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Occurrence of the antidiabetic drug Metformin and its ultimate transformation product Guanylurea in several compartments of the aquatic cycle

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Abstract

In 2030, the World Health Organization estimates more than 350 million people will be diagnosed with diabetes. Consequently, Metformin – the biguanide drug of choice orally administered for diabetes type II – is anticipated to see a spike in production.

Unlike many pharmaceutical drugs, Metformin is not metabolized by humans but passes through the body unchanged. Entering aquatic compartments, such as in sewage, it can be bacterially transformed to the dead-end transformation product Guanylurea.

Several studies (including this paper) have shown the presence of both Metformin (Met) and Guanylurea (Gua) in both sewage treatment plants and rivers. Sampling over one week ($n = 5$) from a Southern German plant revealed very high average (AV) concentrations in influent ($AV_{Met} = 111,800 \text{ ng/L}$, $AV_{Gua} = 1,300 \text{ ng/L}$) and effluent ($AV_{Met} = 4,800 \text{ ng/L}$, $AV_{Gua} = 44,000 \text{ ng/L}$).

To provide a more complete picture of this process, a highly sensitive mass spectrometric method with direct injection was used for the measurement of Metformin and Guanylurea in drinking water, surface water and seawater. Limits of quantification (LOQ) ranging from 2-10 ng/L allowed for the detection of Metformin and Guanylurea in locations: Lake Constance ($n = 11$: $AV_{Met} = 102 \text{ ng/L}$, $AV_{Gua} = 16 \text{ ng/L}$), river Elbe ($n = 12$: $AV_{Met} = 472 \text{ ng/L}$, $AV_{Gua} = 9 \text{ ng/L}$), river Weser ($n = 6$: $AV_{Met} = 349 \text{ ng/L}$, $AV_{Gua} = 137 \text{ ng/L}$) and for the first time in maritime North Sea water ($n = 14$: $AV_{Met} = 13 \text{ ng/L}$, $AV_{Gua} = 11 \text{ ng/L}$). Lake Constance is used to abstract potable water which is further purified to be used as drinking water. A first screening of two tap water samples contained 2 ng/L and 61 ng/L of Metformin, respectively. The results of this study suggest that Metformin and Guanylurea could be distributed over a large fraction of the world's potable water sources and oceans. With no natural degradation processes, these compounds can be easily reintroduced to humans as they enter the food chain.

Keywords: pollution, waste water, seawater, pharmaceuticals, water treatment

1. Introduction

The prevalence of diabetes and obesity has reached epidemic dimensions in the 21st century. In 1995, an estimated 135 million people worldwide were impacted by diabetes (King et al., 1998); this value is predicted to reach 366 million by 2030, with the greatest increase in diagnoses coming from developing countries (Wild et al., 2004).

For decades the orally administered biguanide Metformin (Table 1) has proven to be an effective and convenient pharmaceutical treatment of diabetes mellitus type II (Reitman and Schadt, 2007). Typical dosages range from 0.5 to 2.5 g, with annual European per person sales rates of 5.9-12.1 gram, making Metformin among the most widely produced pharmaceutical drugs worldwide (Oosterhuis et al., 2013; Schuster et al., 2008). In 2011, Germany alone prescribed a total of 601 million defined daily doses (DDD), an increase of 3.9 % compared to 578 million DDDs in the year 2010 (Schwabe and Paffrath, 2012). Each DDD accounts for 2 g of pure biguanide, which scales to a total Metformin consumption of 1202 tons in Germany for the year 2011 (WHO, 2013). This number continues to increase as Metformin is more frequently becoming the main active ingredient in combinatorial antidiabetic drugs (more than 110 million DDDs in Germany) (WHO, 2013).

Metformin cannot be metabolized by the human body and passes through unmodified within urine and faeces (Bailey and Turner, 1996). It can be bacterially transformed (mainly in sewage treatment plants (STPs)) to its' dead-end transformation product Guanylurea (see Table 1)(Trautwein and Kümmerer, 2011).

Several studies showed the presence of Metformin and Guanylurea in European STPs and rivers (Nuijs et al., 2010; Scheurer et al., 2012, 2009; Trautwein and Kümmerer, 2011). International screening programs detected high Metformin concentrations ($AV_{Met} = 110 \text{ ng/L}$) in US streams (Kolpin et al., 2002), Malaysian rivers (Al-Odaini et al., 2013) and Swiss ground/surface water samples (Vulliet and Cren-Olive, 2011). Typically, such water sources are potential staging areas for eukaryotic reuptake of xenobiotics.

Recently, it was shown that Metformin (Eggen et al., 2011) and Guanylurea (Eggen and Lillo, 2012) can enter edible plants and be transferred to their seeds. However, a systematic study covering a larger region has yet to be demonstrated.

The purpose of this paper is to investigate the occurrence and distribution of Metformin and Guanylurea in different environments in the water cycle, where dilution factors are high and no detection has been reported previously. These are Lake Constance, the lower rivers Elbe and Weser with their estuaries to the German Bight and North Sea.

- Lake Constance is the third largest lake in central Europe (571 km² surface area), belonging in parts to Austria, Switzerland, and Germany. It is an important drinking water source and is supplied by the river Rhine, in which past studies have detected downstream instances of both target compounds (Scheurer et al., 2012, 2009).
- The rivers Elbe and Weser are heavily impacted by large German cities such as Dresden, Hamburg and Bremen, with the estuaries of both rivers being connected to the German Bight.
- The 3rd area is the coastal region of the German Bight and North Sea, up to 250 km offshore.

The environmental sampling points were completed by a five day screening of 24h mixed STP influent and effluent samples from a Southern German STP (600.000 population equivalent, no advanced treatment techniques). Additionally, two tap water samples of the urban region of Stuttgart were taken, deriving their drinking water from Lake Constance after some treatment (filtration with pumice and quartz, flocculation with iron(III)chloride, oxidation with hydrogen peroxide, disinfection with ozone and chlorine)(ZVBWV, 2013).

2. Materials and Methods

2.1. Water sample collection

Flow rate dependent 24 hour mixed STP influent and effluent samples were obtained from the treatment plant AZV Breisgauer Bucht (600.000 population equivalent) in Forchheim, Germany. Samples were taken in 1 L HDPE bottles from 05/21/2012 to 05/25/2012 and stored at the STP laboratory at -20 °C until transport.

