well as nebulizing gas. The operating gas flow was set to 500 L/h and the source temperature to 150 °C. Needle voltage was adjusted to 3.0 kV, low and high-mass resolution of the quadrupole were set to 12.0, and the ion energy was set to 1.0. Mass range was calibrated and sensitivity of the instrument was tested with 2 mM $NaNO_3$ solution infused at a flow rate of 70 μL/min directly into the MS using a Harvard syringe pump model 22 (Harvard Instruments, Gams, Switzerland). Positive ion mode was utilized for the analysis of the macrolide antibiotics with electrospray ionization. Single ions were monitored (SIM) at cone voltages of 25 and 50 V. The following main ions $[M + H]^+$ and one or more fragment ions for MS determination at 25 and 50V were chosen: for oleandomycin *m*/*z* 688.4 and 544.3; for spiramycin 843.7 and 422.5; for erythromycin 734.5 and 576.5; for erythromycin-H_2O 716.5, 558.4, and 540.4; for tylosin 916.6 and 257.1; for clarithromycin 748.5 and 590.4; for roxithromycin 837.5 and 679.4; and for josamycin, which produced no fragment ion at either cone voltage, only 828.6 was used. Since josamycin is the method standard, this was considered to be sufficient. Analytes and calibration standards behave similarly at the two cone voltages. Fragment ions gave intense signals at a cone voltage of 50 V for all analytes except josamycin. The ratio of the peak areas of the main ion to the peak area of at least one fragment ion was always determined for standard solutions as well as for the sample solutions. Within a variation of 10−20% these ratios were the same for standards and samples, which assured that only the corresponding macrolide antibiotic contributes to the signal.

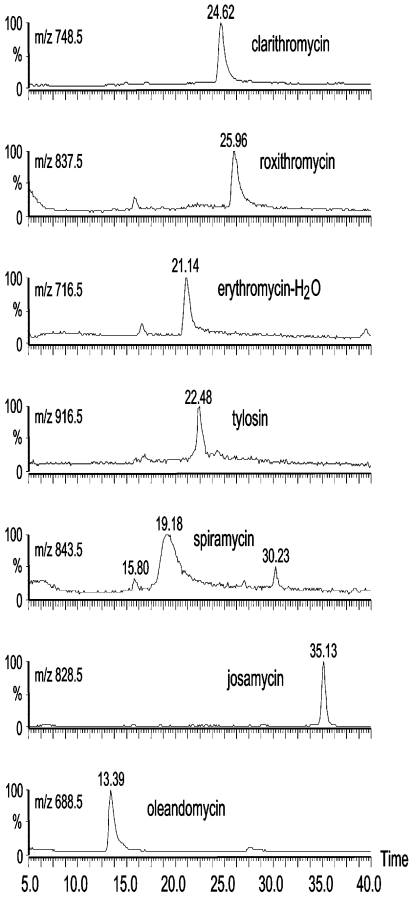

FIGURE 2. SIM results from the analysis of a groundwater sample spiked with 50 ng/L of clarithromycin, roxithromycin, erythromycin-H_2O, tylosin, spiramycin, josamycin and oleandomycin ($[M + H]^+$ ions shown). According to the supplier, spiramycin consists of a mixture of three substances.

Quantification. A chromatogram that shows the separation of seven macrolides is presented in Figure 2. Most samples were run at both cone voltages 25 and 50 V and the setting which yielded the more intense $[M + H]^+$ peak was chosen for quantification. Josamycin and oleandomycin were utilized as the method standards after their absence in water samples had been confirmed. They were added at a concentration of 100 ng/L of water sample. Josamycin was added as a surrogate standard to all samples prior to enrichment to control for possible losses during the analytical procedure. Oleandomycin was added to the solution of evaporated extracts in phosphate buffer as an instrumental standard. Because oleandomycin was found to have matrix interferences due to coelution in some samples, all samples were quantified by correcting for instrumental deviations with josamycin. Since josamycin was added before the enrichment, deviations in extraction recoveries were accounted for at the same time. Duplicate analyses were carried out for each sample. The measured concentrations of macrolides in the samples were corrected by the recoveries.

