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1 20 hydrological models to estimate concentrations of pharmaceutical and natural steroid estrogens in a
2
3 21 water stressed catchment in South Australia alongside a UK catchment and to forecast their
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5 22 concentrations in 2050 based on demographic and climate change predictions. The results show that
6
7 23 despite their differing climates and demographics, modeled concentrations of steroid estrogens in
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9
10 24 effluents from Australian sewage treatment works and a receiving river were predicted (simulated) to be
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12 25 similar to those observed in the UK and Europe, exceeding the combined estradiol equivalent's
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14 26 predicted no effect concentration for feminization in wild fish. Furthermore, by 2050 a moderate
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16
17 27 increase in estrogenic contamination and the potential risk to wildlife was predicted with up to a two-
18
19 28 fold rise in concentrations.

23 29 KEYWORDS:

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26
27 30 Modeling; Steroid Estrogens; Climate Change; Population Growth; Endocrine Disruption; Wastewater
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29 31 Dilution
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35 33 INTRODUCTION

36 34 In the last two decades the steroid estrogens, estrone (E1), 17 β -estradiol (E2) and the pharmaceutical
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38 34 17 α -ethinylestradiol (EE2) have been identified as aquatic pollutants globally¹⁻⁴. Originating from
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40 35 human excretion⁵ as natural steroids and from pharmaceutical use, they are continuously discharged into
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42 36 rivers via sewage treatment works' (STW) effluents, which can constitute up to 100% of river flow
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44 37 during dry periods⁶⁻⁸. As a result, contamination of river networks with steroid estrogens is widespread
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46 38 and there are extensive data to suggest they are the primary endocrine disruptors responsible for
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48 39 feminization of male fish⁹⁻¹¹, particularly downstream of STW effluent discharges. Indeed,
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50 40 environmental concentrations of steroid estrogens can cause feminization effects in fish species
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52 41 maintained under laboratory conditions^{10,12-14}, including the abnormal development of both ovarian and
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54 42 testicular tissue in the gonads. This intersex condition has been well characterized in the UK where it is
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1 44 widespread in the normally dioecious roach (*Rutilus rutilus*)¹⁵ inhabiting freshwater rivers^{6,7,11,16,17}.
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3 45 Since reproductive performance of wild male fish has been negatively correlated with intersex severity,
4
5 46 there has been cause for concern for wild fish populations¹⁸. In fact, during a whole lake experiment
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7 47 with regular dosing of EE2 at concentrations consistent with untreated effluent (mean 4.8-6.1 ng/L), an
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9
10 48 entire fish population collapsed¹⁹. This has led to the recent addition of E2 and EE2 to the list of
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12 49 “priority substances” by the European Commission in December 2012 as the first pharmaceuticals to be
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14 50 considered for regulation under the European Water Framework Directive²⁰.
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17 51
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19 52 In order to map the distribution of steroid estrogen contamination, pioneering hydrological modeling
20
21 53 methods have been used to predict concentrations of these chemicals in effluents and river networks,
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24 54 detecting “hot spots” of potentially at risk areas^{4,21-23}. The results correlate well with measured effluent
25
26 55 concentrations as well as the intersex incidence and severity in wild roach that inhabit the modeled river
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28
29 56 stretches¹¹. Hydrological modeling with Low Flows 2000-WQX has been subsequently used in a risk
30
31 57 assessment of the entire UK river network, predicting that around 39% of the river stretches were at risk
32
33 58 of inducing intersex in wild fish due to steroid estrogen contamination⁴. These modeling techniques
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35
36 59 have since been applied to investigate a range of mitigation options at STWs²⁴ as well as the mixture
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38 60 effects of estrogens and xenoestrogens in a UK river catchment²⁵. They have also been exported
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40 61 internationally for use in national risk assessments in the USA²⁶ and Japan²⁷, as well as for effluent
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43 62 modeling in Chile²⁸.
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47 64 Although the identification of at risk areas in the present day and the future is one of the top 20
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50 65 research questions for pharmaceuticals and personal care products²⁹, in many countries these types of
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52 66 risk assessments for steroid estrogens have not been completed since the hydrological models to enable
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54
55 67 this process have not been developed. In water stressed areas of the world, such models could be highly
56
57 68 informative as lower water availability in these areas potentially reduces the dilution of these
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59
60 69 contaminants in the aquatic environment relative to other areas, increasing their concentrations and their

1 70 risks to aquatic organisms. Moreover, the anticipated global population growth during this century
2
3 71 alone³⁰, coupled with climate induced changes in precipitation³¹, provides an additional need to assess
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5 72 the consequences of changing water availability on future estrogen concentrations and their potential
6
7 73 impacts such that any mitigation options proposed are of an appropriate scale to be effective in the
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9
10 74 longer term. To this end, this study uses predictive modeling techniques to predict effluent and river
11
12 75 concentrations of steroid estrogens in moderately water stressed catchments in the UK and South
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14 76 Australia. In addition, the models were modified to reflect population growth and climate-change
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16
17 77 scenarios, producing the first future projections of steroid estrogen contamination and its potential
18
19 78 impacts in UK and South Australian rivers by 2050 in an approach which can be used as a tool for risk
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21
22 79 management strategies involving large investments in improvements in waste water treatment.
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24 80

26 81 **MATERIALS AND METHODS**

28 82 *Sites*

30
31 83 Four UK STWs (UK1-4) located in the Severn-Trent catchment, typical of the UK's urbanized
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33 84 environment, were compared with 12 STWs in South Australia (Table S1), representing a variety of
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35
36 85 rural and urban scenarios. Both catchments are considered to be moderately water stressed, since the
37
38 86 demand and allocation of water is a high proportion of the total availability³²⁻³⁵. The river hydrology of
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40
41 87 the two catchments contrast with cooler, permanently flowing waters in the UK and warmer more
42
43 88 ephemeral hydrology dominated by winter flow in South Australia.
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47 90 *Modeling Natural Estrogens: Estrone (E1) and 17 β -Estradiol (E2)*

