BASE Project - Implementation of the Baltic Sea Action Plan in Russia

HELCOM

Baltic Marine Environment Protection Commission

Pilot activity

Identifying sources and flow patterns of pharmaceuticals in St. Petersburg to the Baltic Sea

BASE

Pilot Activity	Pilot activity to identify sources and flow patterns of pharmaceuticals in St. Petersburg to the Baltic Sea
Implemented by (Main Consultant)	Institution of Russian Academy of Sciences Saint-Petersburg Scientific-Research Centre for Ecological Safety (SRCES RAS)
Support provided by (EU Expert)	Niina Vieno (Envieno, Finland)
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Back cover	Johanna Laurila
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Summary

St. Petersburg with its population of over five million in 2013 is the largest megapolis on the Baltic Sea. It is also the largest single point of sales, consumption, excretion and, presumably, release of pharmaceutical substances into the Baltic Sea environment.

This study was the first of its kind to be carried out in St. Petersburg. Its aim was to analyse the load of pharmaceuticals entering and passing through the city's sewage system. A comprehensive sampling and chemical analysis campaign was planned and executed. Correlation with medical and pharmaceutical sales statistics, though limited, was also carried out – the available statistical data were found to be in a good agreement with the findings of the chemical analysis.

Initially, just two pharmaceutical substances were to be analysed: Diclofenac (DCF) and Ethinylestradiol (EE2). These two compounds are known to be present in the natural waters and are also known to cause harmful effects on the ecosystem. They are currently being developed by HELCOM as pre-core indicators.

Two sampling series were carried out at St. Petersburg's Central (CWWTP), and one series at the Northern (NWWTP) and the South-Western (SWWWTP) wastewater treatment plants (WWTPs). The first series focused on analysing Diclofenac and on developing and testing the sampling process. The second series was planned for DCF and EE2; however, the pharmaceutical sales statistics revealed a low use of EE2 in St. Petersburg and it was judged that the required detection limit (< 0.1 ng/L) would be difficult to achieve. The focus, therefore, shifted to a more diverse set of some 20 different pharmaceuticals with higher use and to the naturally produced human estrogens: Estron (E1), Estradiol (E2) and Estriol (E3).

The results show that the average concentration of DCF in the effluent varied from 355 ng/L in the summer of 2013 (CWWTP data only) to 510-550 ng/L in the winter of 2014 (all three WWTPs). The upper limit for daily release of DCF from the city can be estimated as 1.1 kg and thus the annual load is approximately 400 kg.

As the River Neva delivers an average annual volume of water of 80 km^3, the expected concentration of DCF in the water flowing into the Gulf of Finland is 5 ng/L.

It was found that the concentration of DCF in the effluent was often higher than that in unpurified sewage water. This phenomenon can be explained by the liberation of DCF from conjugated metabolites during bacterial treatment. Similar observations have been made at a number of WWTPs elsewhere in the world.

From the pharmaceutical sales statistics and population analysis of St. Petersburg, the amount of EE2 excreted into the sewage system did not exceed 315 g per year (assuming that 50% of estrogen excreted was unchanged; this is likely an overestimation). This would correspond to concentration in sewage of 0.4 ng/L. Even if no purification takes place during treatment, the expected concentration of EE2 in the water flowing from St. Petersburg into the Gulf of Finland would not exceed 0.004 ng/L, which is below the EQS of 0.007 ng/L.

Naturally produced human estrogen E1 was found in raw sewage and based on the results of the chemical analysis approximately 40 kg of E1 is excreted annually. This is in a very good agreement with estimation based on medical data for excretion rates in different age and gender groups – 35.2 kg/year.

In the effluent, E1 was detected in only three out of 31 samples; the average concentration in the effluent was therefore judged to be below the detection limit of 10 ng/L. Consequently, the highest possible annual release of E1 from St. Petersburg does not exceed 8 kg and the concentration of E1 in the water flowing from St. Petersburg into the Gulf of Finland does not exceed 0.08 ng/L.

Other naturally produced hormones, E2 and E3, were not detected in either the influent or effluent samples. It indicates the possible degradation of these two less stable hormones during their prolonged travel from the excretion point to the WWTP.

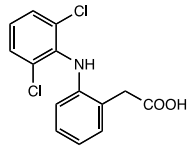
Eighteen other pharmaceutical substances of different classes and chemical nature were found in the raw sewage and effluent. Their levels ranged from tens to hundreds of ng/L. The highest maximal levels in the influent were found for Cyprofloxacin (871 ng/L), Ketoprofen (756 ng/L) and Enalapril (611 ng/L). Concentrations were significantly reduced in the effluent. Significant reduction during treatment was also observed for other pharmaceuticals, with the exception Clarythromycin. Its concentration in raw sewage was in the range of 100-200 ng/L and was rarely reduced by more than 50%.

The project has made a significant contribution to the flagship project “Make the Baltic Sea Region a Lead in Sustainable Management for Pharmaceuticals” under Priority Area 9 of the EU Strategy for the Baltic Sea Region (Hazardous Substances). This report offers reliable information on the levels of a range of pharmaceuticals and detailed data on Diclofenac. The data together with the sample bank collected by SRCES RAS will be a basis for accounting for the role of St.Petersburg, the largest Baltic Sea megapolis, in the management of pharmaceuticals.

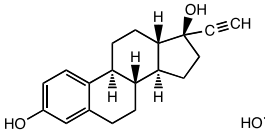
Furthermore, the results of the first series of analyses for Diclofenac were made available to the Ministry of Natural Resources and Environment of the Russian Federation through BASE project partner “Ecology and Business” and were taken into consideration in Russia’s reporting to the HELCOM Ministerial Meeting in 2013.

The data obtained in the project can be used to assess the overall release of pharmaceuticals on the gulf-basin and sea-basin scale. The data can also be used in the assessment of existing water treatment technology in St. Petersburg and in its development.

The comprehensive set of some 100 extracts of the collected samples from the three major WWTPs of St. Petersburg, both influent and effluent taken during different seasons, create a unique specimen bank. The samples are being kept frozen at SRCES (Institution of Russian Academy of Sciences Saint-Petersburg Scientific-Research Centre for Ecological Safety) and can be used for retrospective target analyses of pharmaceutical contaminants should they emerge in the future.



Diclofenac (DCF)



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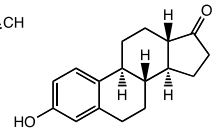
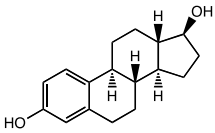
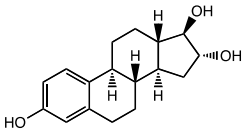


Estron (E1)



Estradiol (E2)



Estriol (E3)

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1. Sampling and the analysis of samples from the Central WWTP for Diclofenac (DCF) during summer 2013

The samples were collected over a three-week period (21.07.2013 – 08.08.2013) at the Central WWTP.

Samples were taken by WWTP personnel. Influent samples were taken over a 24-hour period on which the average was calculated. Effluent samples were grab samples taken on the same morning.

On several days, two samples were taken and analysed independently.

Samples were collected by SRCES personnel immediately afterwards and extracted within 24 hours. At the time of sampling, only ^{13}C-Diclofenac was available as a surrogate standard. It was introduced into the raw sample as it was. ^{13}C-labeled surrogates of steroid hormones became available by the end of the sampling campaign and were used later in several samples for test purposes.

Samples were analysed for Diclofenac on a secondary HPLC-HRMS, IT-TOF. Several of the samples were also analysed for Diclofenac on LTQ OrbiTrap; however, these results cannot be considered quantitative - they were used to confirm the range of concentrations and collect the test data for future analysis on the LTQ OrbiTrap.

Samples were screened for hormones. While no quantitative data can be derived, Estron (E1) was detected in a number of samples.

The data for Diclofenac are summarized in Tables 1 and 2 on the following pages.

Table 1. Primary analytical results for Diclofenac.

Date	Week day	Concentration of Diclofenac, ng/L (values in brackets – on LTQ OrbiTrap)	
		influent	effluent
21.07.13	Sun	396.1	374.7
22.07.13	Mon	253.2	493.8 (326)
		256.4	304.6
23.07.13	Tue	377.7	320.2 (239)
24.07.13	Wed	220.0	513.9 (286)
25.07.13	Thu	332.2	436.5
28.07.13	Sun	373.3	491.1
29.07.13	Mon	413.3 (494)	445.7 (452)
		441.1	321.9
30.07.13	Tue	685.5	369.9
31.07.13	Wed	250.0 (259)	203.2
		251.1	200.3
01.08.13	Thu	154.2	247.1
04.08.13	Sun	481.5 (452)	153.8
		491.8	299.2 (269)
05.08.13	Mon	741.0	344.4
06.08.13	Tue	428.8 (378)	310.2
		487.7	318.6
07.08.13	Wed	400.0	385.4
08.08.13	Thu	555.5	318.3

Table 2. Average concentrations of Diclofenac, athmospheric precipitation, volume of treated water, and the estimation of daily amounts of Diclofenac in raw sewage and discharged in the effluent.

Date	Day	DCF, ng/L		Rain, mm	Volume, 1000 x m³	DCF, est., g/day	
		Influent	**Effluent**			**Influent**	**Effluent**
21.07.13	Sun	396	375	8	1076	426	407
22.07.13	Mon	255	399	18	1216	310	485
23.07.13	Tue	378	320	6	1030	389	330
24.07.13	Wed	220	514	12	881	194	453
25.07.13	Thu	332	437	0	911	303	399
26.07.13	Fri			0	942		
27.07.13	Sat			0	877		
28.07.13	Sun	373	491	0	814	304	400
29.07.13	Mon	427	384	0	869	371	334
30.07.13	Tue	686	370	0	718	492	266
31.07.13	Wed	251	202	0	892	224	180
01.08.13	Thu	154	247	6	894	138	221
02.08.13	Fri			8	1117		
03.08.13	Sat			0	916		
04.08.13	Sun	487	227	0	850	414	193
05.08.13	Mon	741	344	0	830	615	286
06.08.13	Tue	458	314	0	877	402	275
07.08.13	Wed	400	385	0	841	336	324
08.08.13	Thu	556	318	0	905	503	288
Average		**408**	**355**			**362**	**323**

The comparison of results for 'duplicate' samples taken from the same type of water on the same day shows that for the influent, such 'duplicates' are very similar; for the effluent, however, they are often different (three out of five cases).

Concentrations in the influent were 150-740 ng/L with an average of 408 ng/L and concentrations in the effluent were 150-490 ng/L with an average of 355 ng/L. This means that Diclofenac is only removed to a minor extent.

There was no clear weekly trend; however, somewhat lower concentrations were detected on Wednesdays with the most on Mondays and/or Tuesdays.

The influent is a mix of domestic wastewater and rainwater. There is a correlation between meteorological data (precipitation) and the volume of processed water.

Detected concentrations in the effluent are often higher than in the influent (up to 2.5 times).

During the third week of sampling, the WWTP reported a fault (details might be available from Vodokanal upon request), during which time the detected concentrations in the effluent were always lower than in the influent.

This can be an indication of the known property of Diclofenac to form metabolites (conjugates) in the human body and to release them during wastewater treatment. Similar indications were observed in the course of previous work within frame of the BALTHAZAR project[1].

One possible explanation is that standard treatment does not actually remove diclofenac; rather, it destroys its derivatives (conjugates) and liberates diclofenac, thus increasing its concentration in the effluent (Fig. 1).

During the fault (third week), such liberation did not occur or occurred to a lesser extent; moreover, the lower detected concentrations in the effluent reflect sorption/destruction efficiency.

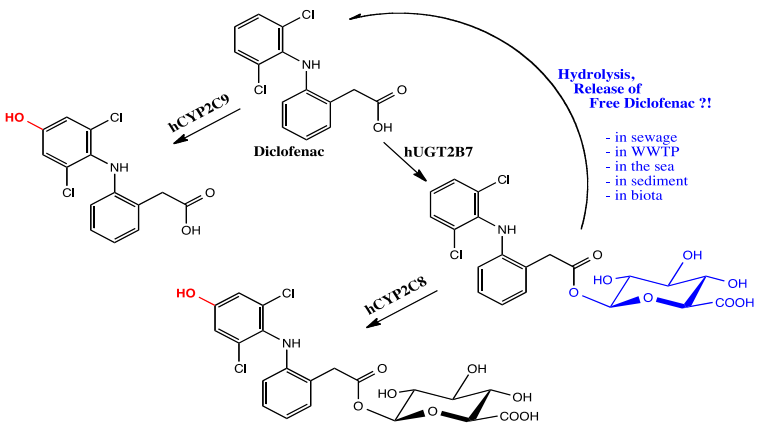

Fig. 1. Metabolic pathways for Diclofenac and how the hydrolysis of a conjugate may occur with the release of DCF.

Another reason for the observed increase in the Diclofenac level after treatment can be the release of DCF from related pharmaceuticals, such as Aceclofenac, which is also registered in the Russian Federation and available at St. Petersburg pharmacies (Fig. 2).