TABLE 1. Recovery, Precision, and Limit of Quantification (LOQ) for the Determination of Macrolide Antibiotics in Groundwater (GW) and in the Effluent of Wastewater Treatment Plants (WWTP)[a]

	recovery ± SD (%)		precision (SD in %)		LOQ (ng/L)	
macrolide	GW ($n = 5$)	WWTP ($n = 5$)	GW ($n = 8$)	WWTP ($n = 6$)	GW	WWTP
erythromycin-H_2O	87 ± 12	69 ± 5	± 5	± 11	8	≈20
clarithromycin	88 ± 11	81 ± 9	± 8	± 13	4	≈10
roxithromycin	97 ± 13	66 ± 6	± 5	± 6	2	≈5
tylosin	84 ± 8	76 ± 10	± 8	± 13	8	≈25
spiramycin	59 ± 10	72 ± 20	± 12	± 10	35	≈70

[a] SD = standard deviation, n = number of samples. LOQ = 10SD for groundwater, LOQ for treated wastewater was estimated for a signal-to-noise ratio of 10.

TABLE 2. Range of Concentrations and Loads of Macrolide Antibiotics Measured in Treated Effluents of Three WWTPs in July 2000[a]

			Erythromycin-H_2O		Clarithromycin		Roxithromycin	
WWTP	inhabitants in catchment area	wastewater discharge m^3/d	concentration [ng/L]	load [g/day]	concentration [ng/L]	load [g/day]	concentration [ng/L]	load [g/day]
Zurich-Werdhoelzli	275 600	170 000	< LOQ		194−328	26−58	< LOQ − 22	up to 2.8
Kloten-Opfikon	25 900	18 000	110−199	1.7−2.9	97−163	1.2−2.7	11−31	0.13−0.50
Duebendorf	35 000	16 000	< LOQ		57−135	0.80−2.4	< LOQ − 17	up to 0.32

[a] <LOQ: lower than limit of quantification.

All quantitative data are based on calculations using an external calibration curve. Concentrations of the analytes relative to the method standard were plotted against peak area ratios. At least five concentration points were used in the expected concentration range, and r^2-values higher than 0.95 were obtained. The linear range of the calibration is from 10 to 1000 ng/L of water sample. The accuracy of this method was checked by comparing the results obtained from external calibration to measurements using standard addition. Therefore, seven WWTP effluent samples were spiked with different amounts of the environmentally relevant macrolide antibiotics, extracted, and analyzed. Variation between the two methods was less than 10% for erythromycin-H_2O, clarithromycin, and roxithromycin.

Method Validation. Blanks were determined by extracting the solid-phase material with 5 × 1 mL methanol followed by the workup described earlier. To evaluate the method reproducibility (precision), arithmetic means, and standard deviations were determined for individual macrolides as follows: 8 L of groundwater samples and 6 L of effluent of a WWTP were spiked in the range of 25−200 ng/L and divided into 1-L aliquots. The aliquots were extracted and analyzed separately. Results are given in Table 1. The method accuracy was determined by recovery studies in the same concentration range. For the polluted WWTP effluent the initial concentration needed to be considered, therefore standard addition was used to determine the recovery. The limits of quantification were calculated from the standard deviations of the measurements for the groundwater samples (LOQ = 10 SD). LOQ for wastewater was difficult to determine, as the samples already contained the analytes erythromycin-H_2O, clarithromycin, and roxithromycin. Therefore, LOQs were estimated from different samples for signal-to-noise ratios of 10. WWTP influents could not be analyzed with LC/MS due to matrix interferences.

Josamycin, the surrogate standard added to the samples before the extraction, was used as method standard for the quantification of the samples as well as to determine the recoveries. Samples quantified relative to josamycin were corrected by the relative recoveries to give accurate amounts. The recovery of josamycin itself was determined in spiked groundwater and was found to be 101±11%. During the measurements, two different batches of SPE material were used. Both batches resulted in the same recoveries.