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50 91 The model was based on an approach provided by Johnson and Williams, which has been applied to
51
52 92 predict environmental concentrations of steroid estrogens in effluents in Europe²³, as well as in
53
54
55 93 hydrological models used for national risk assessments of endocrine disruption in rivers in the UK,
56
57 94 Japan and the USA^{4,26,27}. Our modified model provides a per capita load for E1 and E2 in $\mu\text{g}/\text{day}$
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59
60

95 arriving at a STW, based on the proportions of different estrogen-excreting cohorts within a population.

96 This was calculated as follows:

$$SE2 = 0.5 \sum_{i=1}^n f_i (UE2)$$

$$SE1 = \sum_{i=1}^n f_i (UE1) + 0.5 SE2$$

99 Where S is the per capita load arriving at a STW ($\mu\text{g/d}$), n is the number of cohorts and U is the total
 100 estrogen excreted in urine (in free, glucuronide and sulfate forms) and feces for each cohort percentage
 101 (f_i) of the population. For E2, a factor of 0.5 is incorporated assuming that 50% will be degraded to E1
 102 in transit through the sewerage system to a STW. The mean estrogen excretion of each cohort
 103 percentage is shown in Table 1 and is based on a literature review for the original model that focused on
 104 Caucasian omnivorous women²³. Upper and lower excretion values were also used to provide a range
 105 in the load arriving at a STW. A worked example can be found in the Supplementary Information.

Cohort	Criteria	Mean (range) excretion ($\mu\text{g/d}$)		% of population	
		E2	E1	UK	Australia
Menstrual females	Age 15-50	3.2	11.7	23.5%	24.2%
	(minus pregnant women)	(1.7-4.6)	(7.5-15.4)		
Menopausal females	Age >51	1	1.8	16.1%	13.7%
	(minus menopausal women on HRT)	(0-3.5)	(0-5.7)		
Menopausal females on HRT	7.6% UK and 11.8% Australian menopausal females (>51)	56.1 (51.5-61.5)	28.4 (24-33)	1.3%	1.8%
Pregnant Females	1/22 UK and 1/19 Australian menstrual females	393 (340-445)	550 (432-668)	1.1%	1.3%
Males	Age 15-50	1.8	2.6	39.0%	39.2%
		(1.3-2.4)	(1.4-2.9)		

Table 1. The population breakdown with the estrogen excreting cohorts by criteria and the composition of each census population: UK 2001 and Australia 2006.

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1
2 110 **Cohort Criteria:** The percentages of the populations made up by each cohort were based on age and
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4
5 111 determined from national census data, which was assumed to be relevant to local demographics. This
6
7 112 utilized the national report for England and Wales (age by sex and resident type) from the 2001 census
8
9
10 113 by the Office for National Statistics (ONS) and the Australian 2006 census (age by sex based on place
11
12 114 of usual residence) from the Australian Bureau of Statistics (ABS). Pre-pubescent males and females
13
14 115 were not incorporated since sex steroid production is low until puberty and their inclusion would have
15
16 116 little effect on the final prediction²³. As a result, the male cohort included those from age 15 onwards
17
18
19 117 and menstrual females were assumed to be between 15 and 50 with menopausal females taken from the
20
21 118 age of 51 onwards. The number of females on hormone replacement therapy (HRT) using E2 based
22
23 119 pharmaceuticals was updated for our model where 11.8% of women over 50 were estimated to use HRT
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25
26 120 in Australia³⁶ compared to 7.6% of women in the UK. This was calculated by combining population
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28 121 data from the 2001 census with data on HRT use in the UK in 2004³⁷. These percentages were applied
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30
31 122 to the menopausal female cohort to determine the number of women on HRT, although it should be
32
33 123 taken into account that HRT use has fluctuated in the last decade in both countries^{36,37}. The number of
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35 124 pregnant females was estimated using the census data assuming that the number of live births (people
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37
38 125 aged 0) was representative of the number of pregnant females. Using this model, per capita loads of 3.4
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40 126 (2.7-4.1) and 3.9 (3.2-4.7) $\mu\text{g}/\text{d}$ were produced for E2 in the UK and Australia respectively, as well as
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42 127 14 (10-18) and 16 (12-20) $\mu\text{g}/\text{d}$ for E1.
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47 129 ***Modeling Pharmaceuticals: 17 α -Ethinylestradiol (EE2)***

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49
50 130 The per capita load of EE2 was calculated based on the number of prescriptions in the UK and
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52 131 Australia, which were determined from the National Health Service's Prescriptions Cost Analysis
53
54 132 (2009) for England³⁸ and Wales³⁹ and the Australian Statistics on Medicines (2008)⁴⁰, using a method
55
56
57 133 from Runnalls et al⁴¹. About 17.4kg of EE2 were prescribed in England and Wales in 2009 in
58
59 134 comparison to 5.55kg in Australia in 2008. With populations of 54,809,100 (mid 2009 estimate for
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1 135 England and Wales, ONS) and 22,000,000 (ABS estimation) for Australia and an excretion rate of 40%
2
3 136 of the dose²³, the per capita loads were estimated at 0.35 and 0.28 µg/d for the UK and Australia,
4
5 137 respectively. The higher per capita load in the UK due to the higher prescription level of EE2
6
7 138 contrasted with that of E1 and E2, where the differences in population demographics resulted in a higher
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9
10 139 per capita load in Australia.

