Detection of Estrogenic Hormonal Residues in the Lower Chao Phraya River, Thailand

การตรวจวัดฮอร์โมนเอสโตรเจนตกค้างในแม่น้ำเจ้าพระยาตอนล่าง

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ABSTRACT

Estrogenic hormonal residues in the aquatic system had been considered as an emerging environmental problem worldwide due to the fear of their adverse impacts on human health. Since the common source of these residues were mainly from communities wastewater, samples taken in this study were from 10 locations along the lower part of Chao Phraya River, which ran through the densest habitat in Thailand as Pathumthani, Nontaburi and Bangkok provinces. Water samples were analyzed the physical and chemical properties and two natural estrogenic hormonal residues including estrone and 17β estradiol were also detected by GC-MS. The results found that water quality at the sampling sites in the lower part of Chao Phraya River was classified as class 4 of the standard of water quality. Unfortunately, there was no the estrone and 17β estradiol in all water samples within the analytical detection limit of the GC-MS at 6ng/l. However, prevention guidelines and monitoring system of estrogenic hormonal residues in surface water was purposed to meet an early awareness of this emerging hazard.

บทคัดย่อ

เนื่องจากความเกรงกลัวถึงผลร้ายต่อสุขภาพมนุษย์ทำให้ปัญหาเรื่องฮอร์โมนเอสโตรเจนตกค้างในแหล่งน้ำเป็นเรื่องที่ได้รับความสนใจไปทั่วโลก และโดยเหตุที่ฮอร์โมนตกค้างเหล่านี้มีแหล่งกำเนิดส่วนใหญ่มาจากน้ำเสียชุมชน การเก็บตัวอย่างที่ใช้ในการวิจัยนี้จึงใช้วิธีการเก็บจากแม่น้ำเจ้าพระยาตอนล่างซึ่งไหลผ่านชุมชนหนาแน่นมากที่สุดในประเทศไทย คือจังหวัดปทุมธานี นนทบุรี และกรุงเทพมหานครจำนวน 10 ตัวอย่าง เพื่อทำการตรวจวัดคุณภาพน้ำทางด้านกายภาพและเคมี และตรวจวัดฮอร์โมนเอสโตรเจนธรรมชาติที่ตกค้าง 2 ชนิดคือ เอสโตรนและ17βเอสตราไดออล โดยใช้เครื่อง GC-MS จากผลการศึกษาพบว่า คุณภาพน้ำของจุดเก็บตัวอย่างบริเวณแม่น้ำเจ้าพระยาตอนล่างอยู่ในเกณฑ์มาตรฐานคุณภาพน้ำผิวดินประเภทที่ 4 นอกจากนี้ ไม่สามารถตรวจพบเอสโตรนและ17βเอสตราไดออลในตัวอย่างน้ำทั้ง 10 จุด โดยเครื่อง GC-MS มีการตรวจวัดต่ำสุดได้ที่ 6 นาโนกรัมต่อลิตร จากผลการศึกษาแสดงให้เห็นว่าตัวอย่างน้ำอาจมีปริมาณเอสโตรนและ17βเอสตราไดออลต่ำกว่า 6 นาโนกรัมต่อลิตร แต่อย่างไรก็ตาม การศึกษาครั้งนี้ได้นำเสนอแนวทางในการป้องกันและระบบการติดตามเฝ้าระวังการปนเปื้อนของฮอร์โมนเอสโตรเจนตกค้างในน้ำผิวดิน เพื่อเตรียมพร้อมสำหรับอันตรายจากฮอร์โมนดังกล่าวที่อาจเกิดขึ้นได้ในอนาคต

Key Words : estrogenic hormonal residues, GC-MS, Lower Chao Phraya River
คำสำคัญ : ฮอร์โมนเอสโตรเจนตกค้าง จีซี- เอ็มเอส แม่น้ำเจ้าพระยาตอนล่าง

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Introduction

Studies on low level concentration of estrogenic hormonal residues in the aquatic system indicated the wide spread of such substances in the environment (Desbrow et al., 1998; Halling-Sørensen et al., 1998; Ternes, 1998; Daughton and Ternes, 1999). They and their metabolites were believed to be continually introduced either directly or via incomplete wastewater treatment plants' effluent into the aquatic system. Natural estrogens such as Estradiol (E1) and17β-Estradiol (E2) were normally found in the excretion from human and animals (Shore, Gurevitz and Shemesh, 1993) and were classified as the most potent endocrinal disrupting compounds (Nghiem et al., 2004) due to their biologically active property even at low concentration (Purdom et al., 1994). Their significant effects were in disturbing the endocrine system by mimicking, blocking and disrupting function of hormone, affecting the health of humans and animals species (Bolong et al., 2009) and brought to sexual function interferences in animals by causing feminization, decrease in sperm production and causing developmental abnormalities (Jobling et al., 2004; Roepke et al., 2005).

