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# Parameterization of pharmaceutical emissions and removal rates for use in UK predictive exposure models: steroid estrogens as a case study

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| | |

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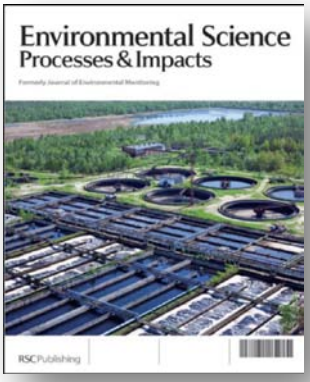


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Dear Professor Stephen Mudge,

I am writing to you in reference of Manuscript ID EM-ART-01-2014-000057
(Title: Developing a modelling approach to predict pharmaceutical discharges from UK sewage treatment works using steroid estrogens as a case study) which was originally submitted to Environmental Science: Processes and Impacts in January 2014.

We wish to thank yourself and the reviewers for providing such detailed feedback on our manuscript (reviewers comments received 23rd April 2014). As suggested, we have fully addressed the concerns of the reviewers and are therefore resubmitting the revised manuscript for publication in Environmental Science: Processes & Impacts. The manuscript is now entitled: Parameterization of pharmaceutical emissions and removal rates for use in UK predicted exposure models: steroid estrogens as a case study. We have detailed our responses to the reviewers' comments below.

Yours sincerely

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**Referee 1, Comment:** The paper is not written that well so in some places it is hard to determine what exactly the authors have done. I suggest the authors re-work the text to make the messages clearer.

It is difficult to address issues of poor writing without more explicit direction, especially when the second reviewer stated the paper was well written. Regardless, we have addressed a number of areas of text to ensure clarity of the message.

**Referee 1, Comment:** I would suggest the title be changed. The paper seems to be about parameterization of emission data rather than developing a new modelling approach. A more accurate title would be something like 'Paramaterization of emissions and removal rates for pharmaceuticals for use in exposure models'.

We particularly value this comment and the guidance given – the title has been modified.

**Referee 1, Comment:** Abstract, Line 2 – I don't think you can say the method is accurate

Addressed in manuscript.

**Referee 1, Comment:** Abstract, Line 8 – 'predictions' not 'predications'

Addressed in manuscript.

**Referee 1, Comment:** Page 1, Introduction, Line 6 – I think this sentence needs some supporting references. What evidence do the authors have that incorrect disposal is important?

Addressed in manuscript.

**Referee 1, Comment:** Page 2, Introduction, column 1, para 1, line 15 – would suggest 'at' rather than 'within'

Addressed in manuscript.

**Referee 1, Comment:** Page 2, Introduction, column 1, para 2 – I think this paragraph (which provides the rationale for the paper) is very misleading and shows a limited understanding of the existing modelling approaches. The models described can be applied to any catchment if the hydrology and STP data are available. The models are also not specific to estrogens and have been applied to other compounds (numerous papers on this exist). It is also possible to parameterise the LowFlows model for specific treatment works using local data on prescriptions and STP-specific removal rates. This part of the introduction should be re-written. The authors are trying to suggest that their method is novel but really it isn't that innovative.

Addressed in manuscript.

**Referee 1, Comment:** Page 2, Introduction, column 2, para 1 – The authors present this study as developing a new model. To me all they have done is use a good quality dataset to parameterise modelling concepts that have been around for some time. I think this section should be toned down.

We feel that this section does not over-state what we have done, nor its relevance to the wider field, as it is simply a generalised description of the modelling approach and its potential application. We believe this section is of value in differentiating our approach, from other approaches seen in the literature i.e. 'per-capita' approach. However, we do appreciate the reviewers comments, and have made some small changes to tone down this section and make it more apparent that we are improving parameterisation, and building upon established modelling concepts (as opposed to creating a new model from scratch) – addressed in manuscript.

**Referee 1, Comment:** Page 2 Model development and parameterisation – To me the authors have not developed a model but just described an approach to parameterise models. I suggest that this whole section is re-titled and reworded.

See above and changes to manuscript

**Referee 1, Comment:** Page 3, column 1, para 1 – I would like to see some background evidence for supporting some of the assumptions, particularly that tourism isn't an issue. Couldn't tourists 'import' pharmaceuticals into the catchment and couldn't this skew the predictions?

The reviewer is absolutely correct that tourism could skew these predictions. However, as detailed, the data used is from outside of the main holiday season and thus influence of tourism is likely to be limited – this has been evidenced in the manuscript. Unfortunately it is not possible to evidence the other assumptions – namely as regards registration of residents, and within catchment excretion. The highly specific information needed to evidence such behaviour in a specific location is not

available – and it is therefore for this reason that the assumptions behind this modelling approach are clearly stated.

**Referee 1, Comment:** Page 5, Figure 2 and accompanying text – The authors state that the results are comparable with measured data but I would like to see some justification for this. From the graph, it appears that the modelling under-predicts E1 and over-predicts EE2. The graph also indicates that the Williams and Johnson estimates for E1 are better than in the current study. The Williams and Johnson data are also not too far off so what is all the fuss about.

The key point is that the relative proportions of E1, E2, EE2 in our predictions, better reflect the measured data than the previous model does. We have also emphasised the accuracy of our EE2 predictions – something very important in relation to the proposed EQS. Hence we have attributed this to use of local prescription data, rather than per capita i.e. therefore supporting our approach.

**Referee 1, Comment:** Page 6, Table 2 – I am intrigued as to why the authors didn't focus on a catchment where monitoring data were available. The paper would have been much stronger if it had been applied to a catchment that had some real data. The authors should consider doing this.

This is a valid point, and we agree that section of a catchment where monitoring data were available would have benefited this study. However, there are a number of reasons why monitoring data were not available/used

1) Little water quality data is available for estrogens in UK river waters owing to the low limits of detection needed and cost of analysis meaning that there is little data available anyway.
2) The study location was selected due to its interesting demographics and rural location – as many models (and monitoring studies) are applied to high population density urban areas, we wished to tackle a generally unconsidered location.
3) The CIP data provides a comprehensive, quality controlled dataset with which to calibrate the model.
4) Additionally very few, if any, UK catchments have undergone in-depth routine monitoring of STW influents and effluents i.e. at every STW in a given catchment at an appropriate regularity to overcome issues around variability in removal performance; and therefore there is limited availability of data for comparison. Therefore (as discussed in the abstract) the general lack of measured sewage works data necessitates the development of an approach to allow comparison of local predictions with average national measurements – hence our use of data from the recent UK wide STW survey.

Words to this effect have been provided in the text.

**Referee 2, Comments:** The authors suggest a new approach to estimating pharmaceutical concentrations entering and being discharged from STW

The paper is well written and the results given by the authors are satisfactory. The authors did a good job of motivating the work.