14 *Predicting Concentrations of Steroid Estrogens in STW Effluent*

16
17 142 The linear emission model was used to predict effluent concentrations (µg/L) reflecting a 24-hour
18
19 143 composite sample of effluent. The total load arriving at a STW (the per capita load (*pc*) (µg/d) of each
20
21 144 estrogen multiplied by the population (*pop*) serviced) was divided by the total flow (*Q*) (L/day) through
22
23
24 145 the STW (domestic plus non-domestic flow). Removal rates (*R*) of 69% and 83% were incorporated for
25
26 146 E1 and E2 respectively, based on a review of removal during the activated sludge process (ASP)⁴².
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28
29 147 However, it should be recognized that in reality removal rates vary, even in a single STW, based on the
30
31 148 treatment process and environmental conditions⁴³. Flow and population data for the STWs were
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33 149 provided by Severn Trent Water, UK and SA Water Corporation, Australia (Table S1).

$$35 \quad C_{effluent} = \frac{pc \cdot pop}{Q_{STW}} \cdot (1 - R)$$

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37
38
39 150 Average, upper and lower effluent concentrations for E1 and E2 were produced by varying the per
40
41 151 capita loads with the upper and lower excretion values (Table 1), whilst for EE2 different removal rates
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44 152 during the activated sludge process (83%, 71.2% and 94.8%) were assumed.

46 *The Relevance to Real World Effluents*

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49 154 To determine the relevance of modeled data to real world steroid estrogen concentrations in effluent,
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51 155 modeled concentrations were compared with measured data from UK2, where data from 19 24-hour
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53 156 composite samples of its activated sludge treated effluent were available from a previous study⁴⁴. These
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56 157 were collected between July and December 2009 and analyzed by liquid chromatography-tandem mass
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1 159 spectroscopy (LC-MS/MS) as described previously⁴⁵. These data were compared with daily average
2
3 160 modeled concentrations based on flow data from UK2 provided by Severn Trent Water from the day of
4
5 161 sampling (Table S2). Up to 96 flow measurements were taken daily so concentrations were produced
6
7 162 for each flow rate based on the average per capita loads.

11 *Predicting River Concentrations*

12 164 **UK, Low Flows 2000-WQX:** The Low Flows 2000 (LF2000) WQX (Water Quality eXtension)
13
14 165 model (Wallingford Hydrosolutions) was used to predict concentrations of steroid estrogens in the River
15
16 166 Erewash as described in William's et al⁴. LF2000-WQX provided a map of interconnected river
17
18 167 reaches, with artificial influences (e.g. abstractions and discharges) incorporated, where the magnitude
19
20 168 and variability of flows at ungauged sites were estimated from runoff and generalized against gauged
21
22 169 catchments. Steroid estrogens were assumed to enter the system continuously via the eight STWs on
23
24 170 the Erewash including UK2 and 4. The per capita load arriving at these STWs was based on the effluent
25
26 171 model with serviced populations updated with new estimates from Severn Trent Water. The dry
27
28 172 weather flows (DWF) through the STWs in the LF-2000 WQX model were updated in line with the
29
30 173 population to maintain the per capita flow, whilst removal at each STW was based on the ASP review
31
32 174 used in the effluent model⁴². The average concentrations of steroid estrogens on a given stretch were
33
34 175 then determined based on an exponential decay model incorporating in-stream temperature dependent
35
36 176 degradation (Table S3)⁴⁶ and dilution based on the spatial variability in flow. Loss through absorption
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38 177 to sediment was not included since it is not a cause of significant removal⁴⁷. Degradation of E2 to E1
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40 178 was also incorporated based on 1 mol E2 degrading to 1 mol of E1.
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52 181 **South Australia, Source Catchments:**

55 182 A point source hydrological model of the Onkaparinga River in South Australia was implemented and
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57 183 run in Source Catchments version 2.0.4 (eWater CRC)^{48,49} to predict steroid estrogen concentrations on
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59 184 a 16 km stretch downstream of the STW SA2. The river itself is vital to the water supply of the city of
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1 185 Adelaide, supplying the Mount Bold and Happy Valley Reservoirs. The model provided a node-link
2
3 186 system representing a series of interconnected river stretches with artificial influences incorporated,
4
5 187 where flow through the stretches (links) was calculated based on the SIMHYD rainfall-runoff model
6
7 188 with laurenson flow routing⁴⁹. Steroid estrogen input was simulated with an inflow function at the node
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9
10 189 representing SA2 based on a time series of daily concentrations modeled using the daily flow rates from
11
12 190 the STW in 2008 to simulate a continuous influx. Another inflow function was incorporated at a node
13
14 191 downstream representing the inter-basin transfer of raw River Murray water from the Murray Bridge-
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16
17 192 Onkaparinga pipeline by adding flow only as no STWs discharge within 500 km from this additional
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19 193 water source. The steroid estrogens were transported through the interconnected stretches from their
20
21
22 194 source with their concentrations calculated on each stretch based on the available dilution from
23
24 195 simulated flows and a simple exponential decay model using half-lives based on their typical
25
26 196 degradation rates in UK rivers at 20°C water temperature⁴⁶ (Table S3). However, this was not
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28
29 197 temperature dependent and it should be recognized that their degradation could differ in Australian
30
31 198 rivers due to different environmental conditions. However, no data are available to support this
32
33 199 possibility. Again, no loss to sediment was assumed and in contrast to LF2000-WQX, the conversion of
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35
36 200 E2 to E1 was not included, which could result in a small underestimation in concentrations of E1. In
37
38 201 addition, the model does not incorporate the farm dam directly downstream of the STW which abstracts
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40
41 202 some water for irrigation, potentially affecting the concentrations of estrogens entering the main river
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43 203 stretch, below this point particularly during the summer months. However this could not be quantified.
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47 ***Risk Assessment of the Equivalent Estrogenic Activity*** 48 205

