Comparison of Steroid Hormone Concentrations in Domestic and Hospital Wastewater Treatment Plants

B. Pauwels[1]; H. Noppe[2]; H. De Brabander[3]; and W. Verstraete[4]

Abstract: Influent and effluent samples originating from two wastewater treatment plants (WWTPs) (treating hospital wastewater and domestic wastewater, Belgium) have been analyzed in order to estimate their steroid hormone content. The natural estrogens estrone (E1), 17β-estradiol (E2), and the synthetic 17α-ethinylestradiol (EE2) together with other steroid hormones progesterone (P) and testosterone (T) metabolites were detected in these samples. The hormone concentrations in both the hospital and the domestic WWTP samples were not significantly different and ranged from <0.2 ng EE2/L to 114 ng EE2/L, from <0.2 ng E1/L to 58 ng E1/L and from <0.2 ng P/L to >100 ng P/L. E2 was detected once at a concentration of 17 ng/L. In the domestic WWTP which comprises a conventional activated sludge treatment in parallel with a membrane bioreactor, no differences in estrogen removal efficiency could be observed for both treatments. In comparison to chemical analysis data, the Yeast Estrogen Screen (YES) appears to underestimate the influent estrogen concentrations, probably due to influent toxicity for the YES. Effluent estrogen concentrations, on the other hand, were overestimated by the YES test, probably due to the presence of other estrogenic compounds in the effluent.

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Introduction

The natural estrogens estrone (E1), 17β-estradiol (E2), and estriol (E3) and the synthetic 17α-ethinylestradiol (EE2) are steroid hormones which enter wastewater treatment plants (WWTPs) after their excretion in urine. E2 and EE2 are considered as the main contributors to estrogenic activity in environmental samples due to their estrogenic potency, i.e., the relative binding to the human estrogen receptor (Johnson and Williams 2004). In WWTPs these estrogens are removed with variable success.

E2 has been detected in domestic wastewater at a maximum level of 150 ng/L (Vethaak et al. 2002) and in domestic WWTP effluent 64 ng/L was detected (Ternes et al. 1999a). It has been documented that E2 is oxidized to E1 by activated sludge (Ternes et al. 1999b). Maximum levels of E1 detected in WWTP influent and effluent are 115 ng/L (Petrovic et al. 2002) and 76 ng/L (Desbrow et al. 1998), respectively. The highest levels of EE2 that were measured are 7 ng/L in domestic WWTP influent (Cargouët et al. 2004) and 42 ng/L in domestic WWTP effluent (Ternes et al. 1999a). Progesterone (P) has been detected by Esperanza et al. (2007) at WWTP influent and effluent concentrations of 74 and below 2 ng/L, respectively. These authors state that no data are available on the fate of progesterone in wastewater treatment, but their data suggest >80% removal.

Some WWTPs remove estrogens very well (>90%), whereas others do not remove estrogens (EE2 in particular) at all (Clara et al. 2004). Removal of estrogens in WWTPs is based on sorption to the activated sludge and solids and on biodegradation. Andersen et al. (2005) concluded that sorption is not important for the fate of steroid estrogens in WWTPs compared to biodegradation. It is generally accepted that E2 is transformed to E1 by activated sludge (Ternes et al. 1999b). Estrogen removal seems to be positively correlated with the presence of nutrient removal in the WWTP and higher sludge age (sludge retention time, SRT 12–15 days) (Holbrook et al. 2002; Andersen et al. 2003; Joss et al. 2004). As E1 is a biodegradation product of E2, a prolonged hydraulic residence time is needed to remove both the E1 present in the influent and the E1 resulting from E2 biodegradation.

In this study, a method developed to detect steroid hormones in aqueous matrices (Noppe et al. 2005) was applied on samples originating from domestic and hospital WWTPs, in order to reveal possible differences due to the origin of the wastewaters and the influence on the receiving water bodies. The measurements in hospital wastewater are regarded as a first risk assessment of this wastewater type. Three different time points were examined for the two treatment plants. At one time point, chemical analysis data were compared to those of the Yeast Estrogen Screen (YES) test. As EE2 is regarded as the most important contributor to estrogenic activity in environmental samples, we especially focused on this compound.

[1]Doctor, Laboratory of Microbial Ecology and Technology (LabMET), Dept. of Bio-engineering Science, Ghent Univ.-UGent, Coupure Links 653, 9000 Ghent, Belgium. E-mail: http://labmet.ugent.be

[2]Doctor, Laboratory of Chemical Analysis, Research Group of Veterinary Zoonoses, Dept. of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, Ghent Univ.-UGent, Salisburylaan 133, 9820 Merelbeke, Belgium.

