Impact of climate change and population growth on a risk assessment for endocrine disruption in fish due to steroid estrogens in England and Wales

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ARTICLE INFO

ABSTRACT

In England and Wales, steroid estrogens: estrone, estradiol and ethinylestradiol have previously been identified as the main chemicals causing endocrine disruption in male fish. A national risk assessment is already available for intersex in fish arising from estrogens under current flow conditions. This study presents, to our knowledge, the first set of national catchment-based risk assessments for steroid estrogen under future scenarios. The river flows and temperatures were perturbed using three climate change scenarios (ranging from relatively dry to wet). The effects of demographic changes on estrogen consumption and human population served by sewage treatment works were also included. Compared to the current situation, the results indicated increased future risk:the percentage of high risk category sites, where endocrine disruption is more likely to occur, increased. These increases were mainly caused by changes in human population. This study provides regulators with valuable information to prepare for this potential increased risk.

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1. Introduction

The steroid estrogens estrone (E_1, natural hormone), estradiol (E_2, natural hormone) and ethinylestradiol (EE_2, synthetic hormone) were identified as the main chemicals causing intersex in male fish, which is a widespread issue in the UK (Jobling et al., 1998).

These substances may be referred to as "down-the-drain" chemicals as, after disposal/consumption, they enter river waters via sewage treatment works (STWs). The potential risk of fish intersex is therefore highest immediately downstream of STWs (Jobling et al., 2006). In 2012, the European Commission published a proposal suggesting a new annual average environmental quality standard of EQS 0.035 ng/L for EE_2 and 0.4 ng/L for E_2 (European Commission, 2012). Since then, these drugs have been placed on a watch list of priority substances in the field of water policy, which will be reviewed in 2014. The possibility of regulatory action on EE_2 is creating significant debate amongst a wide community (Gilbert, 2012). This debate re-emphasises the need for quantifying

exposure to these substances and an assessment to identify where and to what extent risks might occur today and in the future. Indeed, the identification of regions at risk presently and in the future was identified as one of the top 20 priority questions related to pharmaceuticals and personal care products in the environment (Boxall et al., 2012).

Williams et al. (2009) assessed the risk of endocrine disruption induced by these steroid estrogens for the UK under current flow conditions at a catchment level. The concentrations of E_1, E_2, and EE_2 were estimated using a geographical information system-—based model. The estimated concentrations were combined with effect levels to estimate the risk of endocrine disruption across England and Wales. A river network spreading over 21,452 km (10,313 individual reaches) and including more than 2000 STWs serving more than 29 million people was modelled. The study concluded that a very small proportion of the modelled reaches (1—3%) were predicted to be at high risk, and more than a third (39%) were at risk.

It is widely acknowledged that some level of climate change is unavoidable (Stocker et al., 2013). Climate change will affect river flows (Arnell and Reynard, 1996) and thus impact water quality via the dilution of contaminants leading to direct consequences on

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freshwater ecosystems (Delpla et al., 2009; Whitehead et al., 2009). It is recognised that, as for many other chemicals and in particular "down-the-drain" chemicals, climate change might affect steroid estrogen concentrations and thus the potential risk they might cause to the aquatic environment (Green et al., 2013; Sumpter, 2005). Gouin et al. (2013) explored the influence of climate change in multi-media chemical fate models. While they stressed that likely changes due to climate change would be relatively small (about a factor of 2) compared to the uncertainties in the chain of models required to produce such estimates, the processes determining the fate, persistence and bioaccumulation of chemicals would all likely be affected by at least temperature. A previous study from Green et al. (2013) evaluated the possible impact of future flows and demographics in the Erewash catchment in the UK which includes four STWs and has a catchment area of approximately 200 km^2. The study predicted a moderate increase in steroid estrogen concentrations and concomitant risk for feminisation in wild fish by 2050.

The purpose of this study is to test the hypothesis that climate change will result in an increased risk of endocrine disruption in fish due to steroid estrogens in England and Wales by 2050. Williams et al. (2009) previously reported the proportion of reaches at "high risk" of endocrine disruption in the UK as being small (1–3%). For comparison, the present study reproduced this risk assessment with assumed changes in river flows, water temperature and demography to assess how current risk is likely to change across England and Wales. The potential risk under future conditions is derived by comparing predicted environmental concentrations (PECs) with threshold levels defined by environmental effect levels (Williams et al., 2009).