Samples from the Northern German rivers Elbe and Weser were taken during a cruise with the research vessel *Ludwig Prandtl* from 10/10/2012 to 11/10/2012. The water discharge of the river Elbe was low with an average of ~ 370 m³/s.

Sea water samples from the German Bight and North Sea were collected during a sampling campaign with the research vessel *Heincke* from 10/31/2012 to 11/06/2012.

River and seawater samples were collected by a stainless steel bucket and stored in pre-cleaned 1 L HDPE bottles at -20° before further transport and processing.

Lake Constance and river Rhine samples were collected in 25 mL glass vials from 18/09/2012 to 20/09/2012 except for sampling points in Kressbronn, Fischbach, Uhlidingen and Sipplingen where water was collected in 500 mL HDPE bottles on 10/23/2012. Finally, drinking water samples obtained from two municipal tap waters in Filderstadt, Germany were collected in 250 mL HDPE bottles on 10/23/2012.

2.2. Sample filtration and preparation

After transport to laboratory all samples were pretreated for analysis. Samples from Lake Constance and Rhine River were filtered directly (0.45 µm Nylon filters ; Infochroma AG, Zug, Switzerland), whereas all other samples were cleaned-up with two consecutive filtering steps using first 1.6 µm glass microfiber filters (VWR International, Leuven, Belgium) followed by 0.45 µm cellulose nitrate filters (Sartorius, Göttingen, Germany). After filtration all samples were adjusted to pH 7 ± 0.1 with 1 molar NaOH or HCl (Merck Chemicals, Darmstadt, Germany) and again stored at -20°C until analysis. One mL of pH adjusted and filtered water sample was transferred to a HPLC vial. 10 µL of internal standard stock solution (100 ng/mL) were added. Analysis was performed by HPLC-MS/MS. Direct injection (DI) was used for all samples. STP influent and effluent samples were diluted 100 times before analysis.

2.3. Chemicals, standards and calibration solutions

LC-MS grade methanol (MeOH) was purchased from Scharlau Chemie SA (Sentmenat, Spain), ammonium formate from Fluka (Buchs, Switzerland, LC-MS grade). Mobile phases for HPLC were used without filtration. Water used as mobile phase was purified in an Elix Milli-Q system (Millipore Corp., Volketswil, Switzerland). Standards: Metformin (1,1-dimethylbiguanide hydrochloride, 97% purity) and Guanylyurea (amidinourea, 99% purity) were purchased from Aldrich, Switzerland. The purity of Guanylyurea was determined by ¹H- and ¹³C-NMR spectroscopy (Bruker Avance II 400 IOCSP1 400 MHz NMR spectrometer, University of Bern, Department of Chemistry and Biochemistry, Switzerland). Metformin-d₆ (isotopic purity ≥ 99%) was obtained from Alsachim, Illkirch, France.

A stock solution containing Metformin and Guanylyurea at a concentration of 100 ng/mL in MeOH was prepared. Working solutions of the standards from 50 - 100 ng/mL

in MeOH/water 1:1 were prepared for tuning studies. Calibration standards were prepared by serial dilution of the mixed working solution using Evian® mineral water, resulting in individual concentrations of 0-2100 ng/L. The calibration standards in Evian® mineral water were freshly prepared for every new series of samples. For the determination of Metformin and Guanylurea in seawater, matrix-matched standards were prepared, exhibiting a concentration range of 2-100 ng/L. An internal standard spike solution at a concentration of 100 ng/mL in MeOH was prepared. In sample volumes of 1.0 mL, 10 µL of the internal standard spike solution were added, resulting in a final concentration of 1000 ng/L.

2.4. HPLC and MS/MS conditions

A high-performance liquid chromatography Agilent 1260 system (Agilent Technologies, Wilmington, USA) equipped with a binary pump, a column oven thermostat operated at 50 °C, a high performance well-plate autosampler with integrated needle wash (1 wash for 30s with a water/methanol 4:1 solution) and cooling option to keep the HPLC vials at constant temperature (15 °C) was used. Chromatography was performed on a XBridge Phenyl column, carbon load 15%, pore diameter 130 Å, 150 mm x 2.1 mm, 3.5 µm particle size (Waters Corporation, Milford, Ma, USA), protected with a XBridge Phenyl guard cartridge 2.1 x 10 mm, 3.5 µm. Separation was achieved with a binary mobile phase at a flow rate of 100 µL/min. The eluent flow of the first 4.5 min as well as the reconditioning period of the chromatographic run was sent to waste via a 10 port-2-position valve installed post-column in order to prevent the ion source from contamination with matrix components. The optimized separation conditions were as follows: solvent A: water with 5 mM ammonium formate, solvent B: MeOH 100% with 5 mM ammonium formate. The gradient elution was as follows: 0 – 4.5 min: 95% A, the organic phase B was increased to 95% within 4 min. After that, the column was cleaned with 95%

mobile Phase B for 1 min. Re-equilibration with 5% B took 6 min. The sample volume injected was 100 μ L except for seawater (50 μ L).

The HPLC was coupled to an API 5500 Qtrap mass spectrometer (AB Sciex, Concord, ON, Canada) with an electrospray Turbo V ionization source working in the positive ionization (ESI⁺) mode. Acquisition was performed in the scheduled selective reaction monitoring mode (sSRM) and the protonated ion [M+H]⁺ chosen as a precursor. Source parameters were optimized to the following final conditions: ion spray voltage: 4.5 kV (IS), collision gas: medium (CAD), curtain gas: 172 kPa (CUR), ion source gas 1: 310 kPa (GS 1, nebulizer gas), ion source gas 2: 448 kPa (GS 2, turbo gas) and ion source temperature: 550 °C (TEMP). High purity nitrogen (> 98%) was used as desolvation, nebulizer and collision gas. Optimization of MS/MS parameters was performed by direct infusion at 10 μ L/min flow rate of 50 to 100 ng/mL of individual standard solutions. Declustering potential (DP), entrance potential (EP), collision energy (CE) and cell exit potential (CXP) voltages were established for each analyte and are summarized in Table 1. Data were acquired and processed using the instrument software Analyst 1.6.1 (Applied Biosystems).

