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Excretion of manure-borne estrogens and androgens and their potential risk estimation in the Yangtze River Basin

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ABSTRACT

The Yangtze River is the longest river in China, and the river basin spans one fifth of the area of the whole country. Based on statistical data, the excretion of manure-borne steroid hormones, including steroid estrogens (SEs) and steroid androgens (SAs), in 10 provinces of China within the region has been estimated. The potential environmental and ecological risk of manure-borne steroid estrogens to the surface water in this region was also assessed. The manure-borne SE and SA excretions in the 10 provinces and municipalities vary in the order: Sichuan > Hunan > Hubei > Yunnan > Jiangsu > Anhui > Jiangxi > Chongqing > Qinghai > Shanghai. The highest increase of manure-borne SEs (1434.3 kg) and SAs (408.5 kg) was found in Hunan and Hubei provinces, respectively, and the total excretion in 2013 was 65% more than 15 years earlier in these two provinces. However, the emissions in Anhui and Shanghai decreased in this 15 year period of time. Swine urine, chicken feces, cattle urine, and laying hen feces were considered the dominant sources of manure-borne E1, βE2, αE2, and SAs, respectively. Although Jiangsu province did not have the largest excretion of manure-borne SEs, it had the highest level of predicted 17β-estradiol equivalency (EEQ_s) value of 16.65 ng/L in surface water because of the limited surface water resources. According to the lowest observable effect level of 10 ng/L for 17β-estradiol, the manure-borne SEs in Jiangsu province might potentially pose ecological risk to its wild aquatic organisms.

Introduction

Natural steroid hormones, excreted from all vertebrates, are a series of typical endocrine-disrupting chemicals. Although the environmental concentrations of these contaminants are always low, it has been demonstrated that even at levels as low as nanograms-per-liter (ng/L), a wide variety of aquatic organisms exposed to these pollutants can be adversely affected (Kang et al., 2008; Leet et al., 2011; Örn et al., 2006; Shappell et al., 2010). Two important sources of hormone contaminants entering into the environment are municipal wastewater treatment plants (WWTP) and concentrated animal feeding operations (CAFOs). Recently, the livestock source of hormones has started drawing more and more attention, especially the natural steroids such as estrogens and androgenic, because of their large excretion from animal metabolism (Li et al., 2012b; Liu et al., 2012a). Some researchers have pointed out that the amount of hormones excreted by livestock was on

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the same order of magnitude as that by humans, or in certain cases may be even exceed human contributions (Gadd et al., 2010; Hanselman et al., 2003; Johnson et al., 2006; Kolodziej et al., 2004; Lange et al., 2002; Liu et al., 2012b; Zheng et al., 2008). In the United States, the total daily emissions of 17β-estradiol and estrone from swine and dairy cattle were reported to range from 10 to 30 kg and from 20 to 80 kg, respectively, and the mass was 9 times greater than that from WWTP (Raman et al., 2004). In fact, various manure-borne hormones have been detected at high levels in the soils, streams, wells, and surface waters around animal farms or the places receiving animal wastes (Gall et al., 2011; Liu et al., 2012a). Hence, it is necessary to study the contamination issues associated with livestock wastes.

China is the largest animal farming country in the world. The annual animal feces and urine production were estimated to amount to 4 billion tons (Zhang et al., 2011). However, the Chinese animal farming industry is still at a poor management level compared to that in developed countries. Unlike domestic wastewater, there are no specific treatment requirements in China for livestock farm wastes before discharge (Li et al., 2012b), and only 20% of animal farms have treatment facilities like lagoons or sedimentation tanks (Liu et al., 2012a). Direct discharge into streams and application of livestock wastes on agricultural lands are common practices in China (Li et al., 2012b; Liu et al., 2012a). Once the wastes enter water or soil without thorough treatment, manure-borne estrogens will expand their contamination range, posing a serious threat to surrounding groundwater or surface waters (Arnon et al., 2008; Lee et al., 2007). Unfortunately, many previous studies and management policies are focused only on the manure-borne N and P (Khan et al., 2008; Li et al., 2007), and the few studies that investigated the occurrence and fate of steroids in swine or dairy wastes were limited to animal farms and their surrounding environments (Li et al., 2012a, 2012b; Liu et al., 2012a, 2012b). For example, Liu et al. (2011) observed up to 412 ng/g of 17β-estradiol (17β-E2) from dairy fecal matter or 20,700 ng/L of androsterone in farm wastewater; some steroids appeared in the aqueous phase, particle phase and sediment of the streams receiving farm effluents, and the concentrations were much higher than the pollutants in water receiving wastewater from WWTPs (Liu et al., 2011, 2012a, 2012b). Even though these results were from limited studies on point sources, they still help to understand the excretion and risk of animal manure-borne steroid hormones.

