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A MULTICOMPONENT SNAPSHOT OF PHARMACEUTICALS AND PESTICIDES IN THE RIVER MEUSE BASIN

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Abstract: The river Meuse serves as a drinking-water source for more than 6 million people in France, Belgium, and The Netherlands. Pharmaceuticals and pesticides, both designed to be biologically active, are important classes of contaminants present in this river. The variation in the presence of pharmaceuticals in time and space in the Dutch part of the Meuse was studied using a multicomponent analytical method for pharmaceuticals combined with univariate and multivariate statistical analyses of the results. Trends and variation in time in the presence of pharmaceuticals were investigated in a dead-end side stream of the Meuse that serves as an intake point for the production of drinking water, and 93% of the selected compounds were detected. Highest concentrations were found for the antidiabetic metformin. Furthermore, a spatial snapshot of the presence of pharmaceuticals and pesticides was made along the river Meuse. Principal component analysis was successfully applied to reveal that wastewater-treatment plant effluent and water composition at the Belgian border were the main factors determining which compounds are found at different locations. The Dutch part of the river basin appeared responsible for approximately one-half of the loads of pharmaceuticals and pesticides discharged by the Meuse into the North Sea. The present study showed that multicomponent monitoring in combination with principal component analysis is a powerful tool to provide insight into contamination patterns in surface waters. *Environ Toxicol Chem* 2013;32:2449–2459. © 2013 SETAC

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INTRODUCTION

Surface waters are contaminated with thousands of chemical compounds originating from industry, agriculture, and household uses. In recent decades, knowledge of chemical contaminants in the environment and their possible toxic effects has increased. In addition, rapid improvements in chemical and bioanalytical techniques have led to the discovery of all types of so-called emerging contaminants—for example, pharmaceuticals and sweeteners—in surface waters, at concentrations of (sub)nanograms per liter to micrograms per liter [1]. Also, well-known contaminants such as pesticides are still present. These compounds might be problematic for surface-water functions such as the production of drinking water.

To monitor developments in water quality, to guard surface-water catchments, and to fulfill legal obligations, governments and drinking-water companies intensively investigate surface-water sources for the presence of such contaminants and their fate during purification processes. As numbers of known contaminants are still increasing, there is an urgent need for efficient and sensitive analytical techniques to measure contaminants in a wide range. Therefore, we wanted to demonstrate in the present study the applicability of multicomponent analytical methods combined with univariate and multivariate statistical analyses as a possible means to evaluate contamination pressure in time and space. As a case study, pharmaceuticals supplemented with general water-quality parameters and pesticides were investigated in the Dutch part of the European river Meuse.

River Meuse

The area of the Meuse River basin is almost 35 000 km^2 and has 8.8 million inhabitants (3.5 million [40%] in the Dutch part of the river basin) [2,3]. Between its source in France and its outfall in the North Sea in the Dutch delta, the river stretches out over 900 km. The French part of the river basin is used mainly for agriculture, stock farming, and nature. Throughout Belgium, the river passes the Ardennes and urbanized and industrial areas. The Dutch part of the river basin is characterized by a high population density and intensive agricultural and industrial activities. The Meuse, predominantly fed with rainwater, serves as a drinking-water source for more than 6 million people in France, Belgium, and The Netherlands. In 2010, 527.4 million m^3 of surface water from the Meuse was abstracted for this purpose [2].

Pharmaceuticals and pesticides

Pharmaceuticals and pesticides, both designed to be biologically active, are consumed in large quantities in the European Union and thus form important classes of contaminants present in the river Meuse [2,4]. Pharmaceuticals consumed by humans are excreted in urine and feces and may reach surface waters after incomplete removal in wastewater-treatment plants (WWTPs). Pharmaceuticals administered to and excreted by livestock often are not subjected to wastewater treatment but end up in manure and can reach surface water in the runoff of fertilized land [5]. Numerous studies have reported the detection of pharmaceuticals in wastewater effluents and in surface waters [6–8], including at an intake station for drinking-water preparation along the Meuse [1]. Although

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pharmaceuticals have been found only in low concentrations in drinking water from several countries [9–11], public concern about the possible effects of unintentional exposure to pharmaceuticals is high. In general, studies on pharmaceuticals have focused on a subset of the pharmaceuticals present on the market, often selected for rather simple analytical detectability and previous detection. The presence of individual pharmaceuticals in the environment, however, is largely determined by consumption volume, fractions excreted in nonmetabolized form, and removal in wastewater treatment and the environment [8], implying that pharmaceuticals that have not been investigated previously also might be present in the environment.

Pesticides are used in large quantities. They are emitted into surface waters, for example, via runoff from agricultural land and accidental spills. Pesticides form a group of contaminants that have been found in the Meuse River basin for many years [2,4,12,13].

Aims of the present study

The presence of pharmaceuticals in the Dutch part of the river Meuse was studied in time and space using a multicomponent analytical method combined with univariate and multivariate statistical analyses of the results. First, trends and variation in time of pharmaceuticals were investigated at an intake point for the production of drinking water in a dead-end side stream of the Meuse. A fast, multicomponent analysis method was implemented in 2010 using ultra–high-performance liquid chromatography (ultra-HPLC) separation, followed by tandem mass spectrometry detection (MS-MS). For some of the pharmaceuticals, data could be complemented with monitoring results obtained earlier to generate an 8-yr data set (2005–2012) to study trends.

The second aim was to make a spatial snapshot of the presence of pharmaceuticals along the Meuse. Locations (activities in the river basin) and concentrations of compounds were correlated using multivariate statistical analysis (principal component analysis) to identify uniform or atypical groups of compounds that are detected at different types of locations. Pesticides and general water-quality parameters were also included to provide the analysis with more information about each location. The contribution of the Dutch part of the river basin to the total discharge of pharmaceuticals and pesticides by the Meuse was calculated.

MATERIALS AND METHODS

Chemicals

Solvents were bought from Biosolve and were of ultra-LC/MS quality. Analytical standards of pharmaceuticals were purchased from Sigma-Aldrich, except temazepam and oxazepam (Duchefa, Farma, Lipomed) and iopromide (US Pharmacopeia). All other chemicals, such as those for preparation of eluent buffers, were of pro analysis quality or better (Sigma-Aldrich).

Variation and trends of pharmaceuticals in time

Sampling. Grab-water samples were taken in prerinsed bottles of green glass every 4 wk from August 2010 to August 2012 (27 analyses) at the intake site for drinking-water production in the dead-end side stream of the river Meuse (location 11 from the snapshot study). Samples were stored at 4 °C until processing.

Analysis of pharmaceuticals with the ultra-HPLC/MS-MS multicomponent method. Using solid-phase extraction, 100-mL volumes were extracted (Oasis HLB) and eluted with methanol. Extracts were evaporated to 100 μL, to which 1 mL of MilliQ water was added. Pharmaceuticals were analyzed using ultra-HPLC (Waters Acquity), equipped with a quaternary pump, combined with a Quattro Xevo triple-quadrupole mass selective detector (Waters Micromass) with electrospray ionization. Quantification was performed using an external calibration series of 7 concentrations.