Table 1 - Compound dependent mass spectrometric parameters; T₁: 1st transition (quantifier), T₂: 2nd transition (qualifier), MRM = multi reaction monitoring, DP = declustering potential, EP = entrance potential, CE = collision energy, CXP = cell exit potential

Compound	Molecular structure	Precursor [M+H] ⁺	MRM transitions	DP (V)	EP (V)	CE (V)	CXP (V)
Guanylurea		103.1	T ₁ : 103.1 → 60.1 T ₂ : 103.1 → 43.0	56 56	10 10	19 37	8 8
Metformin		130.1	T ₁ : 130.1 → 60.1 T ₂ : 130.1 → 71.0	36 36	10 10	21 31	10 10
Metformin-d6		136.1	T ₁ : 136.1 → 77.0	110	10	47	12

3. Results and discussion

3.1 Method performance and quality assurance (QC)

Under the applied chromatographic conditions, Metformin eluted at a retention time of $t_{rMet} = 6.6$ min and Guanylurea at $t_{rGua} = 5.7$ min with base line separation. Method performance was evaluated through calculation of linearity, sensitivity, recovery and precision. Results are summarized in Table 2. Quantitation based on peak areas was performed using the internal standard (IS) method. For Metformin, a corresponding deuterated analogue was used, whereas Guanylurea was quantified using external standard quantitation (no internal standard was available). Generally, a five point calibration curve was established by spiking milli-Q water with the analytes at a concentration ranging from 2-100 ng/L for drinking water and seawater, 5-1000 ng/L for lake and surface water and 0-2100 ng/L for 100 times diluted waste water samples. For quantitation of seawater, matrix matched calibration was performed in the NS6 sample (see Table 6).

Table 2 - Limits of quantitation (LOQ), correlation coefficients (R^2), recovery (R) and precision in sea, surface, lake, drinking and waste water of Metformin and Guanylurea

Compound	LOQ 1 R^2	LOQ 2 R^2	LOQ 3* R^2	Sea water (n = 6) R (%) RSD (%)	Surface water (n = 3) R (%) RSD (%)	Lake water (n = 3) R (%) RSD (%)	Drinking water (n = 6) R (%) RSD (%)	Waste water (n = 6) R (%) RSD (%)
Metformin	2 ng/L 0.999	5 ng/L 0.999	500 ng/L 1.0	92/103 ¹ 8.7/3	105 ² 4.2	105 ² 5.1	115 ³ 0.5	110 ⁴ 0.6
Guanylurea	10 ng/L 0.999	10 ng/L 0.999	500 ng/L 1.0	97/98 ¹ 2.5/10.8	53 ² 1.1	44 ² 2.3	39 ³ 10.4	96 ⁴ 1.4

LOQ 1: calculated for drinking water; LOQ 2: for surface and seawater, LOQ 3: for wastewater; *LOQ at a 100 fold dilution of the sample; ¹Recoveries performed at two spike levels in the sea water matrix, 1st level: 40 ng/L, 2nd level: 160 ng/L, ²: spike level 100 ng/L, ³: spike level 50 ng/L, ⁴: spike level 500 ng/L, RSD (%): percent relative standard deviation

Calibration curves were obtained for both transitions (T1, T2) and were linear with coefficients of determination $R^2 \geq 0.999$. Quantitative results obtained by the T2 transition regression line were normally within 10% of the T1 linear regression quantitation.

The LOQs for the target analytes were set to the lowest calibration standard. The calculation of the signal to noise (S/N) ratio at the LOQ was ≥ 9 for both compounds. Recoveries (R) were quantitative for Metformin in all matrices and for Guanylurea in sea water and waste water. Lower recoveries were obtained for Guanylurea in surface, lake and drinking water samples ($R = 39\% - 53\%$). This is in accordance with Scheurer et al. (2012) who found Guanylurea recoveries of 83% in drinking water and 65% in river water.

Environmental detection of low abundant xenobiotics like drugs, usually requires different clean-up and up-concentration techniques in order to be able to measure concentrations in the ng/L range.

A previous study for the detection of Metformin and Guanylurea in sewage treatment, surface water and drinking water used solid phase extraction (SPE) as method of choice and reached LOQs of 10 ng/L for Metformin and 50 ng/L for Guanylurea, respectively (Scheurer et al., 2012).

The analytical method described within this paper improved these thresholds by 5-fold, with no enrichment technique. At the same time, direct injection has several strong advantages: no enrichment of matrix or contaminations, no manipulation of samples and economization in time and money for sample preparation. When additionally the first minutes of the chromatographic run (solvent and matrix front) are sent to waste, lifetime of the analytical column can be extended and the mass spectrometer protected from contamination.

3.2. Transformation rates of Metformin to Guanylurea in sewage treatment

As expected, STP samples contained the highest concentrations of both analytes (Fig. 1) with Metformin dominating in the influent (86-142 µg/L) and Guanylurea in the effluent (28-67 µg/L).

Ratios of Metformin/Guanylurea ranged from 43 to 166 in the influent, whereas effluent ratios were inversely related and ranged from 0.08 to 0.18 (Supp. Table 1). The high abundance of Guanylurea in effluent samples demonstrates the strong degree of Metformin transformation in STPs with elimination rates between 93% and 97% (Supp. Table 1).

The microbiological transformation of Metformin into Guanylurea was shown by Trautwein and Kümmerer (2011). Similar observations regarding concentrations and ratios have been made by other researchers. Scheurer et al. (2012) studied five German STPs with Metformin dominating the influent (18-82 µg/L), whereas Guanylurea was greater in the effluent (18-99 µg/L), which is in accordance with our findings. Al-Odaini et. al (2013) detected Metformin in a STP effluent with a maximum concentration of 34 µg/L. The calculated average Metformin STP elimination rate of 95.5% in this study also matches with Scheurer et al. (2012) who found an average elimination of 91.8 %. Oosterhuis et al. (2013) detected Metformin in two Dutch STPs ($AV_{\text{Influent}} = 79 \text{ µg/L}$, $AV_{\text{Effluent}} = 1.5 \text{ µg/L}$), calculating a removal rate of 98%. Guanylurea was detected in only one STP effluent with an average (n = 7) concentration of 48 µg/L. In this study, the daily total of Metformin and Guanylurea in STP's effluent do not correspond to the amount contained in the influent (Fig. 1). The STP Breisgauer Bucht works with three anaerobic digestion towers. The recent finding that Metformin physically embeds to sewage sludge (range 550-1160 µg/kg dry-weight) and thus leaves the aqueous solution (US EPA, 2009), explains this discrepancy.