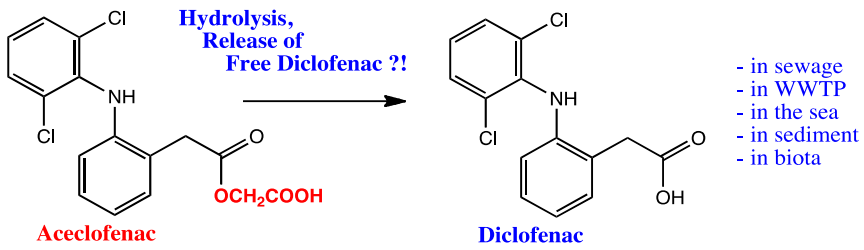

Fig. 2. Hydrolysis of Aceclofenac leading to DCF.

These possible transformations in DCF-related compounds pose very interesting scientific questions and demand careful planning of the future sampling/analysis work on DCF.

[1] Identification of sources for hazardous substances in St. Petersburg area. Balthazar Project Report. HELCOM, 2012.

The conclusion from the first series of analyses is that the concentration of diclofenac in the effluent is hundreds of ng/L and the currently employed method at this WWTP is not satisfactory for the removal of DCF.

The annual release of Diclofenac into the Baltic Sea from St. Petersburg, based on results of the chemical analysis of the first series of samples, was provisionally estimated as 308 kg per year (the population of the Central WWTP collection basin is some 1.95 million; the population of St. Petersburg is 5.1 million; and the average volume of treated water is 2.2 million m^3/day[2]).

The average annual water discharge of the River Neva is 78.9 km^3 (2,500m^3/s).

Thus, the average concentration of Diclofenac in water leaving the St. Petersburg area should not exceed 4 ng/L.

2. Analysis of the sales data and the estimation of consumption of Diclofenac in the Russian Federation and St. Petersburg

Comprehensive information on sales and the consumption of Diclofenac in St. Petersburg is difficult to collect. A total of 87 different preparations containing Diclofenac were registered for use in the Russian Federation in 2009-2010. Moreover, Diclofenac is used in a variety of forms: in pills, in ampoules for injections, in rectal suppositories, in gels and ointments, etc.

In the one-year period from May 2012 to April 2013, sales of Non-Steroid Anti-Inflammatory drugs for external application in Russia totalled RUB 3.9 billion or 33.9 million packages[3]. Voltaren and Diclofenac are the two leaders with 4.7 million packages each. One unit of Voltaren Gel (20 g, 1% costing RUB 190) contains 0.2 g of Diclofenac. Also, 50, 75 and 100 g units are available on the market. Similar packages are also available for the Diclofenac and other brands, estimated at 4.7 million packages altogether. Diklak gel is also available as a 5% preparation in 50 g tubes. Yearly sales of Diklak are RUB 145 million and at a unit price of RUB 170 it corresponds to 2.13 tonnes of Diclofenac substance. It is not known, however, what proportion of Diklak is sold with an active ingredient of either 5% or 1%. Therefore, the total consumption of Diclofenac substance for external application only is estimated from these data as 14 million units per year or 2.8 tonnes. Based on calculations, the use of Diclofenac in St. Petersburg is estimated at 170 kg. In the case that all Diclofenac enters the sewage system intact, its concentration may reach 200 ng/L.

An independent source gives indicative numbers for Diclofenac consumption in the form of pills, injections and suppositories by three leading brands.

[2] SUE “Vodokanal of St. Petersburg”
[3] Sales of NSAID in pharmacies for external application. Moskovskie Apteki. 27.05.2013 (in Russian)

Table 3. Consumption of different brands and forms of Diclofenac in Russia.

	Injections	Pills	Suppositories
Diclofenac	6,731,799	5,958,925	900,616
Ortophen	144,030	6,155,850	26
Voltaren	1,168,177	232,503	489,748
Diclofenac per package, g	0.5 g	1 g	0.5 g
Diclofenac per form	4 tonnes	12 tonnes	0.75 tonnes

The total consumption of Diclofenac in Russia is estimated at 20 tonnes per year. The same source estimates that 27 million Diclofenac units were sold in 2011. The total purchases of Diclofenac by hospitals in 2010 were 900,000 packages for injection and less than 100,000 in pills making a total annual consumption of 20 tonnes.

The share of St. Petersburg would be some 700 kg per year of DCF in all forms. This translates to 850 ng/L in sewage if all purchased Diclofenac enters the WWTP unchanged.

Experimentally determined concentrations are only two times lower than the above estimation. This is a warning. Sources suggest that less than 1% of Diclofenac is excreted unchanged and about 10% in as glucuronide metabolite; therefore, only an externally applied substance can be a significant contributor to the levels in sewage. In this case, the concentration in the wastewater in St. Petersburg would not exceed 200 ng/L.

Possible reasons for the inconsistencies between the pilot market analysis and the empirical data can be: a) a dramatic difference between reported and real data on Diclofenac sales in Russian pharmacies; b) incorrect information on Diclofenac metabolism in pharmaceutical instructions; or c) massive back-transformation of Diclofenac derivatives into free Diclofenac in the sewage and during treatment.

3. Sales of oral contraceptive pills and the estimation of consumption of EE2 in the Russian Federation and in St. Petersburg

Over a four-month period in 2009, sales of oral contraceptive pills in the Russian Federation totalled USD 82.5 million and 5.1 million packages. This corresponds to annual sales of USD 248 million and 15.3 million packages[4]. As a year includes some thirteen 28-day cycles, this number corresponds to approximately 1.2 million women regularly taking oral contraceptive pills.

In the first quarter of 2012, the sales of systemic oral contraceptives in Russia accounted for 2.05% of the total market (USD 4.53 billion)[5]. The estimation for the whole of 2012 is USD 371 million spent on oral contraceptives in Russia. Assuming an average price of a package was USD 24 in that period, the sales of oral contraceptives in Russia in 2012 were 15.5 million packages. As there was no significant increase in the use of oral contraceptives from 2009-2012, the expected consumption of oral contraceptives in 2013 is 15-16 million packages.

The population of the Russian Federation was 143.3 million on 1 January 2013. The percentage of women was 54% in 2009 (77.4 million), of which 1.5% of them used oral contraception pills. Moreover, the number of women of reproductive age (14-49 years) is 38 million with some 3% using oral contraception pills.

The population of St. Petersburg in 2013 was five million. The city has one of the highest rates of female domination in Russia: 1,225 women per 1,000 men; 55% of the city's population are women (2.75 million). An estimated 1.3 million belong to the reproductive age (14-49 years)[6]. According to the nation-wide average, some 39,000 would be regularly taking oral contraceptives in St. Petersburg. However, due to a higher level of education and a higher income level (the price for an oral contraceptive package may cost up to RUB 1,000) we can expect a higher number of consumers.

The recent data on consumer sales of pharmaceuticals in St. Petersburg, including hormones, are summarized in Table 4[7].

Table 4. Total sales of pharmaceuticals and sex hormones in St. Petersburg.

	Jan - Jun 2012	Jan – Jun 2013
Retail market, EUR million	243	301
Retail market, million packages	76.4	77.9
Sales of sex hormones, EUR million	7.3	9.3

Table 4 shows that the sales of hormones in St. Petersburg (mostly oral contraceptives) were EUR 9.3 million in the first six months of 2013, corresponding to annual sales of EUR 18.6 million (RUB 800 million).

There are no domestic hormonal contraceptives on the market. The most popular brands in 2009 were Jarina, Janine and Diane-35 (*Ярина, Жанин, Диане-35*) produced by Bayer, holding 40% of the market.

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[4] Ekaterina Zaichenko. Oral contraceptives. Aptekar. 2009, 9-10 (in Russian).
[5] Market review. InPharmacia, 4(102), 2012, p. 18 (in Russian).
[6] Socio-demographical characterization of population of St. Petersburg (in Russian).
[7] St. Petersburg hospital market, results of 6 months of 2013. Klinicheskaya Farmacia. 02.10.2013 (in Russian).

One package of these pills contains 0.630, 0.630 and 0.735 mg of ethynylestradiol with the current average retail price of RUB 850, 810 and 850, respectively[8]. It can be deduced, therefore, that the average content of ethynylestradiol is 0.665mg per package and average price is RUB 840 per package.

Note: The annual spending for oral contraception is RUB 10,080 per user. This exceeds the average personal pharmacy spending of RUB 4,400 per capita in Russian Federation.

The total annual sales in St. Petersburg are approximately 950,000 packages per year, which corresponds to 73,000 women or 6% of women of reproductive age in the city.

Note: This number is two times higher than the previously derived percentage of women taking oral contraceptives in Russia (3%). Assuming a similar or higher rate in Moscow, these two cities consume about 20% of all oral contraceptives in the country.

The total amount of ethynylestradiol consumed in St. Petersburg is therefore 630 g per year. This is the maximal estimation of possible input into the Baltic Sea from St. Petersburg.

The daily consumption in the city is approximately 1.7 g; therefore, if 100% ethynylestradiol reaches the WWTP, its average concentration in the wastewater might reach 0.8 ng/L.

According to Niina Vieno, the amount of unchanged EE2 excreted by humans is approximately 1/3 of amount taken orally[9]. Therefore, the annual excretion of unchanged EE2 in St. Petersburg is 210 g per year.

This translates to a maximum concentration of 0.33 ng/L in sewage water.

A part of EE2 is known to be excreted as glucuronide conjugates that can release free EE2 during the course of its life in sewage, treatment at the WWTP or in the sea after discharge. In this scenario, the total portion of EE2 is still not more than 50% of the consumed amount, or 315 g per year, which translates to a maximum 0.4 ng/L in the sewage water.

The value of 315 g per year of EE2 can be considered as the highest possible annual discharge from St. Petersburg into the Baltic Sea.

In 2012, the average volume of treated water was 2.2 million m^3/day (98.4% of all water was treated). The annual consumption of water in St. Petersburg is approximately 800 million m^3 per year[10] and the average annual water discharge into the River Neva is 78.9 km^3 (or 2,500m^3/s).

Thus, the average concentration of EE2 in water leaving the St. Petersburg area should not exceed 0.004 ng/L. This is below the very strict EQS (0.007 ng/L). It is unlikely, therefore, that discharges of EE2 from St. Petersburg cause negative effects on the basin-scale (Gulf of Finland).

However, the monitoring of EE2 and its possible effects in the proximity of WWTP discharges is required after routine analytical methods have been established for such low concentrations.

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[8] Diclofenac.
[9] Niina Vieno. Estimation of human excretion and sewage concentrations of estrogens E1, E2, E3 and EE2 in Finland. Envieno, 2013.
[10] SUE "Vodokanal of St. Petersburg"

4. Estimates of the excretion of E1, E2 and E3 in St. Petersburg and their discharges into the Baltic Sea

In addition to EE2, the estrogenic effect can be caused by naturally produced human estrogens; for this reason, an estimation of such release was carried out as part of the project (see Table 5 below).

Table 5. Excretion of estrogens by the population of St. Petersburg.

		Excretion per person, ug/day[11]	Excretion by group, g/day	Annual release, kg/year	E2 equivalent, kg/year	Expected level in sewage water, ng/L
E1	G1	4.9	1.5			
	G2	11	13.8			
	G3	1,300	65	23.7	2.37	
	G4	5.3	6.1			
	G5	4.5	10.1			
	All groups		**96.5**	**35.2**	**3.52**	**48.3**
E2	G1	3.7	1.11			
	G2	5	6.3			
	G3	550	27.5		10	
	G4	2.9	3.3			
	G5	4.5	10.1			
	All groups		**48.3**	**17.6**	**17.6**	**24.2**
E3	G1	2.2	0.66			
	G2	8.1	10.1			
	G3	24,100	1205		4.40	
	G4	2.8	3.2			
	G5	1.5	3.4			
	All groups		**1,222.4**	**446**	**4.46**	**611**
EE2	**G6**	**12**	**0.6**	**0.32**	**0.32**	**0.43**
Total hormones					**25.9**	

G1 – female, age 10-14, 300,000; G2 – female, age 15-49, 1,250,000; G3 - female, pregnant, 50,000; G4 – female, age 50+, 1,150,000; G5 – male, all ages, 2,250,000; G6 – pregnant, 50,000

The relative estrogen activities used for Table 5 were 1 for E2 and EE2; 0.1 for E1; and 0.01 for E3.

The proportion of each group was estimated using statistical data for St. Petersburg's population (Figs. 3 and 4)[12].

[11] Niina Vieno. Estimation of human excretion and sewage concentrations of estrogens E1, E2, E3 and EE2 in Finland. Envieno, 2013.
[12] Demografija. Administration of St. Petersburg, official site.