The analysis method contained 41 pharmaceuticals. In the selection of compounds, specific attention was given to pharmaceuticals with large consumption volumes. Eleven of the 20 most-sold pharmaceuticals were included. Other selection criteria were previous detection, ecotoxicological relevance (e.g., cytostatics, antibiotics, and nonsteroidal anti-inflammatory drugs [14]), and representation of different therapeutic classes. The method was validated by calculating the recovery and standard deviation in surface-water samples from 8 different locations and sampled on different days spiked with pharmaceuticals. The average recovery was $91 \pm 14\%$. Most ($n = 32$) compounds had a minimum reporting limit of 5 ng/L or lower, of which 18 compounds had a minimum reporting limit between 0.1 ng/L and 1 ng/L. The highest minimum reporting limit was obtained for clofibrate (85 ng/L). A detailed description of the method and its validation is provided in the Supplemental Data.

Statistical analyses

Box plot figures representing minimum, first quartile, median, third quartile, and maximum concentrations were made in Excel for pharmaceuticals that were detected in at least 5 samples (20% of the samples). Concentrations less than the minimum reporting limit were artificially set at 25% of the individual minimum reporting limit. The significance of long-term time trends and seasonal variation was tested using the statistical software package Trendanalist (AMO-Icastat). For this purpose, the obtained data set was complemented with archived monitoring results for those pharmaceuticals that had also been monitored with enough sensitivity with LC/MS and gas chromatography (GC)/MS methods at the same location from 2005 to 2010 (the test requires results of a period of at least 4.5 yr). Long-term time trends were tested with linear regression (in case of normally distributed data), and the Mann-Kendall test corrected for seasonal effects (if data were not normally distributed). Seasonal variation was tested with Kruskal-Wallis tests.

Spatial snapshot of pharmaceuticals along the Meuse

Sampling locations. Water from 16 locations was sampled to generate a snapshot of the chemical water quality of the Dutch part of the river Meuse (Figure 1). The following samples were taken from the main stream of the eastern half of the Dutch part of the Meuse (numbering as in Figure 1): "Belgian border" (location 1), "Geleen" (just downstream from a large chemical industrial plant, location 2), "Mook" (location 4), and "Maasdriel" (location 5). Four samples (locations 6–9) were taken from rivers feeding the Meuse, the Dommel and Aa Rivers, because both receive wastewater-treatment effluent (on average, respectively, 38% and 27% of their volume consists of effluent [15]). Samples 6 and 7 were taken from the Dommel (location 6 just downstream of the effluent discharge and location 7 more downstream), and 8 was from the river Aa. Both rivers discharge into the Channel South-William (location 9), which in its turn discharges in the mainstream of the Meuse. Directly downstream from the entrance of this channel into the Meuse, sample 10, "Meuse after entrance D, A&SCW," (location 10) was taken. Farther downstream, the rivers Meuse

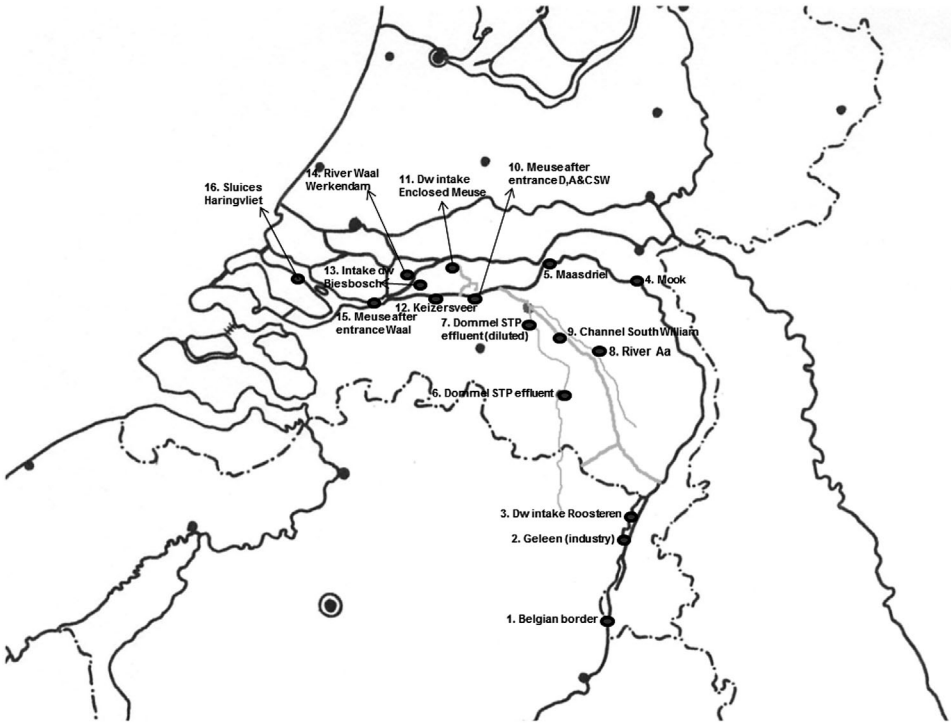

Figure 1. Overview of the sampling locations in the Dutch part of the Meuse River basin. DW = drinking water.

and Waal (the main distributary branch of the river Rhine in The Netherlands), sampled as "Keizersveer" (location 12) and (location 14) "Waal," combine in the Dutch delta, sampled as "Meuse after entrance Waal," (location 15) which discharges into the North Sea via the estuary of Haringvliet, sampled at its sluices (location 16 "Haringvliet Sluices"). Four samples were taken along the Meuse at sites at which water is abstracted for the production of drinking water: "Dw intake Roosteren" (location 3, abstraction via riverbank filtration), "Dw intake Enclosed Meuse" in a dead-end side stream of the Meuse (location 11), "Dw intake Biesbosch" (location 13), and "Haringvliet Sluices" (location 16).

Sampling. Grab samples were collected from the 16 locations in a single sampling campaign between 13 and 16 September 2010. This month had some rain and a low to moderate flow in the river of, on average, 6.8 E6 m^3/d at the Belgian border. From 2 locations (1 and 12) additional samples were taken 1 wk prior (week 1, 9 September) and 1 wk after (week 3, 23 September) the sampling campaign (week 2, 13–16 September) to enable calculation of loads (see section *Loads discharged into the North Sea*) and to gain an understanding of variation in measured concentrations in the semi–long term. Samples were stored at 4 °C and processed within 48 h.

Analysis of general water-quality parameters. Electrical conductivity, pH, and turbidity were measured on a Skalar SP 1000 Robot Analyzer (Skalar Analytical). Ammonium, chloride, and nitrate were analyzed using an Aquakem Photometric Analyzer (Thermo-Fisher). Urea concentration was determined by analysis on the Aquakem of ammonium formed after addition of urease and correction for native ammonium. Total organic carbon (TOC) was analyzed on a TOC-V analyzer (Shimadzu). Hydrogen carbonate and carbon dioxide concentrations were measured potentiometrically by titration with NaOH or HCl, respectively.

