

Occurrence and Fate of the Pharmaceutical Drug Diclofenac in Surface Waters: Rapid Photodegradation in a Lake

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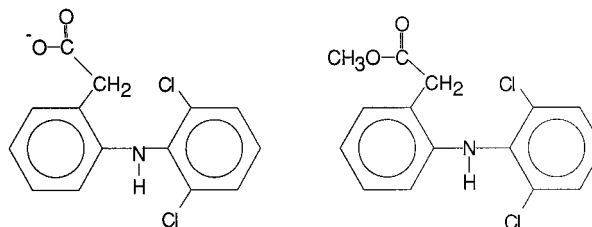
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The pharmaceutical drug diclofenac (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid) was detected in rivers and lakes in Switzerland. The data strongly suggest inputs of diclofenac from human medical use via wastewater treatment plants. Interestingly, the concentrations in a major tributary to a lake (Greifensee) were significantly higher (up to 370 ng/L) than those in the outflow of this lake (up to 12 ng/L). It is estimated that more than 90% of the diclofenac entering the lake is eliminated in the lake, most likely by photolytic degradation. Diclofenac was not detected in the sediments of the lake, and in a laboratory experiment, it showed negligible adsorption onto sediment particles. Incubation of lake water, fortified with diclofenac, showed no degradation in the dark, suggesting minimal chemical and biological degradation. However, when the fortified water was exposed to sunlight, rapid photodegradation was observed with a (pseudo) first-order kinetic and a half-life of less than 1 h (October and 47° N latitude). Modeling these experimental data for the situation of Greifensee, the data indicated that photodegradation can account for the rapid elimination of diclofenac in the lake. Several photoproducts were characterized in the laboratory experiments but were so far not detected under the natural conditions in the lake. Whereas photodegradation is often one among several degradation pathways for environmental contaminants, the photolysis experiments and the computer simulation suggested this process to be the predominant one for diclofenac in the lake.

Introduction

Many anthropogenic compounds have been detected in the aquatic environment. These include industrial chemicals, agrochemicals (pesticides) and fertilizers, detergents and surfactants, plasticizers, and others. Previous studies have also shown that pharmaceutical compounds can reach detectable concentrations in the environment if production and use are sufficiently large and the physicochemical properties are appropriate (1–4). If the compounds are mobile in the aquatic environment, they may then become detectable in rivers and lakes in very much the same way as other environmental contaminants. In this way, e.g., the

CHART 1. Structure of Diclofenac as Anionic Species and Diclofenac Methyl Ester, the Analyzed Species



pharmaceutical compound clofibric acid was detected in rivers, lakes, and in the open sea (5).

During an investigation on the occurrence of pesticides in various lakes in Switzerland we observed the presence of an additional chlorinated acidic compound. This compound is now identified as diclofenac (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid, structure see Chart 1), a popular pharmaceutical drug (6). Diclofenac, mostly used as the sodium salt (diclofenac-Na), is used in human medical care as an analgesic, antiarthritic, antirheumatic compound belonging to the group of the nonsteroidal antiinflammatory drugs (6). It represents an important drug in ambulatory care (7). It is used worldwide and has a production volume estimated to be in the hundreds of tons annually. It is used in the form of tablets, capsules, suppositories, intravenous solutions, and in ointments and gels for dermal application. It is readily metabolized after oral use, but assimilation is lower after dermal application (6).

Diclofenac has been previously identified in effluents from domestic wastewater treatment plants (WWTPs) and in rivers (4). We now confirm these findings and additionally show that diclofenac, in contrast to many other environmental contaminants, is then rapidly degraded in a lake. We also show that the most probable degradation pathway for this in situ elimination is photodecomposition.

Experimental Section

Waters Sampled. The waters from Zürichsee (Lake Zurich), Sempachersee, Hallwylersee, Greifensee, Pfäffikersee, Walensee, and Jörisee (a small mountain lake), all situated in Switzerland, were analyzed (see Figure 1, ref 9). The lakes have been the subject of several previous studies, and their morphologies and hydraulics have been previously described (8–11). Of particular importance for this study is the Greifensee and its catchment area (see map in Figure 1). Greifensee was sampled at one of its major inflows (location L1), the River Aabach, and at its outflow, the River Glatt (location L5), over a period of more than 12 months. Additionally, several samples were collected from selected sites (locations L2–L4) within the main tributary Aabach. Greifensee (435 m above sea level) is located 10 km east of Zurich and has a surface area of $8.49 \times 10^6 \text{ m}^2$, a volume of $1.51 \times 10^8 \text{ m}^3$, maximum and mean water depths of 32 and 17.8 m, respectively, and a mean water residence time (filling time, T) of 408 days (10). The lake is stratified during the warmer season (April–October) with development of an epilimnion (depth, 5 m; volume, $4.2 \times 10^7 \text{ m}^3$) and a hypolimnion. In winter (November–March) the lake is mixed. As pointed out previously, lateral mixing in the lake is fast (within days, see ref 12), and the water at the outflow can be considered to have the composition of the epilimnion (warmer season) or the mixed lake (colder season). There