The paper can be published in the Environmental Science Proceses & Impacts

**Referee 2, Comment – minor points:** Can authors put an error bar for their prediction at figure 1 ? ( i mean 95% conf .int)

We appreciate this comment; however inclusion of 95% confidence intervals for predicted influent loads is not possible without incorporating estimations of error within prescription data sets,

excretion rates, in sewer removal rates, etc. As these types of data are themselves of limited availability, there is no appropriate means to provide sensible estimates of error.

**Referee 2, Comment – minor points:** Johnson and Williams published a model for the prediction of estrogenic compounds in influent and effluent The authors have compared the result of their model with the classic work of Johnson & Williams in terms of influent load at Fig 2. However it would be much more interesting if they had a third column in table 2 with another model ( like J &W --> simplistic population group , or even their model with non update (old) prescription database) in order to demonstrate the superiority of their model even only on the catchment.

We understand why referee 2 has suggested this addition to table 2, to allow further comparison. However, the purpose of this figure is simply to illustrate improvements to the J&W model by taking account of local demographics / data. Therefore comparison of the outputs of this modelling approach with further model outputs (which also do not take account of localised data) is of limited value.

Increasing concern regarding the presence and effects of pharmaceuticals in water has led to changes in European legislation; with a number of pharmaceuticals named on a watch list as part of the Water Framework Directive. The potentially huge cost to the water industry to meet new standards requires accurate predictions of likely concentrations at a catchment scale, in order to target resources. This paper describes an environmental management tool to more accurately predict pharmaceutical loadings to rivers at a catchment scale, using a combination of recently available prescription data, metabolism and fate information and recent monitoring data to develop and calibrate a model to allow all stakeholders to identify sewage treatment works which pose the greatest risk to receiving waters.

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ARTICLE

# Parameterization of pharmaceutical emissions and removal rates for use in UK predictive exposure models: steroid estrogens as a case study

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Newly available prescription data has been used along with census data to develop a localised method for predicting pharmaceutical concentrations in sewage influent and effluent for England, and applied to a case study: the steroid estrogens estrone, 17β-estradiol, and 17α-ethinylestradiol in a selected catchment. The prescription data allows calculation of the mass consumed of synthetic estrogens, while use of highly localised census data improves predictions of naturally excreted estrogens by accounting for regional variations in population demographics. This serves two key purposes; to increase the accuracy of predictions in general, and to call attention to the need for more accurate predictions at a localised and/or catchment level, especially in light of newly proposed regulatory measures which may in future require removal of steroid estrogens by sewage treatment facilities. In addition, the general lack of measured sewage works data necessitated the development of a novel approach which allowed comparison of localised predictions to average national measurements of influent and effluent. Overall in the case study catchment, estrogen predictions obtained using the model described herein were within 95% confidence intervals of measured values drawn from across the UK, with large improvements to predictions of EE2 being made compared with previous predictive methods.

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**Keywords**: steroid estrogens, estradiol, estrone, ethinylestradiol, sewage treatment works, predicted environmental concentration

## Introduction

Human pharmaceuticals are now ubiquitous in the environment and are found as far afield as the Arctic [1]. The vast majority of human medicines enter the aquatic environment in sewage treatment works (STW) effluents, derived from excretion in urine or faeces or via the incorrect disposal of unused medications into household sewerage. Due to the general resistance of patients to taking medication [2], and their tendency to under-medicate [3], with typically 50% of prescribed pharmaceuticals not being administered [4], large quantities of unused pharmaceuticals can be generated. In the UK it is estimated that 85% of these unused medications are disposed of improperly (63% to landfill; 12% domestic waste water stream), and only 22% are returned to pharmacies as recommended [5]. Less significant loadings may be derived from combined sewer overflows, and contributions from landfill leachates are likely to be limited due to advancements in liners [6, 7]. Transport to the environment of these bioactive compounds, which can have deleterious effects upon biota [8,

9], is set to continue and is likely to be further exacerbated over the coming years due to factors such as an aging and increasingly obese population influencing drug usage [10-12]. Furthermore, the occurrence of drugs in drinking water has been an additional cause for concern [13, 14].

In Europe these concerns have led to legislative reform, with selected pharmaceuticals (Diclofenac, 17-α-ethinylestradiol [EE2], 17-β-estradiol [E2]) now included in the Water Framework Directive [15, 16] as substances on a 'watch list' which requires Member States to undertake monitoring programmes and consider reduction in discharges to the environment where appropriate. Initial environmental quality standards (EQS) were proposed as 100, 0.035 and 0.4 ng $L^{-1}$ as annual averages for Diclofenac, EE2 and E2 respectively. Treatment technologies are available to reduce pharmaceutical concentrations in wastewaters, however these raise financial and sustainability concerns [17]. A recent impact assessment estimated compliance costs for the UK water industry to meet proposed EQS for EE2 as over £20 billion [18]. It will therefore be necessary and pertinent to focus limited financial resources at 'pollution hotspots'. One means by which to identify such hotspots is predictive modelling at a small geographical scale. This requires two key numbers to be derived; the concentration being discharged into receiving waters from STW and based on available dilutions [19, 20], predicted environmental concentrations which can be compared against relevant environmental quality standards. This paper sets out an approach for achieving the first objective by improving parameterisation of pharmaceutical usage, and subsequent quantities discharged from STWs. Other developmental work in the UK on modelling water quality [21] has the ability to take discharged loads and predict water quality based on predicted and measured flows.

Catchment models have been developed to predict concentrations of chemicals such as pharmaceuticals in the aquatic environment (e.g. 'Geography Referenced Regional Exposure Assessment Tool for European Rivers' (GREAT-ER) and LowFlow2000-WQX [22, 23] for estrogens). They have been used to assess inputs from specific sources such as hospitals [24] or to predict pharmaceuticals such as estrogens for specific catchments using estimates of excretion rates based on assumptions regarding per capita use of estrogenic medications and demographics [25]. A model has been recently developed (SAGIS) that combines derived loads of contaminants discharged to surface waters from point and diffuse sources which is combined with a water quality model (SIMCAT) via a Geographic Information System (GIS) system to allow accurate predictions of a variety of chemicals in UK water bodies [21]. Generating accurate load data from the main sources such as STWs will generate load inputs for these types of models now being used by the Environment Agency and water industry for planning purposes. Such models rely entirely on the accuracy of the input data and so improvements in estimations of input loads strengthens the decision making process.

To improve estimates of pharmaceutical loads it is essential to take account of the factors that influence endogenous production, exogenous usage, and therefore excretion of steroidal estrogens on a local scale. These include, but are not limited to, the population demographics, prescribing habits of local physicians, pregnancy rates and cultural views on contraception. Incorporation of these influencing factors into a model (as far as is possible) will therefore increase model validity at finer spatial resolution and improve reliability of the outputs.