Multicomponent analysis of pharmaceuticals and pesticides. Pharmaceuticals were analyzed on ultra-HPLC/MS-MS as described above. Concentrations of bisoprolol and propranolol were not included in the snapshot study due to uncertainty in the quantification in some samples caused by matrix effects (ion enhancement). The pesticides were analyzed by Aqualab Zuid, according to their own validated protocols. In short, pesticides were analyzed using a multicomponent method for 65 polar pesticides on ultra-HPLC/triple-quadrupole–MS-MS. A total number of 140 less polar and more volatile pesticides were analyzed with a multicomponent method by means of GC-mass selective detection. The herbicide glyphosate and its metabolite aminomethylphosphonic acid were derivatized and analyzed by HPLC combined with fluorescence detection.

Statistical analysis

A principal component analysis was performed to cluster activities in the river basin according to contamination patterns using XLStat2008 software. Only compounds detected in at least 20% of the measurements were included (10 water-quality parameters, 19 pesticides, and 29 pharmaceuticals). All concentrations less than the minimum reporting limit were artificially set at 0. First, all concentrations were standardized ([concentration at individual location – average concentration]/standard deviation). A matrix was constituted with the 20 samples (16 locations plus the 2 additional samples at both locations 1 and 12) as loadings and filled with the standardized concentrations of general water-quality parameters, pharmaceuticals, and pesticides as observations. Replicates were included to investigate if these measurements would give factor loadings more similar to each other than measurements at other locations. Principal component analysis was performed to check the cumulative variance explained by the first principle component and then repeated with Varimax rotation to reduce the projection of the variance from projection on 20 components to projection on 3 components.

Loads discharged into the North Sea

Daily loads of pharmaceuticals and pesticides passing through the Meuse were calculated from the measured concentrations using flow data at locations 1, 2, 4, 12, and 16, because flow data for these locations could be provided by the Dutch Ministry of Infrastructure and Environment and the Water Board Aa and Meuse. Single measured concentrations for each individual compound were available for locations 2, 4, and 16. Loads for these locations were calculated using the average flow between 2 and 30 September 2010 as follows

$$\text{Load} = Q_{4\text{ wk average}} \times c$$

where Q represents the load and c represents the compound concentration. Three weekly measured concentrations were available for locations 1 and 12. For these locations, average loads were calculated more precisely using the averaging estimators approach [16] with the formula

$$\text{Load} = \left[\sum (c_i \times Q_i) / \sum (Q_i)\right] \times Q_{4\text{ wk average}}$$

where Q_i represents the flow on day i and c_i represents the individual compound concentration on day i.

RESULTS AND DISCUSSION

Variation and trends of pharmaceuticals in time

Presence of pharmaceuticals. Surface water from the enclosed branch of the Meuse (location 11 from the snapshot study, Figure 1) was analyzed every 4 wk from August 2010 to August 2012. Thirty-two compounds were detected at least once in the enclosed Meuse, and 20 compounds were detected in >50% of the samples. Most compounds had median concentrations on the order of 10 ng/L, and variations of concentrations in time were seen in orders of magnitude. Figure 2 provides the concentration characteristics of those pharmaceuticals detected in at least 20% of the samples, represented as a box plot. All results are given in the Supplemental Data (Tables S2 and S3).

Representatives of all investigated therapeutic classes were found during the 2 yr of measurements. Although most individual pharmaceuticals were found in concentrations around 10 ng/L, their combined concentration was between 0.3 μg/L (August 2011) and 1.6 μg/L (May 2012).

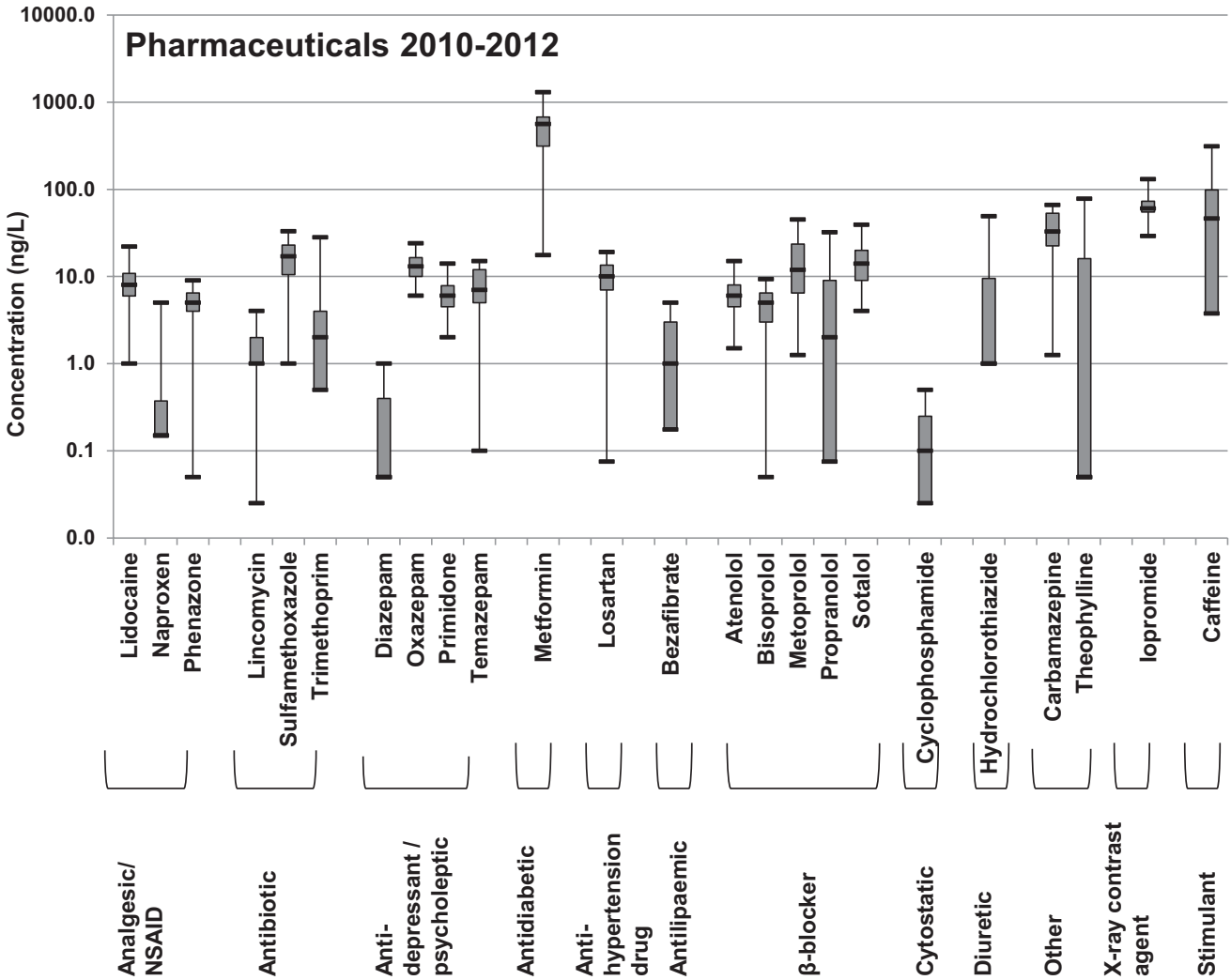

Figure 2. Box plot diagram summarizing the median, minimum, maximum, and 25th and 75th percentile concentrations of 4-wk measured concentrations of pharmaceuticals in the enclosed Meuse between August 2010 and August 2012. NSAID = nonsteroidal anti-inflammatory drug.