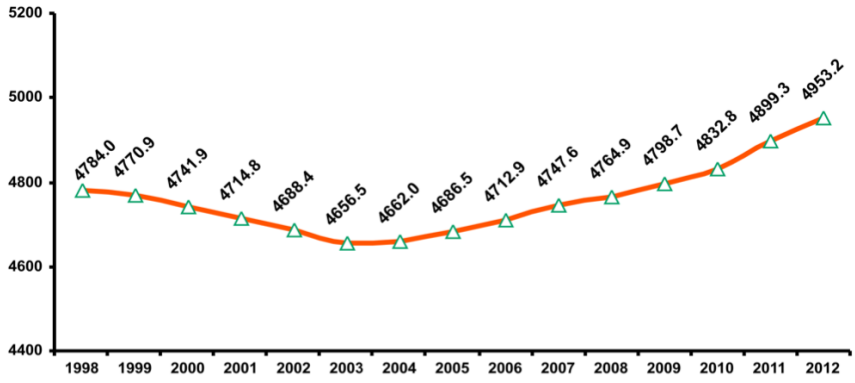
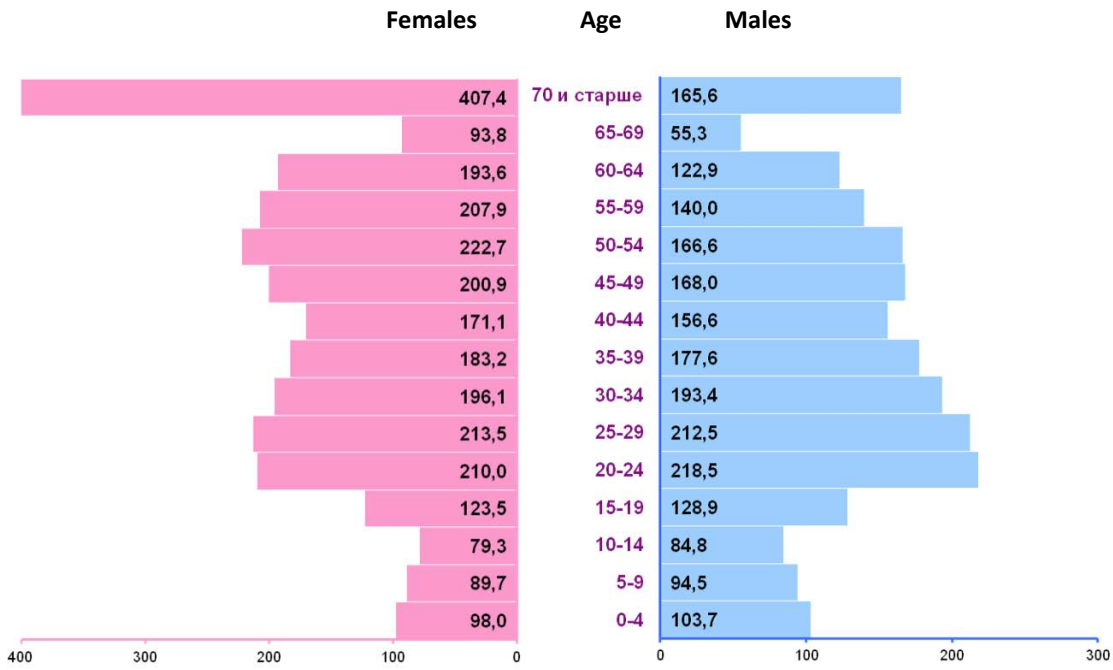


Fig. 3. Population dynamics in St. Petersburg (1998-2012).



Population in thousands (2012).

Fig. 4. Age/gender chart for St. Petersburg (2012).

The number of pregnant women in St. Petersburg was estimated from the annual number of births. There were 56,000 in 2010; 57,000 in 2011; and 62,000 in 2012. The number of births in 2013 is estimated at 65,000. Therefore, the average number of pregnant women in the city is 65x280/365 = 50,000.

It is noteworthy that pregnant women are responsible for the excretion of 67%, 57% and 99% of the total excretion of E1, E2 and E3, respectively. The birth rate in St. Petersburg is growing from the absolute minimum of 29,438 newborns in 1999 to 62,253 in 2012; the total fertility rate changes accordingly (Fig. 5). Presumably, the release of hormones grew substantially in the past decade.

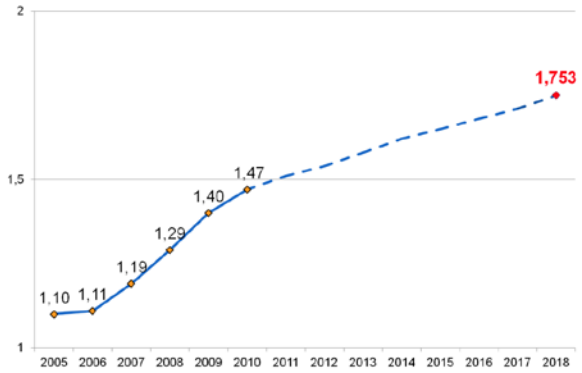


Fig. 5. Total fertility rate dynamics in St. Petersburg and the target value for 2018.

By the Decree of The President of Russian Federation #606 of 07.05.2012, the Total fertility Rate in St. Petersburg must reach 1.753 in 2018[13], which corresponds to 70,000 newborns per year. In this case, a significant increase of estrogen excretion is unlikely.

The record number of newborns registered in St. Petersburg was 73,275 in 1987 with the lowest being 29,438 in 1999. During the period 1993-2001, the number of newborns was below 35,000, roughly two times lower than at present. The estimated estrogen release in E2 equivalent was 17.2 kg/year in 1999, two-thirds of the present value.

The contribution of Ethynylestradiol into the total estrogen activity of sex hormones released into sewage and into the Baltic Sea is negligible (1.2%). Therefore, its importance and relevance as an indicator of pollution status is further doubted. However, the importance of EE2 can be higher if it is proven to be extremely stable.

Further, it is necessary to investigate suggested reversible transformations of all considered hormones into metabolites (conjugates) and back into the active form. The possible formation of more estrogenic derivatives in the sewage, during wastewater treatment or in the environment is a desired subject for scientific studies.

It is also necessary to take into account the release of natural estrogens by other species. Farming is a significant sector in the basin of the River Neva. The scale of farming can be seen from the data for Leningrad region (Fig. 6).

[13] Demografija. Administration of St. Petersburg, official site.

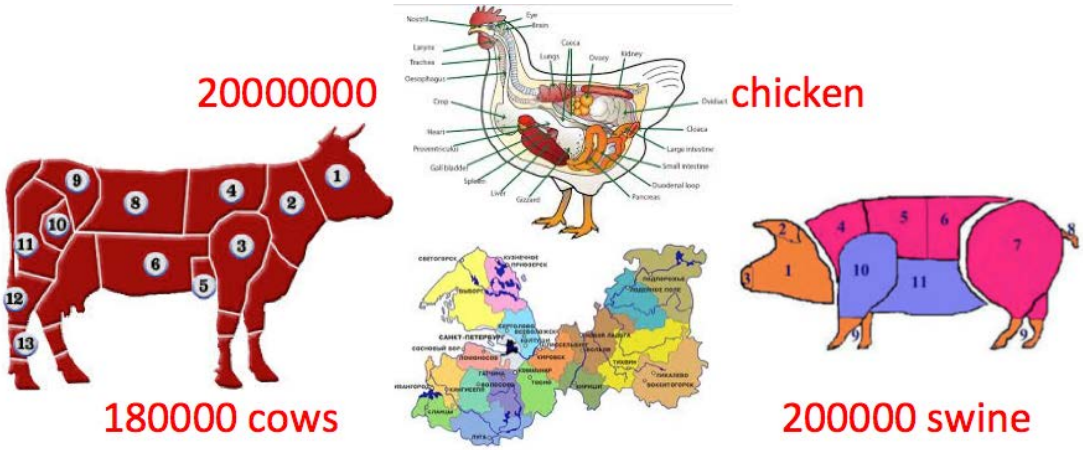


Fig. 6. Cows, swine and chicken in Leningrad Oblast (2013).

The optimal strategy for monitoring this class of compounds would be to use biomethods for total estrogeneicity combined with the monitoring of ecosystem health and target chemical analysis in selected cases.

5. Second sampling campaign: sampling and the chemical analysis of samples from the Central, Northern and South-Eastern WWTPs during February - April 2014

The samples were collected over a three-month period (19.02.2014 – 10.04.2014) at St. Petersburg's three main WWTPs: Central (capacity: 2 million m^3/d), Northern (2 million m^3/d) and South-Western (0.5 million m^3/d).

The area served by these three plants is shown in Fig. 7 on the next page.

Fig. 7. Canalization scheme of St. Petersburg and its suburbs and the sewage collection basins for the main WWTPs.

The WWTPs where research was carried out are shown as large red circles: NWWTP top; CWWTP centre; SWWWTP bottom. The collection areas are shown in light-brown, light-grey and pink, respectively.

The populations of the respective regions are 2,257,000 for the Northern WWTP; 1,958,000 for the Central WWTP; and 540,000 for the South-Western WWTP (as of 1 January 2014).

Samples were taken by WWTP personnel. The second series of samples, both influent and effluent, were collected from 09.00 in the morning until 09.00 the following morning.

At the Central and South-Western WWTPs, influent samples were collected on Mondays and Tuesdays (‘Sunday’ and ‘Monday’ samples respectively). Effluent samples were collected on Tuesdays and Wednesdays (‘Monday’ and ‘Tuesday’ samples respectively). The samples were transferred to SRCES on Tuesdays and Wednesdays after being refrigerated at the WWTPs for 27-30 hours. Upon delivery to SRCES, the lab samples were kept in a refrigerator and extracted within 24 hours.

At the NWWTP, influent samples were collected on Mondays and Wednesdays (‘Sunday’ and ‘Tuesday’ samples respectively). Effluent samples were collected on Tuesdays and Thursdays (‘Monday’ and ‘Wednesday’ samples, respectively). The samples were transferred to SRCES on Tuesdays and Thursdays after being refrigerated at the WWTPs for 27-30 hours. Upon delivery to SRCES, the lab samples were kept in a refrigerator and extracted within 24 hours. On several days, two samples were taken and analysed independently.

The amount of processed raw sewage in the study period is given in Table 6. The values were calculated over a 24-hour period from midnight to midnight; the time does not, however, correspond exactly to the time when the samples were actually taken (09.00 to 09.00 the following morning). Nevertheless, these values are used in the calculations.

The amount of processed water differs significantly on different days (for the Northern WWTP by a factor of 2.6, from 571 m^3 to 1,495,000 m^3; for the Central WWTP by a factor of 2.1, from 647 m^3 to 1,380,000 m^3; for the South-Eastern WWTP by a factor of 1.5, from 206 m^3 to 306,000 m^3). Therefore, such data need to be taken into account for load assessment.

Table 6. Amount of processed water (thousands m^3) (data from Vodokanal).

Date	N WWTP	C WWTP	SW WWTP	Date	N WWTP	C WWTP	SW WWTP
16.02.14	679	1,126	230	22.03.14	1135	687	255
17.02.14	761	1,380	266	23.03.14	1495	908	306
18.02.14	760	1,329	250	24.03.14	1303	736	258
19.02.14	608	1,263	250	25.03.14	1002	674	241
20.02.14	576	1,190	222	26.03.14	881	647	230
21.02.14	577	1,179	213	27.03.14	928	661	223
22.02.14	673	1,137	223	28.03.14	959	687	218
23.02.14	753	1,244	236	29.03.14	821	779	221
24.02.14	995	1,044	241	30.03.14	822	789	227
25.02.14	856	990	233	31.03.14	852	792	226
26.02.14	571	1,054	222	01.04.14	833	780	211
27.02.14	601	1,142	219	02.04.14	833	732	212
28.02.14	629	1,124	215	03.04.14	806	738	224
15.03.14	1056	885	257	04.04.14	816	792	215
16.03.14	1,114	1,000	255	05.04.14	774	826	222
17.03.14	972	893	223	06.04.14	911	861	245
18.03.14	891	835	221	07.04.14	813	826	218
19.03.14	883	876	216	08.04.14	750	804	212
20.03.14	951	881	226	09.04.14	740	797	213
21.03.14	1,173	837	248	10.04.14	754	812	213

Standard retention time of the water at the WWTPs is 18 hours. Thus, for instance, the 'Sunday' influent and the 'Monday' effluent do not correspond to each other exactly. However, in this pilot activity such approximation was deemed to suffice.

6. Second sampling campaign: results of the chemical analysis for Diclofenac

The data for individual samples are summarized in Table 7 on the next page. A number of mass-spectrometrical acquisition methods were employed to avoid the effect of interferences. Also, a number of samples were extracted by two different procedures (LLE and SPE) and analysed independently. The detailed data are given in Appendix 1.