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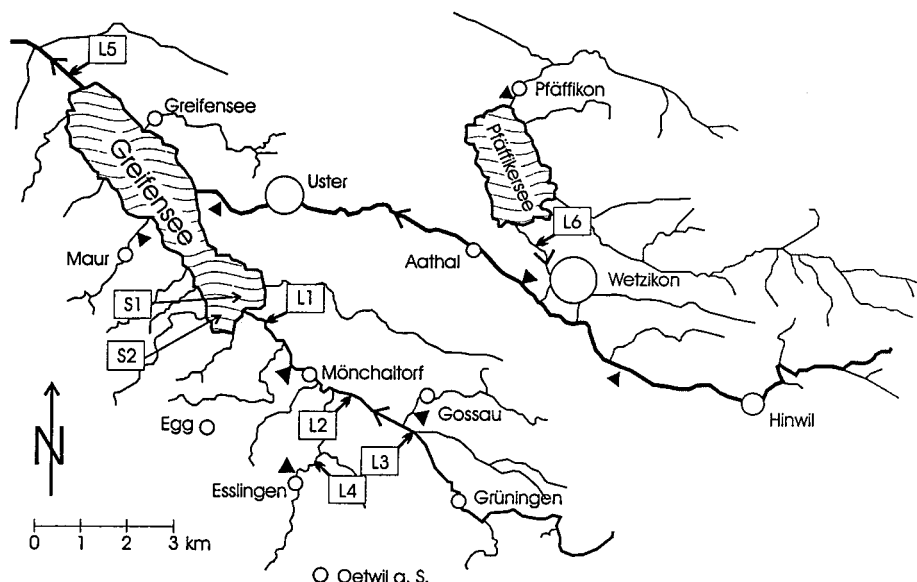


FIGURE 1. Map of Lake Greifensee study area with sampling sites (L1–L6) and location of villages (circles) and wastewater treatment plants (filled triangles).

are about 94 000 inhabitants (85 000 excluding Pfäffikersee) living in its catchment area (160 km²), and eight wastewater treatment plants (WWTPs) are located within this area (13). All sampling sites and the location of WWTPs are indicated in the map in Figure 1.

WWTP Samples Analyzed. Samples (all 24-h flow proportional) were collected after primary sedimentation (influent) and from the effluent emitted to the rivers from the WWTPs of Gossau, Uster, and Pfäffikon (locations, see Figure 1). These installations are modern, three-stage mechanical/biological plants serving populations of about 10 500 (Gossau), 26 000 (Uster), and 8 500 persons (Pfäffikon), respectively (13).

Water Sampling and Analytical Procedures. Surface water from the lakes and rivers and WWTP samples were collected into 1-L methanol-rinsed amber (or green) glass bottles. For lakes and rivers, 1-L samples were fortified with 50 μ L of a 0.4 ng/ μ L all-ring-¹³C₆-(R/S)-2-[2,4-dichlorophenoxyl]propionic acid (¹³C₆-DCPP; Cambridge Isotope Laboratories, Cambridge, MA) in methanol (spike level, 20 ng/L), adjusted to a pH value <2 and extracted using a macroporous polystyrene adsorbent (Bio-Beads SM-2; Bio-Rad Laboratories, Hercules, CA), as previously described (5). In case of the WWTP samples, portions of \approx 300 mL were centrifuged at \approx 13000g using an RC-5B type ultracentrifuge (DuPont Sorvall, Newtown, CT), and 250 mL of the clear supernatants were treated in the same way as the surface waters except that a separate small adsorbent column was used. Diclofenac is eluted from the adsorbent and methylated with diazomethane, and the samples cleaned-up and solvent-exchanged for ethyl acetate, as previously described for phenoxyalkanoic acids (5, 9).

Diclofenac was analyzed as methyl ester (diclofenac-ME) by gas chromatography–mass spectrometry (GC–MS). For this purpose a 1–2 μ L aliquot of the 100–500 μ L extract was used. GC–MS analysis was performed on a VG Tribrid mass spectrometer (VG Fisons, Manchester, England) under electron-impact ionization (EI, 70 eV, 180 °C) and full-scan (m/z 35–435, 1.16 s/scan; mass resolution $M/\Delta M = 500$) or selected-ion-monitoring (SIM) conditions, using the ions at m/z 214.042, 309.032 (quantitation ion), and 311.029 for diclofenac-ME and at m/z 254.021 for ¹³C₆-DCPP-ME. A lock mass of m/z 207.033 from the silicone bleed of the GC column was used in SIM. A 30-m DB-5 fused silica (0.32-mm i.d.) column with on-column injection was used. The column

was temperature programmed as follows: 60 °C, 2-min isothermal, 20 °C/min to 140 °C, and then at 8 °C/min to 280 °C, followed by an isothermal hold at this temperature. Diclofenac-ME eluted at \approx 11.2 min (measured from data acquisition start at 140 °C). The amounts of analyte were determined from peak area ratios relative to the internal standard (¹³C₆-DCPP) and in reference to suitable standard solutions. The reference solutions were prepared from a stock solution of diclofenac-Na (USP, Rockville, MD) in methanol (1 ng/ μ L), which were methylated with diazomethane in diethyl ether after addition of a few drops of trifluoroacetic acid (1% in methanol). The solid-phase extraction and the SIM GC–MS procedure allowed the detection of diclofenac at concentrations of < 1 ng/L with acceptable recovery (50–90%). The WWTP samples with much higher concentrations of diclofenac were quantified using external standardization.

Sediment Analysis. Previously collected (February 27, 1995) lake sediments (Greifensee, sampling sites S1 and S2, see map in Figure 1) were used in the study. The sediments (S1, 0–5 cm; S2, 5–10 cm) were lyophilized and then kept at room temperature until analyzed. One 5-g portion each was treated with \approx 10–20 mL 1 M HCl until gas evolution ceased. Then, 30 mL of methanol was added and the samples shaken vigorously. After centrifugation in a small laboratory centrifuge the clear acidic supernatants were removed and extracted with three 5-mL portions of methylene chloride in a stoppered test tube. The combined extracts were concentrated, methylated, cleaned-up, and exchanged for ethyl acetate (5, 9). The samples were then adjusted to a volume of 1 mL and a 1 μ L aliquot analyzed by SIM GC–MS. A control experiment with 700 ppb of diclofenac added to one of the sediments showed good recovery (90%).