2. Materials and methods

2.1. Overview of the risk assessment method

A risk assessment is available under current conditions, therefore for comparison purposes the same risk assessment method is applied (Williams et al., 2009). The approach adopted in that study was to compare PECs with thresholds levels representative of environmental effect levels.

The LF2000-WQX (LowFlows2000 Water Quality eXtension) model was used to generate PECs of estrogens for each river in England and Wales. LF2000-WQX is a mixed deterministic and stochastic model that combines hydrological models and water-quality models to produce spatially explicit statistical distributions (mean, standard deviation and percentiles) of "down-the-drain" chemicals in surface waters across England and Wales (Williams et al., 2009). The steroid estrogen input loads were determined based on the model described by Johnson and Williams (2004). Within LF2000-WQX, several processes were accounted for whilst estimating PECs, these included: STWs removal (which can vary depending on sewage treatment type), biodegradation and dilution within the water column, and parent to metabolite transformation (E$_2$ transforms to E$_1$). The model outputs consisted of a series of maps and tabulated data providing distributions of PECs (mean, standard deviation and percentiles) for each river reach modelled across England and Wales.

Estrogens occur in the environment simultaneously, it was therefore more appropriate to study their combined biological effect rather than the effect of each estrogen separately. Thus, Williams et al. (2009) applied a combined "toxic equivalent" approach based on estradiol equivalent (EEQ). The PECs of E$_1$, E$_2$, and EE$_2$ were then aggregated to produce an EEQ concentration ([EEQ]) which provided a quantification of the combined exposure of these three steroid estrogens:

$$[EEQ] = \frac{[EE_2]}{0.1} + \frac{[E_2]}{1} + \frac{[E_1]}{3} \tag{1}$$

where [EE$_2$], [E$_2$] and [E$_1$] represent the concentration of EE$_2$, E$_2$ and E$_1$ respectively.

The potential risk for fish endocrine disruption was then assessed based on the EEQ concentrations and each river reach was classified according to one of the following categories: "no risk" ([EEQ] < 1 ng/L), "at risk" (1 ≤ [EEQ] < 10 ng/L) and "high risk" ([EEQ] ≥ 10 ng/L). The threshold between no risk and at risk was based on the predicted no effect concentrations (PNEC) for population level effect endpoints; a full description is given in Environment Agency (2008a). Briefly, for EE$_2$ this was the geometric mean of the lowest observed effect concentration (LOEC, 1.1 ng/L) and the no observed effect concentration (0.3 ng/L) taken from Wenzel et al. (2001) (= 0.57 ng/L) with a safety factor of 5 applied to give a PNEC of 0.1 ng/L. For E$_2$, the PNEC was based on 100% feminisation of medaka fish at 10 ng/L (Nimrod and Benson, 1998) with a safety factor of 10 applied. No suitable data were available for E$_1$ so it was set based on a relative potency to E$_2$ for vitellogenin induction (three times less potent). The high risk thresholds were set to give a high likely hood of a population realted effect were it to be exceeded. They were therefore based on the LOEC from the Wenzel et al. (2001) study for EE$_2$ and the PNEC from the Nimrod and Benson (1998) studies for E$_2$ without any safety factors applied. The value for E$_1$ was again set to be three times that of the value for E$_2$ (Environment Agency, 2008a).

2.2. Predicted environmental concentrations under future conditions representative of the 2050's

Whilst predicting concentrations for down-the-drain chemicals and in particular steroid estrogens, there were two main drivers: population (pollutant emission) and river flows (dilution in receiving waters). Both are likely to change in the future: climate change will give different river flows (Arnell and Gosling, 2013; Prudhomme et al., 2012) and the population of England and Wales is likely to increase (Shaw, 2002). Although the impact of in-stream biodegradation has been shown to have a negligible role in the overall dissipation of a range of pharmaceuticals in several UK rivers (Boxall et al., 2014), the influence of biodegradation was included. It was not expected to be very significant, but it makes a difference for short half life chemicals such as E$_1$ and E$_2$. The influence of in-river temperature changes on decay rates was also included for completeness.