[3]Professor, Laboratory of Chemical Analysis, Research Group of Veterinary Zoonoses, Dept. of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, Ghent Univ.-UGent, Salisburylaan 133, 9820 Merelbeke, Belgium.

[4]Professor, Laboratory of Microbial Ecology and Technology (LabMET), Dept. of Bio-engineering Science, Ghent Univ.-UGent, Coupure Links 653, 9000 Ghent, Belgium (corresponding author). E-mail: willy.verstraete@ugent.be

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Table 1. Measured Concentrations of EE2, E1, P ($n=3$), and Estrogenic Activity (YES; $n=4$) in Different Samples from the Domestic WWTP on Three Different Time Points (EE: Estrogen Equivalents; ND: Not Detected; Dash: Not Measured)

Date	Sample	EE2 (ng/L)	E1 (ng/L)	P (ng/L)	YES (ng EE2eq/L)	Calculated EE (ng EE2 eq/L)
March 7, 2005	Influent	18.4 ± 3.0	25.6 ± 5.7	18.5 ± 5.0	15.8 ± 1.0	31 ± 7
	MBR effluent	114.0 ± 6.4	ND	ND	—	
	CAS effluent	5.7 ± 1.6	ND	ND	—	
	WWTP effluent	22.6 ± 7.9	ND	ND	36.0 ± 3.9	23 ± 8
	Canal upstream	6.0 ± 1.2	ND	ND	—	
	Canal downstream	10.9 ± 15	ND	ND	—	
April 25, 2005	Influent	4.2 ± 1.6	10.1 ± 2.7	4.8 ± 2.3	—	
	MBR effluent	2.4 ± 1.2	1.6 ± 0.5	ND	—	
	CAS effluent	1.7 ± 0.6	1.4 ± 0.5	ND	—	
	WWTP effluent	ND	1.8 ± 0.6	2.5 ± 0.7	—	
	Canal upstream	1.6 ± 0.1	1.2 ± 0.1	0.9 ± 0.3	—	
	Canal downstream	ND	ND	ND	—	
May 18, 2005	Influent	86.3 ± 22.0	24.0 ± 4.0	33.0 ± 6.9	—	
	MBR effluent	11.1 ± 2.2	ND	ND	—	
	CAS effluent	6.9 ± 1.1	15.1 ± 3.7	ND	—	
	WWTP effluent	83.4 ± 9.6	3.8 ± 0.6	ND	—	
	Canal upstream	9.3 ± 1.2	ND	ND	—	
	Canal downstream	6.0 ± 1.5	ND	ND	—	

Note: ND=not detected (below 0.2 ng/L).

Materials and Methods

Site Description and Sampling Procedure

The domestic WWTP (Belgium) has been upgraded in 2001 by installing a membrane bioreactor (MBR) to meet stringent effluent quality standards, because it is surrounded by a nature reserve. This MBR contains an activated sludge basin and Zeeweed membranes (Zenon Environmental Inc., Oakville, Ontario, Canada) in an external loop which separate the MBR effluent and the concentrated sludge. Subsequently, this sludge is fed back to the activated sludge basin. This process train treats a constant flow of 230 m^3/h. The remainder of the incoming wastewater flow ($500-1{,}000+m^3/h$) is treated in a conventional activated sludge (CAS) system. This WWTP discharges in a canal in a residential area. It was not possible to have composite samplers on all locations. Therefore, grab samples of (1) the influent after primary sedimentation; (2) the CAS effluent; (3) the MBR effluent; (4) the combined WWTP effluent and the receiving canal upstream (5); and downstream (6) the WWTP discharge were taken.

The hospital WWTP (Gent, Belgium) is the only WWTP in Belgium that exclusively treats hospital wastewater. It is operated by the hospital itself and it consists of a CAS system. The flow amounts to 300 m^3/days. Samples of the influent and effluent of the hospital WWTP were collected.

Extraction and Analysis

Grab samples of 2 L were extracted and analyzed with GC-MS-MS detection according to Noppe et al. (2005). We refer to this paper for more detailed information on quality assurance of the data. In brief, 2 L samples were extracted with Bakerbond C18 Speedisks (1 g sorbent, Bakerbond, Deventer, The Netherlands). Elution was performed with acetone and methanol. After evaporation of these extracts to dryness, the extracts were reconstituted in chloroform. Hexane was added and these samples were cleaned up with a combination of Si and NH_2 cartridges (Sopachem, Ochten, The Netherlands). After evaporating these samples to dryness, they were derivatized with a mixture of MSTFA [*N*-methyl-*N*-(trimethylsilyl) trifluoro-acetamide], NH_4I, and ethanethiol prior to GC-EI-MS-MS analysis with a Thermofinnigan Trace GC 2000 with a Polaris ion trap mass spectrometer (Impens et al. 2002). Deuterated estrone (E1-D4) and equilinin (EQ) were used as internal standards (100 ng/2 L sample) to account for losses during extraction and sample handling. Standard curves ranging from 0.2 to 100 ng/L were analyzed for E1, E2, estriol (E3), EE2, and P ($R^2 \geqslant 0.98$; data not shown).