Fig. 1 - Total STP influent and effluent concentrations of Metformin and Guanylurea in a Southern German STP (600.000 population equivalent). Values in boxes represent absolute Metformin (grey boxes) and Guanylurea (black boxes) concentrations.

3.3. Metformin and Guanylurea in Lake Constance and potable water

As to be expected, the concentrations of both target compounds were lower by a factor of around 1000 in surface water compared to STP effluent, which is known for other pharmaceuticals too (Kümmerer, 2008). The detected Metformin concentrations in Lake Constance water samples range from 35 to 150 ng/L, whereas Guanylurea exhibited concentrations ranging from 10 to 22 ng/L (Table 3). In the upper Rhine River, Metformin concentrations of up to 220 ng/L were found with lower amounts of Guanylurea (up to 100 ng/L). These concentrations are similar to Metformin but lower for Guanylurea compared to concentrations measured in Basel in the year 2011 (270 ng/L for Metformin, 660 ng/L for Guanylurea, (Scheurer et al., 2012)). In another monitoring program of the Rhine River in the year 2008, 340 ng/L of Metformin were detected in Basel whereas 130 ng/L were found in Schaffhausen (Scheurer et al., 2009) which is in between sampling points LC11 (Wagenhausen, 143 ng/L) and LC12 (Eglisau, 216 ng/L) of this study (Table 3 and Fig. 2).

Table 3: Metformin and Guanylurea concentrations in Lake Constance (LC) and river Rhine, RSD = Relative Standard Deviation

Sampling Point LC	Geographical locations upper Rhine & Lake Constance	Metformin ng/L (%RSD)	Guanylurea ng/L (%RSD)
LC 1	Hard (tributary Rhine) (47° 49' N, 9° 67' E)	54 (17.1)	41 (3)
LC 2	Kressbronn (47° 58' N, 9° 58' E)	46 (20.3)	< LOQ (7 ng/L)
LC 3	Centre Lake (47° 35' N, 9° 26' E)	150 (8.5)	22 (5.4)
LC 4	Fischbach (47° 66' N, 9° 40' E)	35 (14.5)	10 (12.6)
LC 5	Uhdlingen (upper lake) (47° 70' N, 9° 25' E)	47 (14.9)	< LOQ (4 ng/L)
LC 6	Sipplingen (upper lake) (47° 80' N, 9° 07' E)	83 (6.5)	10 (3.5)
LC 7	Constance (47° 39' N, 9° 11' E)	139 (12.8)	17 (3.6)
LC 8	Herrenpark (lake Rhine) (47° 66' N, 9° 17' E)	143 (6.3)	21 (4.6)
LC 9	Gottlieben (lake Rhine) (47° 66' N, 9° 12' E)	143 (1.8)	21 (8.3)
LC 10	Ermatingen (lower lake) (47° 67' N, 9° 08' E)	136 (1.4)	20 (8.5)
LC 11	Wagenhausen (effluent Rhine) (47° 65' N, 8° 85' E)	143 (2.1)	17 (5.7)
LC 12	Eglisau (47° 57' N, 8° 52' E)	216 (1.5)	93 (5.4)

Lake Constance is an important drinking water supply for large parts of Southern Germany and also Switzerland. Its main tributary is the river Rhine, one of the largest streams in Europe. The Rhine valley is densely populated - even upstream to Lake Constance - and thus human impact on the water quality of this lake is expected to be high. A recent multi-level approach for the integrated assessment of polar organic micropollutants (MP) within the catchment area of Lake Constance revealed 252 compounds to be of interest (Moschet et al., 2013), among them 77 pharmaceuticals or their transformation products (TPs). 40 of these MPs or their TPs (18 pesticides, 13 pharmaceuticals, 5 biocides, 4 other) could be identified in an extensive screening in the year 2009 in the free water of Lake Constance using SPE-LC-MS/MS. The seven most abundant compounds were three pharmaceuticals (Carbamazepine $c_{\max}$ = 14 ng/L, Diclofenac and Sulfamethoxazole $c_{\max}$ = 13 ng/L), two corrosion inhibitors and two artificial sweeteners (Acesulfame and Sucralose). Metformin could be identified according to the experimental section, but “not fully confirmed and quantified” with no further processing. Interestingly, concentrations at the epilimnion

(surface until 1 m depth) of Lake Constance were halved compared to the hypolimnion (20 m – 230 m depending on the spot) values.

In our study we detected both Metformin as well as Guanylurea in nearly all sampling spots with maximum concentrations of 150 ng/L (Metformin, Centre Lake) and 27 ng/L (Guanylurea, Rhine tributary) (Table 3). Samples LC2 and LC4-LC6 were taken directly at the riverside of Lake Constance during a rainy day, so that dilution may have influenced the analytical results. At sampling point LC6 (Sipplingen), we detected 83 ng/L Metformin and 10 ng/L Guanylurea. This spot is of special interest since it is exactly at this location where the Lake Constance drinking water works retrieve their raw water (annually approximately 25 million m³ serving four million citizens)(ZVBWV, 2013). Consequently, two tap water samples from Filderstadt (urban region of Stuttgart), with drinking water from Lake Constance, showed 2 ng/L and 61 ng/L of Metformin, but no trace of Guanylurea.

Fig. 2 - Metformin and Guanylurea concentrations in Lake Constance (LC) and Rhine River

In 2008, the International Association of Waterworks in the Rhine Catchment Area (IAWR) proposed target values for anthropogenic compounds of > 100 ng/L (IAWR, 2008). Concern has to rise if xenobiotics with known biological effects are detected in concentrations > 100 ng/L. Moschet et al. (2013) found no such xenobiotics in their monitoring program. By contrast, our measurements show, for most sampling points, Metformin values around 150 ng/L. These analytical results exceed the proposed IAWR target values by 50%.