2.2.1. Incorporating climate change impact on river flows

Climate change may affect many characteristics of the aquatic environment including river flow, river temperature, fish habitat, and the possible fish response to pollution (Hooper et al., 2013). Landis et al. (2013) recently published a set of recommendations for conducting ecological risk assessment in the context of climate change, and stressed the need to determine to what extent climate change should be incorporated. The authors also recommend the identification of the major drivers of uncertainty, and their quantification both spatially and temporally using methods such as the Monte-Carlo method. It has been acknowledged that dilution is currently the main driver in water quality (Whitehead et al., 2009) and in particular whilst estimating PECs for down-the-drain chemicals (Johnson, 2010; Price et al., 2009). It was therefore crucial to predict changes in river flows resulting from climate change.

Prudhomme et al., (2013; 2012) estimated changes in flow for Britain in the 2050s for the 11 different climate change scenarios

defined by the 2009 UK Climate Projections (UKCP09). The UKCP09 explicitly considered climate model parameter uncertainty, hence the range of climate scenarios (Murphy et al., 2009). The changes in river flow were estimated by comparing the flow duration statistics simulated by the semi-distributed hydrological model CERF (Continuous Estimation of River Flows) (Environment Agency, 2008b; Young, 2006) for a 30 year baseline (1961–1990) to a future 30 year period representing the 2050's (2040–2069). Series of perturbation factors were derived for over 1500 incremental catchments across England and Wales.

Within the present study, three scenarios were selected to generate distributions of perturbed future river flows. The three scenarios selected were afgcx, the initial or average parameter set from the UKCP09 climate change model (called "average" from this point). Scenario afixa was chosen to represent a wet scenario (referred to as "wet"). The third was scenario afixk representing a scenario predicted to be amongst the driest (referred to as "dry"). These scenarios were selected based on a visual inspection of maps of percentage change in seasonal mean flow for the 2050's (Prudhomme et al., 2012), to provide a range of water quality conditions representative of the range of future flow conditions (Fig. 1).

The long term average annual rainfall (AAR) for the current scenario (1961–1990) is 895 mm. For the future scenarios (2040–2069), the long term AAR values are predicted to be 910 mm, 934 mm and 850 mm for the average, wet and dry scenarios respectively. This represents, when compared to the current scenario, an increase in AAR for the average (+2%) and wet (+4%) scenarios, and a decrease for the dry scenario (−5%).

Within LF2000-WQX, flow duration statistics are held for every river reach visible at a scale of 1:50,000 on the UK ordnance survey map series. The perturbation factors calculated for each incremental catchment were then applied to each of these river reaches to give three new databases of values representing the three possible futures.

2.2.2. Incorporating temperature change impact on decay rates

The decay rate of substances in river waters is dependent on the river temperature (Chapra, 1997). Within LF2000-WQX, the decay rate at a temperature T (k_T) was calculated from the decay rate at 20 °C (k_{20}) using a temperature correction factor (θ) (Williams et al., 2009):

$$k_T = k_{20}\theta^{(T-20)} \tag{2}$$

Although not published, Prudhomme and colleagues calculated for each of the 11 UKCP09 climate change scenarios average annual air temperature for the period 2040–2069. From this data, a percentage change was calculated for each of the three selected scenarios: average annual air temperatures increased by approximately 30% for the average and dry scenarios, and approximately 23% for the wet scenario.

The river water mean temperature for the future scenarios was derived assuming the same percentage change would apply for water temperature as for air temperature. The standard deviation was then calculated as half of the mean, to define the in-river temperature distribution. For the current scenario, the river water temperature was assumed to have a mean value of 10 °C (standard deviation, 5 °C). Thus for the future scenarios, the mean river water temperature was set to 13 °C with a standard deviation of 6.5 °C for the average and dry scenarios, and 12 °C for the wet scenario (standard deviation, 6 °C). As a consequence of such changes in mean water temperature between the current and the future scenarios, the decay rates for all three steroid estrogens decreased by approximately 20% for $T = 12$ °C (Equation (2)).