The Yangtze River, which is the longest river in China and the third longest in the world, plays a vital role in China's economic development and ecological environmental conservation. The drainage basin of the river accounts for one fifth of Chinese land area and the river flows through 11 provinces and municipalities. In recent years, more and more issues related to animal farming pollution were reported, but few concerning manure-borne hormones. The aim of this study is to estimate the excretion of manure-borne steroid hormones in 10 provinces of the Yangtze River basin (with the exception of the Tibet autonomous region) and assess the potential environmental and ecological risks from manure-borne hormones in the study region using Chinese statistical data and reported data in the literature.

1. Materials and methods

1.1. Study region

The Yangtze River is about 6380 km long and has water resource quantity of 9.5×10^{11} m^3/year, with a watershed of over 1.8 million km^2. The main stream flows through 11 provinces and municipalities. The Tibet autonomous region, Qinghai, Sichuan, Yunnan provinces, and Chongqing municipality belong to the upper reaches of the Yangtze River. The middle reaches contain Hubei, Hunan, and Jiangxi provinces. Anhui, Jiangsu provinces, and Shanghai municipality are in the downstream reaches of the river. As the animal farming in Tibet is too small and statistic data is lacking, Tibet was excluded from this study.

1.2. Mass estimation of manure-borne steroid hormones

1.2.1. Selection of livestock and hormones

Based on the statistics of animal production and manure production in these 10 provinces and municipalities (Table 1), it is clear that pigs, dairy and beef cattle, and chickens are dominant types of livestock and poultry, accounting for 94.1% of animal production and 95.2% of manure production. Since the populations of other species such as sheep, horses and rabbits are extremely small, they are not representative enough to be considered. In addition, since the majority of studies on manure-borne hormones have been focused on pigs, dairy and beef cattle, laying hens and broilers, there has been sufficient and systematic data for the estimation of these 5 species. Therefore, pigs, dairy and beef cattle, laying hens and broilers were selected in this study.

The steroid estrogens (SEs) and the steroid androgenic (SAs) are frequently studied sex hormones, because they are emitted in huge quantities by animals and are the most probable cause of endocrine disruption observed in aquatic organisms (Arnon et al., 2008; Barbosa et al., 2008; Gadd et al., 2010). The manure-borne endogenous SEs mainly include estrone (E1), 17α-estradiol (αE2), βE2 (17β-estradiol), and estriol (E3). While the range of steroid estrogens and their metabolites varies among vertebrates, βE2 and E1 appear to be

Table 1 – Animal[a] and manure[b] production in the Yangtze River basin in 2013.

Livestock category	Animal production (million head/year)	Percentage (%)	Manure production ($\times 10^4$ ton/day)	Percentage (%)
Beef cattle	25.2	2.3	53.1	27.9
Dairy	1.3	0.1	7.2	3.8
Swine	214.3	19.4	111.3	58.4
Chicken	799.3	72.3	9.9	5.2
Other categories	65.6	5.9	9.1	4.8
Total	1105.7	100.0	190.6	100.0

[a] Animal production data are from China Agriculture Yearbook (2013).
[b] Manure production was calculated as the mean excreted coefficients suggested by Ma et al., 2006.

common throughout (Johnson et al., 2006). It is reported that E1 and βE2 are the estrogens of major concern because they exert their physiological effects at lower concentrations than other steroids and can be found in the environment at concentrations above their lowest observable effect concentration (LOEC) (Arnon et al., 2008). αE2 is the more common form excreted by livestock such as beef and dairy cattle (Mashtare et al., 2011), and has endocrine-disrupting potency (Shappell et al., 2010). Although E3 can be detected in farm effluents or rivers at relatively high concentrations, it tends to be neglected because of its much lower endocrine disrupting effects (Duong et al., 2010; Johnson and Williams, 2004). Therefore E3 will not be included in this study. As a result, our study will focus on the predicted estrogenic excretion of αE2, βE2, and E1 by farm animals, for which reliable data on estrogenic potency and effects on aquatic organisms are known. Most of the studies reported total excretion of SAs, therefore, our current study mainly estimated the total manure-borne androgens as well.