This research describes the development of a modelling approach that takes account of actual prescription data within specific catchments, alongside local demographic data, resulting in more refined predictions of these populations' contribution to the environmental burden by improving parameterisation. In the following sections a case study, using estrogenic compounds and a selected catchment in England, is presented as an example of the model's application. This case study uses census data [26] and prescription data [27, 28] encompassing all formulations of hormone replacement therapy including both E2 and conjugated estrogens (CE), and contraceptive pills containing EE2, to predict estrogen influent and effluent concentrations for a specific catchment, by building upon established modelling concepts. A generic form of this approach, which may be applied to any river catchment, any STW, and any medication, for which prescription data is available, is provided in the electronic supporting information. The production of a modelling approach such as this, which is novel in its use of prescription data at highly localised scales (i.e. down to individual STW) and can be applied to any human pharmaceutical, is key in the development of strategies for the deployment of interventional approaches, including technological, to reduce pharmaceutical pollution; thus allowing finite resources to be targeted.

## Modelling Approach (Parameterisation) and Calibration

### River Catchment Identification

A specific catchment was selected as a case study based on factors which included varying demographics and low river flows available for effluent to be diluted into (<10), thus indicating a potential risk of elevated levels of pharmaceuticals occurring in the aquatic environment downstream of the STWs [29]. Census data was obtained [26] to estimate population demographics and utilised for calculation of naturally excreted estrogens in the catchment. Whilst census statistical area boundaries do not generally conform to river catchments, Census Output Areas (COAs) are very small geographical units with a mean population around 300. A 'best-fit' selection of COAs to the catchment was made by overlaying the two sets of boundaries in GIS, resulting in a selection of 79 COAs. GIS analyses were conducted using ArcGIS 10 (ESRI, Redlands, CA) to derive population characteristics (Table 1).

***Table 1:*** Estimated Population Statistics for the Selected River Catchment, 2001 Census

| | |
|---|---|
| Total Population | 22,199 |
| Female Population Age 13-49 | 4,737 |
| Female Population Age 50+ | 5,317 |
| Total Male Population | 10,598 |
| Total Female Population | 11,601 |

Data source: UK Census 2001[26]

### Prescribed Synthetic Estrogens in the Selected Catchment

**Isolation of Local Prescription Data** STW discharge locations were plotted (GIS) and overlaid with the location of all General Practitioner (GP) surgeries in the region based upon postcodes; three GP surgeries were identified as serving the selected catchment. It was assumed that all residents were registered with GPs within this catchment and that all medication prescribed by these surgeries was excreted within the catchment. Any commuting to work was considered to have a neutral impact (i.e. as many people may travel to work in the catchment as travel out of the catchment to their place of work). Parts of the catchment are located near the coast, and so some seasonal influences could be expected. However, data obtained from England's National Health Service (NHS) [27] detailed the number of monthly prescriptions written by each GP surgery between September of 2011 and January of 2012 and therefore out of the peak holiday season [30]. Although it would have been beneficial to work with data on a seasonal or yearly average basis, the NHS only began to release data in this format in September 2011, and this case study acts as an illustrative example. The NHS details this prescribing data in the form of British National Formulary (BNF) codes, which is a prescription guide routinely used by pharmacists and GPs in the UK. Details regarding the BNF codes are provided in the SI (S1).

The prescribing data for BNF codes that included estradiol (E2), conjugated estrogens (CE) and ethinyl estradiol (EE2) were isolated from the dataset for analysis. The average number of prescriptions per month for each relevant BNF code and each of the three GP surgeries within the selected river catchment was determined and summed; providing the total average number of monthly prescriptions written in the selected catchment for each relevant BNF code. Further prescription details can be found in the SI (Table S1).

**Conversion of number of prescriptions to prescribed mass** Converting the number of prescriptions into a mass of prescribed estrogens in the selected catchment required the use of another data set. The NHS quarterly prescription cost analysis data for October-December 2011 [28], details the number of prescriptions for each individual formulation within all BNF codes, for the whole UK; datasets that are tied to GP locations only detail prescribing data down to BNF code i.e. not formulation level. This was used to ascertain typical prescribing practices in the UK, which were used with formulation-specific dosing information obtained from the BNF and online sources [31] to convert the localised prescription data for relevant BNF codes, to a mass of estrogens prescribed, while accounting for different formulations. The net result of these steps can be applied to any human pharmaceutical for which prescription data exists. An example of the application of this approach is provided in the SI (Tables S2 and S3).

**Synthetic and Natural Steroid Hormone Excretion**

The model developed by Johnson and Williams outlines a framework that identifies 5 major population groups which contribute to the environmental burden of estrogens [25]: pregnant females; menstrual females; menopausal females; menopausal females using hormone replacement therapy (HRT); and males. The method described herein and further detailed in SI (S1-S5) for calculating the use of synthetic estrogens in a target river catchment can be used to improve the accuracy in determining the contribution to environmental estrogen load from the population group "menopausal on HRT" and menstrual females using estrogen based contraceptives. The following sections summarize the variables considered when predicting excretion of synthetic and natural estrogens for all population groups. It should be noted that data used within models regarding excretion of pharmaceuticals and their metabolites can have a significant impact upon model outputs. For many pharmaceuticals there is a paucity of data regarding excretion rates, and many of the pharmacokinetic studies which generate these figures utilise radio-labelled compounds, and therefore do not account for metabolites. Additionally a number of other factors specific to the individual using the medicine, such as age and disease status, can also influence excretion rates. In the case of steroid estrogens the opposite scenario occurs with a wide range of highly variable and specific studies available from which to draw excretion data. An approach to take account of this variable data to establish an appropriate excretion rates for estrogenic compounds (as used herein) is described by A.C. Johnson and R.J. Williams [25].

**Excretion of Synthetic E2** The source of synthetic E1 and E2 is the excretion of metabolites from HRT. The method described above was used to calculate the ingestion of HRT medication in the selected catchment. The prescription data also allows for HRT usage to be broken down into estradiol (E2) and conjugated estrogen (CE) use, which provides a further increase in the accuracy of the model.

The total daily ingestion of synthetic E2 in the selected river catchment was calculated, and using literature data it was assumed that 2.8% of ingested E2 [25] and 1.5% of ingested CE [32] are excreted in a potentially releasable form; including free E2 and the E2 glucuronide-conjugate (Figure 1). Summing the two provided the total E2 load discharged to sewer derived from estradiol and conjugated estrogen HRT.