Sediment Adsorption Experiment. Water (1 L) of lake Greifensee (October 28, 1997) was fortified with 14 μ L of a 36 ng/ μ L diclofenac-Na solution in methanol, corresponding to a fortification level of \approx 500 ng/L. The sample was shaken vigorously, allowed to stand for 1 h, and then shaken again. Two 80-mL portions were removed, fortified with 10 μ L of ¹³C₆-DCPP (2 ng) each, acidified, solid phase extracted on the small Bio-Beads column, and further treated as above. Then, a 0.84 g sample of one of the lake sediments was added to the remaining water (0.84 L), and the sample shaken vigorously for 15 min. Two portions (\approx 150 mL each) of this water were centrifuged, and 80 mL from each supernatant

was removed and analyzed in the same way as above. The concentrations of diclofenac in water after the treatment (C_{aft}) were then compared to the concentrations before the treatment (C_{bef}) and the relative amount (%) of diclofenac adsorbed on the sediment calculated as

$$(\% \text{ adsorbed}) = 100 \times (C_{\text{bef}} - C_{\text{aft}}) / C_{\text{bef}} \quad (1)$$

Incubation of Fortified Lake Water. Two 2.5-L portions of water from lake Greifensee (August 1997) were fortified with diclofenac at 100 ng/L. The fortification was made by adding 1.0 mL of a 250 ng/mL aqueous solution of diclofenac-Na. The samples were incubated at room temperature for up to 37 days in clear Pyrex glass bottles whereby one bottle was kept in the dark and the other exposed to daylight. Aliquots of 0.25 L were removed from each bottle and analyzed as described above, the first ones immediately ($t = 0$), the next ones 4, 10, 21, and 37 days thereafter.

Photolysis of Diclofenac under Natural Sunlight. Sunlight exposure of aqueous solutions of diclofenac (concentration, 1 $\mu\text{g/L}$) was carried out in 30-mL clear Pyrex glass test tubes equipped with glass stoppers. One of the samples was immediately analyzed ($t = 0$), and the others exposed to full sunlight for 2 and 4 h under clear sky conditions in mid-October 1997 at Wädenswil (47°14' N, 8°40' E), starting at 10 am. The samples were diluted to 250 mL with distilled water (to cope with the extraction procedure above), and analyzed in the same way as described above.

To detect photodegradation products sunlight exposures of more concentrated (36 mg/L) solutions of diclofenac in small (≈ 0.5 mL) quartz vials were carried out on the same mid-October day (10 a.m. to 3 p.m.; temperature, 22–27 °C). After exposure for 0, 1, 2, 3.5, and 5 h, aliquots of 50 μL were evaporated in vacuo, and the residues methylated, analyzed by full-scan GC–MS, and semiquantified using total-ion-chromatograms (TIC), assuming equal response for diclofenac and photoproducts.

Degradation Kinetics and Modeling of Photodegradation. For the quantitative evaluation of the kinetic experiments as well as for the construction of a simple two-box model of Greifensee the program AQUASIM (EAWAG, Dübendorf, Switzerland) was used. This program performs simulation and data analysis from aquatic systems (14); it allows its users to define the spatial configuration of the system to be investigated as a set of compartments, which can be interconnected. The program can perform simulations, sensitivity analyses, and parameter estimation using measured data.

For computing the theoretical photochemical rates of diclofenac in Greifensee as a function of depth and date we used the computer program GCSOLAR (Environmental Protection Agency, Athens, GA) which has been described in detail by Zepp and Cline (15). This program is also used to estimate the photochemical degradation quantum yield of diclofenac from the rate constants determined in the kinetic experiments, the estimated flux of photons and the UV spectrum of the compound (see below). Then, the daily integrated rate constant was calculated as a function of depth from the quantum yield, the UV spectrum of the compound, and the UV absorbance of lake water, for 47° N latitude and the 15th day of every month.

Results and Discussion

Occurrence of Diclofenac in Swiss Lakes and Rivers. In the methylated acidic fraction of various water samples analyzed by full-scan GC–MS for phenoxyalkanoic acid herbicides (9) we observed a late-eluting (elution temperature, ≈ 230 °C, DB5 column), thus far unknown, dichloro compound. The compound appeared to be related to human activities, as it

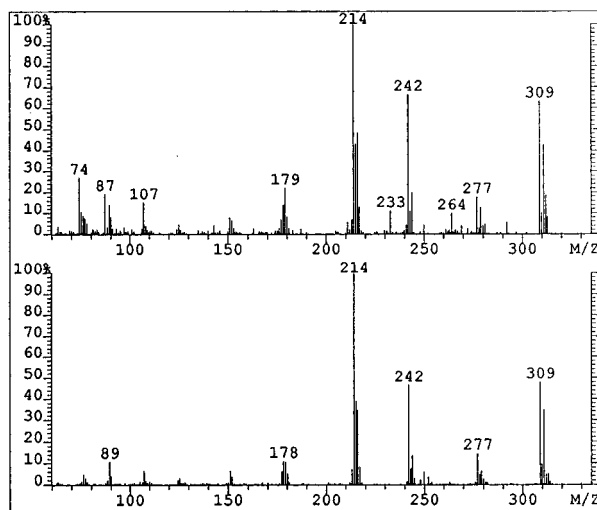


FIGURE 2. EI mass spectra of (upper panel) component observed in the inflow of Greifensee, and identified as diclofenac-ME, and (lower panel) authentic diclofenac-ME ($M^+ = m/z$ 309). Note the virtually identical spectra, except signals from coeluting fatty acid methyl ester in the upper spectrum.

was not observed in lakes from less populated areas such as Walensee or the mountain lake Jörisee. In Figure 2a we show an EI mass spectrum of this compound in a sample from the inflow of Greifensee with a relatively high concentration (310 ng/L). The mass spectrum indicates a dichloro compound with a presumed molecular ion (M^+) at m/z 309 and major fragment ions at m/z 277 (presumable loss of CH_3OH), 242 (loss of $\text{CH}_3\text{OH} + \text{Cl}$), and 214 (loss of $\text{CH}_3\text{OH} + \text{Cl} + \text{CO}$). The compound was subsequently identified as diclofenac-ME by comparison of its mass spectrum and retention time to that of an authentic reference sample (see Figure 2b). Diclofenac ($\text{p}K_a \approx 4$) apparently can be extracted from acidified water using solid-phase extraction with good efficiency, and it cochromatographed as the methyl ester on silica in the cleanup step with the methyl esters of phenoxyalkanoic acid herbicides (9).