Table 7. Concentration of DCF in the raw sewage and effluent at three WWTPs of St. Petersburg

	South-Western WWTP			Central WWTP			Northern WWTP		
	Vol. m^3x10^3	infl, ng/L	effl, ng/L	Vol. m^3x10^3	infl, ng/L	effl, ng/L	Vol. m^3x10^3	infl, ng/L	effl, ng/L
Tue 19.02.14	251	500	430	1,264	590	470	608	630	520
Sun 23.02.14	237	530		1,244	270		753	410	
Mon 24.02.14	242	540	540	1,044	160	470	995		450
Tue 25.02.14	233		600	991		630	856	500	
Wed 26.02.14	223			1,054			571		600
Sun 16.03.14	256	630		1,000	320		1,114		
Mon 17.03.14	224	620	450	894	1,700*	440	972		
Tue 18.03.14	222		540	835		530	891		
Sun 23.03.14	307	490		908	450		1,495	270	
Mon 24.03.14	259	710	580	737	310	520	1,303		190
Tue 25.03.14	242		480	675		500	1,002	430	
Wed 26.03.14	231			648			881		630
Sun 30.03.14	227	630		790	400		822	400	
Mon 31.03.14	226	610	620	793	350	580	852		600
Tue 01.04.14	211		750	781		600	833	600	
Wed 02.04.14	212			733			833		500
Sun 06.04.14	245	740		861	390		911	470	
Mon 07.04.14	218	800	550	827	300	430	813		460
Tue 08.04.14	212		540	804		500	750	530	
Wed 09.04.14	213			798			740		610
Average	**235**	**620**	**550**	**884**	**350**	**530**	**942**	**470**	**510**

* - this value is an outlier and was not taken into averaging and general consideration.

Due to the different amounts of water processed on different days, the data were converted to g/day/WWTP format in Table 8. This table summarizes input and output of Diclofenac at the three WWTPs in grams per day, after correction of experimentally determined concentrations to daily volumes of water processing. It also gives an estimated daily release of Diclofenac per resident of a collection basin.

Table 8. Diclofenac: output of WWTPs in grams per day and the average in micrograms per inhabitant in the WWTP collection area.

	South-Western WWTP			Central WWTP			Northern WWTP		
	Vol. m^3x10^3	In, g/day	Out, g/day	Vol. m^3x10^3	In, g/day	Out, g/day	Vol. m^3x10^3	In, g/day	Out, g/day
19.02.14	251	126	108	1,264	746	594	608	383	316
23.02.14	237	126		1,244	336		753	309	
24.02.14	242	131	128	1,044	167	585	995		339
25.02.14	233		145	991		658	856	428	
26.02.14	223			1,054			571		514
16.03.14	256	161		1,000	320		1,114		
17.03.14	224	139	115	894	1,520	440	972		
18.03.14	222		121	835		474	891		
23.03.14	307	150		908	409		1,495	404	
24.03.14	259	184	178	737	228	472	1,303		284
25.03.14	242		124	675		369	1,002	431	
26.03.14	231			648			881		631
30.03.14	227	143		790	316		822	329	
31.03.14	226	139	141	793	278	400	852		493
01.04.14	211		170	781		476	833	511	
02.04.14	212			733			833		417
06.04.14	245	181		861	336		911	428	
07.04.14	218	174	135	827	248	370	813		419
08.04.14	212		118	804		414	750	398	
09.04.14	213			798			740		458
Average	**235**	**150**	**135**	**884**	**338**	**477**	**942**	**402**	**430**
max		**184**	**178**		**746**	**658**		**511**	**631**
min		**126**	**115**		**167**	**369**		**309**	**284**
per person, µg/day		**278**	**250**		**172**	**244**		**178**	**191**

The average daily amount of diclofenac entering the three major WWTPs was 890 g. The total amount of diclofenac in the effluent discharged daily is 1,042 g, which corresponds to an annual discharge of 380 kg. Based on the results of the second sampling campaign and assuming proportional discharge from other smaller WWTPs, the total annual discharge from St. Petersburg can be estimated at 400 kg. This value is higher than the estimation obtained after Phase 1 (308 kg). One reason for this could be a higher daily discharge observed in the cold season (Phase 2) than in the warm season (Phase 1) - 323 g/day and 477 g/day, respectively. This can be explained by an increased use of diclofenac during the cold period. Of note is a similar input of Diclofenac into the CWWTP (experimental values – 362 g/day and 338 g/day in the warm and cold seasons). A plausible explanation is that in summer, the use (and excretion) of Diclofenac is lower and a significant part of glucuronide metabolites were hydrolyzed back to Diclofenac while traveling in the sewage system. A small part of Diclofenac was degraded or absorbed during the course of treatment; the result is a 10% decrease in the observed concentration in the effluent.

In winter, the use of Diclofenac is higher; also, fewer glucuronides are hydrolyzed during travel in colder temperatures. In the course of the treatment process, a significant part of glucuronides are hydrolyzed into Diclofenac, which leads to an increase of observed concentration in the effluent (> 40%). At the NWWTP, such increase is moderate being 7%. One explanation could be a longer

residence time in a sewer system and the degradation of a major part of glucuronide before entering the WWTP. However, if per person discharge is considered, it suggests that this parameter is 25% lower at the NWWTP. Therefore, it is likely that the water treatment process at the NWWTP leaves a significant part of glucuronides intact. Of course, a lower use of Diclofenac in the NWWTP as compared to the CWWTP can also be a factor.

A record number of amount of DCF per person in both raw water and in the effluent was observed at the SWWWTP. The net result of treatment is a 10% decrease in the amount of Diclofenac. This high input per person is difficult to explain: the collection area is rather small and the residence time in the sewer is the shortest of all three plants. For this reason, the highest proportion of glucuronides is expected with the highest increase of concentration in the effluent, which is not the case.

The results clearly indicate the need for a better definition of Diclofenac as an environmental contaminant. Correct consideration of its conjugates is required for an accurate assessment of the total load and its possible effects on the ecosystem.

An example of mass-chromatographic detection of Diclofenac in the sewage extract is given in Fig. 8 on the next page (CWWTP, untreated influent, March 31, 2014).

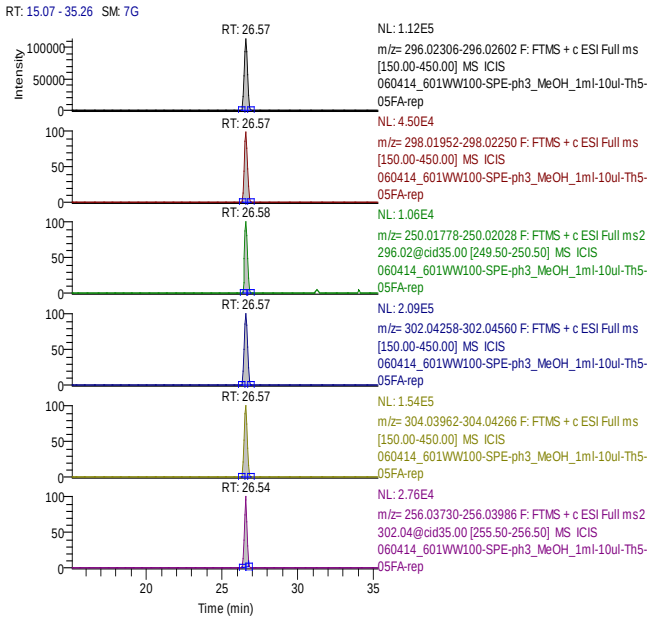
DCF, SIM, m/z 296.025	
DCF, SIM, m/z 298.021	
DCF, MRM, m/z 296.02 → m/z 250.019	
$^{13}C_6$-DCF, SIM, m/z 302.044	
$^{13}C_6$-DCF, SIM, m/z 304.041	
$^{13}C_6$-DCF, MRM, m/z 302.04 → m/z 256.039	

Fig. 8. DCF and $^{13}C_6$-DCF detected in different MS modes.

7. Second sampling campaign: results of the chemical analysis for Estrogens

The same samples that were analysed for DCF have been studied for the presence of hormones. As was expected, Ethinylestradiol (EE2) was not detected in any of the samples. Surprisingly, no Estradiol (E2) or Estriol (E3) were detected either, despite the estimated concentrations in sewage of 24 and 610 ng/L, respectively. The plausible explanation is the degradation of these two compounds in the sewage system or, in the case of E2, its possible transformation to Estrone (E1).

E1 was found in almost every influent sample; the maximal observed concentration being 122 ng/L. The data are summarized in Tables 9 and 10 (g/day/WWTP).

With two exceptions (both at the SWWWTP), E1 was not detected in the effluent. From this, it can be concluded that the average concentration of E1 in the effluent does not exceed 10ng/L (typical detection limit for this series of samples) making the total discharge from the population of St. Petersburg lower than 8 kg per year; and that the concentration of E1 in the water streaming from St. Petersburg area into the Gulf of Finland is below 0.1 ng/L.

Table 9. Concentration of E1 in the raw sewage and effluent at three WWTPs in St. Petersburg.

	South-Western WWTP			Central WWTP			Northern WWTP		
	Volume m^3x10^3	infl, ng/L	effl, ng/L	Volume m^3x10^3	infl, ng/L	effl, ng/L	Volume m^3x10^3	infl, ng/L	effl, ng/L
Tue 19.02.14	251	28	<10	1,264	61	<10	608	38	<10
Sun 23.02.14	237	37		1,244	49		753	64	
Mon 24.02.14	242	122		1,044	72	<10	995		<10
Tue 25.02.14	233		49	991		<10	856	42	
Wed 26.02.14	223			1,054			571		<10
Sun 16.03.14	256	115		1,000	79		1,114		
Mon 17.03.14	224	54	349*	894	59	<10	972		
Tue 18.03.14	222		<10	835		<10	891		
Sun 23.03.14	307	83		908	43		1,495	62	
Mon 24.03.14	259	<10	<10	737	33	<10	1,303		<10
Tue 25.03.14	242		<10	675		<10	1,002	63	
Wed 26.03.14	231			648			881		<10
Sun 30.03.14	227	27		790	28		822	21	
Mon 31.03.14	226	<10	<10	793	<10	<10	852		<10
Tue 01.04.14	211		<10	781		<10	833	21	
Wed 02.04.14	212			733			833		<10
Sun 06.04.14	245	38		861	47		911	95	
Mon 07.04.14	218	25	<10	827	31	<10	813		22
Tue 08.04.14	212		<10	804		<10	750	58	
Wed 09.04.14	213			798			740		76*
Average	**235**	**48**	**<15**	**884**	**46**	**<10**	**942**	**46**	**<12**
max		**122**	**49**		**79**	**<10**		**42**	**22**
min		**<10**	**<10**		**<10**	**<10**		**19**	**<10**

* This value is an outlier and is not taken into averaging and general consideration

There were significant differences in the concentrations of E1 in the raw influent on different days at each of the three WWTPs. The average concentrations, however, were nearly identical being 48, 46 and 46 ng/L. Moreover, the average daily input per person was also found to be similar at the three WWTPS: 22.4, 23.1 and 24.4 micrograms/per/day (Table 10 on the next page).

Table 10. Input of Estrone into the WWTPs in grams per day and the average in micrograms per person residing in the WWTP collection area.

	South-Western WWTP		Central WWTP		Northern WWTP	
	In g/day	Out g/day	In g/day	Out g/day	In g/day	Out g/day
Tue 19.02.14	7.0	n/a	77.1	n/a	23.1	n/a
Sun 23.02.14	8.8		61.0		48.2	
Mon 24.02.14	29.5		75.2	n/a		n/a
Tue 25.02.14		11.9		n/a	36.0	
Wed 26.02.14						n/a
Sun 16.03.14	29.4		79.0			
Mon 17.03.14	12.1	89.3	52.7	n/a		
Tue 18.03.14		n/a		n/a		
Sun 23.03.14	25.5		39.0		92.7	
Mon 24.03.14	n/a	n/a	24.3	n/a		n/a
Tue 25.03.14		n/a		n/a	63.1	
Wed 26.03.14						n/a
Sun 30.03.14	6.1		22.1		17.3	
Mon 31.03.14	n/a	n/a	n/a	n/a		n/a
Tue 01.04.14		n/a		n/a	17.5	
Wed 02.04.14						n/a
Sun 06.04.14	9.3		40.5		86.5	
Mon 07.04.14	5.5	n/a	25.6	n/a		20.0
Tue 08.04.14		n/a		n/a	43.5	
Wed 09.04.14						57.0
average	**12.1**	**1.1**	**45.2**	**nd**	**55**	**7.4**
max	**29.5**	**11.9**	**40**	**n/a**	**42**	**57.0**
min	**n/a**	**n/a**	**n/a**	**n/a**	**19**	**n/a**
resident, µg/day	**22.4**	**2.2**	**23.1**	**-**	**24.4**	**3.3**

The total amount of E1 arriving at the WWTPs is 112.3 g/day. Corrected for the population outside the combined collection basin of the three WWTPs, it gives an amount of 118 g/day of E1 excreted by the residents of St. Petersburg. This corresponds with our estimation of 96 g/day for Phase 1, based on medical data on the excretion of hormones. However, the difference between experimental and estimated values can be an indication of a higher pregnancy rate in 2014 or of a higher proportion of pregnancies covering the period February-April.

Examples of mass-chromatograms for the determination of E1, E2 and EE2 are given in Figure 9.