3.4. Distribution of Metformin and Guanylrurea in North Sea tributaries

The German rivers Elbe and Weser are the two main tributaries of the German Bight and North Sea. Whereas no pharmaceutical contamination of the Weser has been reported to date, the river Elbe and its tributaries have been subject of extensive screening programs (Wiegel et al., 2004). At sampling points close to the estuary of the Elbe into the North Sea, several prominent pharmaceuticals have been detected: Clofibric acid 4-18 ng/L, Diclofenac 3-8 ng/L and Ibuprofen 0.6 ng/L (Weigel et al., 2002; Wiegel et al., 2004). The analytical results of this study for both target compounds are orders of magnitude higher (river Elbe: $AV_{Met} = 472$ ng/L, $AV_{Gua} = 23$ ng/L, river Weser: $AV_{Met} = 349$ ng/L, $AV_{Gua} = 274$ ng/L). Scheurer et al. (2009) detected at one countryside sampling point on the river Elbe, concentrations of Metformin of 1700 ng/L. This value is about three times higher than the findings of this study (Table 4), however can be explained by influence of local STP effluents and smaller dilution factors than the estuary region.

The highest recorded concentrations of Metformin and Guanylrurea in the Elbe occurred at sampling point EW3, located at the end of the Hamburg urban area (Table 4 and Fig. 3). Beyond point EW3, concentrations decrease slightly. Elimination by sorption to suspended particulate matter or volatilisation into the atmosphere can be ignored because of the physical and chemical properties of

Metformin. To verify the dilution by seawater, hypothetical Metformin concentrations were calculated from the salinity gradient (Guanylurea was excluded since it was only detected in half of all sampling points). The results are shown in Fig. 3.

Fig. 3 - Saltwater dilution corrected Metformin concentrations in river Elbe

The calculations show an increase of Metformin concentrations from EW1 to EW3 at which point the trend reverses and decreases until EW8. After EW3 no relevant tributary rivers or STP effluents exist (Ahrens et al., 2009; Bollmann et al., 2012; Wolschke et al., 2011), explaining maximum values at EW3. The consecutive Metformin decrease until EW8 could be attributed to degradation or transformation within the highly microbial contaminated Elbe River. EW8 is the last sampling point without any salinity influence, after which point the calculated values show a constant course. This indicates that under fresh water conditions some further microbial transformation of Metformin can be expected while salt water prevents any Metformin degradation process.

This work reports the first ever evidence of pharmaceuticals occurring in the Weser River, which is a critical watershed for Northern Germany. In general, Metformin concentrations in the Weser River were lower but similar to the Elbe River (Fig. 4, Table 4). A noticeable difference between these two rivers was identified for Guanylurea which was detected at three Weser sampling points (EW14, EW16 & EW18) with concentrations exceeding 200 ng/L (Table 4). These findings are

unexpected, since at sampling points EW15, EW17 and EW19 no Guanylurea was detected. A check of the instruments raw data and repetition of measurement didn't reveal any analytical inconsistencies. One explanation for this discrepancy is dilution changes from changing tides during sampling. Further investigations are necessary for verification.

Table 4: Metformin and Guanylurea concentrations in the German rivers and estuaries of Elbe and Weser (EW), PSU = Practical Salinity Unit, RSD = Relative Standard Deviation

Sampling Point EW	Geographical locations rivers Elbe & Weser (Latitude, Longitude)	Salinity (PSU)	Metformin/ corr Metformin ng/L (%RSD)	Guanylurea ng/L (%RSD)
EW 1	Elbe 1 (53° 33' N, 9° 56' E)	-	553/553 (3,5)	< LOQ (4 ng/L)
EW 2	Elbe 2 (53° 33' N, 9° 53' E)	-	638/638 (1,4)	29 (3,9)
EW 3	Elbe 3 (53° 34' N, 9° 44' E)	-	643/643 (3,5)	30 (3,6)
EW 4	Elbe 4 (53° 36' N, 9° 36' E)	-	612/612 (1,4)	18 (0,5)
EW 5	Elbe 5 (53° 40' N, 9° 31' E)	-	583/583 (1,4)	no peak
EW 6	Elbe 6 (53° 44' N, 9° 26' E)	-	498/498 (6,4)	12 (1,6)
EW 7	Elbe 7 (53° 49' N, 9° 22' E)	-	522/522 (0,7)	24 (1,8)
EW 8	Elbe 8 (53° 53' N, 9° 15' E)	-	463/463 (1,4)	no peak
EW 9	Elbe 9 (53° 53' N, 9° 6' E)	4,5	409/468 (11,3)	no peak
EW 10	Elbe 10 (53° 51' N, 8° 50' E)	10,0	326/454 (2,8)	no peak
EW 11	Elbe 11 (53° 52' N, 8° 44' E)	11,8	299/448 (0)	no peak
EW 12	Elbe 12 (53° 58' N, 8° 31' E)	26,6	114/453 (0,7)	no peak
EW 13	German Bight (53° 59' N, 8° 11' E)	29,6	76/459 (0,5)	31 (1,3)
EW 14	Weser 1 (53° 41' N, 8° 22' E)	8,8	176 (0,7)	223 (21,2)
EW 15	Weser 2 (53° 33' N, 8° 33' E)	-	313 (4,9)	no peak
EW 16	Weser 3 (53° 23' N, 8° 31' E)	-	471 (2,8)	391 (7,1)
EW 17	Weser 4 (53° 28' N, 8° 31' E)	-	430 (2,8)	no peak
EW 18	Weser 5 (53° 29' N, 8° 31' E)	2,6	388 (2,1)	207 (4,2)
EW 19	Weser 6 (53° 32' N, 8° 35' E)	8,6	315 (1,4)	no peak

In summary, we detected high concentrations of Metformin in the rivers Weser and Elbe, exceeding the proposed IAWR thresholds for anthropogenic compounds by 5 times (IAWR, 2008). Guanylurea could be detected in less than half of the sampling points, with moderate values in the Elbe and very high concentrations in the Weser River.

Fig. 4 - Metformin and Guanylurea concentrations in Elbe, Weser and their estuary areas

Because of the specific usage of Metformin as long-term daily treatment drug, the assumption of a daily constant discharge was done. The mass flux estimations are based on the sampling points EW8 for Elbe and EW17 for Weser. These are the last sampling points upstream salt water influences. Daily mass fluxes were calculated by multiplication of concentrations with daily water discharge (Table 5).