Study Area of the Glatt Valley Watershed. The Glatt River watershed is a relatively densely populated region (260 km^2; 264 900 inhabitants) in the northern part of Switzerland, which includes part of the agglomeration of Zurich. The river is 36 km long and has a water residence time of 15−20 h from Greifensee outlet to the influent into the Rhine River. It receives considerable amounts of treated wastewater from mechanical and biological WWTPs. The overall percentage of treated wastewater discharged at the river mouth for the investigated periods was 20% in winter and about 13% in summer. The river bed slope ranges from 1 to 7% resulting in an average flow velocity of approximately 0.5 m/s (*20*). Water flow rates fluctuate in the range of 3−12 m^3/s. Three sampling points are located in the river: at Lake Greifensee outlet, at Oberglatt, and at Rheinsfelden just before the Glatt enters the Rhine River. Samples of effluents of the biggest WWTPs feeding into the Glatt River (WWTPs Duebendorf, Zurich-Glatt, Kloten-Opfikon, and Faellanden) were collected. A total of 195 600 inhabitants are living in the catchment area of these four WWTPs. The two sampling stations at Oberglatt and Rheinsfelden are located below the discharge of WWTP Zurich-Glatt.

Results and Discussion

Macrolide Antibiotics in WWTP Effluents. Ranges of concentrations and loads of three WWTP effluents analyzed in the canton of Zurich are presented in Table 2 and Figure 3. From all the measured macrolides (see Figure 1), only clarithromycin, roxithromycin, and the metabolite of erythromycin were detected. Erythromycin, tylosin, and spiramycin were not found at significant concentrations. Only the metabolite of erythromycin, erythromycin-H_2O, was present in environmental samples. No degradation of erythromycin was observed during sample treatment. Tylosin is used only in veterinary medicine in Switzerland, and, therefore, its absence in WWTP effluents is not surprising. Spiramycin is also used in human medicine, but was probably not detected because its LOQ in wastewater is relatively high (70 ng/L). Daily variations of about 50% can be observed for clarithromycin, the most abundant macrolide antibiotic, but no trend or pattern could be recognized. Loads and relative amounts of the antibiotics vary between the WWTPs. In WWTP Werdhoelzli, a roughly 20 times higher load of clarithromycin in grams per day was found than in the other

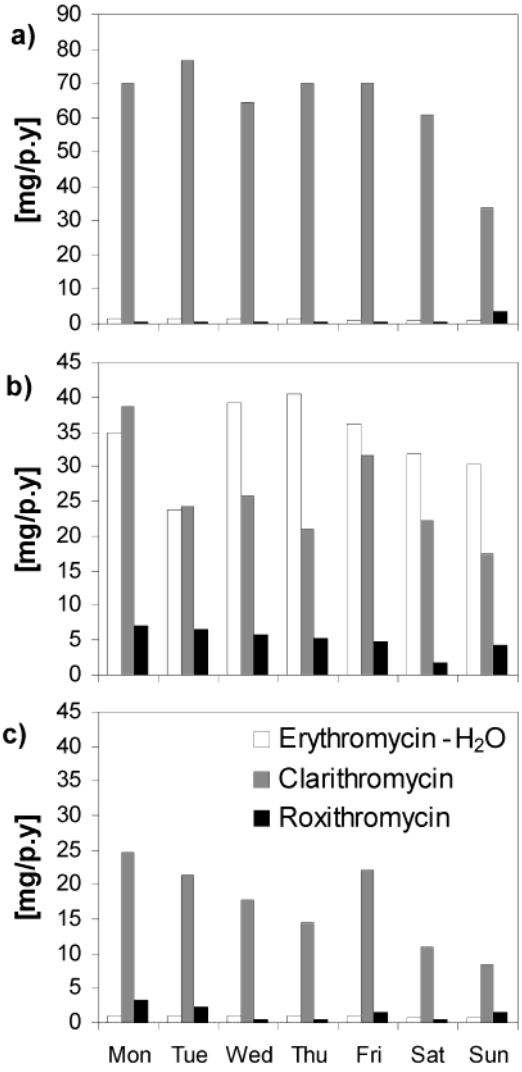

FIGURE 3. Weekly load profiles of macrolides in three treated effluents of WWTPs (a) Zurich-Werdhoelzli, (b) Kloten-Opfikon, and (c) Duebendorf, in mg per year and inhabitant living in the catchment area. Samples were collected in July 2000.