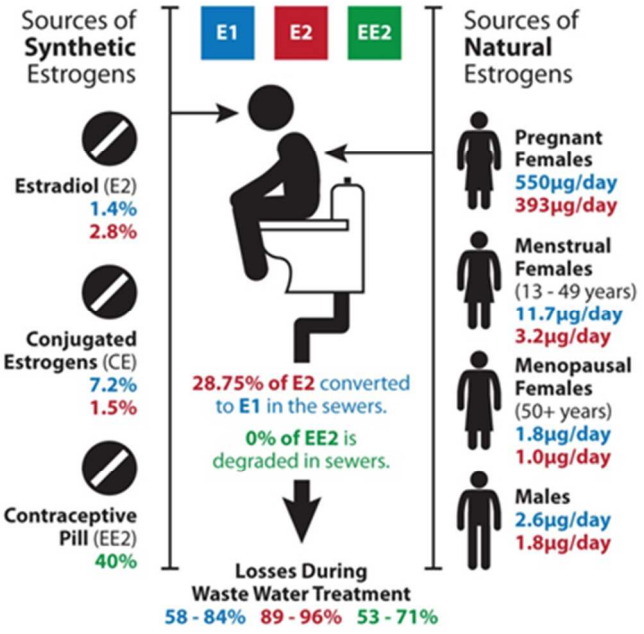


***Figure 1:*** Summary schematic of synthetic and natural estrogen inputs, and removal during transport and treatment. (Note: Presented percentage removals during sewer transport and STW processing are refined removal rates - see S9, Table S5).

**Synthetic E1 Excretion** Estradiol and conjugated estrogen hormone replacement therapies are also a source of E1

excretion and can therefore be calculated from women on HRT in a similar manner to synthetic E2 excretion. Using localised prescription data along with previously reported excretion rates, it is assumed that 1.4% of ingested E2 [25], and 7.2% of ingested CE [32] is excreted as potentially releasable E1 (Figure 1). Adding these loads together provides the contribution of E1 excretion from estradiol HRT and CE therapy to the sewerage system.

**Excretion of Natural E1 and E2**

**Pregnant Females** Although pregnant women account for only a small proportion of the total populace, their per capita contribution to estrogen load can be upwards of 100 times greater than that of a menstruating, non-pregnant female [25]. Accurate demographic and birth rate data are therefore essential when predicting loads to sewer. There are many factors that influence the rate of pregnancy, which may include culture, socio-economic status, religion, views on contraception, and population demographics. By using localised census data the confounding effect of these variables, on regional changes in pregnancy rates, could be accounted for.

Fertility rates are available annually for the UK [33] and are broken down into districts which include any given catchment of interest [34]. In 2010 the UK as a whole had a birth rate per capita of 1.3% and the selected district had a birth rate per capita of 0.88%. During the same period the Waltham Forest district outside of London had a rate of 2.1%. This disparity highlights the importance of using local data to account for societal and demographic differences in pregnancy rates. It should be noted that longitudinal studies demonstrate temporal variation in birth rates [35], as is exemplified by the UK baby boom of the 1960s, which can be due to a range of factors which can feature as a component of socio-economic status [36], such as social norms and financial incentives; thus it is necessary to update assumptions within models to take account of current trends. Since districts encompass areas larger than catchments, relevant district birth rates were broken down by age category and were used alongside corresponding census data for the identified catchment in order to improve accuracy. This derived a per capita rate of 1.0% live births for the selected catchment, which was used in further calculations. An additional factor (0.76) must then be applied to take account of multiple gestations and average gestational period of 40 weeks, i.e. on any given day there are approximately 23% less pregnant women than the yearly number of pregnancies [34]. It should be noted that this approach measures live births, which include all babies that are born with signs of life. Because of the incidence of multiple gestations, there are inherently more live births than maternities in a given population. In the UK, this equates to approximately a 1% difference. Having ascertained the number of pregnant women in the selected catchment, it was possible to determine this population group's contribution to excreted potentially releasable E1 and E2 (free estrogens and glucuronated conjugates [25]; Figure 1).

**Menstrual Females** Women of childbearing age that are not pregnant are the second most important contributors to naturally excreted estrogens. To accurately assign estrogen excretion from this population, the age range of females menstruating has to be defined. Previously the average for menarche was placed at 13.5 years [37]. A more recent study showed that the average age of menarche for girls in the US was 12.5 years, while also demonstrating an association between earlier age at menarche and factors such as increasing body mass index and black racial background [38]. On the other end of the spectrum, multiple reports support an age of 51 as the natural age of menopause, with factors such as education, marital status, education, race and smoking status being implicated as independent predictors of either an increase or decrease in age at menopause [39, 40]. This is clear evidence that changing societal factors such as demographics and socio-economic conditions are an important to consider with respect to the accuracy of model outputs. Before the onset of menopause, there is also a period of "perimenopause", which is characterized by irregularity of menstrual periods that begins on average at the age of 47.5 [40]. Thus for the purposes of this model, women between and including the ages of 13 and 49 and who are not pregnant were considered as part of the "menstrual female" population group. Applying this information to census information allowed excretion amount to be calculated (Figure 1).

**Menopausal Females** Based upon the discussion of menstrual females, it follows that the population group menopausal females is defined as women of age 50 plus. However, when considering only the natural estrogen contribution from this population group, menopausal females using HRT must be excluded as their contribution has already been accounted for. Thus, this group is more accurately portrayed as "non-medicated menopausal females".

In order to estimate the percentage of women in a population using HRT, recent trends in HRT use must be accounted for. In 1994, it was estimated that 22% of postmenopausal females were using HRT pills; the statistic used within the Johnson and Williams (2004) model. Multiple studies have since shown that the use of HRT has decreased by an estimated 40-50%, due to concerns over associations with cancer and heart disease [41-43]. Based on a conservative estimate of a 40% reduction, it can be estimated that only 13.2% of postmenopausal women currently use HRT. It should be noted that over 45% of the female population of the selected catchment was considered menopausal, compared with a UK average of 35% (reflecting the region's attraction to retirees). It can therefore be expected that the synthetic estrogen load is increased owing to greater HRT use, whilst natural estrogen load would be lower because estrogen excretion among non-medicated menopausal females is considerably lower than that of menstrual females (Figure 1).

**Males** Localised census data for the selected catchment allowed for more precise estimates of the male contribution to estrogen excretion (47.7% of the population) residing in the catchment. The excretion rate of potentially releasable estrogens for males has been reported elsewhere [25] (Figure 1); allowing calculation of E1 and E2 excreted by males within the catchment.

**EE2 Excretion** Since the contraceptive pill is the only source of EE2 excretion, targeted prescription data is an exceptionally valuable tool for estimating EE2 in the environment. A load to sewer can be calculated on the basis of reported excretion of potentially releasable EE2 of 40% of the consumed mass [25] (Figure 1).

**Transformations and losses during sewer transport**

**In-sewer transformations: previous studies** Once within the sewerage system the concentration of pharmaceuticals is

 

amended by a complex set of physico-chemical and biological processes which result in de-conjugation of steroid sulphates and glucuronides, partitioning between solids and aqueous phases, and degradation of E2 to E1. Many of these effects are themselves heavily influenced by a variable range of factors including residence time, degree of oxygenation, suspended solids levels and bacterial assemblages present. The dynamics of sewerage systems have therefore influenced previously reported observations of steroid fate which show wide variance depending on ambient conditions. Previous studies have estimated and measured typical retention times for most sewer systems to be between 2 and 6 hours; this is considered too short and of insufficient biodegradation potential to significantly impact on E1 and EE2 concentrations [25, 44, 45]. However, it is widely accepted that E2 will degrade to some extent under these timescales and conditions to E1. Values vary between an estimated 50% degradation to E1 [25] to a between only 6 and 8% based on measured data [45]. These values are largely controlled by factors such as temperature, de-conjugation rates and suspended solids levels.