Table 5: Mass flux analysis of Metformin into the North Sea; ^a = FGG Elbe (2013), ^b = FGG Weser (2013)

River & sampling point (Latitude, Longitude)	Metformin [ng/L]	Water discharge [m ³ /s]	Calculated daily mass flux [kg/d]	Calculated annual mass flux [t/a]
Elbe (EW 8) (53° 53' N, 9° 15' E)	463	^a 380	15.2	5.5
Weser (EW17) (53° 28' N, 8° 31' E)	430	^b 173	6.4	2.3

The estimated mass fluxes are enormous for a pharmaceutically active compound and reach comparable values as industrial pollutants. Ahrens et al. (2009) calculated for polyfluoroalkyl substances (PFAS) 0.91 t/a for the Elbe River. For organophosphorus flame retardants (OPEs) 5 t/a (Bollmann et al., 2012) and benzotriazoles 7 t/a (Wolschke et al., 2011) were estimated.

3.5 Metformin and Guanylurea in the marine environment

Whereas the distribution of pharmaceuticals in STPs, lakes and rivers has been extensively studied in the past (Kolpin et al., 2002; Ternes, 1998), marine environments have been mostly ignored.

This study shows for the first time the presence of Metformin and Guanylurea in marine ecosystems. Whereas Metformin was detected in all seawater samples (except one sample >250 km offshore), Guanylurea was present in only half of the samples (Table 6, Fig. 6). Both target compounds were mainly present in the coastal area, with concentrations up to 33 ng/L for Metformin (NS2) and 23 ng/L for Guanylurea (NS1). Towards the open North Sea, concentrations as a matter of dilution decrease and Guanylurea was not detectable in most of the samples anymore.

The main current conditions of the investigated coastline are dominated by the eastern current of the “Continental Coastal Water” (Turrell, 1992). This current flows from the English Channel along the Dutch coast, the East Frisian Islands to the North Frisian Islands. The water masses in front of the East Frisian Islands (sampling points NS 1,6 and 7) are mainly contaminated from discharges of the Rhine and Scheldt River (Ahrens et al., 2009). This can be an indication for the high detected Guanylurea concentrations in that region.

Table 6: Metformin and Guanylyurea concentrations in the German Bight and North Sea (NS), PSU = Practical Salinity Unit, RSD = Relative Standard Deviation

Sampling Point NS	Geographical locations North Sea & German Bight (Latitude, Longitude)	Salinity (PSU)	Metformin ng/L (%RSD)	Guanylyurea ng/L (%RSD)
NS 1	Island Borkum (53° 71' N, 6° 6' E)	32,0	8 (0,4)	32 (18,9)
NS 2	Island Eiderstedt (54° 22' N, 8° 25' E)	31,5	33 (0,9)	23 (0,8)
NS 3	Island Amrum (54° 68' N, 8° 11' E)	29,5	29 (1,4)	21 (0,6)
NS 4	Island Langeoog (53° 84' N, 7° 5' E)	n.d.	24 (0,1)	no peak
NS 5	Island List (55° 5' N, 8° 25' E)	29,9	31 (0,1)	no peak
NS 6	German Bight Fino (54° 0' N, 6° 6' E)	34,2	< LOQ (1 ng/L)	16 (1,1)
NS 7	German Bight Ems (54° 22' N, 6° 36' E)	34,7	< LOQ (2 ng/L)	19 (4,7)
NS 8	German Bight Centre (54° 23' N, 7° 5' E)	33,8	8 (0,3)	24 (1,8)
NS 9	North Sea 1 (55° 4' N, 6° 32' E)	34,7	10 (0,2)	19 (2,8)
NS 10	North Sea 2 (54° 68' N, 7° 5' E)	33,5	10 (0,1)	no peak
NS 11	North Sea 3 (54° 68' N, 6° 36' E)	34,5	7 (0,1)	no peak
NS 12	North Sea 4 (55° 15' N, 7° 5' E)	33,9	12 (0,6)	no peak
NS 13	North Sea 5 (54° 68' N, 5° 3' E)	34,5	5 (0,1)	no peak
NS 14	North Sea 6 (55° 17' N, 5° 4' E)	34,8	no peak	no peak

Fig. 6 - Metformin and Guanylyurea concentrations in the German Bight and North Sea

The German Bight and North Sea have so far only been scarcely investigated regarding the presence of pharmaceuticals. Weigel et al. (2002) detected in 15 sampling points of the North Sea Clofibric acid with an average concentration around 0.5 ng/L. Diclofenac and Ibuprofen which still were present in the estuary region of river Elbe (Weigel et al., 2002; Wiegeler et al., 2004), could not be detected anymore at open sea. Wille et al. (2010) investigated 13 drugs in the coastal region of Belgium and found Salicylic Acid ($AV = 141 \text{ ng/L}$, $n = 22$) and Carbamazepine ($AV = 12 \text{ ng/L}$, $n = 10$) as most abundant drugs in the open sea (Wille et al., 2010). The latter compound was shown to be taken up by marine mussels and detected in two Mediterranean samples ($n = 10$) with concentrations higher than the LOQ (1.5 ng/g dry weight). This is of special interest, since the herein reported Metformin ($AV_{Met} = 13 \text{ ng/L}$) and Guanyldurea seawater concentrations ($AV_{Gua} = 11 \text{ ng/L}$) are in the same range and thus present the question: can both compounds be enriched by aquatic organisms, in a similar fashion to what was shown for plant and seed uptake (Eggen and Lillo, 2012; Eggen et al., 2011)?

4. Conclusions

- Metformin was firstly identified as a high-potential persistent environmental contaminant in 2006, because of its high prescription rate (Schuster et al., 2008). Half of the total pharmaceutical load into STPs is expected to correspond to Metformin (KWR, STOWA, 2013). The economic boom in many developing countries is often followed by the adoption of an obese western lifestyle, thus increasing strongly the prevalence of diabetes (Wild et al., 2004). Metformin is a proven antidiabetic treatment and new pharmaceutical applications (e.g. against cancer (Leone et al., 2014)) will increase prescriptions for Metformin even higher in the near future.

