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ESTROGEN CONTAMINATION FROM WASTEWATER TREATMENT PLANTS AND ANIMAL WASTES

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ABSTRACT: One important pollution source of estrogen contaminants is the effluent and sludge from wastewater treatment plants. Another important pollution pathway is from agricultural animal wastes. In our work, the occurrence of select estrogens in wastewater, sludge and animal waste were tested. The leaching property of the estrogen compounds from the sludge and animal wastes were also examined. 17β-estradiol, 17α-dihydroequilin, 17α-ethinyl estradiol, estriol and estrone were detected in effluent wastewaters. The concentration of the detected estrogen hormones ranged from 11 to 24 ng/L in the effluent wastewater. 17α-estradiol, 17β-estradiol, 17α-dihydroequilin, and estrone were detected in the final dewatered sludge, with concentrations ranging from 98 to 463 ng/g dry solid. The leaching process is simulated in the lab by mixing the sludge with clean water. The results show that not more than 1% of estrogens were leached from the dewatered sludge. Cow manure and chicken manure were sampled for analysis of estrogen hormones. 17α-estradiol, 17β-estradiol, 17α-dihydroequilin and estrone were detected in the cow manure with concentrations ranging from 6 to 27 ng/gDS. After mixing with water, about 18% to 48% of estrogens enter the aqueous phase. 17α-estradiol, 17β-estradiol and estrone were detected in chicken manure with concentrations ranging from 44 to 150 ng/gDS. About 10 to 100 % leaching of these chemicals was observed. The results demonstrated that animal waste could have more serious estrogen leaching problems as compared to sludge from wastewater treatment plants. Advanced management methods should be studied to control the pollution from animal wastes.
KEY TERMS: estrogen; wastewater; sludge; animal waste; leaching; emerging contaminant.

INTRODUCTION

Occurrence of estrogen hormones as a category of emerging contaminant in environmental systems has gained widespread attention due to their adverse effects to the ecosystem and potential risks to human health (Halling-Sorensen, 1998; Khanal, 2006). Estrogens are either produced naturally in the body, or are synthetically created for use in birth control pills and hormone replacement therapy. Their presence has been reported in surface water at many different areas (Chimchirian, 2005, Kolpin, 2002). Estrogen hormones can interfere with reproductive systems by producing an unnatural response of the endocrine system. There have been reports on adverse effects of trace level estrogen hormones in aqueous systems on organisms (Routledge, 1998; Sanderson, 2004).

One important pollution source of these estrogen contaminants is the effluent and sludge from wastewater treatment plants (WWTPs). The naturally produced estrogens and synthetic estrogens excreted from the human body enter into the municipal wastewater treatment plant and typically undergo the primary and secondary treatment, and disinfection. After treatment, the effluent is generally discharged directly into the surface water systems. Most estrogens are hydrophobic compounds and have high affinity to attach to the solid particles in the treatment systems (Ternes, 2004). Therefore, the discharged sludge from the wastewater treatment plant can be an important pollution source of estrogens. After being disposed or land applied as agricultural fertilizer, the sludge could represent a pollution source due to the leaching of estrogens into the water systems. Another important pollution pathway is from the agricultural animal wastes, which are typically land applied. Estrogen hormones have been detected in animal wastes (Hanselman, 2003). Leaching of estrogens from the animal waste that is directly land applied could also cause pollution of soils, surface water and groundwater.

In this study, the estrogen contamination from the wastewater treatment plant, both in wastewater effluent and sludge, were examined. The estrogen hormones present in animal manure (cow and poultry), and their leaching potential were also tested.

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EXPERIMENTAL

Materials

Estrogen Hormones were obtained from Sigma Aldrich and from pharmaceutical companies. 3-O-methylestrone was used as an internal standard. All solvents (High Perform Liquid Chromatography grade) and other chemicals were purchased from Fisher Scientific. Millipore nitrocellulose filters (47 mm white) with various pore size and amber glass bottles were obtained from Fisher Scientific. Varian Bond Elute 3 ml/500 mg solid phase extraction (SPE) cartridges were obtained from Varian, Inc. Prior to use, all glassware was silanized with 5% dimethyldichlorosilane in toluene.