2.2.3. Incorporating demographic change: impact on chemical consumption and STWs sizes

Within LF2000-WQX, the emission of chemicals was derived from mean influent load and the characteristics of the STWs (population served and dry weather flow (DWF)). In the original database, the population served data were representative of the early 2000's.

Estimates of per-capita influent were originally derived using the model described by Johnson and Williams (2004). This model was based on the assumption that the rate of excretion of these steroid estrogens varies for different cohorts of population. For example, EE_2 is a synthetic hormone and therefore is only excreted by females taking the contraceptive pill (Johnson and Williams, 2004). The influent load mean of a given steroid estrogen excreted per day (F_T, μg/cap/d) was given by.
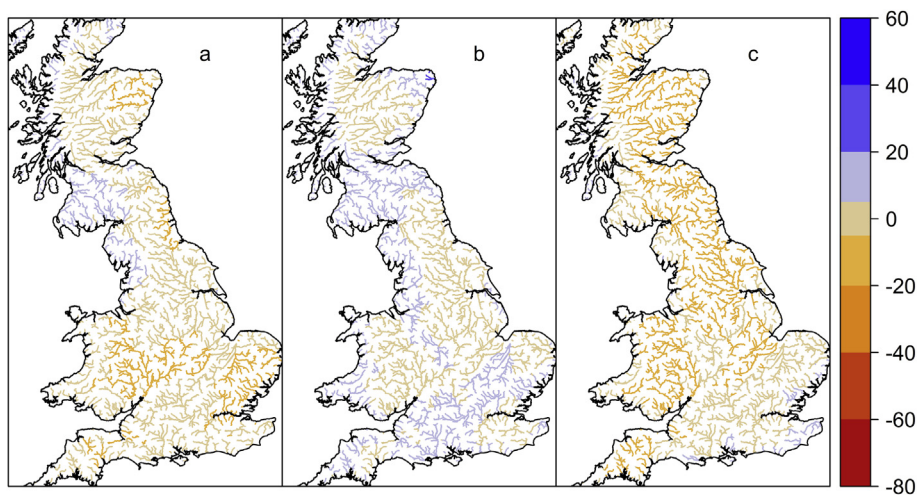
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Fig. 1. Percentage changes in mean flow as simulated by CERF for the 2050's, for the 3 selected climate change scenarios defined by the 2009 UK Climate Projections. a) is the average scenario, afgcx, b) the wet scenario, afixa and c) the dry scenario, afixk. Blue shows flow increase and red shows flow decrease.

$$F_T = \sum_{i=1}^{n} f_i F_i \tag{3}$$

where F_i is the amount of estrogen secreted (μg/cap/d) by the ith fraction (f_i) of the population.

The total influent load for each STW was then estimated within LF2000-WQX by multiplying the per capita excretion amount (F_T) by the population served by the STW.

In Europe (EU27), the total population is projected to rise gradually between 2008 and 2060. Although some countries (mostly eastern countries) are predicted to have a smaller population in 2060, for others, like the United Kingdom, the population is projected to rise continuously (Giannakouris, 2008). Population ageing is a currently observed phenomenon expected to continue into the future in Europe and other parts of the world (Christensen et al., 2009). Such demographic changes will therefore have an impact on the population served by STWs (changes in total population) and on the steroid estrogen load influent mean (changes in the shape of the population pyramid). Increases in population might also affect the DWF of the STW. Within the LF2000-WQX database, STWs were attributed their consented DWF due to lack of measured DWF data. As a consequence, it was assumed that the DWF values used in the current model would remain unchanged for the 2050's.

The population served by STWs was adjusted to reflect expected increases in total population using population projections for England and Wales in 2050 generated by the Office for National Statistics (2011). Amongst several projections available, the principle projection was selected as the most suitable for this study. These projections are based on the year 2010 and were available for both England and Wales. Thus the STW population served from the original risk assessment was increased by 2.03% for Wales and 4.47% in England to account for population growth between 2000 and 2010 (Office for National Statistics, 2002). The resulting population served was then increased by 8.35% for Wales and 28.74% for England to represent the projected population served in the 2050's (Office for National Statistics, 2011). The population density per region based on STW population served was calculated for the 2050's (Table 1).