- Laboratory biodegradation tests according to OECD guidelines revealed Guanylurea as bacterial dead-end transformation product (Trautwein and Kümmerer, 2011), which was stable against common water treatment techniques like UV light irradiation and ozonation (Gartiser et al., 2011; Trautwein and Kümmerer, 2011). Treatment with activated carbon which is the favored advanced treatment technique (no formation of TPs!) did not show consistent removal of both compounds (Scheurer et al., 2012). Only chlorination with subsequent formation of TPs or riverbank filtration and groundwater recharge showed encouraging filtration results (Scheurer et al., 2012). Since these techniques are not applied in wastewater treatment or drinking water preparation, huge amounts of Metformin and Guanylurea are released into and have been detected in various aquatic environments (Kolpin et al., 2002; Scheurer et al., 2012, 2009; Vulliet and Cren-Olive, 2011).
- This study showed the presence of Metformin and Guanylurea in multiple fresh and salt water sources, where both compounds had not been detected before. The acquired analytical results show a complete distribution over the entire regional water cycle: from highest concentrations in a STP ($10^3 - 10^5$ ng/L range) to medium values in discharging rivers and surface waters ($10^1 - 10^2$ ng/L range) to low amounts in drinking water and seawater ($10^0 - 10^1$ ng/L range). Calculated annual Metformin loads of 5.5 t (river Elbe) and 2.3 t (river Weser) into the North Sea are unique for a pharmaceutical compound and reach same magnitudes as industrial pollutants. Some inhomogeneous results in the river Weser and North Sea demand for further screening programs. The newly developed, cheap, efficient and fast detection technique with direct injection provides a suitable tool for this task.
- First time detection of Metformin and Guanylurea in seawater and tap water demonstrates the absence any efficient degradation process in ocean

environments or drinking water preparation; which suggests high persistence and the potential for worldwide distribution.

- Even though Metformin is reputed to have only minor side effects in humans, contradictory reports cite negative impacts such as Vitamin B12 deficiency (O'Loughlin et al., 2013) and increased formation of amyloidic Alzheimer plaques (Chen et al., 2009). Upcoming research should thus focus on risk assessments upon environmental impact and human health, thus considering in detail: bioconcentration and toxicity.

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References

- Ahrens, L., Plassmann, M., Xie, Z., Ebinghaus, R., 2009. Determination of polyfluoroalkyl compounds in water and suspended particulate matter in the river Elbe and North Sea, Germany. *Front. Environ. Sci. Eng. China* 3, 152–170. doi: 10.1007/s11783-009-0021-8
- Al-Odaini, N.A., Zakaria, M.P., Yaziz, M.I., Surif, S., Abdulghani, M., 2013. The occurrence of human pharmaceuticals in wastewater effluents and surface water of Langat River and its tributaries, Malaysia. *Int. J. Environ. Anal. Chem.* 93, 245–264. doi: 10.1080/03067319.2011.592949
- Bailey, C.J., Turner, R.C., 1996. Metformin. *N. Engl. J. Med.* 334, 574–579.
- Bollmann, U.E., Moeler, A., Xie, Z., Ebinghaus, R., Einax, J.W., 2012. Occurrence and fate of organophosphorus flame retardants and plasticizers in coastal and marine surface waters. *Water Res.* 46, 531–538. doi: 10.1016/j.watres.2011.11.028

- Chen, Y., Zhou, K., Wang, R., Liu, Y., Kwak, Y.-D., Ma, T., Thompson, R.C., Zhao, Y., Smith, L., Gasparini, L., Luo, Z., Xu, H., Liao, F.-F., 2009. Antidiabetic drug metformin (GlucophageR) increases biogenesis of Alzheimer's amyloid peptides via up-regulating BACE1 transcription. *Proc. Natl. Acad. Sci.* 106, 3907–3912. doi: 10.1073/pnas.0807991106
- Eggen, T., Asp, T.N., Grave, K., Hormazabal, V., 2011. Uptake and translocation of metformin, ciprofloxacin and narasin in forage- and crop plants. *Chemosphere* 85, 26–33. doi: 10.1016/j.chemosphere.2011.06.041
- Eggen, T., Lillo, C., 2012. Antidiabetic II Drug Metformin in Plants: Uptake and Translocation to Edible Parts of Cereals, Oily Seeds, Beans, Tomato, Squash, Carrots, and Potatoes. *J. Agric. Food Chem.* 60, 6929–6935. doi: 10.1021/jf301267c
- FGG Elbe, 2013. Flussgebietsgemeinschaft Elbe, Dataportal FIS ELBE [WWW Document]. URL <http://www.fgg-elbe.de/elbe-datenportal.html> (accessed 11.1.13).
- FGG Weser, 2013. Flussgebietsgemeinschaft Weser, Weserdatenbank [WWW Document]. URL <http://datenbank.fgg-weser.de/weserdatenbank/index.php> (accessed 11.1.13).
- Gartiser, S., Hafner, C., Kronenberger-Schaefer, K., Happel, O., Trautwein, C., Kuemmerer, K., 2012. Approach for detecting mutagenicity of biodegraded and ozonated pharmaceuticals, metabolites and transformation products from a drinking water perspective. *Environ. Sci. Pollut. Res.* 19, 3597–3609. doi: 10.1007/s11356-012-0925-x
- IAWR, 2008. IAWR Danube, Meuse and Rhine Memorandum 2008 [WWW Document]. URL http://www.riwa-maas.org/uploads/tx_deriwa/Donau-Maas-Rijnmemorandum_02.pdf (accessed 10.25.13).
- King, H., Aubert, R.E., Herman, W.H., 1998. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 21, 1414–1431. doi: 10.2337/diacare.21.9.1414
- Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000: A National Reconnaissance. *Environ. Sci. Technol.* 36, 1202–1211. doi: 10.1021/es011055j
- Kümmerer, K., 2008. *Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks*, 3rd enlarged ed. ed. Springer, Berlin.
- KWR, STOWA, O., 2013. KWO Watercycle Reserach Institute & STOWA Foundation for Applied Water Research: Human pharmaceuticals in the water cycle [WWW Document]. URL <http://www.stowa.nl/upload/publicaties/STOWA%202013%2023.pdf> (accessed 10.28.13).
- Leone, A., Di Gennaro, E., Bruzzese, F., Avallone, A., Budillon, A., 2014. New perspective for an old antidiabetic drug: metformin as anticancer agent. *Cancer Treat. Res.* 159. doi: 10.1007/978-3-642-38007-5_21
- Moschet, C., Goetz, C., Longree, P., Hollender, J., Singer, H., 2013. Multi-Level Approach for the Integrated Assessment of Polar Organic Micropollutants in an International Lake Catchment: The Example of Lake Constance. *Environ. Sci. Technol.* 47, 7028–7036. doi: 10.1021/es304484w