YES was performed according to a modified version of the protocol of De Boever et al. (2001). The modifications in the protocol were as follows: the exposure period of the recombinant *Saccharomyces cerevisiae* yeast to the sample extract for 48 h instead of 24 h in order to obtain a high signal to noise ratio and the wavelengths of the absorbance measurements were 540 and 610 nm instead of 575 and 620 nm. Extraction (without the Si-NH_2 clean up) was performed as described earlier. Methanol/acetone extracts were analyzed in quadruplicate with the YES test. The fitting of the data points to the four parameter logistic curve was performed with Sigmaplot 8.0.

Results and Discussion

The measured concentrations in samples of the domestic WWTP are presented in Table 1. Only the natural estrogen estrone (E1) and the synthetic ethinylestradiol (EE2) were detected, together with the progestagen progesterone (P). The estrogenic activity as measured by the YES is expressed as an EE2 equivalent concentration. In all influent samples testosterone metabolites were identified but not quantified. Testosterone as such was not detected.

The EE2 concentrations measured on the three different time points at the domestic WWTP as presented in Table 1 show a large between-day variation of the EE2 concentration in the in-

Table 2. Measured Concentrations of EE2, E1, E2, P ($n=3$), and Estrogenic Activity (YES; $n=4$) in Different Samples from the Hospital WWTP on Four Different Time Points (EE: Estrogenic Equivalents; ND: Not Detected; Dash: Not Measured)

Date	Sample	EE2 (ng/L)	E1 (ng/L)	E2 (ng/L)	P (ng/L)	YES (ng EE2eq/L)	Calculated EE (ng EE2/L)
February 25, 2005	Influent	ND	0.5 ± 0.0	16.9 ± 9.1	4.3 ± 1.2	—	
	Effluent	27.8 ± 8.4	5.2 ± 1.8	2.5 ± 0.4	0.1 ± 0.2	—	
April 4, 2005	Influent 9:30 am	15.5 ± 1.9	58.3 ± 25.9	ND	15.3 ± 8.1	25.2 ± 0.4	45 ± 13
	Effluent 9:30 am	9.6 ± 4.1	4.0 ± 2.1	ND	3.2 ± 0.8	18.8 ± 7.6	12 ± 4
April 4, 2005	Influent 2:30 pm	18.3 ± 2.5	45.5 ± 14.3	ND	18.5 ± 3.9	—	
	Effluent 2:30 pm	15.3 ± 6.6	3.1 ± 0.3	ND	1.6 ± 0.9	—	
May 2, 2005	Influent	ND	8.1 ± 1.0	ND	>100	—	
	Effluent	ND	ND	ND	ND	—	

Note ND=not detected (below 0.2 ng/L).

fluent and the MBR effluent. The fact that the incoming EE2 concentration in the WWTP has a strong variability can be attributed to dilution by rain water. The lower concentrations encountered on April 25, 2005 can be explained by the weather, the days before were rainy. The CAS effluent shows a relatively stable EE2 concentration. Yet, due to the mixing of MBR and CAS effluent to make up the final WWTP effluent, the EE2 concentration in the effluent reaching the discharge varies with a factor of 100.

A remarkable finding is the presence of a background EE2 concentration in the receiving water, a small canal flowing through a residential area. Most likely some sewers in this residential area discharge in this little canal, or stormwater is discharged via a stormwater overflow. It was not possible to discern an influence of the WWTP effluent on the EE2 concentration in the receiving water.

In the hospital WWTP (Table 2), the natural estrogen E2 was detected once, but also the natural E1, the synthetic EE2, and P. Testosterone metabolites were identified in all influent samples.

These data are to the best of our knowledge the first reports on estrogens and other steroid hormone concentrations in hospital wastewater. It is clear from these data that E1 and P are removed to a high extent, whereas there is only a minor removal of EE2. On April 4, 2005, two sampling campaigns were undertaken, at 9:30 am and at 2:30 pm. The concentrations measured at the two time points do not differ significantly, indicating a good within-day correlation.