By far, the highest concentrations (on average 0.6 ± 0.3 μg/L) were found for the antidiabetic drug metformin (Figure 3B). Because more than 80% of Dutch diabetes type II patients are treated with this drug with daily doses up to 3 g to lower their serum glucose levels, this drug is number 5 in the top list of most prescribed drugs in The Netherlands (http://www.gipdatabank.nl/); and will probably also be among the top-prescribed drugs in Belgium and France. Although previously recognized as possibly environmentally relevant [17], until recently, analysis was challenging because of the drug's polarity (log octanol/water partition coefficient [K_{OW}] −1.4; EpiSuite K_{OWWin}) and charged structure [18,19]. Scheurer et al. [20] were the first to report that metformin was almost ubiquitously present in wastewater and surface waters in Germany. The study generated the first data for the Dutch aquatic environment, indicating a structural presence of metformin (93% detection frequency in the enclosed Meuse) in concentrations of up to 1 μg/L. Metformin is removed well during wastewater treatment (98% removed [21]), but apparently the large consumed volume still causes considerable emission into the environment. To date, early research projects have reported limited to good removal during drinking water–treatment steps [22,23].

The 2 other compounds that were present in concentrations ≥100 ng/L were the stimulant caffeine and the X-ray contrast agent iopromide. Caffeine is administered in combination with analgesics, but large quantities are also consumed as ingredients of beverages such as coffee, tea, and (energy) soft drinks. Another source of caffeine in the river basin is emission by coffee-roasting industries. Both compounds were found with median concentrations (46 ng/L and 60 ng/L, respectively) comparable to those previously found for other European rivers (72 ng/L and 100 ng/L, respectively [4,8]). Six analgesics and nonsteroidal anti-inflammatory drugs were detected. Most prevalent were phenazone and lidocaine (anesthetics, also prescribed as skin ointments or against heart rhythm disorder), present in 96% to 100% of the samples. This is in line with previous findings [11]. Ibuprofen, although belonging to the high–consumption volume compounds, was detected only once (40 ng/L), probably due to its relatively high minimum reporting limit (32 ng/L) and its almost complete removal (99% removed [21]) during wastewater treatment.

The investigated antilipemics belong to the subclasses of inhibitors of cholesterol synthesis (atorvastatin and pravastatin) and fibrates (others). Cholesterol synthesis inhibitors now replace the more old-fashioned fibrates. Of the fibrates (and their metabolites), included because of their previous detection, clofibrate and fenofibric acid were not detected at all, and bezafibrate (the consumption volume is due to large daily doses comparable to that of atorvastatin and pravastatin together) was detected in 52% of the samples. Of the cholesterol synthesis inhibitors, only atorvastatin was detected once, possibly due to its high removal rate in wastewater treatment (85–90% [24]).

All investigated antidepressants/psycholeptics were detected. The benzodiazepines diazepam, oxazepam, and temazepam (psycholeptics) were included in the method because of their high consumption volumes. The highest concentration was found for oxazepam (24 ng/L). The oxazepam detected in the environment can result from application of oxazepam itself but also as a metabolite of diazepam and other benzodiazepines [25]. Of the cytostatics, cyclofosfamide was detected more frequently (52%) than ifosfamide (11%). Both were present at very low concentrations (maximum 1 ng/L) and could be detected only because of a rather low minimum reporting limit in our method for these compounds. The investigated antibiotics clearly divided into 3 (chloramphenicol, oxacillin, sulfaquinoxalin) that were (almost) never found and 3 (lincomycin, sulfamethoxazole, and trimethoprim) that were detected in almost every sample. For both antibiotics and cytostatics the concern about their presence in the environment is largely due to their toxicity (genotoxicity and microbial resistance, respectively). Both classes consist of a far larger and steadily expanding list of compounds than we could include. Further studies should analyze these classes of compounds.

Antihypertension drugs, β-blockers and diuretics, the antiepileptic carbamazepine, and theophylline (drug against chronic obstructive pulmonary disease and asthma) were also structurally detected, with frequencies of 89% for losartan and 67% to 100% for all 5 investigated β-blockers.

Trends in time. Some pharmaceuticals have been monitored at the drinking-water intake in the enclosed branch of the Meuse since 2005. This enabled statistical analysis of temporal trends and seasonal variations. Carbamazepine was the only compound for which a significant temporal trend was found (Figure 3A).

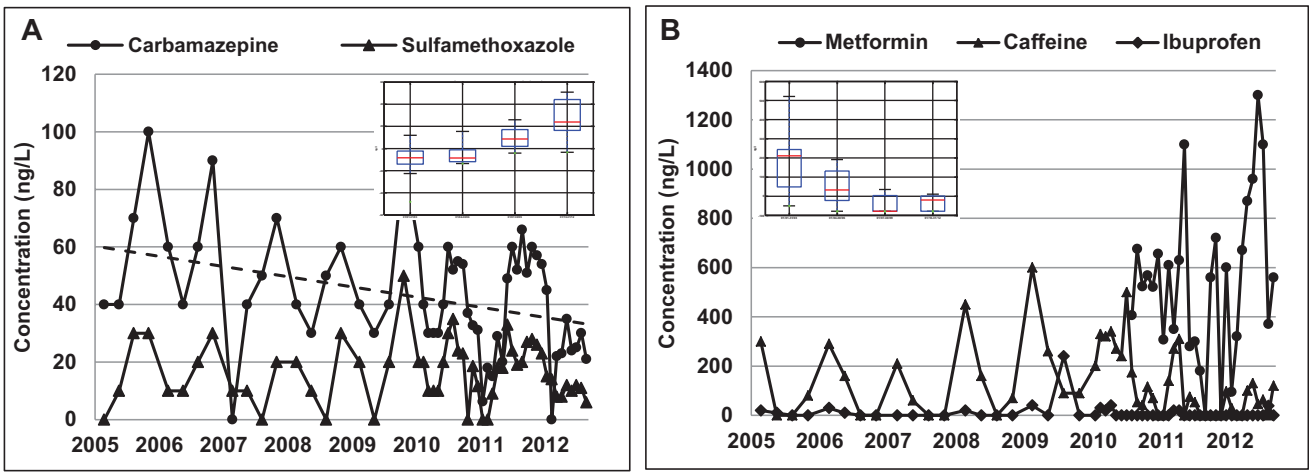

Figure 3. Concentration patterns of pharmaceuticals in surface water from the enclosed Meuse between 2005 and 2011. The dotted line represents the measured trend for carbamazepine. Inserted panes show box-whisker plots of seasonal variations in the concentration of carbamazepine (**A**) and caffeine (**B**) in the 4 periods of January to March, April to June, July to September, and October to December.