Sample Collection

Wastewater samples were collected at different treatment points from two local WWTPs. The wastewater samples included influent, primary effluent, secondary effluent and final effluent. The final dewatered sludge was collected from one of the two WWTPs where a high speed centrifuge was applied for sludge dewatering. The samples were stored at 4^{o}C not more than one week until analyzed. The concentration of the dry solid was tested using the standard method. Animal waste (chicken manure and cow manure) was collected from a local animal farm. The solid waste was frozen until analyzed and experiments were completed within a month from the date of collection.

Filtration of aqueous samples was necessary due to suspended solids and media to prevent clogging of SPE columns. All aqueous samples were filtered using two different size filter papers in a stepwise approach, first through a 0.8 μm filter then a 0.45 μm filter. This provided a consistent flow through the SPE column without causing any clogging.

Leaching Tests

A known amount of solid waste (sludge, animal manure) was collected in a clean silanized centrifuge vial. To this amount a known volume of Milli-Q water was added. The mixture was continuously shaken for 5 hrs on a wrist action shaker. The supernatant from this mixture was collected by centrifugation and filtered using 0.45 μm filters. The concentration of estrogen hormones in the filtrate (leaching solution) was tested following the liquid sample analysis procedures and was used to characterize the leaching properties of the solid samples.

Analysis

The liquid samples, which included wastewater and the leaching solution, were filtered and preceded to SPE and Gas Chromatography – Mass Spectrometry (GC/MS) analysis. The solid samples including dewatered final sludge and animal wastes were firstly analyzed for dry solids content using standard method. The sampled sludge and animal manures were used for the analysis of estrogen hormones. Liquid extraction was performed first on the solid samples to extract the objective chemicals into the liquid solution. Methanol and acetone were used twice for liquid extraction. The liquid extract collected was filtered twice using a 0.45 μm syringe filter. SPE was performed using a Bond Elute C-18 SPE cartridge at a flow rate of 5 ml/min. After loading, the columns were washed one time with 3 ml of Milli-Q water and twice with 3 ml of Milli-Q water/methanol (60:40 v/v) solution. The cartridges were then eluted with 3 ml of methanol. The methanol eluent was collected into clean, silanized test tubes and dried in a Genevac centrifugal evaporator at 45°C and 12 mbar. To the dried sample, 15 μl of pyridine and 65 μl of bis(trimethylsilyl)-trifluoroacetamide containing 1% dimethylchlorosilane were added for derivatization. The sample was vortexed and allowed to react for 15 minutes at room temperature. To the derivatized sample 500 μl of toluene was added and vortexed. The samples were then collected in an amber glass GC vial containing 0.25 μl insert. The head space free vials were placed on the GC/MS for analysis. The GC-MS analysis was performed using an Agilent 6890N GC and a 5973N MS. Splitless auto injections were made onto a Pursuit DB-225MS capillary column (30 m × 0.25 mm × 0.25 μm; J & W Scientific) with an initial temperature of 50°C for 1 min, and a flow of 4.5 ml/min, then ramped to 200°C at 50°C/min with a flow of 4.5 ml/min and held for 95 min. Finally, the oven temperature was ramped to 220°C at 10°C/min and held for 27 min. The post run was held at 240°C for 10 min. with a flow of 4.8 mL/min. Helium was used as a carrier gas, the inlet and source temperature was 240°C and the quad was set at 150°C.

RESULTS AND DISCUSSION

Table 1 show the quality control results using clean water (Milli-Q water, MQ), effluent wastewater as well as the sludge solid. It can be observed from Table 1 that the matrix affects the recovery and analysis significantly. This is due to the

background matters present in the wastewater and sludge solid samples. With the clean water matrix, the recovery efficiencies are higher than 92% except for ethinyl estradiol (EE2) which shows about 84% recovery. The relative standard deviation (RSD) of all tested estrogen compound is not higher than 8%, which means that the reproducibility of the analytical procedures is acceptable. The recovery efficiencies of the estrogens increased except that for EE2 in wastewater. The recoveries of most estrogens in the wastewater are higher than 100%. This might be due to the enhancement effects of the background chemicals during the GC/MS analysis. Similar recoveries of above 100% have been reported by others. The RSD was not more than 10%, except 20% for EE2 and 14% for equilin. This means that this analytical method was acceptable for wastewater analysis. The recoveries of estrogens from sludge varied in a wider range. The RSDs were not more than 10%.