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50 206 Since estrogens exist in the environment in combination and act additively to induce similar
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52 207 biological effects, it is appropriate that a combined “toxic equivalent” is incorporated into any risk
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55 208 assessment⁵⁰. This is presented as the estradiol equivalent (EEQ) in ng/L, calculated based on their
56
57 209 comparative estrogenic activity as $([EE2]/0.1 + [E2]/1 + [E1]/3)$ with a PNEC of 1 ng/L⁵⁰. To
58
59 210 determine the risk to wild fish populations, the hydrological models of the rivers were used to map
60

1 211 potential “hot spots” for estrogen concentrations: categorizing stretches as “no risk”, “at risk” or “high
2
3 212 risk”, based on the EEQ (<1, 1-10 and >10 ng/L EEQ respectively)⁴. This method of predicting the
4
5 213 presence of “risk” stretches from the effluent model and LF2000-WQX has recently been compared
6
7 214 with LC-MS/MS analysis on the Erewash, where modeled and measured concentrations both produced
8
9
10 215 the same risk categories for the river stretches based on the EEQ⁵¹.

14 15 217 *Predicting Estrogen Concentrations in 2050: The Effects of Population and Climate Change*

16
17 218 To determine how levels of steroid estrogens in effluents and rivers could change in the future,
18
19 219 concentrations were modeled based on data relevant to 2050. These were then compared back to the
20
21 220 predictions detailed above, produced from sources dating from 2001-2011, which are henceforth
22
23
24 221 referred to as predictions for the present day. Data on population change was gathered from the
25
26 222 “National Population Projections, 2010-based Projections” publication released in 2011 by the ONS,
27
28
29 223 UK⁵² and “Population Projections Australia, 2006-2101” released in 2008 by the ABS⁵³. Since
30
31 224 projections were available for 2051 for both countries, these were assumed to be representative of 2050
32
33 225 and relevant to the local catchment areas. Three main projections were used for each country based on
34
35
36 226 demographic assumptions of future fertility, mortality and migration to produce different scenarios for
37
38 227 population change. These included a principal projection (B) which assumed that current trends in these
39
40
41 228 demographic assumptions would prevail in the future and high (A) and low (C) population projections
42
43 229 to provide a range.

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45 230
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48 231 Since the data were available on an age by sex basis, new per capita loads for E1 and E2 were
49
50 232 produced based on new estrogen excreting cohorts relevant to 2050 to incorporate the change in
51
52 233 population composition (Table S4). Additionally, the per capita load of EE2 was changed in line with
53
54
55 234 the proportion of menstrual females: the users of the contraceptive pill. The effluent concentrations at
56
57 235 the STWs under each population projection relevant to 2050 were then calculated using the new per
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59
60 236 capita loads and assuming that the populations serviced changed in line with the population change from

1 237 2011-2051 (Tables S5 and 6). No changes were made to the DWF at the STWs, which remained at
2
3 238 present day levels to provide a worst case scenario which assumed that no additional water was
4
5 239 available for dilution.
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7 240
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9
10 241 The river models used the data above at the STW inflows and were modified to incorporate predicted
11
12 242 climate-induced changes to flow. In the UK, the flow on the Erewash in LF2000-WQX was modified
13
14 243 with flow data from the UK Climate Projections (UKCP09) simulation afgcx, which is one of 11
15
16
17 244 physically plausible simulations relevant to a medium emissions scenario in the UK⁵⁴. As a result the
18
19 245 flows were on average 5.2% lower than the 2009 model on each stretch. Estrogen concentrations along
20
21
22 246 the river were again calculated with inflow from the STWs based on the updated population data
23
24 247 relevant to each projection. Again, no changes were made to the DWF. Due to the lack of available
25
26 248 data for South Australia, the Source Catchments model was modified by reducing flow on each stretch
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28
29 249 by 17.5% from its 2008 level to provide a medium range climate model. This was based on a 15-25%
30
31 250 reduction in annual stream flow for the Murray River projected for 2050 using two medium sensitivity
32
33 251 climate scenarios, A1 and B1, from the Special Report on Emissions Scenarios⁵⁵.
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36 252

37 38 253 **RESULTS AND DISCUSSION**

39 40 254 *Predicted Concentrations of Estrogens in STW Effluents*

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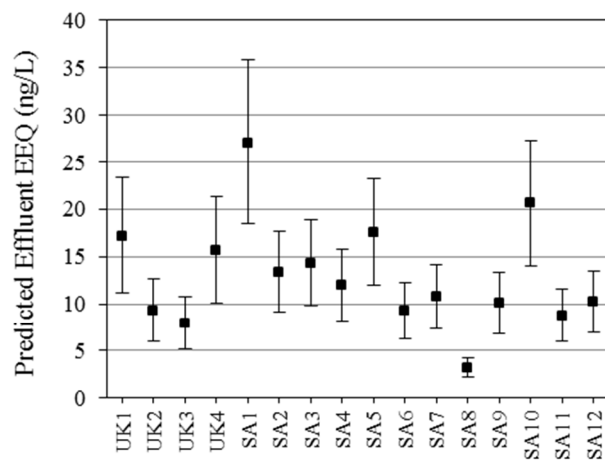