two WWTP effluents. This can be partly explained by the different sizes of the three WWTPs. WWTP Zurich-Werdhoelzli is the treatment plant of the city of Zurich and is the biggest of the three WWTPs (see Table 2 for details). Daily relative loads of macrolide antibiotics measured per inhabitant living in the catchment area are presented in Figure 3. Still, WWTP Zurich-Werdhoelzli exhibits a higher relative load of clarithromycin than the other two WWTPs. It can be inferred that not only the inhabitants contribute to the loads of clarithromycin in the WWTPs, but there are also commuters and hospital patients. For WWTP Zurich-Werdhoelzli, a number of 450 000 inhabitant equivalents including commuters can be estimated, and additionally all the main hospitals of Zurich are also discharging into this WWTP. No hospital discharges its wastewater to the WWTP Duebendorf, and much fewer commuters are registered, what explains the lower clarithromycin loads. In the WWTP Kloten-Opfikon, the relative load of clarithromycin per inhabitant is about 50% higher than in WWTP Duebendorf. This WWTP is a special case, as the main international airport of Switzerland is connected to it, which has an impact on the inputs of macrolides as will be discussed below.

To explain the different relative amounts of antibiotics found in the various WWTPs, consumption data have to be considered. In 1999, on average, 246 mg of clarithromycin

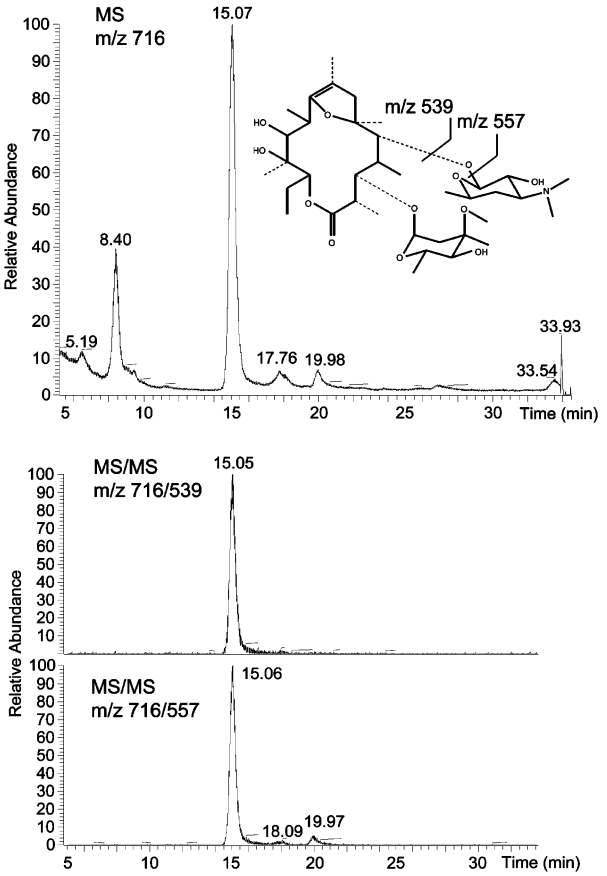

FIGURE 4. LC/MS and LC/MS/MS SIM chromatograms show the major peak for erythromycin-H_2O in the effluent of WWTP Kloten-Opfikon. The MS shows the $[M + H]^+$ ion and the MS/MS shows both fragment ions which are specific for erythromycin-H_2O.