1.2.2. Estimation of manure-borne steroid hormones

The calculations of manure-borne steroids excreted by a unit AU are shown in Eqs. (1), (2), and (3). For aggregating over different types of livestock (Li et al., 2007), the animal unit (AU) is applied to estimate the excretion of animal manure and hormones. Information on the conversion of head counts to animal units, parameters used to calculate animal units and manure are given by Kellogg et al. (2000) and Li et al. (2012a):

Daily excretion of SEs by non-pregnant animals is calculated using Eq. (1).

$$\sum_{i=1}^{n} E_i = \sum_{i=1}^{n} (C_{fi} \times A_{fi} + C_{ui} \times A_{ui}) \quad (1)$$

where E_i (μg/(AU · day)) is the appropriate excreted value of SEs for non-pregnant animal i, which contains cycling dairy, milk cow, cycling beef cattle, bull, calf, cycling swine, laying hen and broiler, thus $n = 8$; C_{fi} (μg/kg) is the concentration of SEs in feces of animal i; A_{fi} (kg/(AU · day)) is the feces excreted by animal i; C_{ui} (μg/L) is the concentration of SEs in urine of animal i; A_{ui} (L/(AU · day)) is the urine excreted by animal i.

Despite the difference in excretion coefficients of SEs between pregnant and non-pregnant stages, the SEs excreted annually by pregnant female livestock are calculated as the total excretions by female animals in pregnant and non-pregnant stage:

$$\sum_{j=1}^{m} E_j = \sum_{j=1}^{m} [(E_{pj} \times D_{pj} + E_{cj} \times D_{cj})] \quad (2)$$

where E_j (μg/(AU · year)) is the appropriate excreted value of SEs in a year for pregnant female j, which contains pregnant dairy, pregnant beef cattle, and pregnant swine, thus $m = 3$; E_{pj} (μg/(AU · day)) is the emission of SEs by female j in pregnant stage; E_{cj} (kg/(AU · day)) is the emission of SEs by female j in non-pregnant stage; D_{pj} (day) is the length of pregnant stage of female j; D_{cj} (day) is the length of non-pregnant stage of female j. In addition, a year consists of 365 days here; E_{pj} and E_{cj} are calculated using Eq. (1).

Daily excretion of steroid androgens for male livestock is calculated using Eq. (3).

$$\sum_{k=1}^{n} T_k = \sum_{k=1}^{n} (C_{fk} \times A_{fk} + C_{uk} \times A_{uk}) \quad (3)$$

where T_k (μg/(AU · day)) is the appropriate excreted value of steroid androgens for male k, which contains bull, boar, laying hen and broiler, thus $n = 4$; C_{fk} (μg/kg) is the concentration of steroid androgens in feces of male k; A_{fk} (kg/(AU · day)) is the feces excreted by male k; C_{uk} (μg/L) is the concentration of steroid androgens in urine of male k; A_{uk} (L/(AU · day)) is the urine excreted by male k.

Data regarding animal production were obtained from the Statistics Yearbook of the ten provinces or municipalities. Since the relative contribution of feces and urine is substantially different between different categories (young or adult, male or female animals), manure production of different animals was calculated according to a previous study (Ma et al., 2006). The contents of hormones in feces and urine were calculated based on the data in the cited literature (Table 2).

Table 2 – Excretion of steroid hormones from different animals (unit: μg/(AU · day)).