The concentrations of diclofenac in the various lakes and rivers are listed in Table 1. The concentrations in the lakes themselves or at their outflows ranged from <1 –12 ng/L. However, higher concentrations (11–310 ng/L) were observed in the river Aabach (site L1), one of the major inflows of Lake Greifensee. Interestingly, the concentrations at this inflow were usually much higher than those at the outflow of the lake, sampled at the same time. Whereas there is no obvious seasonal pattern for the inflow concentration to Lake Greifensee, there appears to be a tendency toward higher concentrations in the outflow during winter. In Figure 3a,b EI SIM chromatograms show the elution of diclofenac-ME in two of these samples, one from the inflow and the other from the outflow, taken in May 1997. The data in Table 1 document the occurrence of this compound in surface water and suggest sources that likely represent human medical use.

Identification of WWTPs as a Source of Diclofenac.

During a sampling campaign (October 7, 1997), additional samples from the tributary Aabach of Greifensee were collected (see map in Figure 1 and data in Table 1). At sampling sites L1, L2, and L4 the concentrations were 200–370 ng/L; only site L3 showed a very low concentration of diclofenac (≈ 5 ng/L), and this result may have even stemmed from cross contamination in the laboratory with one of the higher level samples, analyzed at the same time. Except for L3, all sites are located downstream of WWTP installations (see map in Figure 1). Thus, the only sample with little or

TABLE 1. Concentrations of Diclofenac in Lakes and Rivers in Switzerland

| lake, river | location (depths) ^a | date | concn ^b (ng/L) |
|---------------------------|--------------------------------|-------------------|---------------------------|
| Greifensee outflow, Glatt | L5 (grab, 0.2–0.5 m) | Aug 2, 1996 | <1 |
| | L5 (grab, 0.2–0.5 m) | Aug 29, 1996 | 3 |
| | L5 (grab, 0.2–0.5 m) | Sep 18, 1996 | 1 |
| | L5 (grab, 0.2–0.5 m) | Dec 12, 1996 | 5 |
| | L5 (grab, 0.2–0.5 m) | Mar 3, 1997 | 3 |
| | L5 (grab, 0.2–0.5 m) | May 6, 1997 | 7 |
| | L5 (grab, 0.2–0.5 m) | Jul 2, 1997 | 1 |
| | L5 (grab, 0.2–0.5 m) | Aug 12, 1997 | 4 |
| | L5 (grab, 0.2–0.5 m) | Oct 7, 1997 | na |
| | L5 (grab, 0.2–0.5 m) | Dec 5, 1997 | 12; 11 |
| Greifensee inflow, Aabach | L1 (grab, 0.2–0.5 m) | Aug 2, 1996 | 74 |
| | L1 (grab, 0.2–0.5 m) | Aug 29, 1996 | 11 |
| | L1 (grab, 0.2–0.5 m) | Sep 18, 1996 | 71 |
| | L1 (grab, 0.2–0.5 m) | Dec 12, 1996 | 34 |
| | L1 (grab, 0.2–0.5 m) | Mar 3, 1997 | 20 |
| | L1 (grab, 0.2–0.5 m) | May 6, 1997 | 310 |
| | L1 (grab, 0.2–0.5 m) | Jul 2, 1997 | 75 |
| | L1 (grab, 0.2–0.5 m) | Aug 12, 1997 | 78 |
| | L1 (grab, 0.2–0.5 m) | Oct 7, 1997 | 200 |
| | L1 (grab, 0.2–0.5 m) | Dec 5, 1997 | na |
| Aabach | L2 (grab, 0.2–0.5 m) | Oct 7, 1997 | 270 |
| | L3 (grab, 0.2–0.5 m) | Oct 7, 1997 | ≈5 |
| | L4 (grab, 0.2–0.5 m) | Oct 7, 1997 | 370 |
| | L6 (grab, 0.2–0.5 m) | Aug 12, 1997 | <1 |
| Pfäffikersee (outflow) | (grab, 0.2–0.5 m) | Aug 1996–Jul 1997 | <1–2 (10 samples) |
| Sempachersee (outflow) | (grab, 0.2–0.5 m) | Nov 4, 1996 | <1 |
| Hallwilersee (outflow) | (1 m) | Jul 1, 1996 | 3 |
| Zürichsee (center) | (1 m) | Dec 12, 1996 | 2 |
| Walensee, Jörisee | (1 m) | Jul 96 | <1 |

^a See map in Figure 1. ^b na, not analyzed.

