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THE RISK OF ENDOCRINE DISRUPTION TO FISH IN THE YELLOW RIVER CATCHMENT IN CHINA ASSESSED USING A SPATIALLY-EXPLICIT MODEL

Running title: Endocrine disruption risk in the Yellow River catchment

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Abstract: The GWAVA model, incorporating regional water abstractions and reservoir information, was used to model human-sourced steroid estrogens: estrone (E1) and estradiol (E2) in the Yellow River catchment. The river flows in the main stem were calibrated using gauged flows. Following a review of Chinese data on estrogen discharge from a range of sewage treatment plants, low, median and high discharge rates were identified and used as best, expected and worst case scenarios, respectively. For any given location, the time-variation of modelled estrogens levels was summarized using the mean and upper 90th percentile (90%ile), which is where the model predicts 90% of values would be below this concentration. The predicted means and 90%iles for E1 were comparable with previous E1 measurements reported in the river. For the whole catchment, only 19% (mean value) of the river system by length was predicted to exceed 1 ng/L E2 equivalents (EEQ) using expected estrogen sewage discharge. Only 3% of the network by length was predicted to exceed the dangerously high 10 ng/L EEQ when considering 90%ile concentrations. The highest exposures were in the Fen and Wei tributaries. Endocrine disruption risk from estrogens was predicted to be minimal in the main stem. Only in the worst case discharge scenario and 90%ile predicted concentrations, were the most downstream river reaches of the main stem predicted to be at risk. Reservoirs appeared to be helpful in reducing estrogen concentrations thanks to longer water residence facilitating biodegradation. This article is protected by copyright. All rights reserved

Keywords: Steroid estrogens, Flow modelling, GWAVA, Risk assessment
INTRODUCTION

Natural and synthetic endocrine disrupting compounds (EDCs) continue to be a cause of concern in the aquatic environment [1]. Of these compounds, steroid estrogens are generally present in environment at nanogram per liter concentrations, and have been identified to have endocrine disrupting potencies over three orders of magnitude above other nonsteroidal endocrine-disrupting compounds [2, 3]. These steroid estrogens with high potencies are estrone (E1), 17β-estradiol (E2) and 17α-ethynylestradiol (EE2). These compounds have been widely identified to cause the feminization of male fish and adversely affect individual fish [4]. The feminization or severe intersexuality of fish was associated with older fish [5] which might suggest chronic exposure to estrogens causes greater effects. Other studies suggest that early life exposure to estrogen affects subsequent breeding success when the fish are mature [6, 7].

Effluents from domestic STPs have been identified as the major contributor of estrogens in aquatic environments [8]. These compounds, with the exception of EE2, have now been widely detected in Chinese STP effluent [9-19] and rivers [20]. This apparent absence of EE2 is similar to findings in Japan [8, 21]. Male fish in some Chinese surface waters have exhibited evidence of feminization believed to be linked to exposure to estrogens [22, 23]. Given China’s rapid economic development, fast urbanization, and development of sewage treatment facilities there are concerns that endocrine disruption may become a problem. A comprehensive measurement campaign for estrogens in this large country would be very costly and labour intensive. Model-based predictions might be an economic and efficient alternative to estimate endocrine disruption risks over the big river networks of China. Modelling also has the advantage of having no lower limit of detection, which can be a struggle with conventional analysis at the sub-ng/L level [24]. Examples of this approach can be found for the UK [25], Switzerland [26], South Australia [27], the European Union [28], US [29], and Japan [8]. A previous estrogen risk assessment for China, used a fugacity model to predict a uniform estrogen
concentration for the water compartment of each Chinese river basin [30]; but predictions along the lengths of rivers to find potential ‘hot spots have not so far been attempted.

For this study a spatially-explicit model: GWAVA [31, 32] was used to predict the concentrations of E1 and E2 in the surface water bodies of the second largest river catchment in China: the Yellow River catchment. The Yellow River provides the drinking water source for 12% of the total population (140 million people) and 15% of the total farmland irrigation in China [33]. Increasing water abstraction has reduced discharge to the Bo Sea [34, 35]. More than 30 percent of fish species in the Yellow River have gone extinct in recent years [36]. The identification of river reaches exposed to high estrogen concentrations could assist regulators and environmental scientists on endocrine disruption and on wider water quality issues of exposure to sewage effluent. The objectives of this study were for the Yellow River catchment:

- To model river flow based on existing water abstractions and corroborate with existing gauged river flow data.
- To predict the concentrations of E1 and E2 based on the estrogen discharge rates in STP influent and effluent in China and examine how close the predicted concentrations are to previous measurements.
- To assess the endocrine disruption risks based on the human-sourced contribution of E1 and E2.