Livestock category		E1		βE2		αE2		SAs	Reference
		Feces	Urine	Feces	Urine	Feces	Urine	Feces and urine	
Swine	Pregnant	67.4	2436.4	55.6	–[1]	–	–	–	Gall et al., 2011; Liu et al., 2011
	Cycling	109.1	745.4	81.8	–	–	–	–	Gall et al., 2011; Liu et al., 2012a; Liu et al., 2012b
	Boar	–	–	–	–	–	–	4874.3	Örn et al., 2006; Raman et al., 2004
Dairy	Milk cows	16.3	11.1	47.4	3.3	83.9	45.4	–	Li et al., 2012a; Lu et al., 2010; Lu et al., 2011
	Cycling	88.0	59.8	256.0	18.0	453.6	245.2	–	Li et al., 2012b; Lu et al., 2010; Lu et al., 2011
	Pregnant	47.9	739.8	139.1	221.8	246.2	3036.0	–	Gall et al., 2011; Liu et al., 2011
Beef cattle	Cycling	25.1	17.0	73.0	5.1	129.3	69.9	–	Li et al., 2012b; Lu et al., 2010; Lu et al., 2011
	Bulls	39.6	27.0	115.2	8.1	204.1	110.7	1068.5	Lu et al., 2010; Ma et al., 2006; Shi et al., 2013
	Calves	13.2	9.0	38.4	2.7	68.0	36.9	–	Lu et al., 2010; Ma et al., 2006; Örn et al., 2006
Chickens	Pregnant	58.1	897.2	168.7	269.0	298.6	3681.9	–	Gall et al., 2011; Liu et al., 2011
	Laying hens	787.5	–	3610.0	–	–	–	2330.0	Li et al., 2012b; Liu et al., 2011; Shappell et al., 2010
	Broilers	227.5	–	505.1	–	–	–	873.6	Li et al., 2012a; Li et al., 2012b; Liu et al., 2011

"–" No data.
E1: estrone; βE2: 17β-estradiol; αE2: 17α-estradiol; SAs: steroid androgens.

1.3. *Prediction of manure-borne estrogens in surface water*

In a study concerning estrogenic pollution risk of sewage effluent, the predicted aquatic estrogen concentrations for all of England and Japan had been determined and compared using the predicted emission of population and flow data by Johnson et al. (2011). We applied their estimation method into our study and try to predict the pollution risk of manure-borne estrogens to the surface water in the Yangtze River basin. The equation was as follows:

$$\mathrm{PEC} = \left(\sum_{l=1}^{n} E_l \times \eta \times 10\right) \div r \tag{4}$$

where PEC is the predicted estrogenic concentration in surface water, E_l (kg/year) is the manure-borne estrogens excreted by livestock l, η (100 million m^3/year) is the loss rate of pollutants into surface water and r is the regional surface water resource, 10 is the coefficient of magnitude conversion.

There have been numerous studies of nonpoint source pollution in the Yangtze River basin. The loss rate of 30% has been frequently used in the studies for manure-borne N, P or COD in southern China (Liu et al., 2002). Therefore, 30% was selected as the loss coefficient of manure-borne estrogens in this study. The regional surface water resources (r) were obtained from the Statistic Yearbook of the provinces and municipality.

To simplify the assessment, and facilitate comparison with the literature, the concentrations of estrogens in surface water are converted to βE2 equivalency (EEQ) as their estrogenic potencies normalized to that of βE2 (Chen et al., 2010):

$$\mathrm{EEQ}_s = \sum_{i=1}^{n} (\mathrm{EEF}_i \times \mathrm{MEC}_i) \tag{5}$$

where EEQs (ng/L) is the concentration of βE2 equivalency, EEF_i is the βE2 equivalency factor of estrogen i, MEC_i (ng/L) is the measured environmental concentration of estrogen i, $n = 3$. It has been reported that E1 has 1/3 of the estrogenic potency of βE2 (Wise et al., 2011), and the estrogenic potency of βE2 is almost 8 times that of αE2 (Shappell et al., 2010). Therefore, the EEFs of βE2, E1, and αE2 are 1, 1/3, and 1/8, respectively. The PECs of βE2, E1 and αE2 from Eq. (4) were used as their MECs in the EEQ calculations, respectively.