Table 1: Quality control: Average recovery efficiencies (RE%) and relative standard deviation (RSD%) of the analysis on different matrix samples. Based on triplicate analysis.

ANALYTE	Milli-Q water		Final Effluent		sludge	
	RE %	RSD%	RE%	RSD %	RE%	RSD %
17 α-estradiol	100	6.0	132	8.4	47	3.3
17 β-estradiol	98	4.9	116	6.6	37	5.6
17 α-dihydroequilin	95	4.2	108	9.8	11	0.6
17 a-Ethinyl estradiol	84	5.6	58	20.2	70	1.9
Estriol	95	7.6	104	6.8	50	8.4
Estrone	97	2.7	123	10.1	59	5.7
Equilin	92	5.8	97	14.2	185	6.5

Wastewater could be an important pollution source of estrogen contaminants. Table 2 shows the concentrations of estrogens in the wastewater sampled at different locations in two local municipal WWTPs whose effluent was directly discharged into the surface water. The WWTP 1 uses activated sludge process, and WWTP 2 uses the Sequential Batch Reactor (SBR) process.

Table 2: Concentration of estrogens in ng/L, detected at different locations of two WWTPs. ND: not detected.

ANALYTE	WWTP 1 (activated sludge)				WWTP 2 (SBR)		
	Influent	Primary effluent	Secondary effluent	Final effluent	Influent	SBR effluent	Final effluent
17 α - estradiol	ND	9.6	ND	ND	ND	ND	ND
17 β - estradiol	198.3	37.7	ND	ND	ND	ND	ND
17 α - dihydroequilin	20.4	ND	7.5	11.9	23.8	19.4	23.6
17 α - ethinyl estradiol	16.4	ND	ND	ND	ND	ND	ND
Estriol	709.8	351.0	ND	ND	ND	ND	ND
Estrone	49.2	43.1	15.0	ND	59.0	ND	11.5
Equilin	ND	ND	ND	ND	ND	ND	ND

It can be observed from Table 2 that 17β-estradiol, dihydroequilin, ethinyl estradiol, estriol and estrone were detected in the influent of WWTP 1 with notable concentrations. 17α – estradiol was not detected in the influent, secondary effluent or final effluent but was detected in the primary effluent with a concentration of 10 ng/L. 17β – estradiol was detected in the influent with high concentrations as 198 ng/L but after primary treatment its concentration decreased to 38 ng/L. It was not detected in the effluent. 17α – dihydroequilin was present in the influent with a concentration of 20 ng/L which decreased to 12 ng/L in the final effluent. 17α - ethinyl estradiol was only detected in the influent with a concentration of 16 ng/L. The treatment seemed to be significantly effective for estriol reducing its concentration from 710 ng/L in the influent to non detect in the final effluent. Estrone concentration was also significantly reduced from 49 ng/L in the influent to 15 ng/L in the secondary effluent and non detect in the final effluent. After treatment, only dihydroequilin was detected in the final effluent of WWTP 1. 17α-dihydroequilin was also detected in WWTP 2 with 24 ng/L in the influent, 19 ng/L in the SBR effluent and 24 ng/L in the final effluent. Another estrogen detected in WWTP 2 was estrone with 59 ng/L in the influent, its concentration decreasing to 11 ng/L in the final effluent. 17α-estradiol, 17β-estradiol, 17a-ethinyl estradiol and equilin were not detected in the wastewaters at WWTP 2.

Comparison between the removal effects of estrogens in two treatment plants demonstrated that activated sludge system shows better removal of estrogens than SBR system. After activated sludge treatment, only dihydroequilin and estrone were detected in the effluent with lowered concentration. Dihydroequilin shows more difficulty in removal in both treatment

systems. Estrogens were discharged from both treatment plants, meaning the biological treatment systems can not completely remove the estrogen contaminants. The wastewater effluent represents important pollution of estrogen hormones. Advanced treatment systems are needed for complete destruction of the remaining estrogens in the wastewater.