EE2-equivalent concentrations of EE2 (estrogenic potency=1) and E1 (estrogenic potency=0.5) were calculated (Johnson and Sumpter 2001) following the principle of estrogenic activity additivity (Tables 1 and 2). If these calculated estrogenic equivalent (EE) concentrations are compared to the YES data, a discrepancy can be seen between influent concentrations of both WWTPs. The YES seems to underestimate the real estrogen equivalent concentration with a factor of about 2, probably due to influent toxicity for the yeast strain applied. The testosterone metabolites present in the influent samples can exert an antiestrogenic effect, which could explain this observation as well. The opposite phenomenon is observed when comparing the effluent concentrations. An overestimation by the YES with a factor of about 1.6 is the case, probably due to the presence of other estrogenic compounds which were present in the effluent.

The estrogen concentrations observed in both WWTPs are consistent with literature data (Stumpf et al. 1996; Sattelberger et al. 1998; Adler et al. 2001; Schullerer et al. 2002; Cargouët et al. 2004). For progesterone, our data (4–>100 ng P/L) are in concordance with those described by Esperanza et al. (2007) for domestic WWTP samples.

For E1, E2, and P, much lower concentrations were measured in the effluents than in the influents at all times, which suggests that sorption and biodegradation take place in the WWTPs. For EE2, however, the removal is poor. Whereas the MBR produces an effluent of superior quality in terms of the generally measured physicochemical parameters such as chemical oxygen demand or suspended solids (data not shown), the observed MBR effluent concentrations are in the same range as the CAS effluent concentrations. This is in concordance with the findings of Clara et al. (2005), who found that the EE2 removal in MBRs is not better than in CAS systems. It indicates the need for a further EE2 removal treatment of WWTP effluent to warrant safe discharge into the receiving water bodies. Possible technologies such as ozonation (Huber et al. 2005) or activated carbon adsorption (Fuerhacker et al. 2001) have been suggested, but they might entail high costs and/or the production of potentially harmful side products (Jones et al. 2007).

It is generally accepted in the literature that E2 is converted into E1 in wastewater, be it chemically (at lower redox potentials) or microbiologically by activated sludge bacteria (Ternes et al. 1999a; Andersen et al. 2003). If the wastewater has a long residence time in the sewer, or if the wastewater is stored in a buffer tank prior to treatment, this conversion might take place. The data of both the domestic WWTP and the hospital WWTP suggest that the E2 to E1 conversion takes place before the wastewater reaches the WWTPs, as previously suggested by Ternes et al. (1999b). The wastewater of the hospital is stored in 100 m^3 submerged buffer tanks prior to treatment, which could explain the observed phenomena. The sewers leading to the domestic WWTP however, are short and account for a retention time of only a few hours. E2 has been detected once in the hospital WWTP influent at a concentration of 17 ± 9 ng/L. This E2 occurrence coincided with a low E1 influent concentration (0.47 ± 0.02 ng/L), which further corroborates the hypothesis that the greatest part of the measured E1 in wastewater is a conversion product of E2, and that only if this conversion does not take place in the sewer, then E2 will be measured in the wastewater.

Conclusions

This measuring campaign allowed assessing the concentrations of steroid hormones in both a domestic WWTP and a hospital

WWTP. Natural steroid hormones were detected in the effluents at nanograms per liter concentrations and are not considered as an environmental threat. These natural estrogens are removed in both a MBR and a CAS system, and no significant difference could be observed between household wastewater and hospital wastewater. The principal steroid hormone detected in the WWTP effluents and hence entering the environment is EE2. It can be regarded as an anthropogenic marker in the environment. Further treatment options should be examined and implemented to warrant a safe discharge of these WWTP effluents.

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References

Adler, P., Steger-Hartmann, T., and Kalbfus, W. (2001). "Distribution of natural and synthetic estrogenic steroid hormones in water samples from southern and middle Germany." *Acta Hydrochim. Hydrobiol.*, 29(4), 227–241.

Andersen, H. R., et al. (2005). "Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment." *Chemosphere*, 61(1), 139–146.

Andersen, H. R., Siegrist, H., Halling-Sørensen, B., and Ternes, T. A. (2003). "Fate of estrogens in a municipal sewage treatment plant." *Environ. Sci. Technol.*, 37(18), 4021–4026.

Cargouët, M., Perdiz, D., Mouatassim-Souali, A., Tamisier-Karolak, S., and Levi, Y. (2004). "Assessment of river contamination by estrogenic compounds in Paris area (France)." *Sci. Total Environ.*, 32(1–3), 55–66.

Clara, M., et al. (2005). "Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants." *Water Res.*, 39(19), 4797–4807.