2. Results and discussion

2.1. *Distribution of typical manure-borne steroid hormones*

The emissions of manure-borne hormones along the Yangtze River are shown in Fig. 1. The excretions varied significantly among the ten provinces and municipalities. In the upper reaches of the Yangtze River, the emissions in different provinces were all large except in Qinghai, and increased from 1998 to 2013. The average annual excretion in this region reached 2715.3 kg in 2013. Sichuan had the largest emission of manure-borne hormones, reaching 5383.6 kg in 2013. Among these provinces the excretion of SEs reached 4789.5 kg. In the middle reaches, the average annual emission was 3474.3 kg in 2013. Of all provinces in this region, Hunan had the largest emission, at 4506.0 kg in 2013. Hunan also had the fastest increase rate of emission in the entire basin. The emission in 2013 was 36% larger than that 15 years earlier. The emission of SAs in 2013 was nearly three times that in 1998. The main reason is the fast growth of animal agriculture other than beef cattle. The laying hens increased more than 60 times compared to the data from 15 years earlier. In the lower reaches, the average annual emission was 1907.3 kg in 2013. The largest excretion was in Jiangsu, which reached 3215.3 kg in 2013. Jiangsu also had the greatest annual emission of manure-borne SAs, at 758.7 kg in 2013. The excretions of manure-borne hormones in Qinghai, Sichuan, Yunnan, Hunan, Hubei, Jiangxi, and Jiangsu provinces and Chongqing municipality all increased from 1998 to 2013, while the emissions in Anhui and Shanghai decreased in the same period of time. Compared to Shanghai, Anhui had the most significant reduction of emission of manure-borne hormones; the excretion in 2013 was only 93% of that in 1998, and the amount of reduction reached 171.2 kg, which was mostly contributed by decreased SE emission. The statistical data showed that the quantity of beef cattle in Anhui decreased greatly, with the number of beef cattle at the end of

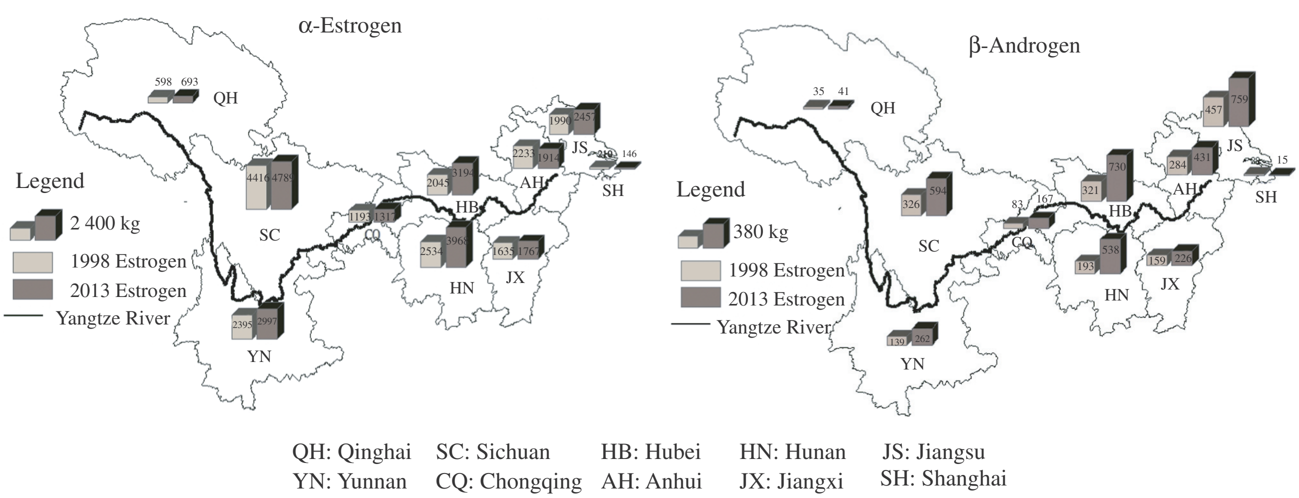

Fig. 1 – Distribution of manure-borne hormones in provinces of the Yangtze River basin.

Table 3 – Composition of manure-borne steroid hormones in three representative provinces of the Yangtze River basin in 1998 and 2013 (kg/year).