MATERIALS AND METHODS

Overview of the Yellow River catchment

The Yellow River catchment covers an area of approximately 753,000 km², and the length of the main stem is 5,464 km [35]. The Yellow River head waters are in the Qinghai-Tibet Plateau, and flows through the Loess Plateau and North China Plain (Figure 1). There are two major sub-catchments: Fen river sub-catchment and Wei river sub-catchment with population densities of 343 person/km² and
258 person/km\(^2\), respectively, which is higher than the average density of 170 person/km\(^2\) in the Yellow River catchment as a whole. Much of the population in the catchment lives in arid and semi-arid areas, thus water abstraction is relatively high. Irrigation uses more than 80\% of the total water abstracted in the Yellow River catchment [35]. There are 15 large-scale reservoirs along the main stem of the river with 66.3 billion m\(^3\) of water capacity [35] (Table S1). Finally, at the bottom of the Yellow River, water is abstracted and transferred out of the catchment to other regions. As a result, the river discharge is heavily impacted. The mean discharge at the tidal limit was 1500 m\(^3\)/s in 1953, but has dropped to 611 m\(^3\)/s in recent years (2003-2012) [35, 37].

**Modelling estrogens in the river networks of the Yellow River catchment**

To model the concentrations of natural estrogens E1 and E2 in the surface water of the Yellow River catchment the GWAVA model and its water quality module [31, 32] was selected. Gridded water flows were estimated by the GWAVA’s hydrological components. Subsequently, pollutant concentration was calculated by the water quality module by incorporating estimated gridded pollutant load releases to surface water.

The GWAVA’s hydrology is driven by monthly climate data of 1950-2000 (available at 30 arc minute resolution) [38, 39]\(^1\) and simulates water discharge in rivers, reservoirs and lakes. The model was applied at a spatial resolution of 30 arc minutes (approximately 50 km × 50 km). Thus, each cell would generate 612 results based on this historic climate data (51 years each of 12 months climate data). For an accurate simulation of surface water discharge, water abstractions and reservoir information in this catchment (annual census data; [35]) were incorporated into the GWAVA model. The physical parameters including soil class and land cover were obtained from the EU WATCH project dataset (http://www.eu-watch.org/data_availability) and the river flow directions at 30-arc minute spatial resolution originated from Vörösmarty et al. [40]. A calibration of water discharge was conducted based on the measured river discharge at eight gauging stations along the main stem of the

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\(^1\) Data from Weedon et al. (2010, 2011) was aggregated from daily to monthly resolution

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The modelled pollutants are transported downstream with water discharge from one cell to the next. The time of travel through the gridded network was accounted for through estimated water volumes and river discharge (Eq. 5 in Dumont et al. [31]). The model included the pollutant loss through water transfer and abstraction (see Dumont et al. [31] for details). In addition, estrogen loss also includes the biodegradation process following first-order kinetics [41]. The mean annual river temperature of the Yellow River is about 10 °C. In the absence of measured biodegradation rates for the Yellow River, the estimated biodegradation rates for E1 and E2 were derived from UK biodegradation rates at 20 °C [41] and extrapolated to 10 °C using Williams et al.’s equation [42] (Table S2).

Thus, the main temporal variables influencing river estrogen concentrations were changes in runoff (less dilution leading to higher concentrations) and residence time (lower flow velocities leading to longer residence times leading to more biodegradation) both driven by the 51 year climate data set. Therefore, the higher, 90th percentile predicted river concentrations will be linked to lower flows but not necessarily the lowest flow since the greater opportunities for biodegradation are offered at longer residence times.

*Estimating estrogen discharge to rivers based on the resident human population*

To reflect current Chinese society the model assumed three different estrogen routes for the catchment: firstly, STP treated effluent (78.1%, [43]), and secondly, urban untreated sewage waters directly discharged via sewer (5.5%), [43]. The remaining human sewage generated by urban residents not connected to sewers was assumed to be disposed to land and not enter the water course. The third route is from rural untreated sewage waters directly discharged (6%, [44]) via flushing toilets (Figure S1). The urban population is believed to be 47.4% of the total [45]. Most of the sewage from rural
residents is generally treated in septic tanks and this source to rivers was not considered. Here this assumption may be acceptable as villages in northern China are rarely located alongside water courses [46]. Overall, the gridded estrogen load (μg/d) was calculated by population and their estrogen discharge rate:

\[ \text{Load} = P_{\text{stp}} \times R_{\text{stp}} + P_{\text{sewer}} \times R_{\text{sewer}} + P_{r, flushing} \times R_{\text{flushing}} \]

where \( P_{\text{stp}} \) is gridded population connected to STPs (person); \( P_{\text{sewer}} \) is gridded population connected to sewer but not treated in STPs (person); \( P_{r, flushing} \) is rural population connected to flushing toilets (person); \( R_{\text{stp}} \) is discharge rate of estrogen for population connected to STPs, which is per capita estrogen load in STP effluents (μg/capita/d); \( R_{\text{sewer}} \) is discharge rate of estrogen for population connected to sewer but not connected to STPs, which is per capita estrogen load in STP influent (μg/capita/d); \( R_{\text{flushing}} \) is discharge rate for rural population connected to flushing toilets, which is per capita estrogen load directly excreted by human(μg/capita/d).

*Estimating spatial location of the population contributing estrogens to the river network*

The only publicly available geographic data for population is the gridded population of the world (GPW) in 2005 [47]. A list of STPs in China is publicly available [48]. All listed STPs in the Yellow River catchment were manually digitised in this study (Figure 1). The method developed to divide the population on a geographic basis between urban STP connected, urban sewer connected (untreated sewage) and rural with flushing toilets directly connected to rivers is described in the supplementary information.

*Estimating estrogen discharge rates based on measured estrogens in Chinese effluents*

There were 43 separate data sets of influent and effluent concentrations found for Chinese STPs [9-19]. Thus, a per capita release of estrogens was derived from the measured influent and effluent concentrations. In each case a per capita value was calculated by the concentration divided by the population connected to STPs (\( P \)) and taking into account effluent water discharge (\( D \)).

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For the urban population connected via sewers directly to rivers (untreated sewage), the estrogen discharge rates \( R_{\text{sewer}} \) were estimated from the estrogen concentration in influent \( C_{\text{in}} \) using the following equation:

\[
R_{\text{sewer}} = (D \div P) \times C_{\text{in}}
\]

This value already accounts for any losses/transformations that took place along the sewer. For the proportion of the rural population which directly discharges to rivers (also untreated sewage), we assumed that what arrives in the river is essentially what is excreted by the humans without further transformation. In this case the loading was calculated from the literature sewage influent concentrations. However, to do this a ‘back-calculation’ was required since the estrogens in the influent have been modified during their transit in the sewer network following excretion. Using the approach of Johnson and Williams [49] we assumed that the influent values were a result of 50% conversion of E2 to E1. Using this, the probable original discharge rate for the rural population was back-calculated.

For E2
\[
R_{\text{flushing--E2}} = R_{\text{sewer--E2}} \div (1 - 0.5)
\]

For E1
\[
R_{\text{flushing--E1}} = R_{\text{sewer--E1}} - R_{\text{flushing--E2}} \times 0.5
\]

The value 0.5 in these equations is the fraction of excreted E2 that is converted to E1 along the sewer. For the urban population connected to sewers and STPs, estrogens were removed during the treatment process. The discharge rates \( R, \mu g/\text{capita/d} \) were derived from the estrogen concentration in effluent \( C_{\text{eff}}, \text{ng/L} \) by the following equation (Table S3):

\[
R_{\text{stp}} = (D \div P) \times C_{\text{eff}}
\]

Among these estimated discharge rates, eight outliers were excluded in statistical analysis (Table S2).

In order to encompass the apparent variety in estrogen removal performance (Table S3), three scenarios: best, expected and worst scenarios were used to reflect the range of likely discharge to rivers.

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The best scenario considered the lowest quartile (25th percentile) of discharge rates, the expected scenario the median values, and the worst scenario the upper quartile (75th percentile) (Table 1).

RESULTS AND DISCUSSION

River flow calibration and verification

The modelled flows (without considering anthropogenic influence) were in relatively good agreement with gauged flow for the 1952-60 period (Figure S2). But when modelling river flows for the more recent 1991-2000 period, the flow was overestimated when artificial influences were not included (Figure S3). Over recent years, river flows in the Yellow River catchment have been denuded by irrigation, reservoirs, industry and domestic demand [50]. So when water abstraction and reservoir influence were incorporated, the estimated mean annual flows improved in agreement with the measured flows [35] (with absolute errors less than 10%) along the main stem of the Yellow River for the period of 2003-2012 (Figure 2). Thus, the hydrological model could be considered adequate in simulating river flow based on mean annual flow.