To reflect changes in the population pyramid, the excreted amount (F_i) was estimated using Equation (3) and adjusted values of f_i derived from the 2010-based projections for 2050 for the UK available from the Office for National Statistics. In the absence of historic data in the excretion of these hormones, it was assumed that the amount of estrogen F_i in each ith fraction of the population (Equation (3)) would remain unchanged in the 2050's. Although significant changes in demographics are predicted with a significant increase in menopausal women and a decrease in menstrual

Table 1
Characteristics of regions in England and Wales.

Region	Population[a] density in 2000 (cap/km²)	Population[a] density in 2050 (cap/km²)	Length of river modelled (km)
Anglian	141	190	5094
Southern	164	221	1499
Thames	447	601	1660
Wales	50	55	2731
Midlands	587	789	3070
North East	219	295	2683
North West	106	143	1786

[a] Steroid relevant population i.e. the population served by the STWs included in the model divided by the total modelled area, from Williams et al. (2009).

Table 2
Population repartition for the 2000 baseline and future projection for 2050's.

Population Category	2000	2050's
Males	50[a]	50[b]
Menstrual females	30[a]	26.1[b]
Menopausal females	13.5[a]	20.9[b]
Menopausal females on HRT	2[a]	2[b]
Pregnant	0.88[a]	0.88[b]

[a] Value available from Johnson and Williams (2004).
[b] Value calculated from 2050's projections, principle projection (Office for National Statistics, 2011).

women (Table 2), the effects on the per capita loads were relatively low (Table 3).

Although it is possible that, depending on economic and environmental policies, sewage treatment efficiency in removing steroid estrogens will improve, percentage removals in sewage treatment were assumed unchanged in the future. This assumption will provide a worse case scenario.

2.3. Risk classification for the 2050's

To allow for comparison with the previous study, the risk thresholds used within this study are those defined in the original risk assessment and related to reproductive endpoints (Williams et al., 2009). For each scenario, all river reaches within England and Wales were classified into risk categories based on potential steroid estrogen impacts: "no risk", "at risk" and "high risk", as defined previously in the methodology overview.

3. Results and discussion

3.1. Predicted risk assessment from fish endocrine disruption for the 2050's

The risk assessment for England and Wales was conducted region by region for each scenario. The demographic characteristics and the lengths of rivers modelled in these regions are presented in Table 1, other regional characteristics (e.g. annual average rainfall and population density) are available from Williams et al. (2009).

A cumulative frequency curve was drawn to highlight the differences in concentrations between the different scenarios. Risk maps were then generated to identify changes in areas of potential risk and compared with the current risk map. As an example, the cumulative frequency curve (Fig. 2) for the Thames region is shown. The Thames region has a surface area of about 12,900 km², and a high population density of about 447 inhab/km² (based on 2000 data) with a projected density for the 2050's of about 601 inhab/km². The mean estradiol concentration curves for all three future scenarios were shifted to the right compared to the current risk assessment, but there was little obvious difference between the three future scenarios themselves. For example, 50% of river

Table 3
Estrogen influent load mean (μg/cap/day) for the 2000 baseline and future projection for 2050's.

Steroid estrogen	Influent load mean for 2000	Influent load mean for 2050's
Estrone (E₁)	3.3[a]	3.3[b]
Estradiol (E₂)	13.8[a]	13.4[b]
Ethinylestradiol (EE₂)	0.89[a]	0.89[b]