were consumed per person in Switzerland, whereas erythromycin and roxithromycin were consumed in amounts of only 24 and 21 mg/person, respectively (*5*). These numbers only account for the private consumption without the applications of antibiotics in hospitals. The ratio of roughly 10:1 between the consumption of clarithromycin and roxithromycin is reflected in the measurements of the effluents of WWTP Duebendorf and WWTP Zurich-Werdhoelzli, whereas erythromycin-H_2O occurred in concentrations below the limit of quantification. In contrast, in the effluent of WWTP Kloten-Opfikon, erythromycin-H_2O was found in the same concentration range as clarithromycin. LC/MS/MS analysis was performed with the effluent samples of WWTP Kloten-Opfikon to verify that matrix effects did not interfere with the results. MS/MS analysis revealed both fragment ions *m*/*z* 557 and 539, which are specific for erythromycin-H_2O (Figure 4), confirming its occurrence qualitatively. Quantitative estimates show that the effluent concentration of erythromycin-H_2O is indeed much higher in WWTP Kloten-Opfikon than in the other two WWTPs, but precise quantitative measurements could not be performed with LC/MS/MS.

As mentioned above, the main airport of Switzerland, Zurich Unique, is connected to WWTP Kloten-Opfikon, and therefore the excretions from the passengers in the airport and the airplane toilets are discharged into this WWTP. The macrolide consumption pattern among passengers from different countries can vary substantially and has to be considered for an estimation of the antibiotic excretion by the passengers. The only available information concerning the passengers' nationalities is the origin and destination of the flights arriving at and departing from Zurich Unique.

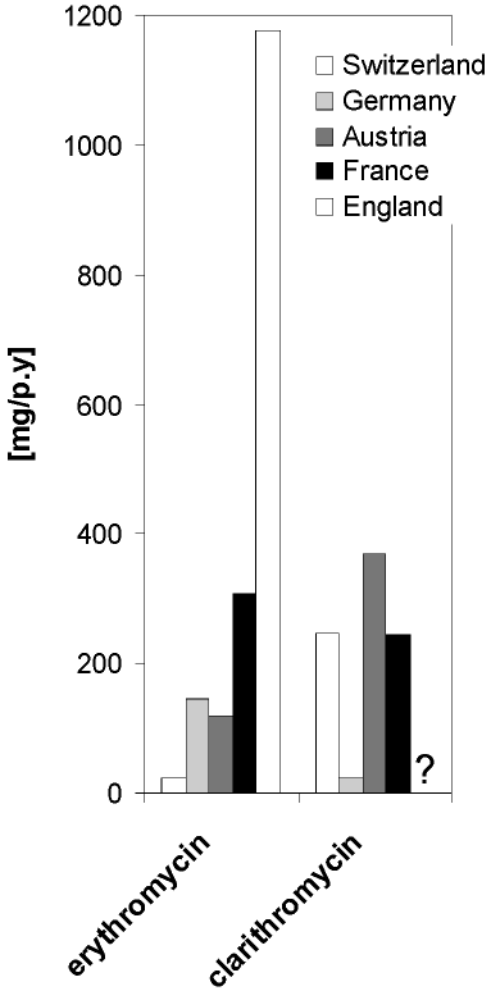

FIGURE 5. Consumption amounts of erythromycin and clarithromycin in Switzerland, Germany, Austria, France, and England (the consumption of clarithromycin in England is not known).

Germany (10.7%), North America (9.9%), England (8.1%), Spain (7.3%), Asia (7%), Switzerland (6.1%), France (5.4%), Middle East (4.9%), Italy (4.7%), Africa (4.3%), and Austria (3.6%) are the destinations with most of the landings and takeoffs in the year 2000 (*21*). Consumption patterns of macrolide antibiotics are only available for Germany in 1995 (*14*), England in 1995 (*22*), France in 1998 (*23*), and Austria in 1997/98 (*24*). In these countries, accounting for 28% of all flight origins, erythromycin is consumed in amounts 4 to 50 times higher per person and year than in Switzerland (average 436 compared to 24 mg/person and year in Switzerland), whereas clarithromycin is used in about the same or lower amounts (average 212 compared to 246 mg/person and year in Switzerland). Numbers are presented in Figure 5. This fact can probably explain the high concentrations of erythromycin-H_2O found in the effluent of WWTP Kloten-Opfikon.