The concentration decreased by an average of 7.5% (3 ng/L) per year. To investigate if the absolute amount of carbamazepine present in the enclosed Meuse had decreased, calculation of loads is necessary. Unfortunately, suitable flow data were not available for this location. However, flow data of the main stream of the Meuse in the same period (Supplemental Data, Figure S1) indicated that, on average, the flow decreased between 2005 and 2012 (–7%/yr). This means that the temporal decrease in the concentration is probably not caused by an increase in the flow, which would have led to more dilution of the emitted carbamazepine. Subsequently, calculated loads of carbamazepine (Supplemental Data, Figure S2) indicated that the load had even a slightly stronger decreasing trend (–12.4%/yr) than the concentration. This suggests that the decreasing concentration is most probably caused by a decrease of the emission of carbamazepine in the river basin in the investigated years. A possible explanation for part of the decrease is a decrease in the use of carbamazepine in the Meuse River basin in this period (a yearly decrease of 4% in this period is documented for The Netherlands, according to www.gipdatabank.nl).

The concentrations of caffeine ($p = 0.2\%$), carbamazepine ($p < 0.1\%$), ibuprofen ($p < 0.1\%$), and sulfamethoxazole ($p = 1.0\%$) varied significantly between seasons. Carbamazepine and sulfamethoxazole (Figure 3A) showed highest concentrations in fall. Caffeine and ibuprofen (Figure 3B) showed highest concentrations (up to 600 ng/L) in winter and spring. The flow in the Meuse (Supplemental Data, Figure S1) was highest in winter and lowest in summer, leading to corresponding seasonal patterns for loads as for concentrations (Supplemental Data, Figure S4). The higher loads in fall and winter can possibly be explained by higher consumption of caffeine-containing beverages and pharmaceuticals (such as painkillers and antibiotics) in this season in combination with decreased activity of environmental or WWTP biodegradation in this season. Seasonal variation in concentrations of ibuprofen has been reported previously for the river Rhine [8,26].

Snapshot of the river Meuse

Concentrations along the Meuse River basin. A snapshot was made of the chemical water quality along the river Meuse with respect to general water-quality parameters, pharmaceuticals, and pesticides. General water-quality parameters indicated that locations were on first sight comparable in TOC, pH, and nutrients with concentrations normal for the Meuse [2]. Samples taken along the small rivers Dommel and Aa had higher TOC, lower pH, higher CO_2—possibly due to different soil composition—and higher NH_4^+ and urea concentrations, in agreement with the WWTP effluent emitted in these rivers.

Thirty-five pharmaceuticals were detected during the sampling campaign in the Meuse (Figure 4B and Supplemental Data, Table S4). The observed pharmaceuticals were largely similar to those found in the enclosed Meuse and present in concentrations on the same order of magnitude (from approximately 0.1 ng/L to more than 1.0 μg/L). Remarkably, a high concentration of 442 ng/L of unknown cause of the antilipemic pravastatin was detected in the Meuse at Maasdriel.

Twenty-eight pesticides were detected. Concentrations varied between less than the minimum reporting limit (10–20 ng/L for most pesticides) to 1.3 μg/L for aminomethylphosphonic acid at location 2 (Figure 4A). Pesticides have long been the most important group of contaminants of concern to drinking-water companies using the Meuse as a water source [2]. In contrast to pharmaceuticals, which are generally of point-source origin to watersheds (e.g. via WWTP outfalls), herbicides are mostly of non–point-source origin because they are applied directly to the land for agricultural purposes [27]. The fact that only 14% of 205 analyzed pesticides were detected might be partly explained by the fact that the multicomponent methods used for pesticides contained many pesticides that are not frequently found in Dutch surface waters anymore but for which monitoring is still obligatory according to European Union or national legislation. Only 4 insecticides were detected: diazinone, bromophos-ethyl, dichlofenthione, and *N,N*-diethyl-meta-toluamide. All were found once, except *N,N*-diethyl-meta-toluamide, which was found in 60% of the samples. The main use of *N,N*-diethyl-meta-toluamide is not in agriculture but as an insect-repellent by the public. Two fungicides were detected: carbendazim and 2,6-dichlorobenzamide. Both were present in more than 75% of the samples. Nineteen detected pesticides belong to the class of herbicides. Among them were glyphosate and aminomethylphosphonic acid (its degradation product). They are notorious contaminants in the river Meuse. The main emission pathways to the Dutch part of the Meuse are runoff from pavements. Glyphosate is not well degraded in WWTPs. Degradation to aminomethylphosphonic acid takes place mainly in the environment [28]. Glyphosate and aminomethylphosphonic acid were the only pesticides found in all samples.

Relatively high concentrations of pharmaceuticals and pesticides were found in samples from the WWTP effluent receiving rivers feeding the Meuse (locations 6, 7, and 8). However, as can be seen from Figure 4, total concentrations and those of individual pharmaceuticals and pesticides did not differ largely between locations along the Meuse (i.e., up to a factor of 4.3 for pharmaceuticals and 3.4 for pesticides but in most cases much less). This agrees with the fact that most locations are mutually correlated by being part of the same river. Therefore, it is difficult to link specific contamination patterns and locations on first sight, and we decided to apply multivariate statistics to this aim.

Principal component analysis—factor loadings. Principal component analysis was performed with a data matrix consisting of 20 samples (locations) as variables and 58 parameters as observations (10 water-quality parameters, 29 pharmaceuticals, and 19 pesticides that were detected in at least 20% of the measurements). The analysis showed that of the 20 principle components, the first accounted for 17% of the total variance, the second for 16%, and the third for 14% of the total variance of the data set. Collectively, the first 3 components could thus explain 47% of the total variance. The factor loadings for principal components 1, 2, and 3 after the Varimax rotation are shown in Supplemental Data, Table S5. Figure 5 shows the factor loadings to the first and second principal components.

Figure 5 reveals that the 3 replicate samples taken 1 wk apart at the Belgian border (location 1) give very similar loadings. Those at Meuse Keizersveer (location 12) show more diversity. This indicates that water quality can be quite constant for 1 mo at 1 location but that it also may vary during this period. Therefore, we concluded that the replicates could be included in the principal component analysis as individual factors.