Studies from many countries found that they were not uncommon to find such natural estrogenic compounds in the aqua systems like rivers, lakes or waterways that located in the highly populated areas especially those received the drainages from municipal treatment plants. The researches on the discharges particularly from municipal STP in Europe and USA, frequently found the residues of 17β-Estradiol and 17α ethinylestradiol (from contraceptive pills) at the concentration of few pg/l to few ng/l (Ternes et al., 1999a). They brought to the conclusion that municipal wastewater discharge was the major contributor of estrogenic hormonal contamination of urban river waters due to the increase of population and inadequate of wastewater treatment facilities. Theoretically, most of estrogenic hormones when excreted were in the form of conjugated estrogens which were not active forms, but in practice, the active unconjugated forms were also found in the effluents from the waste water treatment plants (WWTP). Baronti et al. (2000) assumed that deconjugation process of estrogens occurred mostly in sewers and were later released into the environment. Therefore, a good understanding of their physiochemical, biodegradable and sorption properties was a necessity for improving the knowledge of their fate in the environment.

This study was designed to make a screening investigation of these two estrogenic hormones in the lower Chao Phraya River which ran through the densest habitat in Thailand. Ten samples were collected along the river and analyzed for the hormones by GC-MS. Since it was the first study of its kind in Thailand, the methods developed for this study would be the starting point for further studies about micro-pollutants in the aquatic environment.

Materials and methods

1.1 Study area and sample collection

The Chao Phrya basin was the most developed and most important basin in Thailand. It covered 30% of the country's land and was the most crowded place where the lower Chao Phrya basin, which comprised of Bangkok Metropolitan Area

(BMA) and its vicinity of Samut Prakan, Nonthaburi and Patum Thani, being the highest populated area with population about 11.5 million and the highest population density area of 1,497 inhibitants/km^2 (the office of Natural Water Resources Committee (ONWC) of Thailand, 2005). A daily wastewater of Bangkok alone was around 2.5 m^3 (Department of Drainage and Sewage, 2003) and approximately 40% being treated in wastewater treatment plants with the remaining wastewater and all WWTP effluents being discharged into the lower Chao Phraya River. The river ran from north to south about 372 kilometres (231 mi) long from the central plains to Bangkok and exit to the Gulf of Thailand at Samut Prakarn province.

where community wastewater and hospital discharges were expected (Fig. 1). They were collected along the river from up to downstream and a distance of the discharging point and collecting site not exceed 1 km. apart.

Sampling was performed in August 2009 which was a high flow season in Thailand. They were obtained from bridges at sites a, b and c and from the boat in the middle of the river at the rest of the sites (d-j) at the depth of < 1 m from the water surface. They were collected and stored in 2 L plastic bottles and were transported in boxes packed with ice. When arrived at the laboratory, they were all kept in the refrigerator at 4^o C.