**In-sewer transformations: this study** The case study catchment and its attendant STWs were selected on the basis of available dilution within the watershed, identifying the catchment as a potential 'hotspot' for pharmaceuticals entering surface waters. This provided the opportunity to use it as a case study for the application of the model. Like most STW in the UK, there were no available routinely measured steroid data for crude sewage or effluents in the case study STW. Time and budget precluded sampling and determination of steroid estrogens in STW present within the case study catchment and therefore direct comparison between measured estrogen concentrations and concentrations modelled for the select catchment could not be performed. However, the application of English demographic data to the model (details provided in Supporting Information, page S6 and Table S3) enabled comparison with measured data from a recent detailed UK survey by generating predicted excretion data for England as a whole; thereby allowing optimisation of removal rates. Modification of model inputs to allow predictions for England are detailed in the supporting information (Page S6 and Table S3).
The UK water industry has recently undertaken an extensive research programme to determine priority chemicals, including estrogens entering and being discharged from 25 STWs [46] (Figure 2). This dataset was generated under carefully controlled sampling and analysis conditions, with thorough quality assurance applied throughout. This has generated a valuable dataset with statistically rigorous summary statistics regarding concentration ranges and removal rates with which to compare the model outputs.

Although the total E1 + E2 influent predicted for England by the model described herein (~16 µg capita$^{-1}$ day$^{-1}$) is less than the measured E1 + E2 influent (~22 µg capita$^{-1}$ day$^{-1}$), it was possible to utilise measured data to refine the value of the in-sewer degradation rate of E2 to E1. When a 50% in-sewer degradation of E2 to E1 is assumed, the result is a predicted ratio of E1:E2 that differs considerably from measured values. Reduction of the degradation rate within the sewerage system will decrease E1 influent predictions, whilst increasing E2 predictions, which allows for the ratio of the compounds in predicted data to calibrate with the measured data. In measured influent an E1:E2 ratio of 2.72 is observed, an identical E1:E2 ratio is achieved in the model with a 28.8% transformation rate; thus providing a balance between observed and predicted E1 and E2 concentrations entering STWs. Details of this ratio-approach for the case study are detail in the SI (Pages S7-8; Table S4).

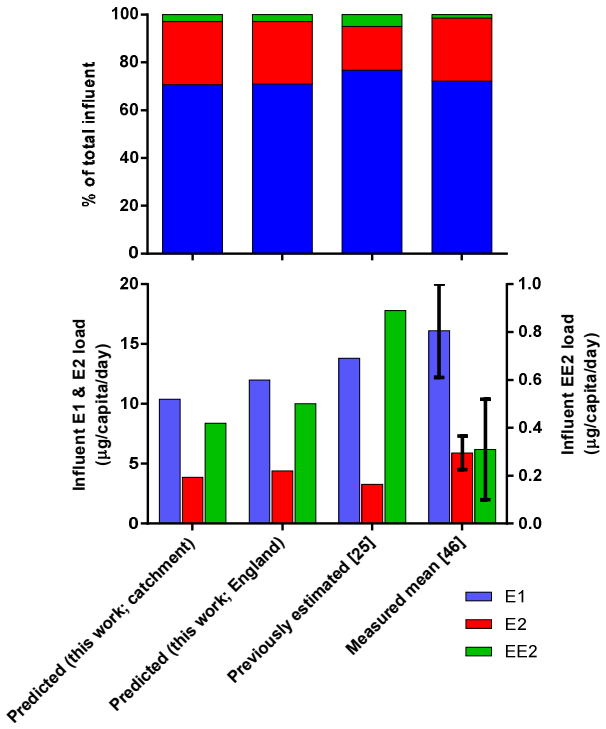


***Figure 2:*** Comparison of predictions of influent steroid loads between this study, previous predictions and measured data drawn from across the UK. Error bars represent ± 1 standard deviation. Predictions for this work (catchment and England) were performed with sewer removal rate of 28.75% (E2 to E1 transformation).

The data in Figure 2 above and Table S4 of the SI shows the outcome of the calibration exercise and clearly illustrate that predicted influent quantities of the three compounds were proportionally comparable with those measured across a number of UK STWs. Due to the ratio calibration approach used this is hardly surprising for E1 and E2, however EE2 influent predictions were not adjusted (i.e. assumed no in-sewer losses) and despite this the relative proportions of all 3 compounds when taken together align well with measured data versus previous model outputs. Although there are variations between predicted influent concentrations for the catchment and England they are not significant and can be explained via differences in demographics (detailed below). For other pharmaceuticals much greater variation in local versus national estimates may be expected as drug use may be more polarised. For example use of beta blockers and statins in aging populations in retirement biased locations, elevated antidepressant use in socially deprived areas and antibiotics use on a seasonal basis or in the event of an outbreak of an infectious disease. It is therefore important to consider the benefit of locally derived estimates across the range of pharmaceutical use, not just estrogens.

Predicted E1 influent loads for England (10.4 μg capita$^{-1}$ day$^{-1}$) were within two standard deviations of the measured mean (mean of 10.4 with ± 2σ = 8.3 to 24 μg capita$^{-1}$ day$^{-1}$). The observed variation may, in part, reflect the demographics of the catchment. Pregnant women dominate the amount of E1 and E2 excreted and so any small variations in catchment demographics could impact significantly upon predicted influent loads to the STWs. The equivalent E1 predictions for England (12.0 μg capita$^{-1}$ day$^{-1}$) are also less than measured values, and could indicate that an alternative source of E1 has not been accounted for in the model which could include prepubertal children [47, 48] and individuals not registered on the census (including holidaymakers). Although animals such as cattle, sheep, pigs, poultry and domestic animals naturally excrete E1 and E2 [49, 50]; their contribution to the sewerage system would not be expected to be significant, with the exception of possible veterinarian discharges.

A similar situation exists for E2 where estimates from this research generate STW influent loads of 3.9 μg capita$^{-1}$ day$^{-1}$ for the selected catchment, and 4.4 for England as a whole; similar to the value of 3.3 predicted previously [25], but less than that measured in the UK survey of 5.9 μg capita$^{-1}$ day$^{-1}$. The data in the model shows the significance of regional variations in demographics associated with proportion of pregnant females and age variations.