The occurrence of estrogens in the final dewatered sludge from municipal WWTPs was also investigated. After being disposed or land applied as agricultural fertilizer, the sludge could still represent a pollution source due to the leaching of pharmaceutical compounds into the water systems. The estrogen hormones are known to be hydrophobic compounds and therefore have high sorption affinity to solids. The leaching process was simulated in the laboratory by mixing the sludge with clean water. The concentrations of leached hormones in the aqueous phase were then examined. It can be seen from Table 3 that 17α – estradiol, 17β – estradiol, 17α – dihydroequilin and estrone were detected in the final sludge with noteworthy concentrations. After mixing with water, not more than 1% estrogens were leached into liquid phase, which meant the leaching is not important for sludge pollution. However, a longer leaching test is needed to verify this.

Table 3: Presence of estrogen hormones in final dewatered sludge* and their leaching properties.

ANALYTE	Concentration (ng/g Dry Solid)	Leaching (ng/g Dry Solid)	Leaching Percentage (%)
17 α - estradiol	462.6	3.0	0.7
17 β - estradiol	229.7	0.1	0.1
17 α - dihydroequilin	152.1	0.5	0.4
Estrone	98.5	0.3	0.3

* Solid content in final dewatered sludge is 29%; leaching test: liquid/solid ratio: 16.2:1 (w/w); continuously shaken for 5 h.

The results shown above demonstrate that two kinds of pollution sources exist at municipal wastewater treatment plants. Wastewater, which after treatment, still contains notable concentration of estrogens and is discharged directly into the surface water. Another pollution pathway from the wastewater treatment plant is through the disposal and land application of sludge which contains significant amount of estrogens. Although the leaching percentage of estrogens from the sludge is very small, the long-term contacting with water could mean potential risks to the ecosystems. The leached estrogens might transfer to soils, sediment as well as the groundwater.

The estrogens detected in the animal manure are shown in Tables 4. It can be observed that 17α – estradiol, 17β-estradiol and estrone were detected in the chicken manure with concentration as high as 93, 150 and 44 ng/g dry solid (DS), respectively. 17 α – estradiol, 17 β - estradiol , 17 α – dihydroequilin and estrone were detected in the cow manure with concentrations of 6, 17, 27 and 16 ng/g DS, respectively. The estrogens present in the animal waste could leach and enter the environment with runoff from farm land where manure was stored, as well as from land where it is applied as fertilizer. The leaching characteristics of estrogens in the two types of animal manure were tested and also shown in Tables 4. It can be observed that some estrogens have high leaching characteristics. For example estrone and 17 α – estradiol showed high leaching percentage: estrone with 100% from chicken manure and 48 % from cow manure; and 17 α – estradiol with 16 % from chicken manure and 41% from cow manure. The results demonstrate that animal waste could represent an important estrogen pollution source. Significant amount of estrogen was leaching from the animal waste, which could cause the contamination to the soils, surface water as well as groundwater if the animal waste is not well managed to control the estrogen concentration and leaching.

Table 4: presence of estrogen hormones in chicken manure and their leaching properties

ANALYTE	**Chicken manure [1]**			**Cow manure [2]**		
	Concentration (ng/gDS)	Leaching (ng/gDS)	Leaching percentage (%)	Concentration (ng/gDS)	Leaching (ng/gDS)	Leaching percentage (%)
17α - estradiol	92.716	15.029	16.2	6.202	2.542	41.0
17β - estradiol	149.856	15.877	10.6	16.573	3.031	18.3
17α - dihydroequilin	ND	9.017	--	27.419	4.881	17.8
Estrone	44.221	59.422	134.4	16.129	7.729	47.9

1. Chicken manure sample with dry solid of 25.95%; leaching test: liquid/solid ratio: 3.85:1 (w/w); continuously shaken for 5 hr; use the aqueous phase at 0.45μm for test.
2. Cow manure sample with dry solid of 15.2%; leaching test: liquid/solid ratio: 16:1 (w/w); continuously shaken for 5 hr; use the aqueous phase at 0.45μm for test.

CONCLUSION

The estrogen contaminants could be from the municipal wastewater treatment plants (wastewater and sludge). The conventional biological treatment systems can not completely remove the estrogens and notable concentrations of estrogens are discharged into the surface water. The final discharged sludge contained high concentrations of estrogens. Its pollution potential through leaching is of concern if land applied. Noticeable estrogens were detected in the animal wastes and in their leaching solutions. Agricultural animal waste is another important estrogen contaminant source.

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