Figure 5 shows 3 clusters grouping the locations according to local influences. Principal component 1 groups locations according to the extent to which they are influenced by WWTP effluent, giving negative scores. This is found for the locations along the rivers Aa and Dommel and at location 13. Although the impact of WWTP effluent at this last location is not known, the finding is in line with previous work in which we observed estrogenic effects, which are often caused by exposure to

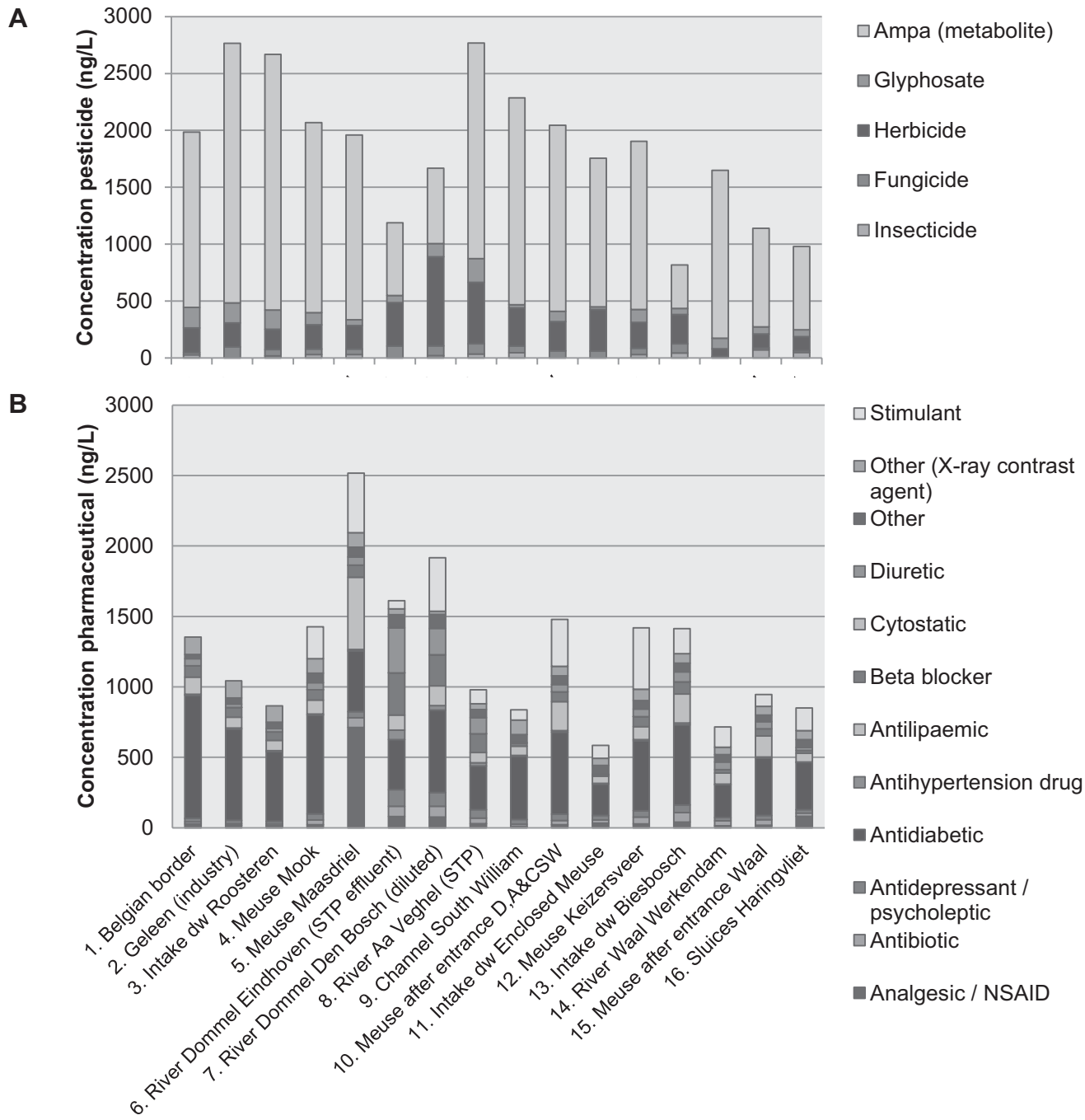

Figure 4. Pesticides (**A**) and pharmaceuticals (**B**) in 20 water samples taken in September 2010 in the Dutch part of the Meuse River basin. Combined concentrations of all pharmaceuticals and pesticides are shown according to their class per location. Strictly speaking, glyphosate is a herbicide; however, because its concentration is so high and as such so determinative for the total concentration of herbicides, it is shown separately. NSAID = nonsteroidal anti-inflammatory drug; DW = drinking water.

effluent, in breams at this location [15]. Locations with a positive score on principal component 1 are less influenced by WWTP effluent due to strong dilution (locations 14–16 are situated in the large river Waal and in wide parts of the Meuse) or environmental degradation (e.g., the residence time of water in the enclosed Meuse [location 11] is about 6 wk). Principal component 2 groups samples mainly according to their geographical location in the river basin. A positive loading is found for locations in the first part of the river basin downstream from the Belgian border. It represents the Meuse water in its composition as it enters The Netherlands at the Belgian border. Negative loadings are found for samples taken in waters feeding the Meuse (locations 6–9 and 11) and in the western part of the river basin (locations 13–16). They contain diluted water or water not originating from the Meuse. No clear trend was observed in the loadings on principal component 3. This principal component apparently reflects projection of a combination of diffuse factors that could not be straightforwardly interpreted. Therefore, interpretation of scores was done only for principal components 1 and 2.

Principal component analysis—factor scores. Figure 6 shows the factor score plot for principal component 1 versus principal component 2. It gives an impression of the extent to which types of locations are predictors of the compounds found somewhere. The components belonging to the group of pesticides have factor scores most to the center of the plot and are scattered throughout