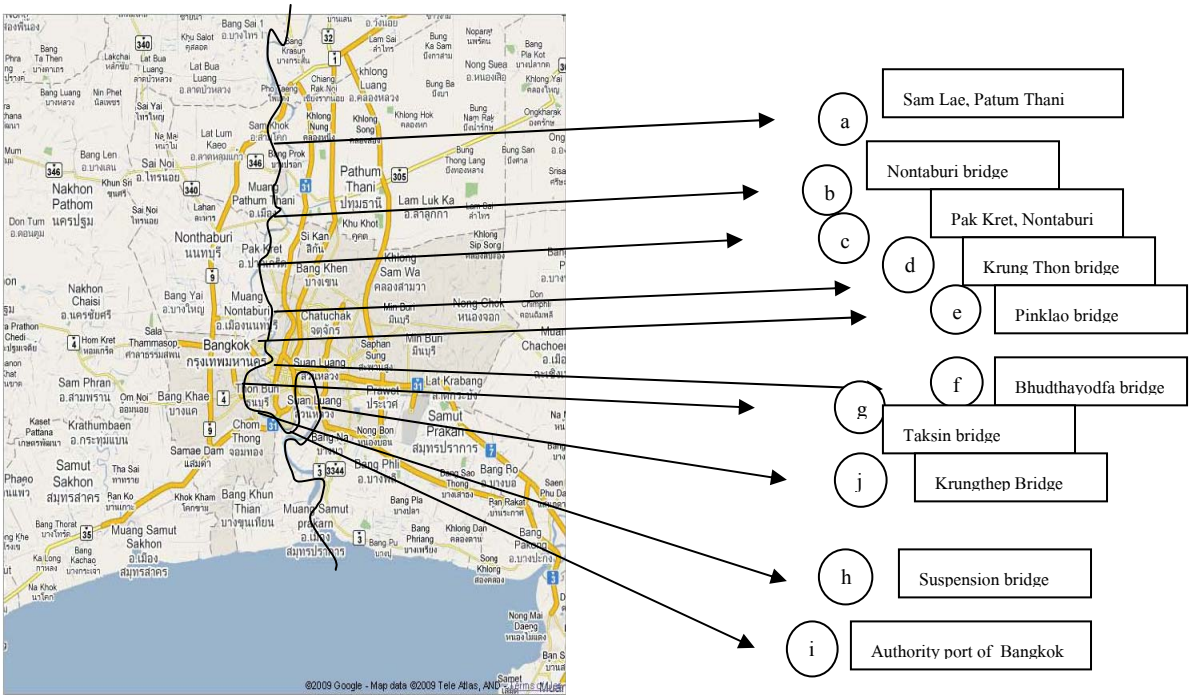

Figure 1 Sample collection site map

Ten water samples were collected from the middle of the lower Chao Phraya River at the points

1.2 Extraction and analysis

Water samples were examined for physical parameters at the collecting sites with probes and meters. The analysis methods were based on standard methods for examination of wastewater by APHA, AWWA and WPCF as in table 1.

Table 1 Analysis Methods for water parameter

Parameters	Analysis Method
pH	pH meter Electrometric method
DO	Azide Modification, P20 by number and period of sampling
BOD	Azide Modification at 20°C 5 days, P80 by number and period of sampling
Conductivity	conductivity meter
SS	Dry the sample at 103°-105°C then weigh the solid

For estrogenic hormonal detection method, a 500 ml sample (or control) was spiked with the internal standard (ISTD, 25 μl of 17α-methyltestosterone, 25 μg/mL in methanol). The solution was passed through a Sep-Pak® C18 cartridge at the flow rate of about 5 ml/min and the estrogen eluted off with 2 ml methanol. The methanol extract was dried with a flow of nitrogen gas at 55°C. The dry residue was then derivatized with 50 μl of MSTFA/NH_4I/2-mercaptoethanol (1000:2:6,v/w/v) for 20 min at 60°C on a heating block. After that, a 3 μl aliquot was injected with CTC PAL Autosampler into the GC-MS system which was HP 6890 Series GC System, MSD 5975. The injection mode was split at ratio 10:1 with the injection temperature at 280°C. An Ultra 1, Cross-linked Methyl Siloxane column (17m x0.2mm, film thickness 0.11 μm) was used for chroma tographic separation while Helium acted as a carrier g as at constant pressure adjusted so that the retention time of ISTD was 15 ± 0.3 minute. The oven was ini tially set at 180°C, then rose by 3 °C/min to 230 °C and finally by 40 °C/min to 310 °C and hold for 3 minutes. It took 21.67 minutes run time for the process. Mass Selective Detector, MSD 5973 detector was set for tr ansfer line at 280 °C while solvent delay time was 2.50 minutes. The 70 eV electron impact was determ ined as ionization mode with the acquisition mode as SIM.

The analytes were quantified by using an int ernal standard method with calibration against absolute standard solutions which was 500 mL tap-water spiked with 20 ng of standard estradiol as control. The charac teristic ion results were read by Hewlett Packard Chem Station which had method detection limits (MD Ls) of 6 ng/L. The characteristic ions of estrone and 17β es tradiol were shown in table 2.