Clara, M., Strenn, B., Ausserleitner, M., and Kreuzinger, N. (2004). "Comparison of the behaviour of selected micropollutants in a membrane bioreactor and a conventional wastewater treatment plant." *Water Sci. Technol.*, 50(5), 29–36.

De Boever, P., et al. (2001). "Optimization of a yeast estrogen screen and its applicability to study the release of estrogenic isoflavones from a soygerm powder." *Environ. Health Perspect.*, 109(7), 691–697.

Desbrow, C., Routledge, E. J., Brighty, G. C., Sumpter, J. P., and Waldock, M. (1998). "Identification of estrogenic chemicals in STW effluent 1. Chemical fractionation and in vitro biological screening." *Environ. Sci. Technol.*, 23(11), 1549–1558.

Esperanza, M., et al. (2007). "Fate of sex hormones in two pilot-scale municipal wastewater treatment plants: Conventional treatment." *Chemosphere*, 66(8), 1535–1544.

Fuerhacker, M., Dürauer, A., and Jungbauer, A. (2001). "Adsorption isotherms of 17 beta-estradiol on granular activated carbon (GAC)." *Chemosphere*, 44(7), 1573–1579.

Holbrook, R. D., Novak, J. T., Grizzard, T. J., and Love, N. G. (2002). "Estrogen receptor agonist fate during wastewater and biosolids treatment processes: A mass balance analysis." *Environ. Sci. Technol.*, 36(21), 4533–4539.

Huber, M. M., et al. (2005). "Oxidation of pharmaceuticals during ozonation of municipal wastewater effluents: A pilot study." *Environ. Sci. Technol.*, 39(11), 4290–4299.

Impens, S., De Wasch, K., Cornelis, M., and De Brabander, H. F. (2002). "Analysis on residues of estrogens, gestagens and androgens in kidney fat and meat with gas chromatography-tandem mass spectrometry." *J. Chromatogr, A*, 970(1–2), 235–247.

Johnson, A. C., and Sumpter, J. (2001). "Removal of endocrine-disrupting chemicals in activated sludge treatment works." *Environ. Sci. Technol.*, 35(24), 4697–4703.

Johnson, A. C., and Williams, R. J. (2004). "A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works." *Environ. Sci. Technol.*, 38(13), 3649–3658.

Jones, O. A., Green, P. G., Voulvoulis, N., and Lester, J. N. (2007). "Questioning the excessive use of advanced treatment to remove organic micropollutants from wastewater." *Environ. Sci. Technol.*, 41(14), 5085–5089.

Joss, A., Andersen, H., Ternes, T., Richle, P. R., and Siegrist, H. (2004). "Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: Consequences for plant optimization." *Environ. Sci. Technol.*, 38(11), 3047–3055.

Noppe, H., et al. (2005). "Development and validation of an analytical method for detection of estrogens in water." *Anal. Bioanal. Chem.*, 382(1), 91–98.

Petrovic, M., Solé, M., Lopez de Alda, M. J., and Barcelo, D. (2002). "Endocrine disruptors in sewage treatment plants, receiving river waters, and sediments: Integration of chemical analysis and biological effects on feral carp." *Envir. Toxicol. Chem.*, 21(10), 2146–2156.

Sattelberger, R., Hartl, W., Lorbeer, G., and Scharf, S. (1998). "Steroidhormone in der aquatischen Umwelt-erste Untersuchungsergebnisse aus Österreich." *Wiener Mitteilungen*, Band 153, 59–70 (in German).

Schullerer, S., Spengler, P., and Metzger, J. W. (2002). "Influence of different water treatment steps in sewage plant technology on the concentration of estrogens in sewage." *Vom Wasser*, 98, 65–80.

Stumpf, M., Ternes, T. A., Haberer, K., and Baumann, W. (1996). "Determination of natural and zynthetic estrogens in sewage plants and river water." *Vom Wasser*, 87, 251–261.

Ternes, T. A., et al. (1999a). "Behaviour and occurrence of estrogens in municipal sewage treatment plants. 1: Investigations in Germany, Canada and Brazil." *Sci. Total Environ.*, 225, 81–90.

Ternes, T. A., Kreckel, P., and Müller, J. (1999b). "Behaviour and occurrence of estrogens in municipal sewage treatment plants. 2: Aerobic batch experiments with activated sludge." *Sci. Total Environ.*, 225, 91–99.

Vethaak, A. D., Lahr, J., and Kuiper, R. V. (2002). "Estrogenic effects in fish in The Netherlands: Some preliminary results." *Toxicology*, 181, 147–150.