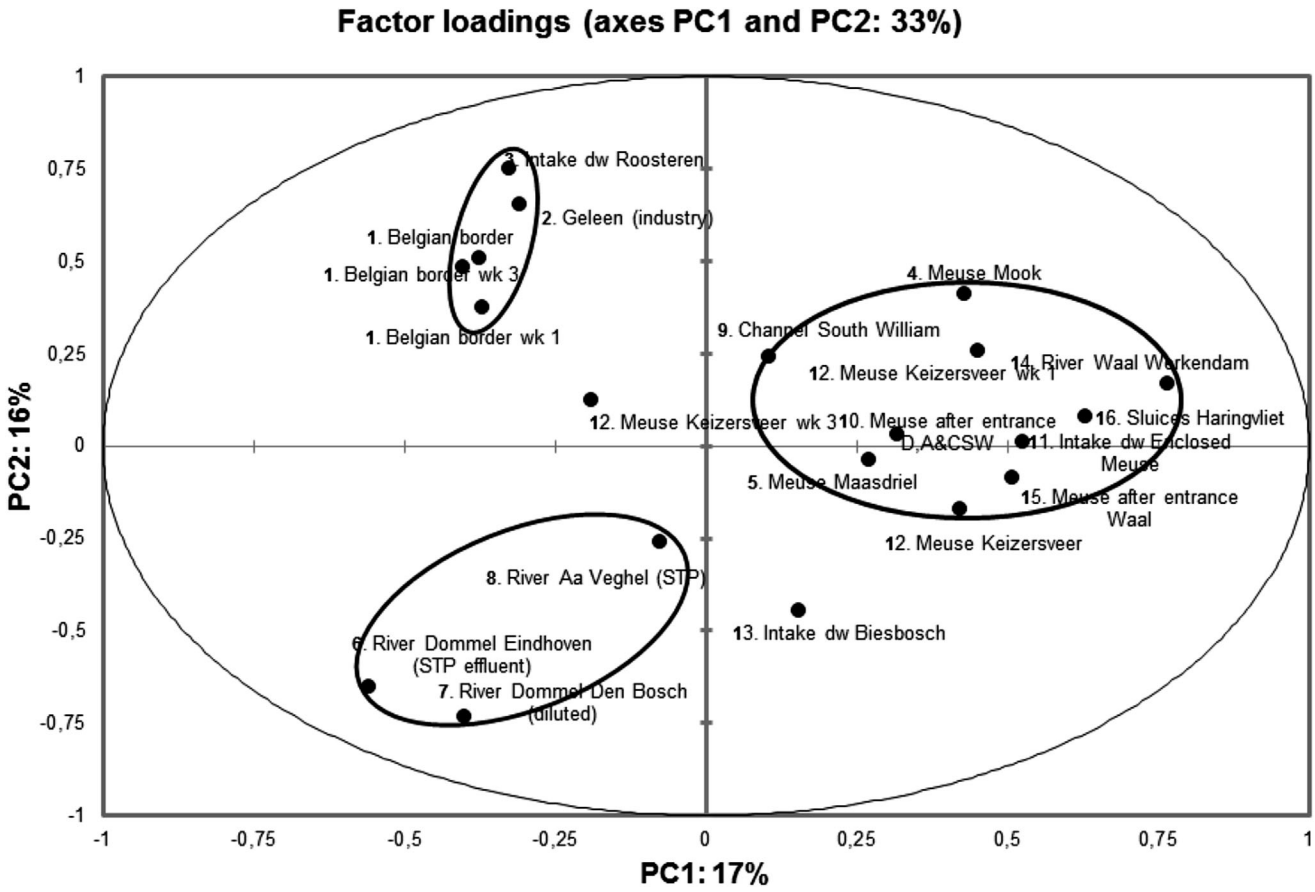

Figure 5. Factor loadings (samples of the snapshot study) on the first and second principal components (PC1 and PC2, respectively) by principal component analysis. STP = sewage-treatment plant; DW = drinking water.

the plot. This indicates that contamination with pesticides as a group occurs throughout the Meuse River basin and is not very location-specific within or is not projected enough on the first 2 components of the principal component analysis to elucidate a specific clustering of individual pesticides. Water-quality parameters and pharmaceuticals, however, do show distinct clustering and separation.

On the left in Figure 6, the water-quality parameters (circles) CO_2, NH_4^+, TOC, and urea are found. Indeed, NH_4^+, TOC, and urea are known to be markers for WWTP effluent, especially during rainy periods and sewer overflows [29]. In addition, the majority of pharmaceuticals detected in the present study (18, 62%) are found in this same cluster. This is in agreement with the fact that WWTPs are important sources of pharmaceuticals in surface waters [1,30]. Comparison of literature values of WWTP removal rates indicated that the pharmaceuticals inside the cluster had on average lower removal rates ($35 \pm 29\%$) in wastewater treatment than the other 11 outside the cluster ($73 \pm 14\%$). Although this result should be taken with caution as removal rates were only available for 9 pharmaceuticals inside and 5 outside the cluster, and values were obtained for other WWTPs that might differ from the ones in the Meuse River basin, this result might nevertheless indicate that principal component 1 especially clustered the pharmaceuticals most persistent in wastewater treatment. Besides lack of persistence, for some compounds, such as sulfamethoxazole, sulfaquinoxalin (used in veterinary pharmaceuticals), and iopromide (only used in hospitals), scores outside the cluster can be explained because they have emission routes other than WWTPs. The score of caffeine, also not in the cluster, agrees with its high water solubility and low persistence, which make it a suitable marker for anthropogenic influence but not specific for WWTP effluent [31,32].

Conductivity, HCO_3^-, pH, and chloride cluster positively on principal component 1. This is explained by the fact that HCO_3^- and pH are chemically correlated via the carbonate equilibrium. A decrease of HCO_3^- thus leads to higher concentration of CO_2, which was indeed found on the negative part of principal component 1. The highest pH and chloride were measured at locations in the delta area due to influence of intruding seawater and mixing with water from the river Waal.

Principal component 2 was found to represent the water composition of the Meuse at the Belgian border. In the upper part of the score plot, a remarkably high positive score on principal component 2 is found for nitrate and for some pesticides (glyphosate and its metabolite aminomethylphosphonic acid and diurone). This may be explained by leaching of these compounds from the sandy soils in the province of Limburg, which are used for intensive chicken and pig farming and treated with manure [33].

Calculated loads. The snapshot study was performed in September at low-flow conditions, just before the seasonal rise of flow in the river Meuse occurred. Water flows at the Belgian border were comparable during the first 2 sampling weeks (respectively, 8.2 E6 m^3/d and 8.4 E6 m^3/d) and much lower in the third sampling week (3.4 E6 m^3/d). Therefore, it was important to use all the replicate samples for the calculation of loads. At location 12, Meuse Keizersveer, the flow varied less between the sampling weeks, with flow rates of 16 E6 m^3/d, 15 E6 m^3/d, and 12 E6 m^3/d, respectively.