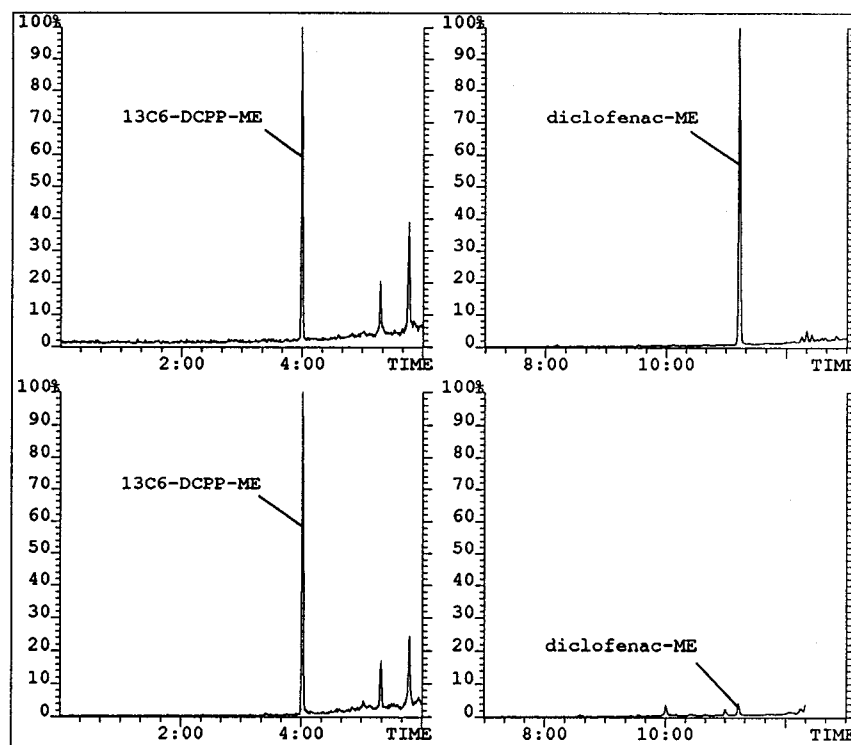


FIGURE 3. EI SIM chromatograms showing elution of $^{13}\text{C}_6$ -DCPP (left-side panels; m/z 254, retention time, 4.0 min) and diclofenac (right-side panels; m/z 309, retention time, 11.2 min), analyzed as methyl esters, in samples from the inflow (River Aabach; upper panels), and the outflow of Greifensee (River Glatt; lower panels). Note the greatly reduced level of diclofenac at the outflow of the lake.

no diclofenac present was from a site without an upstream WWTP installation.

After this finding, influent and effluent of the WWTP installation at Gossau and later from those at Uster and Pfäffikon were sampled and analyzed. The results in Table

2 indicate concentrations of several hundred ng/L from all installations. These concentrations are in the range of those reported from municipal WWTP installations in Germany (effluent concentrations up to $1.59 \mu\text{g/L}$, see ref 4). The differences we observed in concentration between influents

TABLE 2. Concentrations and Amounts of Diclofenac Emitted from WWTP Installations

| installation, location | population served (persons) | date | throughput <i>Q</i> (m ³ /day) | influent | | effluent ^a | | normalized output (g/day per 10 000 persons) |
|---------------------------|--------------------------------|--------------|--|----------|-------|-----------------------|-------|---|
| | | | | ng/L | g/day | ng/L | g/day | |
| Gossau | 10 500 | Oct 28, 1997 | 3 236 | 1920 | 6.2 | 930 | 3.0 | 3.0 |
| | | Nov 11, 1997 | 2 912 | 860 | 2.5 | na | na | na |
| | | Dec 5, 1997 | 3 917 | 470 | 1.8 | 310 | 1.2 | 1.2 |
| | | Feb 2, 1998 | 15 440 | 655 | 10.1 | 575 | 8.9 | 3.4 |
| Pfäffikon | 8 500 | Feb 2, 1998 | 3 161 | 720 | 2.3 | 686 | 2.2 | 2.6 |

^a na, not analyzed.

and effluents may not necessarily reflect actual degradation of diclofenac during treatment, as the samples were never from exactly the same parcels of water moving through the plants (there are time delays of up to 24 h, depending on water load conditions). Furthermore, incubation of influent with activated sludge in a preliminary experiment under aerobic conditions showed no degradation of diclofenac (data not shown). The findings made it almost certain that these installations are the source for diclofenac in this tributary.

The concentrations of 310–930 ng/L observed for the 24-h averaged effluents, and the known outputs of wastewater, amount to total outputs of 1.2–3.0 g of diclofenac per day (average, 2.1 g/day) for the plant at Gossau and to 8.9 and 2.2 g per day for the plants at Uster and Pfäffikon, respectively (see Table 2). When normalized for the populations serviced by these plants, the outputs were 1.2–3.4 g/day per 10 000 persons (average, 2.6 g/day per 10 000 persons; see Table 2). These outputs are not unreasonably high. Overall utilization of diclofenac in two studies was reported at 10.8 (16) and 18.6 defined daily doses/1000 persons (calculated from data in ref 4), respectively. With therapeutic doses of 100–150 mg/day this would amount to 11–28 g/day for a population of 10 000. Assuming similar outputs from the other installations as the ones we measured, the total input of diclofenac into Greifensee from WWTPs (excluding the one located on Pfäffikersee, see Figure 1) would amount to 10–29 g/day (average, 22 g/day) or 3.7–11 kg/yr (average, 7.9 kg/yr) (85 000 persons).

Evidence for a Rapid Elimination Process for Diclofenac in Greifensee. The much higher concentrations of diclofenac in one of the main inflows of Greifensee (contribution estimated at ≈40% of all inflows), compared to the lower concentrations at the outflow of this lake, suggest the presence of an efficient elimination process for diclofenac with an elimination rate (k_e) that is higher than the effective water exchange rate (k_w , see below) of the lake, as we will outline next. The data in Table 1 suggest that the inflow concentrations are more than 10 times higher than the outflow concentrations and that thus over 90% of the diclofenac is eliminated in the lake. In the unlikely case that there are no inputs from other tributaries than the one analyzed, the elimination would still amount to >75%.