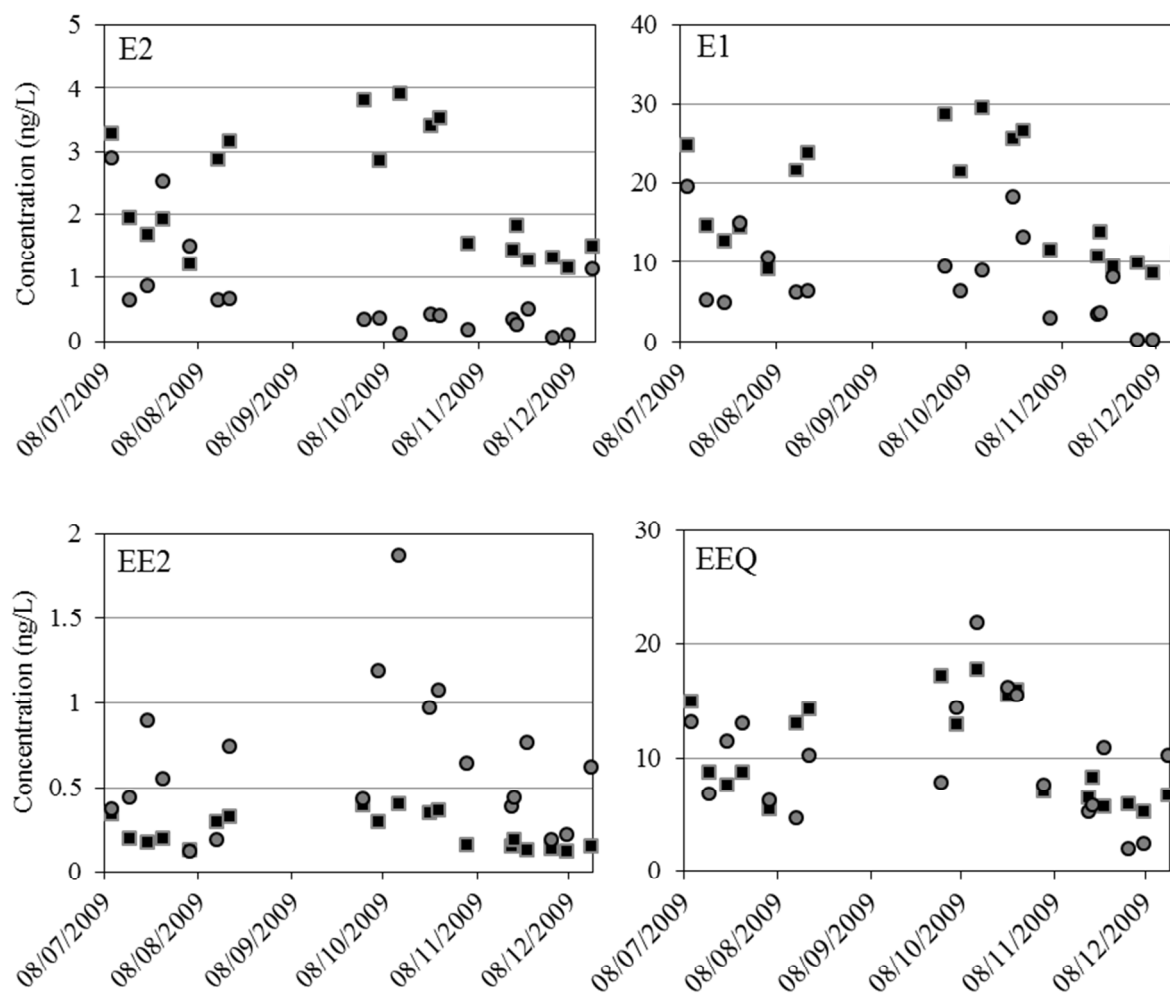
Figure 1. The predicted EEQs of effluents from UK and South Australia STWs. Boxes represent the predictions based on the average per capita loads (squares) with error bars extending to predictions based on the upper and lower per capita loads for E1 and E2 and excretion rates for EE2.

With lower populations served on average, the predicted total estrogen load arriving at South Australian STWs in the present day was lower than the UK. However, the lower flow through South Australian STWs produced a similar dilution factor (the per capita flow) to those in the UK (Table S1). As a result, the predicted concentrations of E1, E2, EE2 (Figures S1) and the EEQ were similar in both the UK and South Australian effluents (Figure 1), corresponding with the measured data range from the two countries and a review of effluents globally⁵⁶. The deviations in concentrations between STWs resulted from their differing per capita flows, demonstrating the importance of dilution in predicting estrogen concentrations at a given STW.

The Relevance to Real World Effluents

In previous studies predictive modeling has been shown to produce environmentally relevant estimations for STWs⁴³. On a national scale the range of concentrations predicted by this study for both the UK and South Australia were within the range of measured concentrations from 43 UK STWs⁵⁷ and over 70 STWs in Australia^{3,56,58-64} (Table S7). The exception to this was SA1, which exceeded the 54 ng/L reported maximum observed concentration of E1 in Australia⁶³. Although the range provided by

278 the assessment of 70 effluents is relatively extensive, it only represents a small proportion of Australian
 279 STWs and it is plausible that higher concentrations could occasionally occur in some of the older STWs.
 280



281
 282 **Figure 2.** The daily average modeled (squares) and measured (dots) estrogen concentrations with the
 283 EEQ (ng/L) in effluent from UK2 over 19 sampling points from July to December 2009. A data gap
 284 exists between 27.8.09 and 21.9.09 due to the lack of available flow data to produce modeled
 285 concentrations.