Predicted influent loads of EE2 for England were 0.5 μg capita$^{-1}$ day$^{-1}$ compared with a measured average value of 0.31 μg capita$^{-1}$ day$^{-1}$. However, this is closer to the measured data than achieved in a previous model (0.89 μg capita$^{-1}$ day$^{-1}$), and is also within +1 standard deviation of observed values. Variations may be owing to the assumption that all prescribed medication was consumed, which is not necessarily the case [51] which would lead to over estimation of actual loads. In consideration of this influencing factor, it would also be logical to argue that E1 and E2 influent concentrations should also be modified to reflect HRT compliance rates. However, drug adherence in HRT users is high (98.9% compliance [52]) and so would have a limited impact on excretion and influent predictions. Other physico-chemical characteristics of EE2 such as its higher log Kow [50] may also mean a degree of partitioning to biofilms present in the sewerage system thereby reducing levels at the STW. The different predictions of EE2 effluent concentrations seen in a previous study compared with measured and modelled data presented here (Figure 2) may be explained by assumptions used for estimating populations of women using the contraceptive pill. From 2006-2010, the Center for Disease Control and Prevention reported that use of oral contraceptives was 17% of *women aged 15-44* in the US [53]. As of 2011, 39.8% of the total UK female population is between 15 and 44 [34]. Assuming that use of the pill in England does not differ from the US, only 6.8% of all females in the UK (i.e. regardless of age) are using the contraceptive pill. Previous estimates also cited the same CDC source, but used a figure of 17% of *all women* using the pill [25]. The difference between 17% and 6.8% is approximately 60%, remarkably similar to the 56% difference in EE2 influent predicted by both methods.

**Transformations and losses during sewage treatment**

From a risk assessment point of view the wide ranging removal levels observed during different types of STW processes generate a large degree of uncertainty, which needs to be accounted for when assessing likely effluent concentrations. This uncertainty can be quantified by substituting different removal rates and comparing predicted effluent concentrations with those measured across UK STWs. STW removal rates could be modified to ensure that predicted and measured effluent values match, however this would not take account of the differential influent concentrations seen, nor variability in the relative proportions of the three compounds in predicted influent. Therefore a ratio-approach similar to that used during sewer transformation calculations was used to refine STW removal rates for use within the model, and considered removal rates across all treatment types and under specific processes. This exercise is detailed in the supporting information (Page S9, Table S5) and resulted in refined removal rate ranges of 58-84, 89-96 and 53-71% for E1, E2 and EE2 respectively.

The variability in the measured effluent levels (Table 2) is significantly higher than that of the influent loads, reflecting the variations in removal rates within the STW (S8, Figure S1) leading to a wider range of observed effluent concentrations (Table 2). This demonstrates that there is still a significant amount of research required to identify (and eventually model) specific mechanisms within STW which control the removal processes. Nevertheless, accepting the inevitable uncertainty, predicted ranges of effluent concentrations (for England and the selected catchment) and measured effluent concentrations (expressed as 95 percentile confidence intervals around the means) overlap for all steroids. Predictions for the catchment are different from measured data due to population differences; highlighting the importance of accounting for local demographics in such risk exercises.

***Table 2:*** Summary of predicted data for select catchment and England, using refined removal rates

| | Selected Catchment | | | England | | |
|---|---|---|---|---|---|---|
| | **E1** | **E2** | **EE2** | **E1** | **E2** | **EE2** |
| Load to sewer per capita (μg d$^{-1}$) | 8.82 | 5.44 | 0.42 | 10.22 | 6.19 | 0.50 |
| Catchment population | 22199 | 22199 | 22199 | 49138832 | 49138832 | 49138832 |
| Load to Sewer (mg d$^{-1}$) | 196 | 121 | 9.4 | 502051 | 303925 | 24587 |
| % loss in sewer | +29% (from E2) | -29% (to E1) | 0 | +29% (from E2) | -29% (to E1) | 0 |
| Influent load (mg d$^{-1}$) | 230.5 | 86.0 | 9.4 | 589429 | 216546 | 24587 |
| Influent flow to STW (m$^3$ d$^{-1}$) | 5550 | 5550 | 5550 | 12284708 | 12284708 | 12284708 |
| Predicted influent (ng L$^{-1}$) | 41.5 | 15.5 | 1.7 | 48.0 | 17.6 | 2.0 |
| Range of removal during treatment (%) | 58 – 84 | 89 - 96 | 53 - 71 | 58 – 84 | 89 - 96 | 53 - 71 |
| Predicted range* for effluent (ng L$^{-1}$) | 6.6 - 17.5 | 0.6 - 1.8 | 0.5 - 0.8 | 7.7 - 20.2 | 0.7 - 2.0 | 0.6 - 0.9 |
| Measured range* for effluent (ng L$^{-1}$) | | | | 10.9 – 24.7 | 1.3– 3.9 | 0.33– 0.78 |

* 95% confidence interval around the mean

## Implications

With predicted concentrations generated, it was possible to assess potential compliance with water quality standards. Currently in the EU pharmaceuticals are not included in water quality assessments. However, predicted no effect concentrations have been derived in the UK [54], and include a provisional value of between 3 and 5 ng $L^{-1}$ for E1. At an EU level provisional EQS have been derived for E2 and EE2 (0.4 and 0.035 ng $L^{-1}$ respectively) but have not been currently implemented in lieu of additional data being sought [15].

Comparing ranges for predicted effluent quality, for the catchment and England, with these values demonstrates that for E1 a dilution of 2.2 to 5.8, and 2.6 to 6.7 respectively, would be required to ensure compliance with the downstream EQS assuming there is no E1 present upstream of the effluent discharge. For E2 a similar situation arises with dilution of between 1.4 and 4.4 for the catchment, and 1.6 and 5.0 for England required. For EE2, however, as the EQS is so low, then dilutions of 14 to 23, and 17 to 27, for the catchment and England respectively would be necessary. Given that the selected case study catchment was chosen owing to its low available dilution (<10) the risk assessment suggests that downstream concentrations of EE2 may exceed the derived EQS. Recent reported data [29] suggests that between 1 and 5% of STW in the UK (depending on STW removal rate) would not have sufficient dilution to guarantee compliance with the EE2 EQS after mixing of effluent with receiving waters, leading to an estimate of £26 billion [16] in additional treatment to reduce effluent concentrations.

In 2004 Johnson and Williams published a model for the prediction of estrogenic compounds in influent and effluent. The simplistic population group approach used for predicting excretion of these compounds requires a minimum of data and hence has been a popular approach used in a number of different models. The modelling approach described herein updates many of the parameters used to correspond with the socio-economic and cultural changes seen since its publication. Significantly more accurate estimates of EE2 achieved using the modelling approach described herein, demonstrate the importance of using available up-to-date information and databases (in this case prescriptions in particular), in order to update and refine models which can be used for screening purposes to assess possible compliance with new legislation. Additionally the provision of new prescription data sets within England, have allowed predictions for the excretion of synthetically derived compounds to be more accurately incorporated (as evidenced in particular by the improved EE2 predictions generated herein), for specific geographical locations. Furthermore, the approach for calculating usage of synthetic compounds can be used in isolation, to predict excretion rates for pharmaceuticals which do not have a natural source. The availability of highly localised census data has also allowed more refined predictions of natural excretion of estrogen compounds, and highlights the importance of performing these types of risk assessment at small geographical scales e.g. catchment level. Improvements in sample processing and trace analysis of drugs in complex matrices have resulted in recent provision of measured data on pharmaceutical concentrations for both influent and effluent. The use of such measured data has allowed improvements to removal rate assumptions (during sewer transport and within STWs) used within this modelling approach, by utilising novel ratio-approaches, thereby further increasing opportunities for calibration and thus predictive accuracy.