The elimination rate, k_e , is estimated using the following considerations. The rate equation for a process involving dilution (water exchange) and elimination in a system, and assuming first-order kinetics, is

$$d[C]/dt = k_w C_{in} - (k_w + k_e) C_{out} \quad (2)$$

whereby C_{in} and C_{out} are the inflow and outflow concentrations of diclofenac, respectively. In a steady-state situation ($d[C]/dt = 0$) it follows

$$k_e = k_w (C_{in} - C_{out}) / C_{out} \quad (3)$$

Since the average water residence time (filling time, T) in Greifensee is 408 days, the water exchange rate ($k_w = 1/T$) is 0.0025 day⁻¹ in the mixed lake and higher (0.009 day⁻¹) in

its epilimnion during stratification (April–October). Assuming that >90% of the diclofenac is eliminated from Greifensee, the elimination rate, k_e , as estimated from eq 3, is >0.022 day⁻¹ (half-life, $\tau = \ln 2/k_e < 30$ days, mixed lake situation in winter), and even higher (>0.081 day⁻¹; $\tau < 8$ days) when the lake is stratified during summer.

In the following we will try to identify this elimination process. As potential elimination processes for diclofenac in the lake we considered (i) removal by sorption into the sediments, (ii) biotic degradation, and (iii) abiotic (chemical, photochemical) degradation. Volatilization, as a further possibility, is less likely for diclofenac due to its polar, anionic character at the pH of the lake (pH = 7.6).

Negligible Adsorption of Diclofenac onto Lake Sediments. As a first possibility for the elimination of diclofenac we considered its binding to particles and the subsequent removal from the water column into the sediments. Two sediment samples from Greifensee, taken near the inflow of the river Aabach, where we expected the highest concentrations of diclofenac, were analyzed (locations S1 and S2, see Figure 1). No diclofenac was detected (estimated detection limit, <10 ng/g). In Greifensee, with a sedimentation rate of 1.25 kg/m² × yr (17), and a maximum concentration of 10 ng/g in the sediment, the total amount of diclofenac sequestered into the sediments would amount to <12 μg/m² × yr or <0.11 kg/yr and thus to <1–3% of the estimated total input of diclofenac to the lake.

To further substantiate negligible adsorption of diclofenac to the sediments, a sediment adsorption experiment was conducted. One liter of water from Greifensee was fortified with diclofenac at a concentration of 500 ng/L, analyzed, equilibrated for 15 min with 1 g of sediment per liter of water, and then reanalyzed. The particle concentration of 1 g/L in this experiment is ≈200-fold above that actually observed in the lake and should thus exaggerate the adsorption conditions in the lake. The aqueous phase after centrifugation and removal of the sediment particles showed no decrease in concentration (<1%), again indicating negligible adsorption of diclofenac to sediment particles. All these facts indicate that adsorption to suspended particles and sequestration into the sediments are unimportant for the elimination of diclofenac from the lake.

No Evidence for Chemical and Biological Degradation of Diclofenac from the Incubation Experiments. As a next possibility we considered removal of diclofenac by chemical and/or microbiological degradation. Fortified water (100 ng/L) from Greifensee was kept in the dark at room temperature and periodically reanalyzed. The data in Figure 4 (curve a) for this sample show little degradation of diclofenac for up to 37 days, whereas diclofenac in lake water, when exposed to sunlight, showed rapid degradation (see below). Assuming first-order kinetics in the initial phase (up to 21 days), the overall degradation rate constant (k) thus estimated was ≈0.004 day⁻¹ ($\tau \approx 170$ days). The experiment thus gave no evidence for an efficient elimination of diclofenac via chemical degradation (e.g. hydrolysis), and it suggested, because of the nonsterile conditions, minimal microbial

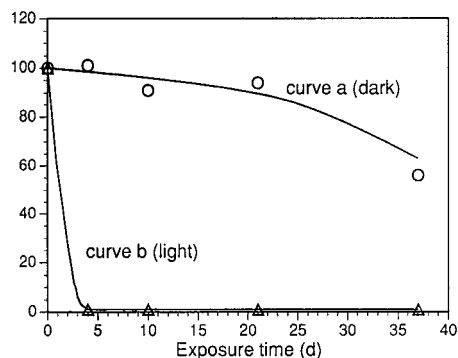


FIGURE 4. Degradation of diclofenac in natural lake water fortified at 100 ng/L, incubated under dark conditions (curve a, circles) and under light conditions (curve b, triangles). Note rapid decomposition of diclofenac upon exposure to sunlight.

degradation of diclofenac.

Evidence for a Photochemical Degradation Process from Laboratory Experiments. The data from the daylight exposed, 100 ng/L of fortified lake water showed rapid degradation of diclofenac to a level <1% of the initial concentration in 4 days (see Figure 4, curve b). The result suggested virtually complete degradation of diclofenac via a photochemical pathway at a fast rate ($\tau < 1$ day).

A second experiment was carried out to confirm the fast decomposition of diclofenac on exposure to sunlight. Dilute aqueous solutions of diclofenac (1 $\mu\text{g/L}$) were exposed to natural sunlight in Pyrex glass test tubes for 0, 2, and 4 h. Pyrex glass transmits light with wavelengths $\lambda > 290$ nm and thus is transparent for tropospheric sunlight. In this experiment, diclofenac rapidly decomposed to a level of $\approx 4\%$ of the initial concentration in only 4 h of exposure, indicating a first-order reaction rate of 0.80 h^{-1} ($\tau \approx 0.9$ h).