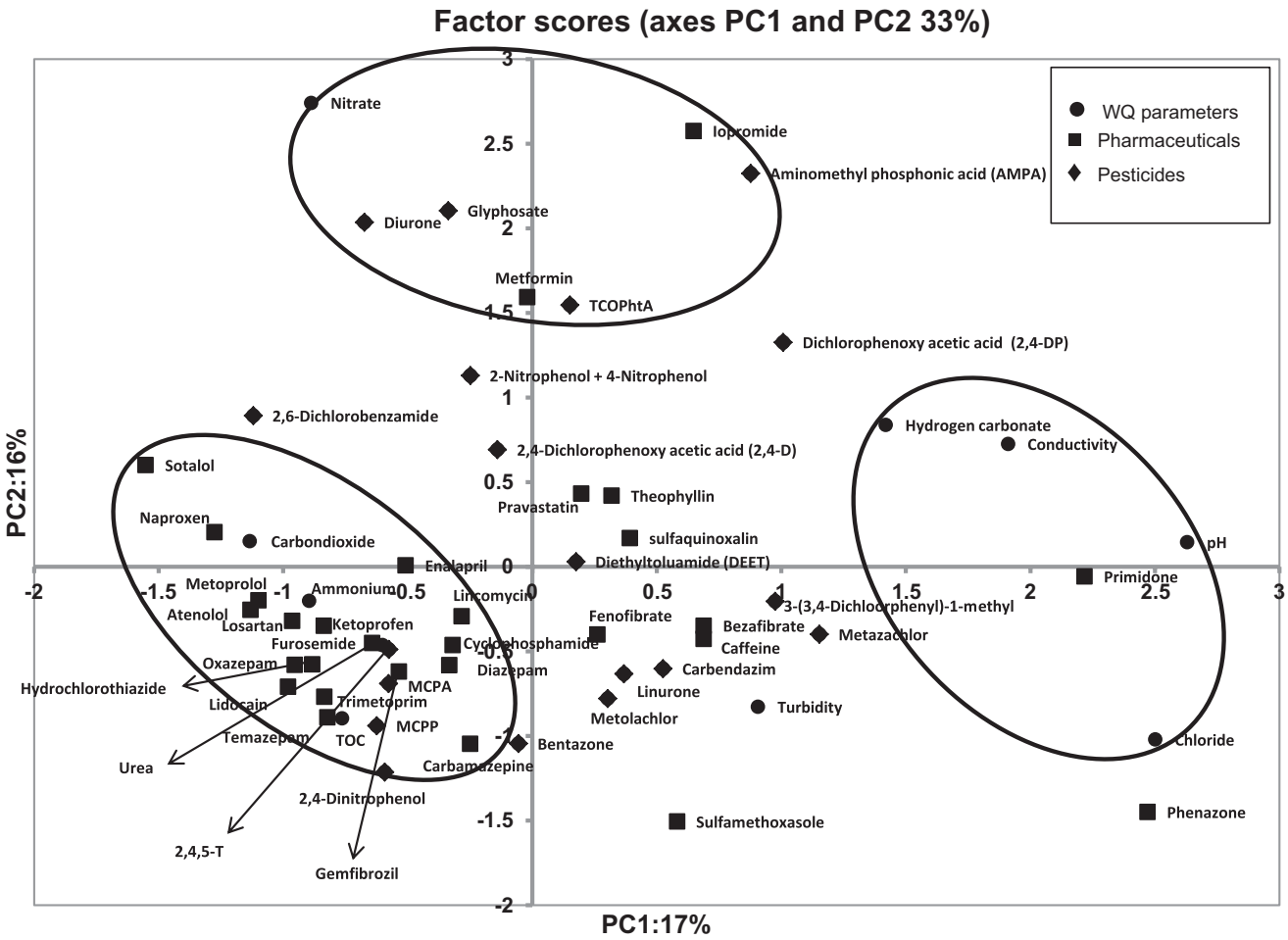

Figure 6. Factor score plot of measured parameters of the snapshot study on principal components 1 and 2 (PC1 and PC2, respectively) after Varimax rotation by principal component analysis. The factor scores indicate how the processes projected on the first and second principal components predict the contamination pattern of individual parameters (compounds). WQ = water quality; MCPP = 2-(2-methyl-4-chlorophenoxy) propanoic acid; MCPA = (4-chloro-2-methylphenoxy) acetic acid.

Loads were calculated to determine the contribution of the Dutch part of the river basin to the total discharge of pharmaceuticals and pesticides by the Meuse. The average water flow and the loads of pesticides and pharmaceuticals are shown in Supplemental Data, Figure S5. The flow doubled along the river basin between the Belgian border (6.8 E6 m^3/d) and Keizersveer (13.3 E6 m^3/d). Concentrations did not decrease proportionally (Figure 4), however, so loads of 18.3 kg/d (6.7 t/yr) of pharmaceuticals and 25.6 kg/d (9.2 t/yr) of pesticides are found at Meuse Keizersveer, indicating an increase in The Netherlands by a factor of 2.0 and 2.6, respectively, between the Belgian border and the Meuse at Keizersveer. In the delta area between Keizersveer and Haringvliet Sluices, a further increase in loads was observed (Supplemental Data, Figure S5). However, as water in Haringvliet consists of an average 1:4 mixture of water from the rivers Meuse and Waal [2], concentrations measured here are more representative for the Waal than for the Meuse. The calculated contribution of The Netherlands is higher than expected based on the area of the river basin (23% of the area is situated downstream from the Belgian–Dutch border) and on the population density (40% in The Netherlands). A possible explanation could be a higher consumption of pharmaceuticals and pesticides in The Netherlands in comparison with upstream countries. Another explanation might be that compounds emitted in the French and Belgian parts of the river basin have more time for environmental degradation before they reach the Belgian border and, as such, concentrations in the upper part are less clearly related to emission than those downstream.

CONCLUSION

Multicomponent methods were successfully applied to investigate the presence of pharmaceuticals in time and space in the river Meuse. Among the detected compounds were those included in the method because of their large consumption volumes and those that were not investigated in the Meuse basin previously, such as metformin and benzodiazepines, confirming the relevance of consumption volume as a selection criterion for analysis of pharmaceuticals in the aquatic environment. It can—ideally, if combined with data on metabolism and degradation—serve to anticipate what can be expected to penetrate into surface waters [34] and thus escape the pattern of focusing environmental monitoring only compounds previously detected (such as carbamazepine).

The principal component analysis applied in this snapshot study revealed that emission of WWTP effluent and the composition of Meuse water as it enters The Netherlands at the Belgian border were the most important factors predicting the presence of compounds at locations in the Dutch part of the

Meuse River basin. Multicomponent monitoring in combination with principal component analysis thus proved to be a powerful tool to provide insight into the relation between locations (activities in river basin) and compounds. However, pesticides especially occurred throughout the river basin and behaved mutually very differently in the principal component analysis. Therefore, it is not possible without considerable loss of information to select only 1 or a few compounds for monitoring that could represent a large group of environmental contaminants. Monitoring a broad range of compounds thus remains essential to investigate the quality of surface waters, especially if the water functions in the production of drinking water.

Several studies have concluded that measured traces of individual pharmaceuticals in water are too low to give rise to concern [35–38]. Nevertheless, the structural presence of low concentrations of multiple pharmaceuticals in water abstracted for drinking-water production is an issue requiring further attention. A toxicological risk assessment of the mixture of compounds detected in water sources is the next step of our work.

Pharmaceuticals and pesticides were found throughout the Meuse River basin. Because rivers often run through several countries, upstream activities can influence surface-water quality in other countries downstream. A good quantitative view of discharges was lacking for the Meuse [34]. Our study showed that it is not appropriate to speak of the Dutch delta as Europe's "sewage drain," because approximately one-half of the discharged pesticides and pharmaceuticals appear to be added in The Netherlands itself. This result stresses the necessity of international collaboration in the protection of water quality in rivers crossing national boundaries.

SUPPLEMENTAL DATA

Tables S1–S5.
Figures S1–S5. (516 KB DOC).

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