This new approach to estimating pharmaceutical concentrations entering and being discharged from STW moves modelling capability forward in the UK. The case study data derived for the steroid estrogens provides excellent comparison with measured influent and effluent concentrations with significant improvements in accuracy of EE2 loads and concentrations. This is particularly important as future river water standards are likely to be set at very low (potentially sub ng $L^{-1}$) values, which emphasises the importance of being able to accurately predict concentrations when considering programmes of measures to improve water quality.

The development of a modelling approach, such as that presented herein, which can predict influent and effluent concentrations at scales as small as individual STW will be important in targeting limited remediation resources when legislation regarding priority substances comes into force. This is particularly important as environmental improvements under the Water Framework Directive are being focussed on a catchment scale with stakeholders such as sewage treatment companies needing to identify works at risk of contributing to EQS exceedances. The fact that local demographics could lead to significantly varying influent/effluent concentrations which may also be seasonal in character emphasises the necessity of being able to model this variability, particularly in areas where tourism is the main industry. Water companies hold information on holiday populations within sewer catchments which could be used to adjust the excreted loads based on the assumption for example that the incoming population were of similar demographics and being prescribed similar medication.

The applicability of this modelling approach to any pharmaceutical is reliant upon data or estimates of excretion rates, removal rates in sewer and STW. With improving analytical methodologies being developed and an increasing concern regarding the discharge and fate of pharmaceuticals in the environment, it is anticipated that an increasing dataset will be generated in the coming years, driven by legislation such as the Water Framework Directive in the EU, which will provide input data for modelling approaches such as the one presented here.

The NHS prescription database details millions of prescriptions per month which makes large scale (e.g. whole of England) assessment of 'hotspots' very challenging. However, this could be considered in the future when sufficient data is available (this database has only been available since September 2011). This may be even more significant where local demographical variations in pharmaceutical use may vary more significantly than for use of steroid estrogens. Examples could include use of anti-depressants in socially deprived areas, beta blockers and statins in retirement biased populations or the seasonal use of antibiotics.

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## Notes and references

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Electronic Supplementary Information (SI) available: The Supporting Information, including 5 Tables and 1 Figure, provides a description of the method used, by detailing the datasets used, and using example data from the case study to illustrate the steps involved in predicting steroid usage, excretion and removal during sewer transport and within STWs to derive predicted influent and effluent concentrations. Additionally, a generalised formula for use in other case studies is provided, along with an explanation of how modifications were applied to derive estimations for England, along with data and discussion regarding removal rates and associated references. See DOI: 10.1039/b000000x/

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**SUPPORTING INFORMATION**

**Manuscript Title:** Parameterization of pharmaceutical emissions and removal rates for use in UK predicted exposure models: steroid estrogens as a case study

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**Contents:**

**Predicting Synthetic Estrogen Usage: A Case Study**

*Understanding the dataset: British National Formulary (BNF) codes*

The BNF is split into chapters (i.e. endocrine system), and further divided into multiple sections (i.e. sex hormones, female sex hormones, oestrogens and hormone replacement therapy), which are then further classified as codes for specific pharmaceuticals and combinations of pharmaceuticals (i.e. Combined Ethinylestradiol 30mcg"). These codes are often comprised of a number of different formulations, which vary by brand, dose and/or route of administration; no specific coding or labelling is given for individual formulations. For example, in the UK there are 16 different formulations within the BNF code for "Combined Ethinylestradiol 30mcg" (BNF code 070301F0). The mass of active ingredient in each formulation may differ over a range of brands, routes of administration, or combinations with other drugs (see S1 for case study example).

| | **Average prescriptions per month ($R_t$)** | | | |
|---|---|---|---|---|
| Pharmaceutical name & BNF Code | GP Surgery 1 | GP Surgery 2 | GP Surgery 3 | Total for selected catchment |
| Estradiol (systemic) 0604011G0 | 48.6 | 22.8 | 25.4 | 96.8 |
| Estradiol Valerate 0604011K0 | 2.6 | 7.2 | 3.4 | 13.2 |
| Estradiol with Progestogen 0604011L0 | 28.2 | 15 | 21 | 64.2 |
| Estradiol (topical) 0702010Ga0 | 35.2 | 14 | 28.2 | 77.4 |
| Estradiol Val & Estradiol Val + Dienogest 0703010R0 | 0 | 0.2 | 0 | 0.2 |
| Oestrogens Conjugated 0604011P0 | 4.6 | 6.8 | 6.4 | 17.8 |
| Oestrogens Conjugated with Progestogen 0604011Q0 | 0.8 | 5 | 5.2 | 11 |
| Combined Ethinylestradiol 20mcg 703000 | 1.6 | 2.2 | 11.4 | 15.2 |
| Ethinylestradiol 0604011D0 | 2.2 | 0 | 1.4 | 3.6 |
| Combined Ethinylestradiol 30mcg 070301F0 | 59.8 | 39.8 | 70.8 | 170.4 |
| Combined Ethinylestradiol 35mcg 070301G0 | 19.6 | 7.6 | 7.4 | 34.6 |
| Phased Formulations Of Ethinylestradiol 070301P0 | 0.8 | 1.8 | 5 | 7.6 |
| Co-Cyprindiol (Cyprote Acet/Ethinlestr) 1306020C0 | 2 | 1 | 3.2 | 6.2 |
| Etonogestrel/Ethinylestradiol 070301A0 | 0 | 0 | 0.2 | 0.2 |

**Table S1: Estrogen Prescription Data for the Selected River Catchment**

Mean number of prescriptions calculated across 01.09.11 to 31.01.12. Source: National Health Service, Prescribing by GP Practice, The Information Centre for Health and Social Care, 2011 [1].

*Conversion of number of prescriptions to prescribed mass*

In the UK there are 16 different formulations within the BNF code for "Combined Ethinylestradiol 30mcg" (BNF code 070301F0; table S1). The mass of active ingredient in each formulation may differ over a range of brands, routes of administration, or combinations with other drugs, but in this particular case all formulations contained 30 micrograms of EE2. NHS data [2] detailed for the UK as a whole the number of prescriptions written for each individual formulation ($I_f$) and the quantity of drug dispensed ($Q_f$). The units for $Q_f$ depend upon how each individual formulation is dispensed, and can be a "pill", "pack", "millilitre", "gram", "capsule", etc. To illustrate this point, data for "Loestrin 30_Tab" (which is a formulation within BNF code 070301F0) is discussed (see table S2). There were 24,528 prescriptions written and 2,380,457 pills of Loestrin 30_Tab dispensed in the UK between October and December of 2011. Therefore each prescription of Loestrin 30_Tab contained on average 97 pills, which equates to 2.91 mg of active ingredient (Table S2). The average mass of active ingredient per prescription for each formulation of EE2 ($M_{i,f}$), across the whole of the UK was calculated in this manner (Eq S1; Table S2); however this did not account for regional (or catchment level) prescribed mass.