In a third experiment diclofenac was exposed in small quartz vials to natural sunlight at higher concentrations (36 mg/L) in order to facilitate the detection of photoproducts. Diclofenac was decomposed to <1% in 5 h of exposure. Three photolysis products were observed, designated as PH1 (most abundant), PH2, and PH3 (see Figure 5). They were characterized by EI mass spectrometry after methylation (see below) and semiquantified using TIC GC-MS.

Photoproduct PH1 (EI MS: m/z 239, 207, 179) is unchlorinated with a suggested molecular weight (M^+) of 239. Photoproduct PH2 (EI MS: m/z 273, 241, 213) is likely a chloro anlog of PH1. It is only observed as an initial photolysis product. PH1 and PH2 likely correspond to the methyl esters of carbazole-1-acetic acid and its 8-chloro derivative, respectively, previously identified as photoproducts of diclofenac in aqueous buffer or methanol on irradiation with UVA light (18). Photoproduct PH3 (EI MS: m/z 195, 167, 166) is unknown. Attempts to detect these photoproducts in the outflow of Greifensee were so far unsuccessful.

The degradation of diclofenac, and the formation and subsequent degradation of the photoproducts, is plotted in Figure 6. Diclofenac is degraded in a first-order reaction to PH1 and PH2, both of which were then further degraded to products including PH3. The rate eqs 4 and 5 used for the fitting of the experimental data are

$$d[\text{D}]/dt = -k_1[\text{D}] \quad (4)$$

$$d[\text{PH1}]/dt = \chi k_1[\text{D}] - k_2[\text{PH1}] \quad (5)$$

whereby [D] and [PH1] are the concentrations, and k_1 and k_2 are the photodegradation rates of diclofenac and photoproduct PH1, respectively. The coefficient χ ($0 \leq \chi \leq 1$) is introduced to account for the unknown stoichiometry of the reaction with respect to PH1 and is fitted along with k_1

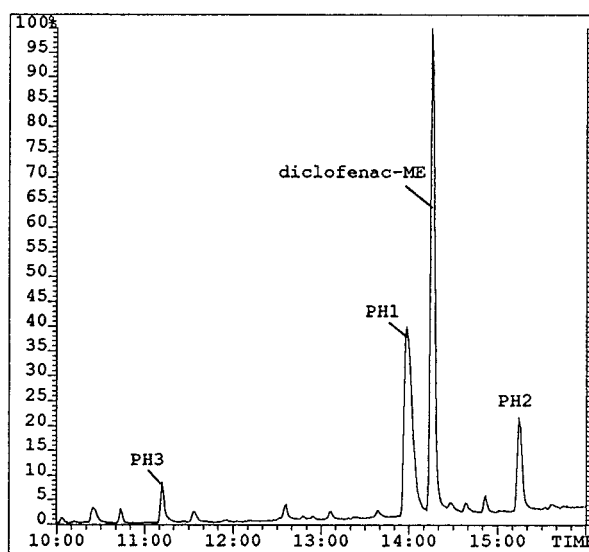


FIGURE 5. EI TIC GC-MS chromatogram of photolyzed diclofenac (60-min sunlight exposure in quartz) showing formation of photoproducts PH1, PH2, and PH3, analyzed as methyl esters (see text). Note that about 66% of the diclofenac is decomposed under these conditions. Also note that data acquisition was started at 80 °C (retention time + 3 min, compared to Figure 3).

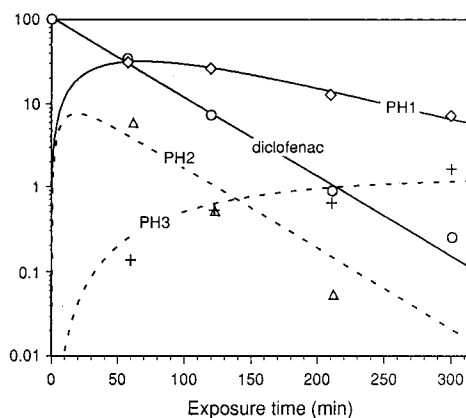


FIGURE 6. Degradation of diclofenac and formation of photoproducts PH1, PH2, and PH3 (see text). Normalized concentrations ($100 \times C/C_0$, logarithmic scale; TIC data) are plotted versus exposure time (min). Diclofenac is photolyzed with a first-order rate of $1.31 \pm 0.03 \text{ h}^{-1}$ ($\tau \approx 32$ min).

and k_2 in Figure 6. Also plotted (but not fitted) are the data for photoproducts PH2 and PH3. The modeled concentrations for diclofenac and PH1 correspond well with the experimental data, indicating that the mechanistic considerations are valid. The first-order photodegradation rate of diclofenac (k_1) determined in this way is $1.31 \pm 0.03 \text{ h}^{-1}$ ($\tau \approx 0.53$ h), and that for the major photoproduct PH1 (k_2) is $0.54 \pm 0.17 \text{ h}^{-1}$ ($\tau \approx 1.3$ h) or ≈ 3 times slower ($\chi \approx 0.5$).

The data from all these experiments indicate that diclofenac is rapidly decomposed in dilute aqueous solution at a fast rate on exposure to natural sunlight. The UV spectrum of diclofenac does in fact show a high-intensity absorption band at 275 nm ($\epsilon \approx 10,000$), well extending into an absorption region of >290 nm (data not shown), and diclofenac thus absorbs in the tropospheric range of sunlight. Diclofenac therefore appears to be susceptible to direct photolysis. The quantum yield of the photochemical decomposition of diclofenac was estimated at $\Phi \approx 0.13$ using the program GCSOLAR. This value corresponds well with the one ($\Phi = 0.22$) reported for the photolysis under artificial sunlight conditions (18).