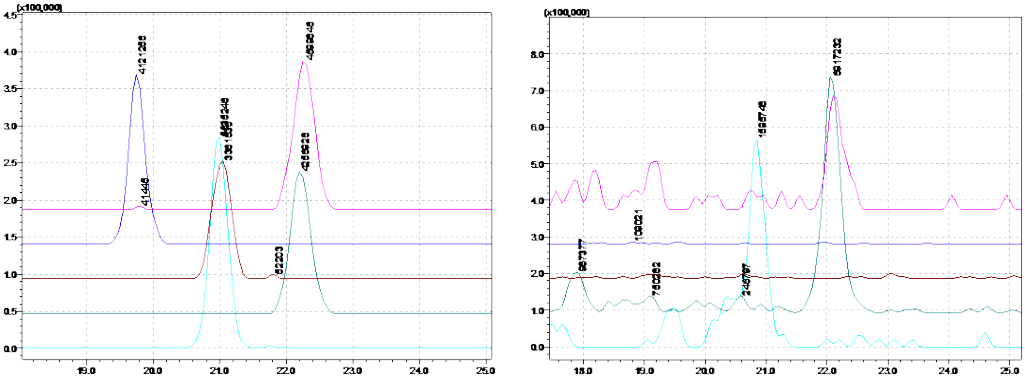


Fig. 9. Reconstructed ion chromatograms for hormones.

Left*: standard mixture in methanol (100 ng of each compound) inject 10 µl.*

Right*: sample 585 - influent water, NWWTP, 24.03.2014 (100 ng of $^{13}C_2$-EE2, and $^{13}C_6$-E1 each compound added into 0.5 L). Estrone was detected in the sample (Peaks of E2 and EE2 are absent).*

MS traces from top to bottom:

SIM m/z 269.15 for Estrone (E1). RT=22.1

SIM m/z 271.17 for Estradiol (E2). RT=19.7

SIM m/z 295.17 for Ethynylestradiol (EE2). RT= 20.9

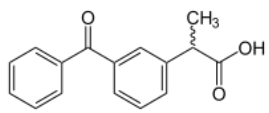
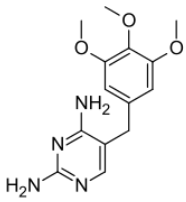
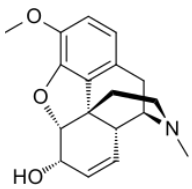
SIM m/z 275.15 for $^{13}C_6$-E1, RT= 22.1

SIM m/z 297.17 for $^{13}C_2$-EE2, RT=20.9

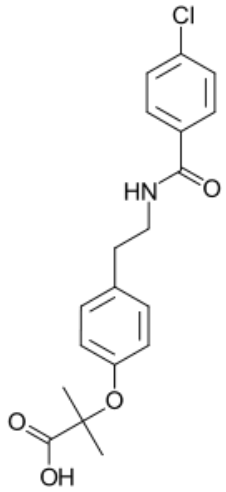
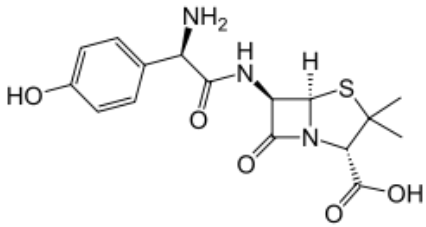
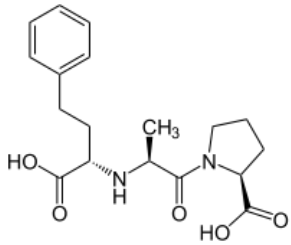
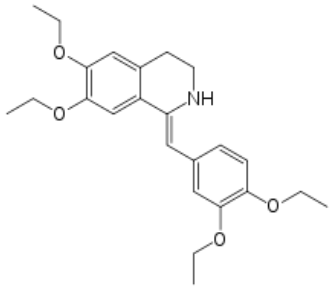
8. Second sampling campaign: results of the chemical analysis and consumption assessment for a variety of common pharmaceutical substances

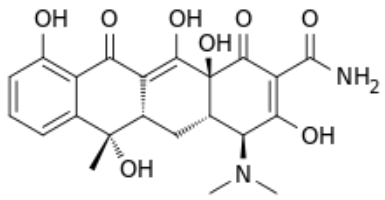
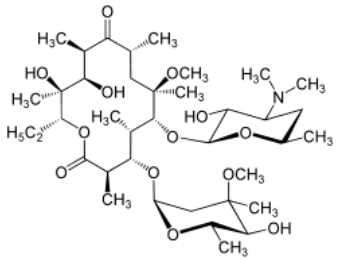
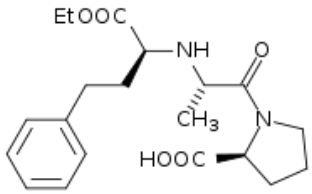
All samples collected in the second series (February-April 2014) were analysed for the presence of a range of common pharmaceuticals (see Table 11). While concentrations significantly varied from sample to sample, there were no clear differences between the different WWTPs. Due to this high variation of observed concentrations - probably caused by insufficient extraction or inappropriate quantification (no isotope-labelled standards were available for the listed substances) - the data in Table 12 provide frequencies of the presence of substances in either the influent or effluent on a given day. The original data are presented in Appendix 2.

Table 11. Common names, chemical names and chemical structures of the observed pharmaceutical substances.

Ketoprofen NSAID, analgesic, antipyretic (RS)2-(3-benzoylphenyl)-propionic acid CAS 22071-15-4 $C_{16}H_{14}O_3$	
Trimethoprim Bacteriostatic antibiotic 5-(3,4,5-Trimethoxybenzyl)pyrimidine-2,4-diamine CAS 738-70-5 $C_{14}H_{18}N_4O_3$	
Codeine Analgesic, antitussive, sedative, hypnotic (5α,6α)-7,8-Didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan-6-ol CAS 76-57-3 $C_{18}H_{21}NO_3$	

Ranitidine Histamine receptor antagonist *N*-(2-[(5-[(dimethylamino)methyl]furan-2-yl)methylthio]ethyl)-*N'*-methyl-2-nitroethene-1,1-diamine CAS 66357-355-5 $C_{13}H_{22}N_4O_3S$	
Norfloxacin Antibacterial 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1*H*-quinoline-3-carboxylic acid CAS 70458-96-7 $C_{16}H_{18}FN_3O_3$	
Ciprofloxacin Antibiotic 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid CAS 85721-33-1 $C_{17}H_{18}FN_3O_3$	
Ampicillin Antibiotic (2*S*,5*R*,6*R*)-6-([(2*R*)-2-amino-2-phenylacetyl]amino)-3,3-dimethyl-7-oxo-4-thia- 1-azabicyclo[3.2.0]heptane-2-carboxylic acid CAS 69-53-4 $C_{16}H_{19}N_3O_4S$	

Bezafibrate Fibrate drug 2-(4-{2-[(4-chlorobenzoyl)amino]ethyl}phenoxy)-2-methylpropanoic acid CAS 41859-67-0 $C_{19}H_{20}ClNO_4$	
Amoxicillin Antibiotic (2*S*,5*R*,6*R*)-6-{[(2*R*)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-24-carboxylic acid CAS 26787-78-0 $C_{16}H_{19}N_3O_5S$	
Enalaprilat ACE inhibitor (2*S*)-1-[(2*S*)-2-{[(1*S*)-1-carboxy-3-phenylpropyl]amino}propanoyl]pyrrolidine-2-carboxylic acid CAS 76420-72-9 $C_{18}H_{24}N_2O_5$	
Drotaverine Antispasmodic (*Z*)-1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline CAS 985-12-6 $C_{24}H_{31}NO_4$	

Tetracycline Antibiotic (4*S*,6*S*,12a*S*)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide OR (4*S*,6*S*,12a*S*)-4-(dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide CAS 60-54-8 $C_{22}H_{24}N_2O_8$	
Clarithromycin Antibiotic (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*S*)-6-{[(2*S*,3*R*,4*S*,6*R*) -4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy} -14-ethyl-12,13-dihydroxy-4-{[(2*R*,4*S*,5*S*,6*S*)-5-hydroxy -4-methoxy-4,6-dimethyloxan-2-yl]oxy}-7 -methoxy-3,5,7,9,11,13-hexamethyl -1-oxacyclotetradecane-2,10-dione CAS 81103-11-9 $C_{38}H_{69}NO_{13}$	
Enalapril ACE inhibitor (2*S*)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino} propanoyl]pyrrolidine-2-carboxylic acid CAS 758847-73-3 $C_{20}H_{28}N_2O_5$	

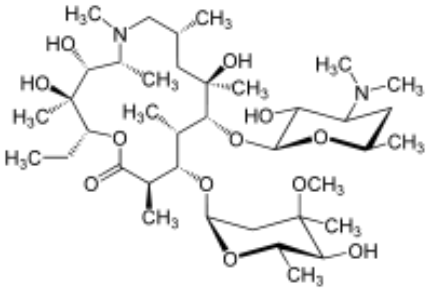
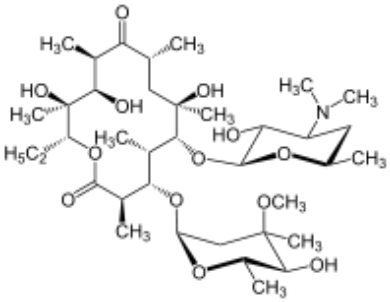
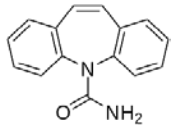
Azithromycin Antibiotic (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo- 11-{[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy}-1-oxa-6-azacyclopentadec-13-yl 2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranoside CAS 83905-01-5 $C_{38}H_{72}N_2O_{12}$	
Erithromycin Antibiotic (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione CAS 114-07-8 $C_{37}H_{67}NO_{13}$	
Carbamazepine Anticonvulsant 5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide CAS 298-46-4 $C_{15}H_{12}N_2O$	

Table 12. Number of detected pharmaceuticals at the three WWTPs in St. Petersburg and the maximal observed concentrations (C_{max}).

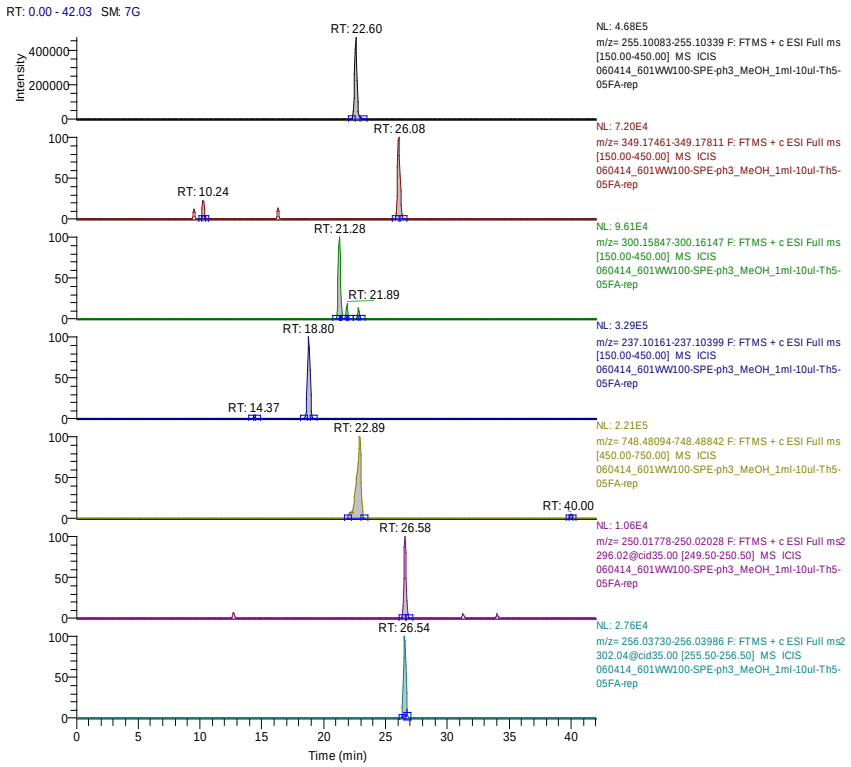
	WWTP (number of sampling days)			C_{max}, ng/L
	SWWWTP (11)	**CWWTP (9)**	**NWWTP (11)**	
Dibazol	0	0	0	nd
Ketoprofen	11	9	11	756
Trimethoprim	7	4	9	457
Codein	7	8	5	191
Ranitidine	2	1	1	252
Norfloxacin	5	3	6	502
Ciprofloxacin	11	5	11	871
Ampicillin	0	1	1	32
Bezafibrate	0	1	0	48
Amoxicillin	5	0	2	525
Enalaprilate	11	8	10	461
Drotaverin	11	7	9	452
Tetracycline	3	1	2	124
Clarithromycin	10	8	11	230
Enalapril	11	9	10	611
Azithromycin	11	9	11	332
Carbamazepine	11	9	11	76
Erythromycin	11	9	11	216

Ketoprofen, Azithromycin, Erythromycin and Carbamazepine were found in every sample. In addition to these four, Trimethoprim, Codein, Ciprofloxacine, Enalaprilate, Drotaverin, Clarithromycin and Enalapril were detected in more than 50% of the samples.