Table 2 Classification, physicochemical properties and characteristic ions of estrone and 17β estradiol

Compound	CAS RN	Classification	Ions (m/z)
Estrone	53-16-7	Natural estrogen	342, 257
17β-Estradiol	50-28-2	Natural estrogen	416, 285

Results and discussion

The water samples were collected in August 2009 which was high-flow season. Their parameters were tested at site except for BOD, TDS and SS which were sent to DPC's lab whilst for hormone detection lab, samples were separated, stored in the ice box and transferred to the lab immediately afterwards. The result of the water samples were shown in table 3.

The water parameters showed that river water quality was fit for standard category 4 with BOD $\leq$ 4.0 mg/l (0.8-1.7 mg/l) and DO $\geq$ 2.0 mg (0.5-4.2mg/l). The suspended solid appeared to be quite high (24-66 mg/l) and the salinity was slight (0-0.2 ppt). Due to many studies, these parametric values and their physiochemical properties were considered to have affects on the existence of estrogenic hormonal residues in the aquatic environment. Their sorption correlated directly with the organic carbon content and salinity in the water that the more salinity in water the easier was their sorption (Lai et al., 2000). William et al. (1999) found that most of the estrogenic hormonal residues especially estrone bound more easily to sediment than water and was kinetically occurred at all time, his investigation of bed sediments in three English rivers showed that hormonal loads accounted between 13% and 92%. High suspended sediment found in this study might also affect the fate of estrogen in the aqua environment for it was clear that high suspended sediment (SS) content could cause these estrogenic steroids' biodegradation (Liu et al., 2009) and lesser chance of the hormones' finding. Though was not examined in this study, high concentration of bacteria was also considered to be an important cause of estrogenic hormone's biodegradation in the environment (Matsuoka et al., 2005; Williams et al., 1999). Moreover, a result of an experiment done by Ramon et al. (2001) on acidity to estrogenic hormone biodegradation showed that 17β–estradiol concentrations decreased rapidly in non–acidified samples especially during the first day as much as 90%. While at the temperature of 30^{o}C, 40% of the total estrogens were lost over the 7 day period with the largest portion of estrogen remaining was estrone from which gave a strong suggestion of the need of acidification and low temperature in keeping of environmental samples for estrogens testing. Another factor that could give impact on this study was dilution effect. Since the sample collecting time in this study was during rainy season, dilution effect was unavoidable. It was possible that the trace amount of estrogen might be diluted to lower than the detection limit of the equipment and therefore could not be detected by the instrument. As from the experiments by Masoka et al. (2005), collected water samples were done throughout one year and found that the levels of estrogenic substances in river water changed daily, weekly and monthly and the highest 17β-estradiol level (E2 equivalent) was obtained in summer.

It was undeniable that estrogenic hormonal concentration report in this study was found less than method detection limit but it was not a firm prove of their absent. It might be related to the fact that estrogenic hormones in the environment was trace and checking instruments might need to have a detecting limit to as low as 0.1 ng/l as in other studies elsewhere. Also, the correlation between their sorption, biodegradation and the dilution effect might affect the findings as mention above.

Table 3 Water samples' parameters and estrogenic hormonal residues value by GC-MS

Station	Time	Water temperature (°C)	pH	Turbidity (NTU)	Conductivity (µmho/cm)	Salinity (ppt)	DO (mg/l)	BOD (mg/l)	TDS (mg/l)	SS (mg/l)	Estrone(E1) (ng/l)	17βEstradiol (E2) (ng/l)
A	10.00	31.6	8.8	40	25	0.0	4.2	0.9	189	25	<MDL	<MDL
B	11.00	31.7	8.7	37	198	0.1	2.6	0.9	198	24	<MDL	<MDL
C	11.30	31.4	8.8	36	328	0.0.2	4.0	0.8	202	28	<MDL	<MDL
D	13.10	30.5	8.8	41	225	0.1	2.2	0.9	216	39	<MDL	<MDL
E	13.30	30.7	8.7	45	354	0.2	1.9	0.9	212	50	<MDL	<MDL
F	13.45	30.8	8.7	38	371	0.2	1.9	1.3	226	38	<MDL	<MDL
G	14.00	30.7	8.1	47	375	0.2	1.2	1.4	226	36	<MDL	<MDL
H	14.10	30.4	8.2	31	379	0.2	0.7	1.7	221	40	<MDL	<MDL
I	14.25	30.2	8.3	26	159	0.1	0.5	1.2	228	37	<MDL	<MDL
j	14.40	30.4	8.8	21	160	0.1	0.8	1.6	225	66	<MDL	<MDL
Average		30.8	8.6	36	257	0.1	2	1.2	214	38	<MDL	<MDL