Modeling Photodegradation of Diclofenac for the Situation of Greifensee. To extrapolate photolysis rates determined from laboratory experiments to the situation in a lake, several factors have to be considered, namely (i) the seasonal variation of the sunlight available for photolysis, (ii) the light attenuation by water and dissolved natural organic compounds in the lake, and (iii) the light attenuation by clouds and fog. The first two factors were addressed by the computer program GCSOLAR, a program that estimates the average flux of photons as a function of latitude and date, and then calculates photolysis rate constants for different water depths using only the UV spectrum of the compound of interest, the photolysis quantum yield, and the UV absorbance of water as input. The resulting photolysis rates follow a sinusoidal curve with a maximum in June and a minimum in December. For diclofenac the photolysis rates (integrated over a full day, taking the diurnal variation of sunlight intensity into account) thus estimated for Lake Greifensee (average depth, 17.8 m) ranged from 0.02 (December) to 0.20 day⁻¹ (June).

The rates are calculated by the program GCSOLAR for perfectly sunny skies. Overcast skies, however, significantly reduce the amount of light available for photodegradation. Thus, the fraction of available light was calculated from global radiation (GLR) data measured at a nearby location (EAWAG, Dübendorf, 6 km west of Greifensee), as the ratio of the daily average GLR to the maximum radiation obtained from fitting of a sinus curve to the top 3% data points. For the sunlight conditions to be used with our model we selected a long-term average of the years 1989–1993 for which continuous and consistent data was available. From daily averages of these years, monthly averages for the fraction of available light were calculated, which varied from 36% in winter to 72% in summer. The so corrected photodegradation rates then varied from 0.006 to 0.14 day⁻¹, with an average annual rate of 0.068 day⁻¹. This rate is ≈ 3 times faster than the predicted minimal rate (0.022 day⁻¹), calculated from concentrations in the in- and outflow of Greifensee and is thus clearly sufficient to account for the observed elimination of diclofenac from the lake.

The photolysis rate constants were then used to construct a simple model for diclofenac in Greifensee, assuming photolysis and flushing as the only processes for the elimination of diclofenac from the lake. A constant flushing (1.35×10^8 m³/yr, or 4.2 m³/s) and a constant input of 25 g/day (≈ 9 kg/yr) were used for the calculation. For the model the lake was considered perfectly mixed from November to March and stratified from April to October, assuming a constant epilimnion depth of 5 m. The resulting concentration profile is shown by the upper limit of the shaded area in Figure 7. According to this model, the concentrations are highest in January (≈ 10 ng/L) and gradually decrease until April (≈ 4 ng/L). Beginning in May, the lake is stratified and all input of diclofenac (and flushing) is to the epilimnion, where the concentration further decreases due to the high light intensity (minimum ≈ 1.5 ng/L in June). Starting in August, the diclofenac concentrations in the epilimnion increase again, as the sunlight intensity decreases. The concentration in the hypolimnion is constant (no input, no photolysis) until November, when the lake is overturned (dashed line, Figure 7). In Figure 7 we also show the modeled concentrations when assuming 100% sunlight conditions by the lower limit of the shaded area. As shown, these concentrations range from 6 ng/L (maximum in January) to ≈ 1 ng/L (minimum in June). Also shown in Figure 7 are the measured diclofenac concentrations in the outflow of Greifensee. At a first glance there is significant scattering of the experimental data. Deviations of these data from the modeled data can result from varying inputs, a varying flushing rate, different sunlight conditions, and inhomogeneities in the lake. Nevertheless, the experimental data is in

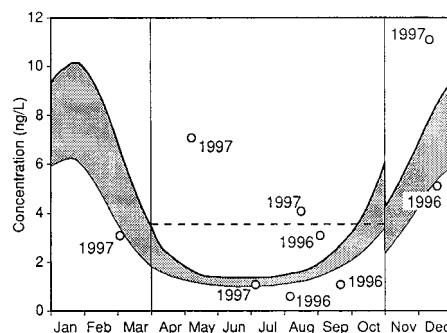


FIGURE 7. Seasonal variation of the concentration (ng/L) of diclofenac in Lake Greifensee, as predicted by computer simulation, assuming photolysis and flushing as the only processes for elimination (for parameters, see text). Shaded area, range of concentrations (ng/L) expected from maximum sunlight conditions (lower limit) and long-term average sunlight conditions at Dübendorf (upper limit), as predicted by GCSOLAR. Vertical lines: transition from mixed to stratified lake model. Full lines, concentrations in surface water, dotted line, concentrations in hypolimnion during stratification. Also shown are the experimental data (circles) for the years 1996 and 1997.

the range of that predicted by the model (1–11 ng/L), and there are upward trends in concentration from summer to winter in both 1996 and 1997. Assuming no other elimination than by water exchange the concentration of diclofenac would increase to 67 ng/L in the lake.

Concluding Remarks. The data strongly suggest inputs of diclofenac into rivers and lakes from human medical use via WWTPs. The study, however, also showed that diclofenac is not very persistent in a lake (Greifensee) and that it is rapidly degraded, most likely via direct photolysis. The results are important because they document the rapid elimination of this compound in surface water under field conditions. The findings have transfer character because the same process can be expected in other water bodies (lakes, streams) with a sufficient time constant, as suggested by the consistently low concentrations observed in the outflows of other lakes. This rapid elimination of diclofenac in the lake is different from the behavior of many other environmental contaminants.

So far, the evidence for photodegradation is from laboratory experiments and kinetic considerations. Direct evidence, such as from photodegradation products in the lake, is still lacking and additional research is required for verification. Vertical concentration profiles under different conditions (stratified and mixed lake conditions) together with more refined model calculations may serve to substantiate these findings further. Whether photolysis is the only available pathway for the degradation of diclofenac in the environment remains to be investigated. The detailed behavior of diclofenac in WWTP installations should also be further investigated.

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