Predicting estrogen concentrations in the main stem of the Yellow River based on human contributions

The predicted concentration of E1 in the Yellow River generally increased from the head waters to the catchment outlet (Figure 3). It was considered that the many reservoirs (Figure 1) increased the water residence time in the main stem by 10-20 d in the wet season and 20-30 d in the dry season [51]. Therefore, enabling further estrogen biodegradation [41]. Concentrations in the head waters were close to zero, which was expected being mountainous areas with low population density. Towards the outlet of the catchment, the concentration again declined, linked to minimal additional input and the impact of degradation along the river.

These E1 predictions were compared to measurements (Figure 3) previously made along the main stem of the Yellow River in 2008 [52] in May (wet season) and November (dry season) 2008. Where E1 was below the limit of quantification (LOQ = 0.5 ng/L), so a value of half the LOQ was used to represent these concentrations. The measured E1 values for May were mostly within the predicted

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range (within 0.25-0.5 ng/L) (Figure 3). The higher measured values in the drier November period (associated with low flows) were generally within the predicted 90th percentile concentrations (Figure 4). However, the measured E1 values at the upstream Lanzhou site (circled) were unexpectedly higher by about 1.5 ng/L than those predicted even assuming worst case estrogen discharge. However, these samples taken at Lanzhou were very close to some major STPs (Figure S4 and Table S4), which may explain the higher E1 than expected concentrations. Overall, the predictions were broadly acceptable, but with a tendency to underestimate in the expected case scenarios compared to previous measurements.

There are a number of possible explanations for the tendency to slightly underestimate E1 in the main stem of the Yellow River. One may be a higher proportion of untreated domestic sewage reaching the river and the other is the discharge of estrogens from agriculture which may have by-passed the soil. Some studies have suggested livestock might be a major contributor of estrogens in surface water in China [30, 53]. Poor water quality in the Tai Lake Basin has been strongly linked with nutrients and organics from livestock [54]. A particular source of concern is concentrated animal feeding operations but these account for only around 20% of the total livestock [55] (Table S5). The biggest contributor from livestock is the small scale livestock farms with around 80% of the total livestock [55] (Table S5). The location and count of livestock in the Yellow River basin is unknown.

*Estrogenic risk in the Yellow River catchment*

To assess estrogen based endocrine disruption in the UK, the Environmental Agency for England and Wales, used risk categories based on estradiol equivalent (EEQ) concentration ([EEQ]): no risk ([EEQ] < 1 ng/L), at risk (1 ng/L < [EEQ] < 10 ng/L) and high risk ([EEQ] > 10 ng/L) [25] [56]. With the roach (*Rutilus rutilus*), the no-observed-effect concentration (NOEC) reported is 0.3 ng/L EE2 [56], which could be viewed as equivalent to 3 ng/L EEQ if EE2 is assumed to have a potency 10 times higher than E2 [25]. If chronic and lifelong exposure to estrogens are the highest concern, so annual average exposure would be the most relevant value to study. In the rivers of the Yellow River
catchment, based on the expected scenario, the mean value of overall EEQ was 0.56 ng/L. This value was lower than English rivers with 0.9 ng/L EEQ but is higher than that predicted for Japanese rivers with 0.1 ng/L [8]. The major difference between these nations/regions is the available dilution per capita which is 3.8 m³/cap/d for England, 6.7 m³/cap/d for the Yellow River catchment and 19.6 m³/cap/d for Japan.

Taking the catchment as a whole with respect to mean concentrations per cell, 92% (best case) and 54% (worst case) of river reaches were predicted to have an EEQ below 1 ng/L with the expected outcome being 81% (Figure 5). For the 90th percentile concentrations, 63% (best case) and 43% (worst case) of river reaches were predicted to have an EEQ below 1 ng/L with the expected outcome being 53% (Figure 5). Whilst some endocrine disruption phenomena, such as vitellogenesis in males, might occur in water containing 1 ng/L EEQ, much more severe intersex conditions would be expected in fish when the EEQ reaches 10 ng/L [4]. With the mean and 90th percentile predicted estrogen concentrations only 1.2-21% (worst case) of river reaches reached this higher risk level of 10 ng/L EEQ.

For the main stem of the Yellow River, for the mean annual concentrations, there was no exposure >1 ng/L EEQ, even in the worst case scenario (Figure 6 and Figure S5). Examining the highest possible exposures (90th percentile predicted concentrations), then 11% of main stem river reaches in the worst case scenario exceeded the 1 ng/L EEQ initial risk level (Figure S6), mostly in the downstream Yellow River (Figure S7). This low Yellow River main stem risk is related to the relatively modest population compared to the flow together with the long residence times.