[a] Value available from Johnson and Williams (2004).
[b] Value calculated from Johnson's model (Johnson and Williams, 2004) and 2050's projections (Office for National Statistics, 2011).
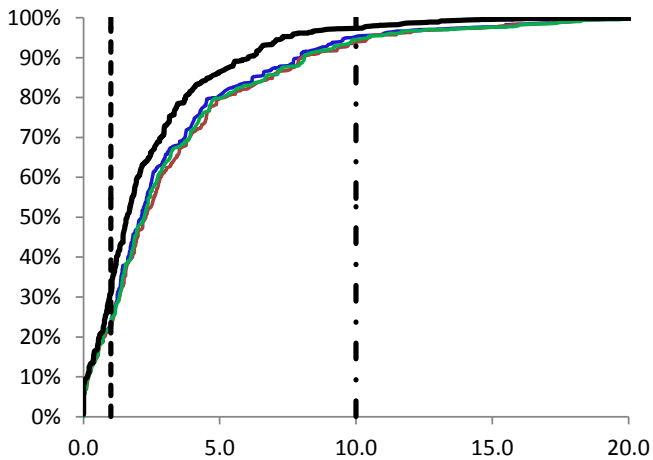
Fig. 2. Cumulative frequency curve of predicted estradiol equivalent concentrations in the Thames region. The black line is from the risk assessment under current conditions; the blue line is for the wet scenario, the green line the average scenario and the red line the dry scenario. The vertical dotted lines show the concentrations that correspond to the change from "no risk" to "at risk" and from "at risk" to "high risk". The *x*-axis has been shortened to emphasize the difference between the results for each scenario. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
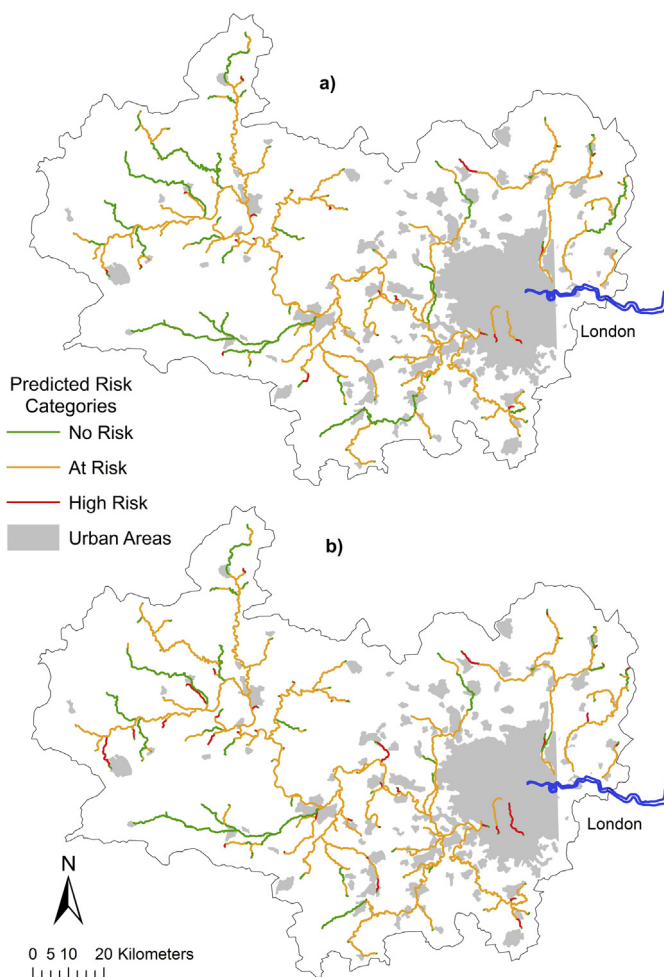

Fig. 3. Spatial distribution of predicted risk categories in the Thames region a) current risk assessment, b) dry scenario.

reaches exceed 1.6 ng/L under the current risk assessment, which increases to 2.1, 2.2 and 2.3 ng/L under the wet, average and dry scenarios respectively. Because of the similarity in future scenario results, only the risk map derived from mean concentrations for the dry scenario is presented alongside the current risk map (Williams et al., 2009) for the Thames region (Fig. 3).

Within the Thames region, the spatial distribution of the predicted risk categories were also fairly similar between all three future scenarios with many reaches being "at risk" (data not shown). Compared to the spatial distribution of the risk under current conditions, these data project an increase in the number of stretches "at risk" of endocrine disruption by 2050. The reaches at "high risk" under future scenarios seemed to occur mainly downstream of the STW already causing "high risk" under the current scenario, however the river length at "high risk" downstream of these STWs appears to have significantly increased: 91 km, 84 km and 108 km under the average, dry and wet scenarios respectively compared to 44.5 km in the original risk assessment. The dry scenario showed the greatest increase in risk from the current risk assessment with increases in "at risk" and "high risk" sites in the North East and South West parts of the catchment (Fig. 3).

The extent of the risk level in each region of England and Wales was quantified in terms of percentage of total length (TL) of river modelled (Table 4).