MDL: Method Detection Limit = 6ng/l

However, prevention measures of the estrogenic hormonal residues contamination in the aquatic system in Thailand were still crucial. There were many regulatory mechanisms that could use to manage municipal wastewater and industrial chemical discharges in order to reduce the chance of releasing estrogenic hormones into the environment. For example, increase the efficiency and number of the wastewater treatment plants to make cleaner effluents before discharging into the river, control of industrial emissions through discharge consents which was an useful measure for point sources or used of voluntary initiatives measure to create a self control process (Gross-Sorokin et al., 2006) etc. Nevertheless, environment agency in collaboration with government and the water industry should play a major role in this issue in conducting the sewage treatment processes assessment program to explicit these estrogenic hormonal residues, to improve the effectiveness of the WWTPs in removing or reducing these hormones and to provide information for future decision on how to control this estrogenic contamination. The program should involve an effluent-testing program comprising a combination of chemical determination and biological activity monitoring and studied the possible treatment methods to remove steroid estrogens and to reduce estrogenic activity of final effluents. In addition, environmental monitoring (e.g., histology and population parameters) and population modeling should be done in supplementary research programs to evaluate effectiveness of the present technologies in providing important information on changes to environmental quality (Gross-Sorokin et al., 2006).

For Thailand, the most suitable treatment scheme had to be both efficient and low cost, so the optimizing existing treatment technology was more considered. The study from Koh et al. (2008) showed that secondary biological treatment in the conventional treatment plants was a key process in removing most of the estrogenic residues. Transformation and biodegradation were considered important for hormone removal as some microorganisms possessed the potential to utilize steroid estrogens as carbon sources for metabolism. The suggested retention time was at least 10 to 12.5 days to assured for the growth of micro-organisms that decomposed E2 and E1.

Servos et al. (2005) also reported in their study of the significant effect on nitrification in the biological treatment system for its potential on estrogens removal.

Conclusions

Estrone and 17β Estradiol were two natural estrogenic hormones excreted merely by human and animals. They entered the aquatic envi ronment through discharge of municipal wastewater and animals waste. Even though they were detected in trace amount and no strong evidence, up till now, of the adverse effects of these residues to human health but their endocrinal disrupting properties brought much concern on the finding. Many studies, though limited, indicated that their fate in the environment depended on their physiochemical properties and environmental media. More understanding of their changes and their metabolites throughout their life cycle would certainly benefit the prediction, prevention and treatment plans to manage this unwanted problem in the future.

The experiment designed in this study was aimed for detecting these two estrogenic hormonal residues in the Lower Chao Phraya River. Since all sampling and investigating methods were the first time in Thailand, some of them needed to be adjusted in the future. They were : sampling time that might change to summer time to avoid dilution effect or conducted year round sampling to compare for the seasonal effect, sample acidification to prevent biodegradation by water micro-organisms and much lower method detection limit of the instrument and more appropriate detection method to enhance chance of detection. The fineness and accuracy of the detecting instrument was one of the key factors to detect these trace substances in many studies by which their affirmation induced alertness among stakeholders.

Anyway, for Thailand, periodic monitoring and prevention plan were recommended for early detection of this emerging hazard. Environment agency in collaboration with government and the water industry should play a major role in this issue and acted through many regulatory mechanisms such as municipal wastewater discharging regulation, industrial waste emission permission, polluter pay principal or voluntary consent among stakeholders. For treatment aspect, optimizing existing treatment technology of the present WWTP might suit Thailand. Biological processes in the secondary treatment would play a major role in estrogenic residues reducing by using biodegradation, biotransformation and adsorption mechanisms together with longer SRT and HRT to achieve the goals.

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