- van Nuijs, A.L.N., Tarcomnicu, I., Simons, W., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2010. Optimization and validation of a hydrophilic interaction liquid chromatography–tandem mass spectrometry method for the determination of 13 top-prescribed pharmaceuticals in influent wastewater. *Anal. Bioanal. Chem.* 398, 2211–2222. doi: 10.1007/s00216-010-4101-1
- O'Loughlin, S., Mahmood, W.W.A., Tun, K.T., McDermott, J.H., Sreenan, S., 2013. Vitamin B12 Deficiency in Diabetes Patients Treated with Metformin. *Ir. J. Med. Sci.* 182, S417–S418.
- Oosterhuis, M., Sacher, F., Ter Laak, T.L., 2013. Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. *Sci. Total Environ.* 442, 380–388. doi: 10.1016/j.scitotenv.2012.10.046
- Reitman, M.L., Schadt, E.E., 2007. Pharmacogenetics of metformin response: a step in the path toward personalized medicine. *J. Clin. Invest.* 117, 1226–1229. doi: 10.1172/JCI32133
- Scheurer, M., Michel, A., Brauch, H.-J., Ruck, W., Sacher, F., 2012. Occurrence and fate of the antidiabetic drug metformin and its metabolite guanyurea in the environment and during drinking water treatment. *Water Res.* 46, 4790–4802. doi: 10.1016/j.watres.2012.06.019
- Scheurer, M., Sacher, F., Brauch, H.-J., 2009. Occurrence of the antidiabetic drug metformin in sewage and surface waters in Germany. *J. Environ. Monit.* 11, 1608. doi: 10.1039/b909311g
- Schuster, A., Hädrich, C., Kümmerer, K., 2008. Flows of Active Pharmaceutical Ingredients Originating from Health Care Practices on a Local, Regional, and Nationwide Level in Germany—Is Hospital Effluent Treatment an Effective Approach for Risk Reduction? *Water Air Soil Pollut. Focus* 8, 457–471. doi: 10.1007/s11267-008-9183-9
- Schwabe, U., Paffrath, D., 2012. *Arzneiverordnungs-Report 2012: Aktuelle Daten, Kosten, Trends und Kommentare*, 1st Edition. ed. Springer, Berlin.
- Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers¹. *Water Res.* 32, 3245–3260. doi: 10.1016/S0043-1354(98)00099-2
- Trautwein, C., Kümmerer, K., 2011. Incomplete aerobic degradation of the antidiabetic drug Metformin and identification of the bacterial dead-end transformation product Guanyurea. *Chemosphere* 85, 765–773. doi: 10.1016/j.chemosphere.2011.06.057
- Turrell, W., 1992. New Hypotheses Concerning the Circulation of the Northern North-Sea and Its Relation to North-Sea Fish Stock Recruitment. *Ices J. Mar. Sci.* 49, 107–123. doi: 10.1093/icesjms/49.1.107
- US EPA, O., 2009. *Biosolids: Targeted National Sewage Sludge Survey Report* [WWW Document]. URL <http://water.epa.gov/scitech/wastetech/biosolids/tnsss-fs.cfm> (accessed 10.28.13).
- Vulliet, E., Cren-Olive, C., 2011. Screening of pharmaceuticals and hormones at the regional scale, in surface and groundwaters intended to human consumption. *Environ. Pollut.* 159, 2929–2934. doi: 10.1016/j.envpol.2011.04.033
- Weigel, S., Kuhlmann, J., Huhnerfuss, H., 2002. Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine

and DEET in the North Sea. Sci. Total Environ. 295, 131–141. doi: 10.1016/S0048-9697(02)00064-5

WHO, 2013. ATC/DDD Index, WHO Collaborating Centre for Drug Statistics Methodology, http://www.whocc.no/atc_ddd_index/ [WWW Document]. URL http://www.whocc.no/ddd/list_of_ddds_combined_products/ (accessed 10.23.13).

Wiegel, S., Aulinger, A., Brockmeyer, R., Harms, H., Löffler, J., Reincke, H., Schmidt, R., Stachel, B., von Tumpling, W., Wanke, A., 2004. Pharmaceuticals in the river Elbe and its tributaries. Chemosphere 57, 107–126. doi: 10.1016/j.chemosphere.2004.05.017

Wild, S., Roglic, G., Green, A., Sicree, R., King, H., 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27, 1047–1053. doi: 10.2337/diacare.27.5.1047

Wille, K., Noppe, H., Verheyden, K., Vanden Bussche, J., De Wulf, E., Van Caeter, P., Janssen, C.R., De Brabander, H.F., Vanhaecke, L., 2010. Validation and application of an LC-MS/MS method for the simultaneous quantification of 13 pharmaceuticals in seawater. Anal. Bioanal. Chem. 397, 1797–1808. doi: 10.1007/s00216-010-3702-z

Wolschke, H., Xie, Z., Moeller, A., Sturm, R., Ebinghaus, R., 2011. Occurrence, distribution and fluxes of benzotriazoles along the German large river basins into the North Sea. Water Res. 45, 6259–6266. doi: 10.1016/j.watres.2011.09.028

ZVBWV, 2013. Zweckverband Bodensee-Wasserversorgung (ZVBWV) [WWW Document]. URL <http://www.zvbwv.de> (accessed 11.1.13).

Supplementary Material

Supp. Table 1 - Measured STP influent and effluent concentrations for Metformin and Guanyurea

Sampling time	Influent Metformin ng/L	Influent Guanyurea ng/L	Ratio	Effluent Metformin ng/L	Effluent Guanyurea ng/L	Ratio	Elimination Metformin (%)
05/21/2012 (Mo)	142333	857	166	5100	67233	0.08	96.4
05/22/2012 (Tue)	86167	1987	43	4993	28233	0.18	94.2
05/23/2012 (Wed)	114333	1563	73	3387	37100	0.09	97.0
05/24/2012 (Thur)	97533	1233	79	6430	49167	0.13	93.4
05/24/2012 (Fri)	118667	850	139	3967	37833	0.11	96.7