286
 287 In a review of comparisons between modeled and measured data, predicted concentrations of
 288 pollutants in effluent were routinely predicted within a factor of 5 of the measured values⁴³. At UK2,
 289 when modeled concentrations were compared with measured concentrations from effluent samples
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1 290 collected between July and December 2009, clear temporal variation was observed in both datasets
2
3 291 (Figure 2). The differences between measured and modeled concentrations at each sampling point also
4
5 292 varied where predictions for E1 and E2 both tended to overestimate the measured by a factor of 0.9-54
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7 293 (median 3.1) and 0.8-33 (median 4.7) respectively. However, modeled concentrations of EE2 tended to
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9
10 294 underestimate the measured by a factor of 0.2-1.5 (median 0.4). These deviations in opposing directions
11
12 295 produced a smaller deviation in the modeled EEQ, which generally overestimated the measured by a
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14 296 factor of 0.5-3.0 (median 1.0). However, it is important to note that every STW is unique and that the
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16
17 297 deviations in the datasets observed at UK2 may be very different in another STW.
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21 299 Based on the linear emission model these deviations cannot be explained by varying flow alone.
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24 300 Indeed, a lower actual per capita load and/or a higher removal rate could explain the overestimation of
25
26 301 E1 and E2 and vice versa for EE2. At UK2, removal rates are reported to be higher than those assumed
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28
29 302 in the model for E1 and E2 (95 and 98% respectively) and lower for EE2 (32%)⁶⁶. When these
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31 303 measured removal values were input into the model, the deviation factor lowered to 0.2-9.4 (median
32
33 304 0.53) for E1, 0.1-3.8 (median 0.55) for E2, 0.7-6.1 (median 1.53) for EE2 and 0.5-3.0 (median 1.1) for
34
35
36 305 the EEQ. This switched the original overestimation of E1, E2 and the EEQ and the underestimation of
37
38 306 EE2, which suggests that the real removal rates are likely to be somewhere between the modeled and
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40
41 307 measured. The deviations between the modeled and the measured data continued to vary across
42
43 308 sampling points and are likely to be caused by variation in removal rates, which can cause 10 fold
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45 309 differences in day to day effluent concentrations⁶⁵. Nonetheless, with these removal rates incorporated,
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48 310 all modeled data was within the measured range, demonstrating that a simple calibration of model
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51 311 parameters with data specific to an STW can improve the model performance. In particular, this could
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53 312 impact risk assessment, since different removal rates will change the proportions of each estrogen in
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55 313 effluent and potentially impact the EEQ. This also implies that river models will be more accurate with
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57 314 up to date removal data, although due to the impact of dilution, modeled estrogen concentrations from a
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1 315 previous study that overestimated concentrations by up to 10 fold still predicted concentrations within
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3 316 the same risk category as measured data⁵¹.
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Predicted River Concentrations in the Present Day and Risk Assessment for Endocrine Disruption

in Fish

LF2000-WQX and Source Catchments were used in the UK and South Australia to identify potential hot spots of “at risk” areas for endocrine disruption in fish based on predicted concentrations of steroid estrogens in the present day. On the River Erewash, UK, in agreement with data from the Johnson and Williams model⁵¹ almost the entire river was categorized as “at risk” of endocrine disruption in wild fish (Figure 3, Figure S2), with an average EEQ of 2 (0-7) ng/L along the entire river. This resulted from the assumption of constant influx of steroid estrogens from the eight STWs along the river which maintained the EEQ above 1 ng/L. On the Onkaparinga River in South Australia, concentrations were also predicted to exceed the 1 ng/L EEQ PNEC (Figure 3, Figure S2) downstream of SA2. Around 9 km of the river was categorized as “at risk,” with concentrations decreasing with the distance downstream due to degradation and dilution from tributaries, eventually dropping below the PNEC upstream of the Mount Bold reservoir. An average EEQ of 3 (0.4-9) ng/L was predicted over these river stretches and individual steroid estrogen concentrations were comparable with those measured at five river sites in Queensland at effluent outfalls and 1 km downstream of STWs⁶⁰. They also compared with concentrations measured globally⁶⁷.

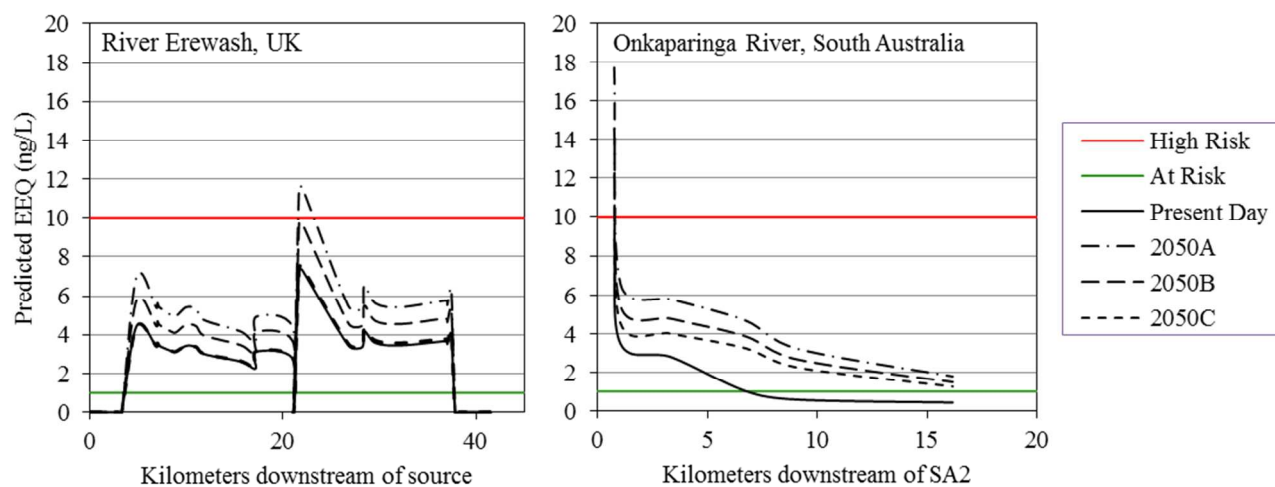
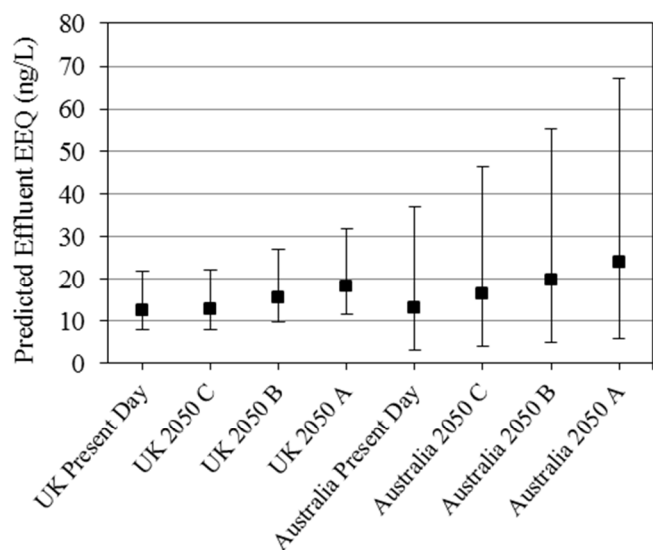