With the consumption data available, one can estimate a load of erythromycin from these passengers. A total of 61 635 person movements are registered per day in the airport (2.4 times more than inhabitants living in the catchment area of the WWTP). Assuming that 50% of all passengers are inhabitants of the destination of their flight, one can calculate the amount of passengers arriving and leaving the airport from the different countries. Multiplying this number with the average amount of erythromycin consumed per day in the four countries, one can calculate an amount of 11 g/day erythromycin consumed by these passengers (for comparison: the same calculation gives an amount of 2.5 g/day clarithromycin consumed). Considering the residence time

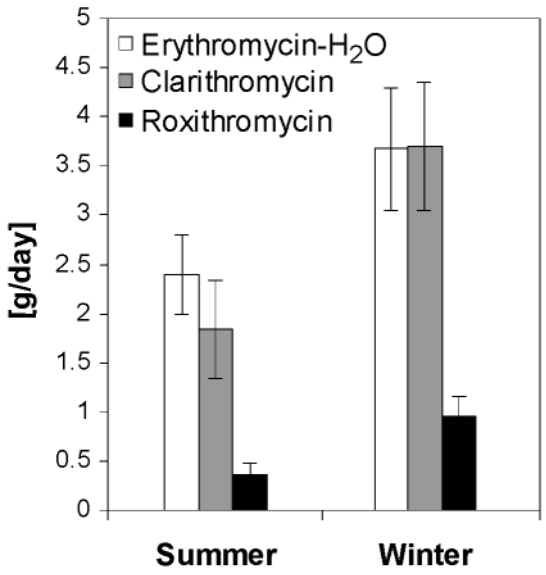

FIGURE 6. Daily loads (average per week with standard derivations) of macrolide antibiotics in WWTP Kloten-Opfikon effluent in summer 2000 and in winter 2001.

of the passengers is not 24 h in the airplane and at the airport, we have to reduce this amount by a factor of maybe 2 or 3. However, we also have to consider that the consumption data are only available for a few industrialized countries which make up only 28% of all flight origins. It might well be that passengers from third world countries consume even more of the old and relatively cheap antibiotic erythromycin. The load of erythromycin from the inhabitants living in the catchment area of the WWTP can be calculated to be 1.7 g/day in the average. The described simple calculations show that effluents from the airport potentially represent a significant point source for antibiotics which are discharged into the WWTP.

Results of two sampling campaigns at the WWTP Kloten-Opfikon are shown in Figure 6. During the winter season loads of macrolide antibiotics were two times higher than those measured in summer. Such seasonal differences can have two reasons. Either, the elimination of macrolide antibiotics in WWTPs is smaller in winter due to lower biological activity, or the input of macrolide antibiotics is higher in winter. Measurements of influent samples would be necessary to study possible elimination differences caused by the lower temperatures in winter. However, monthly sales data show that macrolides are indeed sold in two times higher amounts in January/February than in summer (*25*) because they are mainly used to cure infections of the respiratory tract.

These results indicate clearly that seasonal and regional differences of the macrolide loads exist, and therefore varying concentrations occur in the aquatic environment. Such variations must be considered for in environmental risk assessments.