The highest maximal levels in the influent were found for Cyprofloxacin (871 ng/L), Ketoprofen (756 ng/L) and Enalapril (611 ng/L). Concentrations of these substances were significantly reduced in the effluent. Significant reductions during treatment were also observed for other pharmaceuticals with the exception of Clarythromycin whose concentration in raw sewage was in the range of 100-200 ng/L and was rarely lower than 100 ng/L.

Examples of the detected pharmaceuticals are given in Figure 10 on the following page.

Substance	Sample 601, CWWTP influent (31.03.2014)
Ketoprofen, m/z 255.10211	
Enalaprilat m/z 349.17636	
Codeine m/z 300.15997	
Carbamazepine m/z 237.1028	
Claritromycin m/z 748.48468	
Diclofenac m/z 250.01903	
$^{13}C_6$- Diclofenac m/z 256.03858	



Substance	Sample 615, CWWTP influent (08.04.2014)
Trimethoprim m/z 291.14572	
Drotaverin m/z 398.23315	
Ciprofloxacin m/z 332.14105	
Enalapril m/z 377.20764	
Erythromycin m/z 734.46906	
Diclofenacm/z 250.01903	
$^{13}C_6$- Diclofenac m/z 256.03858	

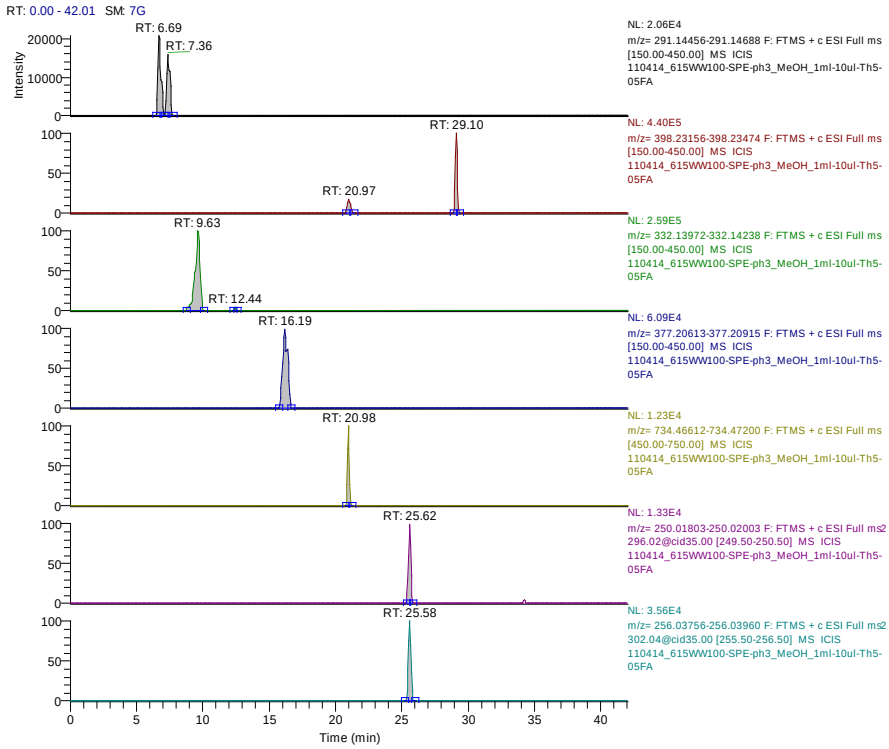


Fig. 10. SIM-chromatograms with the confident detection of pharmaceutical substances in influent extracts.

As the sales and/or medical statistics for St. Petersburg or Russia are not available for this wide variety of pharmaceuticals, for comparison purpose the statistical data from Estonia, Latvia and Lithuania[14] were combined and used to estimate the levels in St. Petersburg's sewage with the assumption of equal consumption (Table 13).

Table 13. Average consumption of selected pharmaceuticals in Estonia, Latvia and Lithuania by Defined Daily Doses (WHO) and the estimation of expected daily consumption, concentration in sewage and annual input into the WWTPs in St. Petersburg

	DDD/ 1,000/ day	DDD, g	g/ 1,000/ day	Eqv. kg/d in SPb	SPb sewage µg/L	SPb kilo/ year
Dibazol	n/a	n/a	n/a	n/a	n/a	n/a
Ketoprofen	0.63	n/a	n/a	n/a	n/a	n/a
Trimethoprim	1.2	0.4	0.48	2.4	1.1	800
Codein	0.4	0.1	0.04	0.2	0.09	65
Ranitidine	6	0.3	1.8	9	4.1	3,000
Norfloxacine	0.23	0.8	1.8	9	4.1	3,000
Ciprofloxacine	0.7	n/a	n/a	n/a	n/a	n/a
Ampicillin	0.37	2	0.75	3.8	1.7	1,200
Bezafibrate	n/a	n/a	n/a	n/a	n/a	n/a
Amoxicillin	5	1	6	30	14	10,000
Enalaprilate	n/a	n/a	n/a	n/a	n/a	n/a
Drotaverin	4	n/a	n/a	n/a	n/a	n/a
Tetracycline	0.03	1	0.03	0.15	0.07	50
Clarithromycin	1.5	n/a	n/a	n/a	n/a	n/a
Enalapril	20	0.01	0.2	1	0.45	300
Azithromycin	0.43	0.5	0.22	1.1	0.5	330
Erythromycin	0.05	1	0.05	0.25	0.11	80
Carbamazepine	1.7	1	1.7	8.5	3.9	2,800
Diclofenac	18	0.1	2	10	4.5	3,300
Ibuprofen	20	1.2	24	120	54	40,000
Paracetamol	7	3	21	105	48	35,000

[14] Baltic Statistics on Medicines 2010-2012, Tartu, 2013. ISBN 978-9949-33-396-7 (print), ISBN 978-9949-33-396-4 (pdf)

The values of DDD/1,000/day in Table 13 are the mean from the corresponding values for the three countries, not corrected for population (DDD/1,000/day = Number of Defined Daily Doses per 1,000 persons per day; the DDD values are taken from WHO[15]). The figures were converted into grams per day per 1,000 people and the equivalent daily use in kg was calculated for St. Petersburg.

These estimations were obtained by assuming a 100% excretion rate. The findings were corrected for the excretion rate where available (different sources give different excretion rates for the same substance; also, excretion may largely depend on the administration method). The corrected values for the expected concentration in sewage were compared with experimentally determined maximal concentrations in the untreated sewage of St. Petersburg (Table 14 on the following page).

The data allow pinpointing the substances that are probably used more often in St. Petersburg than in Estonia, Latvia and Lithuania and, presumably, in other HELCOM countries and members of the EU. Such substances are Codeine, Enalapril, Tetracycline and Erythromycin - the last two substances are antibiotics and might be an issue of concern. On the other hand, such antibiotics like Ampicillin and Amoxycillin were found in lower concentrations than expected from the statistical data but should be interpreted with caution. Further studies on the pharmaceuticals in St. Petersburg's sewage and in the Gulf of Finland are required for load, effect and risk assessments, and for the development of action plans.

[15] Drug and Therapeutics Committees - A Practical Guide. Annex 6.1 Defined daily doses (DDD) of some common medicines. Essential Medicines and Health Products Information Portal. A World Health Organization resource, 2003.

Table 14. Expected concentrations in St. Petersburg sewage based on pharmaceutical sales statistics for Estonia, Latvia and Lithuania, corrected for the excretion rate and experimentally determined maximal concentrations.

	Equivalent consumption in SPb, kg/year	Excretion rate, % of dose	Estimated Concentration in SPb sewage µg/L	Maximal Concentration in SPb, found µg/L
Dibazol	n/a	little	n/a	n/a
Ketoprofen	na	80%(g)	n/a	0.8
Trimethoprim	800	60%	0.6	0.45
Codeine	65	10, 5-15	0.009	0.19
Ranitidine	3,000	30-70%	2.0	0.25
Norfloxacine	3,000	30-60	2.0	0.50
Ciprofloxacine	n/a	50-70	n/a	0.87
Ampicillin	1,200	90	1.5	0.03
Bezafibrate	n/a	50	n/a	0.05
Amoxicillin	10,000	50-70	9	0.53
Enalaprilate	n/a	67	n/a	0.46
Drotaverin	n/a	little	n/a	0.45
Tetracycline	50	80-90	0.06	0.12
Clarithromycin	n/a	80	n/a	0.23
Enalapril	300	26	0.11	0.61
Azithromycin	330	65	0.35	0.33
Erythromycin	80	15-30	0.03	0.22
Carbamazepine	2,800	2-5	0.2	0.08
DCF	3,300	50	2.2	0.8
Ibuprofen	40,000	1 – 10	0.54 – 5.4	n/a
Paracetamol	35,000	3-5	2.0	n/a

Conclusion

St. Petersburg with its population of over five million in 2013 is the largest megapolis on the Baltic Sea. It is also the largest single point of sales, consumption, excretion and, presumably, release of pharmaceutical substances into the Baltic Sea environment.

This study was the first of its kind to be carried out in St. Petersburg. Its aim was to analyse the load of pharmaceuticals entering and passing through the city's sewage system. The loads and expected concentrations for a number of pharmaceuticals were estimated based on the findings.

However, an estimation of the contribution of the River Neva input would not be accurate without taking the whole Neva basin into account.

In addition to St. Petersburg, the whole Leningrad Region (population 1,751,000), Kaliningrad Region (955,000), Pskov Region (661,000) and large parts of Novgorod Region (623,000) the Republic of Karelia (955,000) also belong to the basin of the Baltic Sea. Just under three million inhabitants reside in the basin of the River Neva. In addition to the human population, farming activities can be a significant source of hormones reaching Baltic Sea. However, Lake Ladoga may act as a 'natural WWTP' for waters originating from Novgorod and Pskov Regions and the Republic of Karelia.

Of significance is the fact that three million inhabitants of Belarus (Minsk, Vitebsk and Grodno regions) live within the Baltic Sea basin; however, their awareness of BSAP can be considered minimal. Cooperation of Belarus with the HELCOM countries would nevertheless be beneficial for the Baltic Sea.

On the basis of the analytical results, the average concentration of DCF in the effluent was found to vary from 355 ng/L in the summer of 2013 (CWWTP data only) to 510-550 ng/L in the winter of 2014 (all three WWTPs). The upper limit for the daily release of DCF from the city can be estimated at 1.1 kg making an annual load of some 400 kg.

The River Neva delivers an average annual volume of water of 80 km^3; accordingly, the expected concentration of DCF in the water flowing into the Gulf of Finland is 5ng/L.

It was found that concentration of DCF in the effluent was often higher than that in untreated sewage water. This phenomenon can be explained by the liberation of DCF from conjugated metabolites during bacterial treatment. Similar observations have been made elsewhere in the world.

From the pharmaceutical sales statistics and population analysis of St. Petersburg, the amount of EE2 excreted into the sewage system was found to not exceed 315 g per year (assuming 50% of estrogen excreted remained unchanged, but this is likely an overestimation). This would correspond to a concentration in sewage of 0.4 ng/L. Even if no purification takes place during treatment, the expected concentration of EE2 in the water flowing from St. Petersburg into the Gulf of Finland would not exceed 0.004 ng/L, below the EQS of 0.007 ng/L.

Naturally produced human estrogen E1 was found in raw sewage and based on the results of the chemical analysis some 40 kg is excreted annually. This corresponds with the estimation based on medical data for excretion rates in different age and gender groups at 35.2 kg/year.

As E1 was detected in only three out of 31 samples in the effluent, the average concentration in the effluent was judged to be below the detection limit of 10 ng/L. Thus, the highest possible annual release of E1 from St. Petersburg would not exceed 8 kg. Moreover, the concentration of E1 in the water flowing from St. Petersburg into the Gulf of Finland would not exceed 0.1 ng/L.

Other naturally produced hormones, E2 and E3, were not detected in either the influent or effluent samples. It indicates the possible degradation of these two less stable hormones during their prolonged travel from the excretion point to the WWTP.

Eighteen other pharmaceutical substances of different classes and chemical nature were found in the raw sewage and effluent. Their levels ranged from tens to hundreds ng/L. The highest maximal levels in the influent were found for Cyprofloxacin (871 ng/L), Ketoprofen (756 ng/L) and Enalapril (611 ng/L). Concentrations were significantly reduced in the effluent. Significant reduction during treatment was also observed for other pharmaceuticals with the exception of Clarythromycin whose concentration in raw sewage was in the range 100-200 ng/L and was rarely lower than 100 ng/L.

The data obtained in the project can be used to assess the overall release of pharmaceuticals on the gulf-basin and sea-basin scale.

The data can also be used to assess the existing water treatment technology in St. Petersburg and to improve it.

The comprehensive set of 100 extracts of the collected samples from three major WWTPs of St. Petersburg, both influent and effluent, taken during different seasons form a unique specimen bank. The samples are being kept frozen at SRCES and can be used for retrospective target analyses of pharmaceutical contaminants should they emerge in the future.

Good correlation between experimental findings on Diclofenac (DCF) and Estron (E1) and medical sales/excretion data is evidence that the sample bank is representative enough for the whole population of St. Petersburg throughout the year. With the acquisition of analytical standards and better equipment it would be possible to re-analyse samples for other contaminants of interest without additional sampling. Moreover, the data acquired on an OrbiTrap mass-spectrometer in a High Resolution Full-Scan mode can be used in search of such compounds without further experimental work.