Figure 3. The average predicted EEQs (ng/L) for the present day compared with the three future population projections, high (2050A), principal (2050B) and low (2050C), with river flows reduced for medium range climate change scenarios. Risk levels are indicated.

Scenarios for Concentrations of Steroid Estrogens in 2050

Effluent concentrations: In both countries three population projections representative of 2050, including high (A), principal/medium (B) and low (C) projections, were used to determine how the change in human population size (Figure S3, Table S6) and composition (Table S4) affected modeled estrogen concentrations. Interestingly the population composition had a small impact on the per capita load. A small increase occurred under the high projection and a small decrease occurred under the principle and low projections (Figure S4, Table S5) as a result of changes in the proportions of high estrogen producing menstrual females and pregnant females relative to low estrogen producing menopausal females. Population growth had a much greater impact, resulting in an increase in the total estrogen load arriving at the STW (Figure S5) and an increase in their subsequent concentrations in effluents to be discharged into the environment (Figure 4). The exception to this was the UK projection C, where effluent concentrations reduced since the increase in population was not sufficient to compensate for the lower per capita load. The worst case scenario was observed with the high

353 population projections, where effluent concentrations almost doubled by 2050 under the Australian
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Figure 4. The predicted EEQs of effluents in the UK and South Australia under present day and future projections assuming no change in DWF at the STWs. Boxes represent the mean (squares) with error bars extending to the minimum and maximum concentrations from the four UK STWs and 12 Australian STWs.

River Concentrations: The river models were modified for medium range climate scenarios with reduced flow and used in conjunction with population projections and future estrogen loads to determine how river concentrations may change by 2050 (Figure 3). On the River Erewash, decreased dilution and increased estrogen input in effluent under projections A and B caused increases in the average EEQ on impacted stretches from 3.7 (2.3-7.4) ng/L to 5.9 (3.6-11.6) and 4.9 (3-9.7) ng/L respectively. In addition, two stretches became “high risk” areas in projection A (Table S8). However, in projection C the increase was smaller with an average EEQ on impacted stretches of 3.8 (2.3-7.5) ng/L due to the reduced input of steroid estrogens from the STWs. An increase in average EEQ was also predicted between the SA2 discharge and the Mount Bold reservoir on the Onkaparinga River under all population projections, from 2.9 (0.4-8.9) ng/L to 6.6 (1.8-18), 5.5 (1.5-15) and 4.6 (1.2-12) ng/L EEQ