Clarithromycin in the Glatt Valley Watershed. Only clarithromycin could be detected in the Glatt River during the winter season. Average loads of clarithromycin measured in the river and in treated WWTP effluents are given in Figure 7. The sum of the loads from the lake effluent and the four analyzed WWTPs yields a value of 30.2 g/day. Note that the input of the small WWTP Bassersdorf was not measured. This WWTP has 13 000 inhabitants corresponding to 6% in the discussed catchment area. If we assume that its input is proportional to the input of the average load of all four analyzed WWTPs per inhabitants in the catchment area (41.8 mg per year and inhabitant), then an additional 1.5 g/day

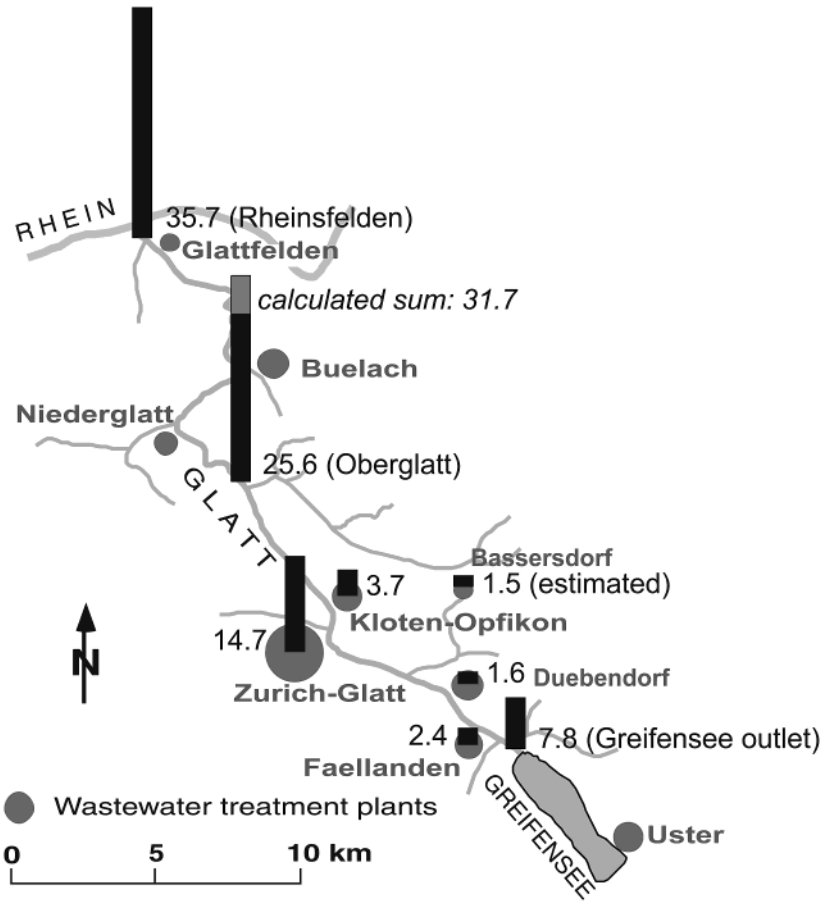

FIGURE 7. Clarithromycin loads in the Glatt Valley Watershed in winter 2001 in g/day. The input from the treated effluents of four WWTP was measured (bars on points representing the WWTPs) and the input of WWTP Bassersdorf was estimated (see text for details). Samples from three stations along the Glatt River were analyzed (bars at Greifensee outlet, Oberglatt, and Rheinsfelden). The input at Oberglatt is given in the Figure as calculated sum.

clarithromycin was added into the river. If we compare this calculated input of 31.7 g/day to the measurement in the Glatt River at Oberglatt where a load of 25.6 g/day was determined, we get an average elimination of clarithromycin in the river between the sampling stations at Greifensee outlet (1.2 km) and Oberglatt (13.2 km) of 19%. This elimination rate must be considered as statistically not significant because of the uncertainties of water flow measurements and chemical analysis.

The three WWTP Niederglatt, Buelach, and Glattfelden (with totally 56 300 inhabitants living in the catchment areas) that feed into the river between Oberglatt and Rheinsfelden were not sampled. Again assuming that their inputs are proportional to the load in the other WWTPs per inhabitants, we can calculate an input of 6.4 g/day clarithromycin from these WWTPs. This estimated input is in fact smaller than the measured input between the sampling stations in Oberglatt (13.2 km) and in Rheinsfelden (35.2 km). The calculations show that the elimination between these two sampling stations can be neglected.