The regions of the Yellow river with the highest exposures were predicted in the mean annual simulation to be in the Fen River (90% river reaches >1 ng/L EEQ) and Wei River (30% river reaches >1 ng/L EEQ) (Figure 6). These two sub-catchments have a higher population density than the average for the whole catchment.
CONCLUSIONS

Modelling of estrogens in the Yellow River remain challenging due to difficulties in assessing anthropogenic influences on river flows, true sewage connectivity and the changing level of sewage treatment in China. However, based on this experience with GIS-based water quality modelling the following statements can be made regarding the Yellow River and endocrine disruption:

- The GWAVA simulation gave E1 values comparable to those from a previous monitoring campaign for the Yellow River catchment.
- For a very long major river with big reservoirs the long residence times make in-stream biodegradation an important process.
- Given the high available dilution and long residence time in the Yellow River main stem, endocrine disruption was not predicted to be widespread for fish based on exposure under mean annual conditions. However, the predicted 90th percentile concentrations, associated with the worst case scenario (high level estrogen discharge), resulted in risks of endocrine disruption in the more downstream reaches.
- The Fen and Wei tributaries with short lengths and dense populations are predicted to be the locations with the greatest potential for endocrine disruption in aquatic wildlife.
- Having shown that this model can predict estrogen concentrations in the Yellow River catchment; this model may prove to be a useful tool for predicting estrogen concentrations and associated endocrine disruption risk in other catchments in China.

SUPPLEMENTAL DATA

Tables S1–S5.

Figures S1–S7. (3MB DOC).

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Data availability—Climate forcing data can be accessed by URL: ftp://rfdata:forceDATA@ftp.iiasa.ac.at or ftp downloads of individual files: ftp.iiasa.ac.at, un=rfdata, pw=forceDATA then: “cwd /WFDEI”. Population data can be accessed by NASA Socioeconomic Data and Applications Center. The water demand data, gauging flow data and census data can be accessed to the published Statistical Yearbook in China.
REFERENCES


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Figure 1. Yellow River Catchment.

Figure 2 Comparisons of the mean annual estimated and measured flow during 2003-2012. The gauging stations are LZ: Lan Zhou; LM: Long Men; TG: Tong Guan; SMX: San Men Xia; HYK: Huan Yuan Kou; GC: Gao Cun; AS: Ai Shan; LJ: Li Jin.

Figure 3 Comparisons of the mean predicted and measured E1 concentrations along the Yellow River main stem in three scenarios: best scenario included 25th percentile estrogen discharge rates, expected scenario 50th percentile estrogen discharge rates, and worst scenario 75th percentile estrogen discharge rates.

Figure 4 Comparisons of the 90th percentile predicted and measured E1 concentrations along the Yellow River main stem.

Figure 5 Predicted mean (A) and 90th percentile concentrations (B) of EEQ in surface waters throughout the Yellow River catchment (based on the best, expected and worst case scenarios).

Figure 6 Predicted mean concentrations of EEQ in surface water based on the expected scenario.
Table 1 Scenarios based on estimated estrogen discharge rates (μg/capita/d)

<table>
<thead>
<tr>
<th>Scenarios</th>
<th>$R_{\text{flushing}}$</th>
<th>$R_{\text{sewer}}$</th>
<th>$R_{\text{stp}}$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>E1</td>
<td>E1</td>
<td>E1</td>
</tr>
<tr>
<td>Best case</td>
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<tr>
<td>Expected case</td>
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<td>3.78</td>
<td>13.3</td>
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<td>0.21</td>
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<tr>
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</tr>
</tbody>
</table>

$R_{\text{flushing}}$ is estrogen discharge rate for rural population which directly discharges sewage to rivers via flushing toilets; $R_{\text{sewer}}$ is estrogen discharge rate for urban population connected via sewers directly to rivers; $R_{\text{stp}}$ is estrogen discharge rate for urban population connected to sewers and STPs.
Figure 1
Figure 2

Mean annual flow for the period of 2003 - 2012 at eight gauging stations

Flow (m³/a)

Gauging station

LZ  LM  TG  SMX  HYK  GC  AS  LJ

Year  2003  2005  2007  2009  2011

Flow (m³/a)

LZ

SMX

HYK

GC

AS

LJ

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Figure 3
Figure 4
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Figure 6