Steroid hormones	Source	Sichuan				Hunan				Jiangsu			
		1998	Percentage (%)	2013	Percentage (%)	1998	Percentage (%)	2013	Percentage (%)	1998	Percentage (%)	2013	Percentage (%)
E1	Cattle feces	38.8	1.3	23.9	0.7	19.8	0.9	15.2	0.5	3.2	0.3	2.2	0.2
	Cattle urine	169.0	5.6	92.8	2.7	85.2	4.0	60.5	2.1	13.1	1.1	8.6	0.7
	Swine feces	213.3	7.1	226.4	6.7	156.0	7.4	187	6.6	82.2	6.6	83	6.4
	Swine urine	2528	84.4	2912.0	85.8	1825	86.5	2435.4	86.0	1007	81.1	968.9	74.6
	Chicken feces	47.2	1.6	140.0	4.1	25.2	1.2	133.8	4.7	135.4	10.9	236.8	18.2
	Total	2996.0	100.0	3395.0	100.0	2111.0	100.0	2832.3	100.0	1241	100.0	1299.3	100.0
βE2	Cattle feces	112.7	21.5	69.4	7.8	57.6	19.1	44.3	5.5	9.4	1.4	6.3	0.6
	Cattle urine	50.7	9.7	27.8	3.1	25.6	8.5	18.1	2.2	3.9	0.6	2.6	0.2
	Swine feces	163.0	31.1	173.6	19.5	119.1	39.5	143.7	17.8	62.9	9.3	63.1	5.7
	Chicken feces	197.4	37.7	620.0	69.6	99.5	33.0	603.2	74.5	601.9	88.7	1038.6	93.5
	Total	523.8	100.0	890.8	100.0	301.8	100.0	809.4	100.0	678.2	100.0	1110.7	100.0
αE2	Dairy feces	1.5	0.2	7.3	1.5	0.16	0.0	5.2	1.6	1.0	1.4	7.8	16.8
	Dairy urine	5.7	0.6	26.8	5.4	0.58	0.1	19.3	6.0	3.9	5.5	28.8	61.8
	Beef cattle feces	198.2	22.2	110.2	22.2	102.0	22.6	69.7	21.7	15.7	22.2	3.3	7.1
	Beef cattle urine	687.7	77.0	351.1	70.9	349.1	77.3	227.0	70.7	50.0	70.8	6.7	14.3
	Total	893.0	100.0	495.4	100.0	451.8	100.0	321.2	100.0	70.6	100.0	46.5	100.0
SAs	Bull feces and urine	74.2	22.7	37.2	6.3	37.6	19.5	24.2	4.5	5.3	1.2	0.6	0.1
	Boar feces and urine	105.6	32.4	134.7	22.7	74.8	38.7	114.0	21.2	43.9	9.6	39.8	5.3
	Laying hen feces and urine	116.0	35.6	387.1	65.2	54.4	28.2	383.3	71.3	377.2	82.6	641.8	84.6
	Broiler feces and urine	30.6	9.4	35.1	5.9	26.4	13.7	16.3	3.0	30.3	6.6	76.4	10.1
	Total	326.3	100.0	594.1	100.0	193.1	100.0	537.7	100.0	456.7	100.0	758.7	100.0

E1: estrone; βE2: 17β-estradiol; αE2: 17α-estradiol; SAs: steroid androgens.

2013 only one fourth of that in 1998. It must be the leading reason for the reduction in excreted hormones. However, the main reason for the declining emission of hormones in Shanghai was that the local government introduced policies to reduce livestock production to protect the environment in 1999. As a result, the total quantities of swine, beef cattle, and chickens had decreased greatly by the end of 2013. The excretions of SEs and SAs in Shanghai were both smaller than those in other provinces. The excretions of manure-borne hormones in the 10 provinces and municipalities of the Yangtze River watershed are ordered from large to small as follows: Sichuan > Hunan > Hubei > Yunnan > Jiangsu > Anhui > Jiangxi > Chongqing > Qinghai > Shanghai.