Our measurements show clearly that there is no significant elimination (<20%) of clarithromycin in the Glatt River within a length of 36 km. The findings can be compared to the results of a study on the fluoroquinolone antibiotics ciprofloxacin and norfloxacin in the same watershed (*19*). Significantly lower loads of ciprofloxacin and norfloxacin (max. 10 g/day in winter) were measured in the Glatt River. Substantial removals along the river of 66% and 48% were found for ciprofloxacin and norfloxacin, respectively.

Impact of the Closure of WWTP Zurich-Glatt. In spring 2001, WWTP Zurich-Glatt has been closed stepwise, and the wastewater was diverted from the Glatt River to the WWTP Werdhoelzli with the Limmat River as receiving water. As a consequence, the input of treated wastewater into the Glatt River was drastically reduced, because WWTP Zurich-Glatt contributed 40% to the total amount of discharged treated wastewater. To follow the reduced input of wastewater into the Glatt River due to the closure of the WWTP, samples were taken for the analysis of macrolide antibiotics in the Glatt River at three sampling locations before and after partial and full diversion.

The results shown in Figure 8 indicate that already at 80% diversion in summer, a clear reduction of clarithromycin loads occurred, which subsequently was confirmed by the measurements after 100% diversion in the following winter. At the sampling station in Oberglatt, which is located below the effluent of WWTP Zurich-Glatt, the average load of clarithromycin was reduced by 13.9 g/day (54%) between winter 2001 and winter 2002. At the sampling station in Rheinsfelden a reduced load of 16.7 g/day (47%) was measured. These lower clarithromycin loads can be explained by the reduced input of wastewater into the River Glatt due to the closure of the WWTP Zurich-Glatt. In winter 2001, 14.7 g/day clarithromycin was measured in the effluent of WWTP Zurich-Glatt (see Figure 7), which accounts for the reduced load of clarithromycin measured in the two sampling locations after the diversion of the WWTP effluent. The differences may be explained by varying inputs from the WWTPs or variations in loads due to different consumption patterns. It should be noted that also in the sampling location at the Greifensee outlet a reduction of the clarithromycin load of 5 g/day was observed. The concentrations determined

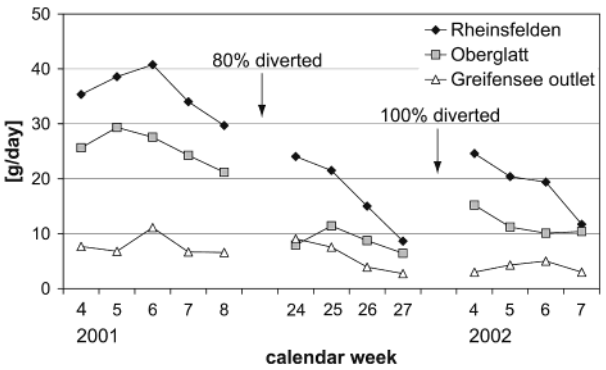

FIGURE 8. Clarithromycin loads at three sampling stations in the Glatt River before (winter 2001), after partly closure (summer 2001, 80% diverted) and after full closure (winter 2002, 100% diverted) of WWTP Zurich-Glatt.

at this location in winter 2001 were around 24−32 ng/L, and in winter 2002 around 4−9.5 ng/L. The latter values include a large quantification uncertainty because of the scatter very close to the limit of quantification (4 ng/L).

Our results clearly demonstrate that macrolide antibiotics are not eliminated completely in WWTPs and the residual amounts reach receiving surface waters. The elimination of macrolide antibiotics in surface water is only small, and a reduction of antibiotics in ambient waters can be achieved only by reduced inputs from WWTPs. Ongoing studies aim to gain more knowledge on the elimination processes of antibiotics within the WWTPs including sorption studies onto sewage sludge (EU project POSEIDON (*26*)).

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