All the future scenarios predicted higher levels of risk than the current risk assessment, overall the percentage of "no risk" sites decreased under all future scenarios. For England and Wales, future scenarios predicted an increased percentage of reaches "at risk":- wet scenario 45%, current 38% and dry scenario 46%. It was however the "high risk" category that showed the largest increase: when compared to the current risk assessment (1% of total length modelled) there is a difference of a factor of three with the wet scenario (3%) and a factor of four with average and dry scenarios (4%). These results are in agreement with the conclusions from Green et al. (2013) who predicted a moderate increase in risk in the Erewash catchment part of the Severn-Trent catchment (England, Midlands Region).

As with the national scale, within most regions, it was the "high risk" category that proportionally increased the most when comparing future scenarios to the current situation. For example in the North East the current risk assessment predicted 1% of total river length modelled at "high risk" compared to 4% for the average and wet scenarios, and 6% for the dry scenario. Future scenarios also predicted an increased percentage of reaches "at risk" in the North East. However, although not negligible, this increase was proportionally less important (Table 2). This pattern is completely different for Wales where the percentage of the total length of river reaches modelled at "high risk" was predicted to be similar in the future and the current scenarios (<1%), and the "at risk" category increases from 5% under the current scenario to between 6% (wet scenario) and 9% (dry scenario) in the 2050's.

Less populated regions including Southern, South West, and Wales mainly showed a rise within the "at risk" category. Amongst all three future scenarios, unsurprisingly, the wet scenario gave the least reason for concern as the proportion of river length within the "at risk" and "high risk" category was lower.

3.2. Biological effects

The risk assessment classification levels were set based on effects on individual fish that might be expected to impact on the ability of affected fish to sustain the population. The "at risk" classification was designed to identify reaches of rivers where effects might be likely to be seen. At "high risk" sites the exposure concentration would be expected to produce endocrine disrupting

Table 4
Percentage of total length of river modelled per risk categories for Thames and Wales in future scenarios and current risk assessment.

Scenario	Risk Category	Anglian	Southern	Thames	Wales	Midlands	North East	North West	South West	England and Wales
Average	No risk	36	54	23	93	28	52	62	77	50
	At risk	59	45	72	7	65	44	35	22	46
	High risk	5	1	5	<1	7	4	3	<1	4
Wet	No risk	37	57	23	94	31	53	63	79	52
	At risk	59	42	72	6	63	43	35	20	45
	High risk	4	1	5	<1	6	4	2	<1	3
Dry	No risk	36	52	22	91	28	47	58	78	50
	At risk	59	47	71	9	66	47	39	21	46
	High risk	5	1	7	<1	6	6	3	<1	4
Current[a]	No risk	48	65	30	95	43	61	65	84	61
	At risk	50	34	67	5	55	38	34	16	38
	High risk	2	1	3	<1	2	1	1	<1	1

[a] Values available from Williams et al. (2009).

effects associated with reproductive fitness of affected individuals. Thus the prediction is that as "high risk" areas will increase in extent the incidence of endocrine disruption and specifically the occurrence of intersex males should rise. In addition, exposure concentrations were also predicted to increase and we would also expect this to increase the severity of intersex (assuming a normal dose-response relationship). Other factors affected by climates change (e.g. temperature) could increase the sensitivity of fish to effects from chemicals due to changes in uptake, metabolism and excretion rates thereby magnifying these effects (Hooper et al., 2013). Whether these effects on individuals would have an effect on the fish population was beyond the scope of this study. A recent study has, however, shown that even in those reaches predicted to be "high risk" (using the same model as used here), and where there were known intersex fish, the resident populations of roach were shown to be self-sustaining (Hamilton et al., 2014).