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Appendix 1: Original results: Diclofenac, determined concentration in ng/L (second series of samples).

Method	South-Western WWTP			Northern WWTP			Central WWTP		
	receipt	in	out	receipt	in	out	receipt	in	out
	19.02.14	**549**	**550**	**20.02.14**	**553**	**554**	**19.02.14**	**551**	**552**
full FT		522	522		668	503 / 554		675	493
MRM		479	334		600	394 / 550		504	442
full Ft(i)					616	- / 600			
LLE full FT		779	730.2		715	416		765	462
LLE MRM		636	557		502	524		501	541
LLE (IT-TOF)		661							
	25.02.14	**562**	**563**	**25.02.14**	**558**	**559**	**25.02.14**	**560**	**561**
full FT		459 / 479	538		359	452 / 558		265	412 / 483
MRM		595 / 556	545		467	553 / 591		334	423 / 596
full Ft(i)		- / 547				- / 513		200	- / 423
LLE full FT		1580			600	642		358	701
LLE MRM		649			486	598		263	757
LLE (IT-TOF)		669	422						501

	26.02.14	568	569	27.02.14	572	573	26.02.14	570	571
full FT		642	601		578	604		185	654
MRM		432	593		418	596		130	608
full Ft(i)									
LLE full FT		735	555			766		331	822
LLE MRM		703	522		652	649		370	493
LLE (IT-TOF)								378	
	17.03.14	**578**	**577**	**24.03.14**	**585**	**586**	**17.03.14**	**575**	**576**
full FT		623 / 724	450		263	218		301	447
MRM		492 / 722	571		295	212		346	459
full Ft(i)		523 / 684	328		272	144		121	422
LLE (IT-TOF)		296							
	19.03.14	**583**	**584**	**26.03.14**	**597**	**598**	**19.03.14**	**581**	**582**
full FT		654 / 662	590		404	781		1775 / 1651	484
MRM		543 / 480	636		547	551		1611 / 1659	624
full Ft(i)		776 / 630	385		311	567		2798 / 1738	479

	24.03.14	589	591	01.04.14	**599**	**600**	25.03.14	587	588
full FT		500	584		397	594		554	610
MRM		432	531		456	692		442	459
full Ft(i)		539	634		359	504		358	478
	25.03.14	**590**		**02.04.14**	**609**	**610**	**24.03.14**	**593**	**594**
	26.03.14		**592**						
full FT		681	514		662	430		286	541
MRM		551	484		639	546		346	247
full Ft(i)		884	421		483	31		146	456
	31.03.14	**605**		**07.04.14**	**611**		**31.03.14**	**601**	**602**
	01.04.14		**606**	**08.04.14**		**612**			
full FT		677	635		512	556		472	634
MRM		676	571		528	382		411	490
full Ft(i)		557	668		358	450		329	614
	01.04.14	**607**		**09.04.14**	**622**		**01.04.14**	**603**	**604**
	02.04.14		**608**	**10.04.14**		**623**			
full FT		527	675		542	621		382	683
MRM		721	880		567	654		305	557
full Ft(i)		521	687		497	560		363	572
	07.04.14	**617**					**07.04.14**	**613**	
	08.04.14		**619**				**08.04.14**		**614**
full FT		661	665					396	483
MRM		678	458					374	342
full Ft(i)		884	541					153	481
	08.04.14	**618**					**08.04.14**	**615**	
	09.04.14		**620**				**09.04.14**		**616**
full FT		820	574					279	517
MRM		812	551					284	528
full Ft(i)		789	497					355	451

Appendix 2: Primary data for concentrations of pharmaceuticals at the WWTPs (second series of samples).

	Date	SWWWTP		Date	NWWTP		Date	CWWTP	
		in	out		in	out		in	out
	19.02. 2014	549	550	20.02. 2014	553	554	19.02. 2014	551	552
Dibazol		<5	<5		<5	<5		<5	<5
Ketoprofen		102.8	<7.5		755.8	121.9		74.6	58.1
Trimethoprim		<7.5	<7.5		<7.5	<7.5		<7.5	85.8
Codeine		<7.5	<7.5		4.0	<7.5		14.9	4.5
Ranitidine		43.9	32.7		<15	<15		<15	<15
Norfloxacine		<15	<15		<15	<15		<15	<15
Ciprofloxacine		534.0	<7.5		12.7	<7.5		489.3	<7.5
Ampicillin		<15	<15		<15	<15		<15	<15
Bezafibrat		<10.5	<10.5		<10.5	<10.5		<10.5	<10.5
Amoxicilin		524.9	<15		<15	<15		<15	<15
Enalaprilat		461.4	<7.5		<7.5	<7.5		94.2	2.8
Drotaverin		451.5	63.4		13.7	<5.5		22.5	5.6
Tetracycline		<15	<15		<15	<15		<15	74.2
Claritromicine		229.9	40.2		<7.5	<7.5		76.7	14.1
Enalapril		180.6	30.3		97.2	47.3		35.2	<7.5
Azithromycin		54.7	43.7		29.9	<10.5		43.2	<10.5
Carbamazepin e		23.1	25.2		32.6	<7.4		27.0	29.3
Erythromycin		108.5	41.6		215.6	<7.5		72.8	12.2
	25.02. 2014	562	563	25.02. 2014	558	559	25.02. 2014	560	561
Dibazol		<5	<5		19.0	<5		<5	6.2
Ketoprofen		189.2	44.9		172.5	148.6		63.4	40.5
Trimethoprim		<7.5	113.5		53.6	17.9		566.9	135.1
Codeine		64.9	<7.5		18.8	<7.5		<7.5	<7.5
Ranitidine		<15	<15		<15	<15		<15	<15
Norfloxacine		<15	<15		<15	<15		<15	<15
Ciprofloxacine		342.8	91.8		<7.5	<7.5		226.6	<7.5
Ampicillin		<15	<15		<15	<15		<15	<15
Bezafibrat		<10.5	<10.5		<10.5	<10.5		<10.5	<10.5
Amoxicilin		<15	<15		<15	<15		16.2	<15
Enalaprilat		247.0	<7.5		65.1	<7.5		<7.5	<7.5
Drotaverin		55.9	5.6		28.3	<5.5		13.2	<5.5
Tetracycline		<15	<15		<15	<15		<15	<15
Claritromicine		128.9	106.4		197.7	158.7		139.3	16.1
Enalapril		13.8	41.9		33.0	36.4		90.8	30.6
Azithromycin		157.4	18.5		136.6	14.7		53.2	<10.5
Carbamazepin e		46.3	14.1		40.2	13.9		26.4	<7.5
Erythromycin		170.4	33.4		107.5	22.7		78.8	<7.5
	26.02. 2014	568	569	27.02. 2014	572	573	26.02. 2014	570	571

Dibazol		<5	6.2		49.9	<5		<5	8.2
Ketoprofen		317.3	69.9		247.8	267.0		107.3	50.0
Trimethoprim		<7.5	111.6		24.8	9.2		456.8	121.5
Codeine		<7.5	<7.5		132.2	46.3		<7.5	7.8
Ranitidine		252.3	<15		<15	<15		<15	<15
Norfloxacine		<15	<15		127.3	<15		<15	<15
Ciprofloxacine		<7.5	119.3		<7.5	<7.5		124.1	<7.5
Ampicillin		<15	<15		<15	<15		<15	<15
Bezafibrat		<10.5	<10.5		<10.5	<10.5		<10.5	<10.5
Amoxicilin		<15	<15		<15	<15		<15	<15
Enalaprilat		<7.5	16.4		415.9	35.8		37.0	21.3
Drotaverin		<5.5	10.0		<5.5	<5.5		43.7	10.1
Tetracycline		<15	<15		74.5	29.6		<15	<15
Claritromicine		<7.5	115.8		168.3	68.4		173.6	19.3
Enalapril		436.6	35.5		121.6	36.4		311.2	25.4
Azithromycin		216.6	12.8		332.4	29.9		108.5	<10.5
Carbamazepin e		52.2	15.1		27.1	<7.5		57.7	34.4
Erythromycin		105.6	24.2		84.9	13.2		87.4	15.0
	17.03. 2014	578	577	24.03. 2014	585	586	17.03. 2014	575	576
Ciprofloxacin		528.1	<7.5		166.7	<7.5		363.1	19.3
Bezafibrate		<10.5	<10.5		<10.5	<10.5		<10.5	<10.5
Dibazol		<5	<5		<5	<5		<5	<5
Drotaverin		<5.5	35.2		<5.5	3.3		<5.5	<5.5
Ketoprofen		167.7	39.0		290.9	7.2		187.4	44.7
Enalapril		128.1	<7.5		105.3	5.8		<7.5	0.0
Enalaprilat		33.1	8.8		<7.5	5.4		86.0	25.8
Ampicillin		<15	<15		<15	<15		15.8	<15
Amoxicillin		30.4	<15		<15	<15		8.3	<15
Norfloxacin		<15	<15		<15	<15		32.3	<15
Ranitidine		<15	<15		<15	<15		16.0	<15
Trimethoprim		7.6	<7.5		<7.5	8.2		22.4	<7.5
Claritromycin		132.1	62.7		190.0	30.4		132.4	129.1
Codeine		191.1	<7.5		<7.5	<7.5		<7.5	<7.5
Tetracycline		<15	<15		<15	<15		<15	<15
Azithromycin		142.1	27.9		49.9	<10.5		24.5	<10.5
Carbamazepin e		22.8	<7.5		13.7	<7.5		23.6	10.1
Erythromycin		26.5	<7.5		46.4	<7.5		46.6	<7.5
	19.03. 2014	583	584	26.03. 2014	597	598	19.03. 2014	581	582
Ciprofloxacin		766.7	<7.5		220.5	26.8		552.7	<7.5
Bezafibrate		<10.5	<10.5		<10.5	<10.5		<10.5	<10.5
Dibazol		<5	<5		<5	<5		<5	<5
Drotaverin		<5.5	10.0		11.8	6.9		<5.5	<5.5
Ketoprofen		554.5	23.5		248.9	115.5		216.5	36.4
Enalapril		592.1	0.0		126.0	0.0		185.1	0.0
Enalaprilat		10.8	0.0		15.2	18.4		26.1	22.7
Ampicillin		<15	<15		<15	<15		<15	<15
Amoxicillin		<15	<15		<15	<15		<15	<15
Norfloxacin		<15	<15		<15	<15		<15	<15

Ranitidine		<15	<15		<15	<15		<15	<15
Trimethoprim		<7.5	<7.5		9.0	57.7		35.5	8.3
Claritromycin		183.3	128.4		108.2	140.1		138.1	131.5
Codeine		<7.5	<7.5		8.6	<7.5		<7.5	<7.5
Tetracycline		<15	<15		<15	<15		<15	<15
Azithromycin		48.1	14.0		53.5	<10.5		62.3	<10.5
Carbamazepin e		26.2	9.8		43.9	16.3		36.4	17.5
Erythromycin		155.8	25.9		176.5	<7.5		171.4	<7.5
	24.03. 2014	589	591	01.04. 2014	599	600	25.03. 2014	587	588
Ciprofloxacin		656.1	<7.5		<7.5	<7.5		475.5	<7.5
Bezafibrate		<10.5	<10.5		<10.5	<10.5		<10.5	<10.5
Dibazol		<5	<5		<5	<5		<5	<5
Drotaverin		30.0	8.0		<5.5	6.7		6.3	<5.5
Ketoprofen		203.3	71.8		162.3	61.5		280.1	39.8
Enalapril		99.5	0.0		71.1	0.0		170.0	0.0
Enalaprilat		31.0	21.3		30.1	18.0		0.0	21.0
Ampicillin		<15	<15		<15	<15		<15	<15
Amoxicillin		<15	<15		<15	<15		<15	<15
Norfloxacin		<15	<15		<15	<15		87.7	<15
Ranitidine		<15	<15		<15	<15		<15	<15
Trimethoprim		23.1	6.1		<7.5	<7.5		75.7	<7.5
Claritromycin		96.9	79.5		88.7	127.0		155.5	165.9
Codeine		22.0	<7.5		8.3	<7.5		<7.5	<7.5
Tetracycline		<15	<15		<15	<15		<15	<15
Azithromycin		69.8	9.9		88.2	36.2		93.3	15.9
Carbamazepin e		37.5	18.7		43.5	15.6		42.8	8.5
Erythromycin		77.4	<7.5		188.1	<7.5		86.4	51.9
	25.03. 2014	590		02.04. 2014	609	610	24.03. 2014	593	594
	26.03. 2014		592						
Ciprofloxacin		471.1	76.7		871.7	<7.5		739.1	68.8
Bezafibrate		<10.5	<10.5		<10.5	<10.5		<10.5	<10.5
Dibazol		<5	<5		<5	<5		<5	2.5
Drotaverin		19.9	5.7		20.2	6.3		19.7	6.1
Ketoprofen		183.2	50.0		611.9	44.1		177.7	18.5
Enalapril		118.1	<7.5		234.7	<7.5		83.3	0.0
Enalaprilat		28.5	26.8		107.1	<7.5		31.8	19.5
Ampicillin		<15	<15		<15	<15		<15	<15
Amoxicillin		23.8	<15		<15	<15		<15	<15
Norfloxacin		349.5	17.6		293.3	<15		292.0	<15
Ranitidine		<15	<15		<15	<15		<15	<15
Trimethoprim		65.3	4.3		38.3	<7.5		41.3	48.4
Claritromycin		77.3	68.8		230.4	142.7		145.9	118.8
Codeine		17.6	<7.5		15.5	<7.5		<7.5	<7.5
Tetracycline		<15	<15		37.0	<15		<15	<15
Azithromycin		64.9	37.8		86.6	11.0		80.6	27.8
Carbamazepin		63.1	16.0		76.4	25.4		40.2	14.0