2.2. Sources of typical manure-borne steroid hormones

As there are great differences among different animals in contributing to the excretion of E1, αE2, βE2 and androgens (Table 2), identifying the major contributor to manure-borne estrogens and androgens is obviously important. According to the analysis of hormone excretions in the ten provinces, Sichuan, Hunan, and Jiangsu, which had extremely large emissions, were selected as the representative districts of the upper, middle, and lower reaches of the Yangtze River to investigate the differences in contributions to the manure-borne hormones by different types of animal farming.

As shown in Table 3, the contributions of different animal feces and urine to E1 excretion vary in the order swine urine > swine feces > chicken feces > cattle urine > cattle feces. The swine urine is always the major source of E1, with the average percentage about 80%. It should be noted that its contribution to E1 in Hunan was up to 86.0% due to the large amount of swine feeding there. The contribution of swine feces to E1 was stable at 6.6%, while the contribution of dairy and beef cattle declined from 1998 to 2013 in the three representative provinces. Compared with Sichuan and Hunan, the sources of manure-borne E1 changed more markedly in Jiangsu, as the contribution of swine urine decreased 6.5%, while the chicken feces accounted for 7% more and reached 18.2% in 2013. Laying hen and broiler feces were the main sources of βE2, with highest percentage close to 93.5%. In the three representative provinces, the contributions to βE2 are ordered from high to low as follows: chicken feces > swine feces > cattle feces > cattle urine. The absence of swine urine above is because there is very low emission of βE2 in swine urine. Hunan had quite different emission sources of manure-borne βE2 in 1998 and 2013. As a result of increasing chicken production in Hunan, the contribution of chicken feces in 2013 doubled compared to that in 1998 and was up to 74.5%. The main source of αE2 is beef cattle urine in the Yangtze River basin, which accounts for 77.3% at the maximum. The most obvious change in sources of manure-borne αE2 was in Jiangsu, as the contribution of dairy urine was 61.8% higher in 2013 than that 15 years earlier, which exceeded the contribution from beef cattle urine and became the largest contributor of αE2. As discussed above, swine urine, chicken feces, and cattle urine are the dominant sources of manure-borne E1, βE2, and αE2, respectively, and should be treated before entering and polluting the environment. Since βE2 has the strongest estrogenic potency, the estrogenic excretion of laying hen and broiler feces should be strictly controlled.

The predicted emission of manure-borne androgens shows that laying hens are the dominant source, accounting for 84.6% of the emission (Table 3). The sources of androgenic excretion differed significantly in Hunan province between 1998 and 2013. The feces of laying hens made the greatest contribution to SAs in Hunan, which was 43.1% higher than 15 years earlier and accounted for 71.3% of the total emission in 2013, while the androgenic emissions of bulls, boars, and broilers all declined. Therefore, the SAs in laying hen manure must be well treated to protect the ecological environment.

2.3. Potential risk of manure-borne estrogens to the surface water

The predicted concentrations of manure-borne estrogens in 10 provinces and municipalities of the Yangtze River basin are shown in Fig. 2. It reveals that the predicted EEQ_s in the districts of the lower reaches are generally higher than in the upper and middle reaches. The average EEQs of upper, middle, and lower reaches were 2.13, 3.49, and 9.36 ng/L in 2013, respectively. This is consistent with the previous study by Shi et al. (2013), who also proved that higher level estrogen effects were detected in the water at lower reaches of Yangtze River than those at upper reaches. Jiangsu province had the highest predicted EEQs at 16.65 ng/L, almost 24-fold higher than the lowest estimation in Qinghai province. Chongqing and Hubei had the highest EEQs in the middle reaches, at 3.67 and 6.69 ng/L, respectively. The extremely huge excretion of SEs and limited water resource are the main reasons for the conspicuously higher EEQs in Jiangsu, because the surface water resource is only two fifths that in Hubei province where has similar estrogenic emissions, which may result in insufficient dilution of the manure-borne estrogens when they enter the surface water.