3.3. Identifying the most influential factor(s) in the risk for the 2050's: Thames region as an example

To predict future risks, several future changes were considered: changes in river flows, in river temperature and in human population. Although variations in river temperature and population were assumed constant across England and across Wales, changes in river flow fluctuated across regions and catchments. Therefore for each region the most influential factor in the risk may differ. The Thames region was selected, as an example, to determine which were the most influential factor(s) across the different scenarios. The risk assessment for the 2050's was thus compared, for each scenario, to two test cases: i) Test case A: included changes in population and river flow only, and, ii) Test case B: incorporated changes in river flow and temperature only. When neglecting the impact of temperature on biodegradation rates (test case A), there was a slight increase in risk (Table 5) when compared to the risk assessment results for 2050's. Such findings imply that the increase in temperature induced more in-stream biodegradation and thus reduced in-stream concentrations. This observation is in line with the fact that concentrations after in-stream removal were modelled with a temperature dependent first-order exponential decay equation in LF2000-WQX (Williams et al., 2009). Population was shown to be the most influential factor in determining changes in the percentage of reaches in the Thames region at risk of endocrine disruption; test case B (no population change) not only showed the most difference with the 2050's risk assessment (Table 5) but it also showed little difference when compared to the current risk assessment (Table 4).

Table 5
Identification of the most influential factor for the 2050's risk assessment in the Thames region. Risk assessment for the 2050's included changes in human population, river flow and temperature, test case A included changes in human population and river flow, and test case B included changes in river flow and temperature.

Scenario	Risk Category	Risk assessment 2050's (%)	Test case A (%)	Test case B (%)
Average	No risk	23	22	30
	At risk	72	71	67
	High risk	5	7	3
Wet	No risk	23	25	33
	At risk	72	70	64
	High risk	5	5	3
Dry	No risk	22	21	30
	At risk	71	71	67
	High risk	7	8	3

These observed changes across test cases and the future risk assessment were in alignment with the percentage changes between the current and the future situation: population was increased by approximately 8% and 29% for respectively Wales and England, the decay rate decreased by around 20% for all scenarios and changes in river flow by ± 5%. For E_1 and EE_2, the influent load per capita for the future was equal to the current value used, however a slight decrease is predicted for E_2 ($\approx 4\%$), due to the predicted ageing of the population in the 2050's.

Although the test cases have not been carried out across all regions, it is reasonable to assume that across England and Wales it was the increase in population that was the primary factor influencing the predicted risk increases for the 2050's. Indeed, the population directly determined the steroid load and hence had a direct link with chemical concentrations. There are a number of different estimates of future population growth for the UK. Only the middle growth path has been investigated here and clearly higher or lower steroid estrogen loads would be generated by the higher and lower population growth projections (Green et al., 2013). The different cohorts that make up the future population might also be important. For example, the change in the number of menstrual females by 2050 has been estimated with an assumed reduction in consumption of EE_2. There are also likely to be more menopausal females taking hormone replacement therapy and again this has been estimated. Clearly only three plausible scenarios have been taken forward in this study from a wide range of others that could influence the future exposure level for fish to steroid estrogens.

4. Conclusions

In this study, the risk assessment for endocrine disruption in fish due to steroid estrogens (E_1, E_2 and EE_2) was reassessed for the

2050s based on changes in human population (increase and ageing), river flows and river temperature. Across all three scenarios considered, there was an increase in risk for endocrine disruption in fish due to steroid estrogens in England and Wales. However this increase varied across regions: regions with a lower population density presented the least changes in risk levels. The risk assessment raising the least reason for concern was from the wet scenario, which had the highest river flows, and therefore highest dilution factor between DWF and river flow. The three scenarios envisaged indicated that climate change should likely make the risk assessment outcome worse for endocrine disruption in fish arising from steroid estrogens. It is nevertheless important to emphasise the fact that only 3 out of 11 climate change scenarios were investigated. Although these scenarios were selected to sweep across the range of climate sensitivity in England and Wales (dry, average and wet), other scenarios might have indicated different levels of risk at the local scale (catchment scale). However, we believe that the national overall change in risk will remain similar to the ones investigated, as climate change is not the most influential factor. The principle factor driving the increase in risk in the future was the human population change, this was illustrated in the Thames catchment. This fact is likely to hold true across England and Wales as population increases at a more substantial rate than the other factors investigated.

This work provides a warning to regulators and policy makers that current risk assessments could look different in the future. However only a small fraction of these changes was attributable to flow changes, human population being the most influential factor. This research has the potential to be refined by considering the impact of improved STW treatment efficiency; if higher levels of treatment are envisaged, then levels of risk in the future would be reduced.

Acknowlededgements

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