e									
Erythromycin		110.6	16.2		166.4	34.1		80.7	13.3
	31.03. 2014	605		07.04. 2014	611		31.03. 2014	601	602
	01.04. 2014		606	08.04. 2014		612			
Ciprofloxacin		835.9	15.2					339.3	<7.5
Bezafibrate		<10.5	<10.5		48.0	15.6		<10.5	<10.5
Dibazol		<5	<5		<5	<5		<5	<5
Drotaverin		42.4	8.2		<5.5	<5.5		<5.5	7.5
Ketoprofen		211.6	87.3		<7.5	8.3		376.8	74.9
Enalapril		145.7	<7.5		278.4	73.7		101.3	<7.5
Enalaprilat		46.5	40.4		108.9	8.0		27.3	22.7
Ampicillin		<15	<15		31.8	<15		<15	<15
Amoxicillin		<15	<15		<15	<15		<15	<15
Norfloxacin		501.6	<15		<15	<15		28.3	<15
Ranitidine		<15	<15		72.0	<15		<15	<15
Trimethoprim		31.5	<7.5		0.0	0.0		8.3	<7.5
Claritromycin		<7.5	<7.5		20.0	92.5		99.2	112.4
Codeine		28.2	<7.5		18.8	11.2		25.2	<7.5
Tetracycline		<15	<15		<15	<15		<15	<15
Azithromycin		168.9	26.6		115.6	<10.5		105.3	17.8
Carbamazepin e		38.9	18.8		44.9	13.9		49.5	15.8
Erythromycin		107.9	<7.5		154.5	<7.5		154.6	21.2
	01.04. 2014	607		09.04. 2014	622		01.04. 2014	603	604
	02.04. 2014		608	10.04. 2014		623			
Ciprofloxacin		409.6	<7.5		426	<7.5		529.5	<7.5
Bezafibrate		<10.5	<10.5		<10.5	<10.5		<10.5	<10.5
Dibazol		<5	<5		<5	<5		<5	<5
Drotaverin		18.5	7.8		12.9	8.1		<5.5	7.1
Ketoprofen		235.0	123.8		120.6	28.4		206.7	48.2
Enalapril		124.1	<7.5		84.9	<7.5		111.2	<7.5
Enalaprilat		76.3	<7.5		25.4	15.5		25.5	21.8
Ampicillin		<15	<15		<15	<15		<15	<15
Amoxicillin		100.1	<15		<15	<15		<15	<15
Norfloxacin		292.0	<15		121.3	<15		142.1	<15
Ranitidine		<15	<15		<15	<15		<15	<15
Trimethoprim		30.5	<7.5		<7.5	<7.5		15.5	<7.5
Claritromycin		117.3	103.3		139.6	91.8		39.8	<7.5
Codeine		14.3	<7.5		12.0	<7.5		10.0	<7.5
Tetracycline		17.8	25.2		<15	<15		18.4	<15
Azithromycin		85.5	20.6		69.0	22.4		117.5	24.7
Carbamazepin e		43.0	24.0		47.4	15.0		40.4	18.4
Erythromycin		169.9	<7.5		65.7	<7.5		90.8	19.6
	07.04. 2014	617					07.04. 2014	613	
	08.04. 2014		619				08.04. 2014		614

Ciprofloxacin		720.2	<7.5					861.3	49.9
Bezafibrate		<10.5	<10.5					<10.5	<10.5
Dibazol		<5	<5					<5	<5
Drotaverin		19.9	8.2					150.2	7.8
Ketoprofen		560.5	85.4					258.7	65.8
Enalapril		304.4	<7.5					115.4	<7.5
Enalaprilat		169.7	30.0					32.5	20.6
Ampicillin		<15	<15					<15	<15
Amoxicillin		<15	<15					<15	<15
Norfloxacin		59.5	<15					0.0	<15
Ranitidine		<15	<15					<15	<15
Trimethoprim		114.5	<7.5					19.3	38.6
Claritromycin		140.1	92.0					90.0	85.6
Codeine		31.9	<7.5					7.5	<7.5
Tetracycline		71.3	<15					<15	<15
Azithromycin		77.4	<10.5					86.7	<10.5
Carbamazepine		45.7	13.8					58.9	18.4
Erythromycin		137.0	<7.5					151.4	<7.5
	08.04.2014	618					08.04.2014	615	
	09.04.2014		620				09.04.2014		616
Ciprofloxacin		790.1	<7.5					477.6	<7.5
Bezafibrate		0.0	0.0					0.0	0.0
Dibazol		<5	<5					<5	<5
Drotaverin		23.3	9.0					24.7	8.2
Ketoprofen		416.0	88.5					203.0	29.6
Enalapril		611.2	<7.5					74.3	<7.5
Enalaprilat		317.7	26.7					50.4	12.3
Ampicillin		<15	<15					<15	<15
Amoxicillin		18.4	<15					<15	<15
Norfloxacin		159.4	<15					137.5	<15
Ranitidine		<15	<15					<15	<15
Trimethoprim		333.0	<7.5					26.5	<7.5
Claritromycin		180.8	71.2					96.7	29.9
Codeine		37.9	<7.5					7.6	<7.5
Tetracycline		124.3	<15					36.6	<15
Azithromycin		78.2	11.6					20.4	<10.5
Carbamazepine		43.9	10.3					19.3	<7.5
Erythromycin		127.5	<7.5					168.2	17.6

Appendix 3: Sampling and instrumental analysis parameters

Diclofenac was extracted separately by two methods: Liquid-liquid extraction (LLE) and Solid phase extraction (SPE).

Sample Preparation Procedure (LLE)

For quantification, an internal standard (100 ng $^{13}C_6$- diclofenac) was added to all samples and blanks.

Sample Volume was 0.5-L.

Spike 100 ng of $^{13}C_6$- diclofenac (as internal standard).

Add HCl to pH 3.

Add 40 ml of acetone and 50 g of $(NH_4)_2SO_4$, mix thoroughly.

Add 30 ml extracting solvent Hexane, stir thoroughly.

Collect upper hexane layers.

Repeat hexane extraction.

Combine hexane layers and dry over Na_2SO_4.

Reduce volume using rotary evaporator near dryness.

Reconstitute the residue in 1 mL of methanol.

Sample Preparation Procedure (SPE)

The received extract by the SPE method also contains other pharmaceutical compounds.

Sample volumes are different for influent (0.1 L) and effluent (0.25 L) water samples.

Spike 100 ng of $^{13}C_6$- diclofenac (as internal standard) into each sample.

Add Na_4EDTA (15 mg).

Add HCl to pH 3

Let stand for 30 min.

SPE cartridge (Oasis HLB, 200 mg (Waters)) is conditioned by 10 mL MeOH and then 10 mL twice distilled water and at the end 10 mL of water pH3.

Extract sample through cartridge (flow rate 5-10 mL/min).

Wash the cartridges with 10 mL of water.

Dry cartridge for 20 min.

After drying, elute cartridges using 10 mL of methanol and 6 ml of mixture of methanol/acetone=1/1.

Reduce the volume to near dryness using a rotary evaporator near dryness, constitute the residue in 1 mL of methanol.

LCMS (for Diclofenac and pharmaceuticals):

The mobile phase solvents for chromatography were A – 0.05% formic acid (FA) in Direct - Q water and B -0.05% FA in MeCN. Separation was achieved in a gradient program on a Thermo Hypersil Gold C_{18}-coloumn (50 x 2.1 mm, 1.9 µm). Flow Rate – 0.2 mL/min, column temperature is 40°C. The gradient program was the following:

Time, min	Conc. B,%
0.01	5
6	5
20	50
28	95
38	95
39	5
43.0	5

The instrument was an LTQ OrbiTrap ('Finnigan') liquid chromatograph (high-resolution mass-spectrometer) in a positive electrospray-ionization mode (ESI+). Mass spectra are recorded in full scan and multiply reaction monitoring (MRM, CID 35%) - regime. Resolution is 30,000; Ion Spray Voltage 3.2 kV; Cone Voltage 18 V; Tube Lens 90 V; Temperature of Ion Capillary - 320°C; Auxiliary gas (N_2) 20 arb.

The identification of target compounds was carried out by retention times and an accurate masses of protonated molecular ions $[M+H]^+$ (accuracy within 5 ppm) (Table. 2); for Diclofenac and $^{13}C_6$-Diclofenac we also used accurate masses of ion-product (m/z 250.01903 and 256.03858).

Quantitative detection of the substances was obtained by methods of the isotopic-label external and internal standard.

List of analysed pharmaceutics and characteristic ions used for detection.

Compound	$[M+H]^+$	Pharmacy group	Instrument detection limits, ng per injection	LOD, Detection limits, ng/L	MS mode
Ranitidin	315,14911	histamine receptor blocker	0,1	15,0	Full scan
Amoxicillin	366,11237	antibiotics	0,3	15,0	Full scan
Trimethoprim	291,14572	spasmolytic	0,1	7,5	Full scan
Ketoprofen	255,10211	anti-inflammatory	0.3	7.5	Full scan
Clarithromycin	748,48468	antibiotics	0.3	7.5	Full scan
Bezafibrate	362,11591	fibrate	0.3	10.5	Full scan
Diclofenac	296,02454	anti-inflammatory	0.2	7.5	Full scan & MRM
Dibazol	209,10788	spasmolytic	0.2	10.5	Full scan
Enalaprilat	349,17636	Fate block	0.3	7.5	Full scan
Enalapril	377,20764	Fate block	0.3	7.5	Full scan
Drotaverine (No-Spa)	398,23315	spasmolytic	0.2	10.5	Full scan
Ampicillin	350,11746	antibiotics	0.3	15.0	Full scan
Norfloxacin	320,14105	antibiotics	0.3	15.0	Full scan
Ciprofloxacin	332,14105	antibiotics	0.2	7.5	Full scan
Tetracycline	445,16107	antibiotics	0.5	15.0	Full scan
Azithromycin	749,51636	antibiotics	0.2	10.5	Full scan
Carbamazepine	237,1028	antibiotics	0.2	10.5	Full scan
Erythromycin	734,46906	antibiotics	0.2	10.5	Full scan

Hormones

Sample Preparation Procedure (LLE)

For quantification, internal standards (mixture of isotope labeled ^{13}C -hormones: E1, E2, EE2 of 100 ng each) were added to all samples, blanks and fortified samples.

For quantification, an internal standard (100 ng of each $^{13}C_6$-E1, $^{13}C_6$-E2, $^{13}C_2$-EE2) was added to all samples and blanks.

Sample Volume was 0.5-L.

Add 100 ml of acetone and 250 g of $(NH_4)_2SO_4$.

Vortex thoroughly until stratification of the sample into two layers.

Collect the upper acetone layer; filtrate through a paper filter.

Add NaOH (10M solution) to pH10.

Extract with Hexane, 30 ml.

Collect upper hexane layer.

Repeat hexane extraction.

Combine hexane layers and dry over Na_2SO_4.

Reduce volume using a rotary evaporator near dryness.

Reconstitute the residue in 1 mL of methanol.

LCMS (for Hormones):

Analysis for the determination of hormones was run using a Shimadzu Hybrid High Performance Liquid Chromatograph-Mass Spectrometer of high resolution (LCMS-IT-TOF equipped with ion trap and time-of-flight mass spectrometer).

LC:

The mobile phase solvents for chromatography were A – Direct-Q water and B - acetonitrile. Separation was achieved on a gradient program on two consequentially combine Thermo Hypersil Gold C_{18}-coloumn (150 x 2.1 mm, 5 µm). Flow Rate – 0.2 mL/min, column temperature was 40°C.

The gradient program was as follows:

Time, min	Conc. B,%
0.01	2
5.0	2
6.0	50
25.0	50
27.0	95
42.0	95
43.0	2
47.0	2

MS:

Ionization was performed in ESI (-) mode and mass spectra were recorded in SIM regime (narrow interval-0.5amu). Interface Voltage was set at –3.5kV. The temperature of the CDL and Heater Bloch was 250^0C; Nebulizing Gas Flow -1$Lmin^{-1}$.

The identification of hormones was carried out by retention times and masses of deprotonated molecular ions $[M-H]^-$ (accuracy within 15-20 ppm).

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