Chen et al. (2010) investigated the estrogenic concentrations along a creek near a concentrated livestock feedlot in southern Taiwan. It was reported that the EEQ levels were 217 ng/L near the feedlot and 25.1 ng/L 15 km away. Another study in Lake Taihu, Jiangsu province, reported that the concentrations of E1

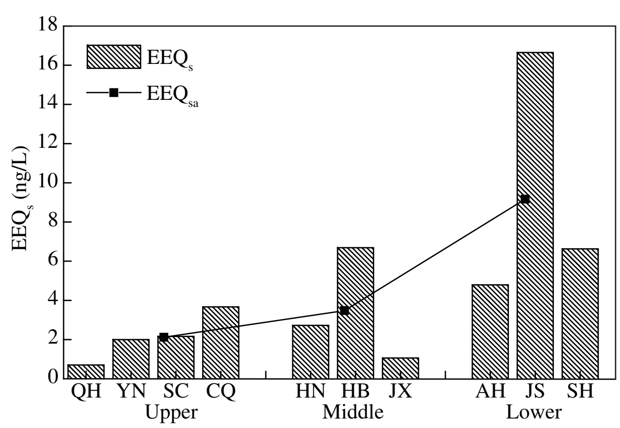

Fig. 2 – Distribution of predicted EEQ_s in 10 provinces of the Yangtze River basin in 2013. QH: Qinghai; SC: Sichuan; YN: Yunnan; HN: Hunan; HB: Hubei; JX: Jiangxi; AH: Anhui; JS: Jiangsu; CQ: Chongqing; SH: Shanghai. EEQ_{sa} represents average values of upper, middle, and lower reaches.

and βE2 were 1.0–15.8 and 1.0–10.8 ng/L, respectively (Lu et al., 2011); the EEQ value would be in the range of 1.3–16.1 ng/L, which is similar to our predicted level in this region. Hu's investigation (Hu et al., 2013) also verified that the EEQ_s level in the Jiangsu section of the Yangtze River ranged from 0.9–12.0 ng/L and βE2 accounted for 99% of the estrogenic activity. The above studies suggested that the high level of EEQ_s in the Yangtze River and its basin should be paid more attention.

The Environment Agency of UK has proposed 1 and 10 ng/L as the predicted no effect concentration (PNEC) and lowest observable effect concentration (LOEC) for βE2, respectively (Shappell et al., 2010). Taking the proposed PNEC as the guideline, then all of the provinces and municipalities in the Yangtze River watershed have risk of estrogenic pollution, with the only exception being Qinghai. Similarly, other studies also reported that the concentrations of βE2 reached 2.0 ng/L in water samples of the Yangtze River, which meant that the detected βE2 equivalents might be higher than the PNEC (Hu et al., 2013 and Shi et al., 2013). Since the environmental threshold is established as the LOEC (10 ng/L), the wild aquatic organisms in surface water very likely suffered from manure-borne estrogen-related endocrine-disruption in Jiangsu province. For example, Lu et al. (2010) and Song et al. (2011) noted individually that the E2 concentration in goldfish blood increased progressively with the increase of environmental steroidal estrogens in the Nanjing section of the Yangtze River. More seriously, manure-borne estrogenic risk potential has occurred not only in the surface water but also in the ground water within the Yangtze River Delta recently, which warns us that hormones must be considered as a priority to control their amount and adverse effects (Shi et al., 2013).

It should be noted that the predictions in this study are from statistical data without considering the adsorption, biodegradation or other processes of manure-borne estrogens in the environmental matrix. So the estimated EEQs may be the highest concentrations possible in surface water. Moreover, the predictions should be verified by a practical sample survey of the study areas.

3. Conclusions

In the studied areas, Sichuan province was estimated to have the largest annual emission of steroid hormones, while the emission in Hunan had the highest rate of increase because of the increase in chicken production. In general, the predicted concentrations of manure-borne estrogens in surface water (calculated in EEQ_s) of the Yangtze River lower reaches were higher than that of upper and middle reaches. In particular, Jiangsu province had the highest EEQ_s and the estimated value exceeded the lowest observable effect concentration (LOEC) for βE2, which meant the potential for ecological risks to the wild aquatic organisms in the surface water.

It is also concluded that swine urine, chicken feces, and cattle urine in the Yangtze River were the main sources of manure-borne E1, βE2, and αE2, respectively, and the dominant contributor of manure-borne androgens is laying hens. We strongly suggest thorough treatment of swine urine, chicken feces, and cattle urine before land application or discharge to assure that the hormones in these wastes would not pollute the surface water and eco-systems.

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