1 371 for projections A, B and C respectively. Importantly, the length of river downstream of the STW
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3 372 considered “at risk” increased under all three projections to include the entire 16 km modeled stretch
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5 373 upstream of the reservoir, whilst in projections A and B the stretch immediately downstream of SA2
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7 374 became “high risk”. However, it is important to note that additional variables exist in the prediction of
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10 375 estrogen concentrations in the future. For example, measures to conserve water may further reduce
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12 376 dilution of estrogens arriving at STWs, whilst increasing anthropogenic control of river flow and the use
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14 377 of recycled wastewater could result in additional changes to their dilution in rivers. Furthermore, an
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17 378 increasing occurrence of extreme weather events could cause greater changes in flow which could have
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19 379 more dramatic implications for estrogen concentrations than our model suggests. Indeed, variation in
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22 380 flow and dilution may be a much greater driver than population change alone, causing increases or
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24 381 decreases in concentrations that may differ from our model, depending on water availability. Since a
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26 382 better understanding of the drivers that cause at risk areas has been called for²⁹, these scenarios may
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29 383 provide interesting subjects for more detailed assessment in the future.
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33 385 Mitigation to combat rising estrogen concentrations may be achieved with increased removal
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36 386 efficiency at STWs with improved uptake of modern treatment technologies, many of which are already
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38 387 used for treating drinking water and recycled wastewater. This has already been demonstrated in the
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41 388 UK^{24,44,66} and similar results have been found in Australia^{68,69}. Indeed, in Western Australia the
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43 389 induction of the estrogenic biomarker vitellogenin was found in male fish downstream of a secondary
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45 390 treated rural effluent but not downstream of tertiary treatment⁶⁸. However, a number of studies have
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48 391 also detected steroid estrogen concentrations which exceed the PNECs upstream of STWs,
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50 392 demonstrating the importance of considering multiple origins of environmental steroid estrogens^{3,60,68},
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52 393 such as agricultural runoff⁷⁰ as well as sewage effluent. In addition, other chemicals with the potential
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55 394 to cause feminizing effects in wildlife, such as the nonylphenol ethoxylates, which have been restricted
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57 395 under EU legislation, are still in use in Australia and have been detected in surface water⁶⁰.
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1 397 This study demonstrates the first use of predictive effluent and river modeling of steroid estrogens in
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3 398 South Australia as a tool for estimating concentrations and predicting the presence of “at risk” areas.
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5 399 The results suggest that effluents discharged in South Australia could cause concentrations of steroid
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7 400 estrogens in rivers to exceed the 1 ng/L EEQ PNEC, implying that there is a risk of endocrine disruptive
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10 401 effects occurring in wild fish. Evidence of feminization of non-native fish has already been observed in
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12 402 effluent contaminated areas^{68,71,72}, whilst native species have been shown to be susceptible to steroid
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14 403 estrogens under laboratory exposure^{73,74}. As a result, further investigation is warranted to determine
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17 404 how susceptible Australian species are to estrogens from all sources, particularly from effluents derived
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19 405 from different levels of sewage treatment, which will allow Australian PNECs to be derived that
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22 406 accurately reflect the risks and mitigation required to protect Australian biota. In the absence mitigation
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24 407 strategies we could anticipate an increase in estrogen concentrations in rivers in both the UK and
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26 408 Australia by 2050 as a result of the growing populations coupled with reductions in river flow through
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29 409 changing climate. Moreover the magnitude of this change may increase further with continued
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31 410 reduction in flow and population rise by 2100 and beyond. This suggests that endocrine disruption in
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33 411 wild fish may be a long-term management issue for which effective investment in preemptive mitigation
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36 412 today may pay off in the future.
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ASSOCIATED CONTENT

Supporting Information Available

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47 416 The supplementary data section includes: a simple worked example of effluent modeling at a South
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50 417 Australian STW; Tables of the parameters for each STW; half-lives for the steroid estrogens used in the
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52 418 Source Catchments model; cohort percentages from census data for the present day and future
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54 419 projections; per capita loads for the present day and future projections; fold change in population
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57 420 between 2011 and 2050; the measured and modeled data range of estrogens from UK and Australian
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59 421 STW effluents; risk categories of river stretches for the present day and future projections; Figures of
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1 422 the predicted effluent concentrations of the steroid estrogens in UK and Australian effluents; Location
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3 423 and heat maps showing risk categories of modeled stretches of the two rivers; population change; per
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5 424 capita load of estrogens and total estrogen load arriving at a STW up to 2050 for all population
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7 425 scenarios. This material is available free of charge via the Internet at <http://pubs.acs.org>.
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50 442 Water Ltd are on the basis that they are for the sole use in connection with “*An integrated approach to*
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53 443 *assess safety of treated wastewaters as environmental flows in the Australian riverine environment: The*
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55 444 *UK-Australia collaboration*” and are not to be used for any other purpose. Any views expressed are
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60 446 of the data remains with Severn Trent Water Ltd.

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ABBREVIATIONS

ABS, Australian Bureau of Statistics; ASP, Activated Sludge Process, DWF, Dry Weather Flow; E1, estrone; E2, 17 β -estradiol; EE2, 17 α -Ethinylestradiol; EEQ, E2 equivalent concentration; GAC, Granular Activated Carbon; HRT, Hormone Replacement Therapy; LC-MS/MS, Liquid Chromatography-tandem Mass Spectroscopy; LF2000-WQX, Low Flows 2000 Water Quality eXtention model; ONS, Office of National Statistics; PNEC, Predicted No Effect Concentration; STW, sewage treatment works, UKCP09, UK Climate Projections 2009.

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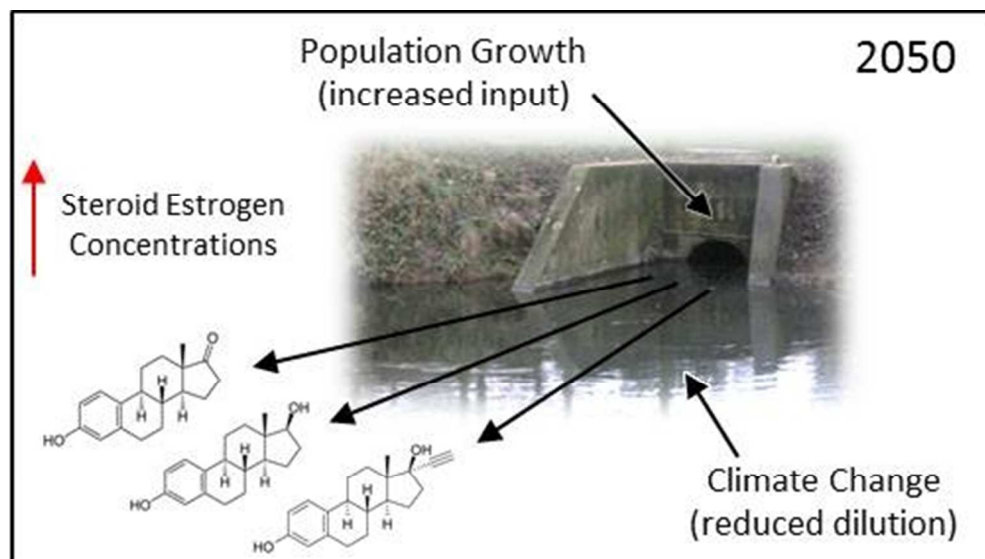
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