As localised prescription data is not detailed down to the individual formulation level (only BNF codes), it was necessary to ascertain the relative proportion of each formulation prescribed within each BNF code to obtain typical UK wide prescribing practices, which could then be applied to localised catchment level data. For example, 3% of the 934,016 "Combined Ethinylestradiol 30mcg" (BNF code 070301F0) prescriptions written in the UK were of the "Loestrin 30_Tab" formulation (Eq S2; Table S2). Assuming that the prescription habits of GPs are uniform throughout the UK, 3% of the 170.4 monthly prescriptions for "Combined Ethinylestradiol 30mcg" in the selected river catchment were the "Loestrin 30_Tab" formulation (Eq S3). This output was then used in conjunction with average mass per prescription (Eq S1) of a specific drug formula to calculate the

total mass of Loestrin 30_Tab prescribed in this catchment per month and per day (Eq S4). A simplified summary formula covering all stages of calculation is presented in Eq S5 summary. This process was repeated for all formulations contained within each BNF code of interest and were then summed to give the total mass prescribed for each chemical of interest (E2, EE2, CE) (Eq S5).

| **Formulation Name** | **EE2 dosage (mg) ($D$)** | **Items (1000's) ($I_f$)** | **Quantity (1000's) ($Q$)** | **Mean mass per prescription (mg) ($M_1$)** | **Proportion prescribed ($P$)** | **Number of prescriptions / month for catchment ($R_f$)** | **Mass prescribed per day for catchment (mg) ($M_D$)** |
|---|---|---|---|---|---|---|---|
| Elevin_Tab 150mcg/30mcg | 0.03 | 2.33 | 222.48 | 2.87 | 0.00 | 0.42 | 0.04 |
| Femodene ED_Tab | 0.023 | 1.20 | 135.42 | 2.54 | 0.00 | 0.22 | 0.02 |
| Femodene_Tab | 0.03 | 36.5 | 3651.70 | 3.00 | 0.04 | 6.66 | 0.66 |
| Gedarel_Tab 30/150mcg | 0.03 | 18.7 | 1800.22 | 2.88 | 0.02 | 3.42 | 0.32 |
| Katya 30/75_Tab | 0.03 | 0.20 | 17.87 | 2.65 | 0.00 | 0.04 | 0.00 |
| Levest 150/30_Tab | 0.03 | 12.10 | 1194.52 | 2.96 | 0.01 | 2.21 | 0.21 |
| Levest 150/30_Tab (Actavis) | 0.03 | 1.18 | 114.67 | 2.92 | 0.00 | 0.22 | 0.02 |
| Loestrin 30_Tab | 0.03 | 24.53 | 2380.46 | 2.91 | 0.03 | 4.47 | 0.43 |
| Marvelon_Tab | 0.03 | 47.99 | 4718.38 | 2.95 | 0.05 | 8.76 | 0.85 |
| Microgynon 30 ED_Tab | 0.0225 | 26.94 | 3171.78 | 2.65 | 0.03 | 4.91 | 0.43 |
| Microgynon 30_Tab | 0.03 | 492.78 | 48562.78 | 2.96 | 0.53 | 89.90 | 8.74 |
| Millinette_Tab 30/75mcg | 0.03 | 2.41 | 231.32 | 2.89 | 0.00 | 0.44 | 0.04 |
| Minulet_Tab | 0.03 | 0.00 | 0.25 | 3.78 | 0.00 | 0.00 | 0.00 |
| Ovranette_Tab 150mcg/30mcg | 0.03 | 46.79 | 4488.25 | 2.88 | 0.05 | 8.54 | 0.81 |
| Rigevidon_Tab | 0.03 | 39.97 | 3953.00 | 2.97 | 0.04 | 7.29 | 0.71 |
| Yasmin_Tab | 0.03 | 180.39 | 17653.28 | 2.94 | 0.19 | 32.91 | 3.18 |
| **Totals** | | **934.02 ($I_T$)** | | | | **170.40 ($R_T$)** | |

**Table S2: Formulation level data for BNF code 070301F0**

*Formulae for a generalised method to predict prescription usage*

A general method for determining daily use of prescription medications in a targeted catchment is presented below.

**Eq S1**. The average mass per prescription for a specific drug formulation, $M_{i,f}$, is given by

$$M_{i,f} = \frac{D_f Q_f}{I_f}$$

where $D_f$ is the mass of a single dose of a given formulation, $Q_f$ is the quantity of doses dispensed of this formation in the UK within a given time frame (e.g. number of pills dispensed from October-December 2011), and $I_f$ is the number of prescriptions written of a given formulation UK wide (within the same time frame as $Q_f$).

**Eq S2**. The relative proportion prescribed of each formulation on a UK wide basis within a given BNF code, $P_f$, is given by

$$P_f = \frac{I_f}{I_t}$$

Where $I_t$ is the sum of all $I_f$ within a given BNF code.

**Eq S3**. The number of prescriptions written of a given formulation in a localised catchment per month, $R_f$, can then be estimated by

$$R_f = P_f R_t$$

where $R_t$ is the average number of prescriptions written per month in the catchment for a given BNF code (from Table S1).

**Eq S4**. The mass prescribed for each formulation in a given BNF code per month in the targeted catchment, $M_m$, can now be calculated by

$$M_m = M_{i,f} R_f$$

which can be converted to the daily mass prescribed of each formulation in a given BNF code, $M_d$, which is given by

$$M_d = \frac{12 M_m}{365}$$

**Eq S5**. The series of equations above can be simplified to the following formula:

$$M_d = \frac{12 D_f Q_f R_t}{365 I_t}$$

**Eq S6**. To ascertain the total mass of a drug prescribed in a given catchment this series of calculations was repeated for each formulation within each BNF code of a given chemical, e.g. all 47 formulations of EE2 which are contained within all 7 BNF codes that include EE2. This method of determining the mass of pharmaceuticals used is contingent on the assumption that all prescribed medications are actually ingested by the patient. Typically, only 50% of prescriptions are taken [3], but data suggests that patient adherence to contraceptive and HRT regimens is much higher [4, 5]. Therefore, assuming all prescribed medications are consumed, the sum of all $M_d$ across all BNF codes containing a given chemical is the total daily consumption for the given chemical in the target catchment, $M_t$, and can be given by

$$M_t = \sum_{f=1}^{n} M_{d,f}$$

where $n$ is equal to the number of formulations of a given chemical and the subscript $f$ denotes the $f$th formulation of a given chemical.

*Modifying case study model inputs to generate predictions for England*

As there was no measured crude sewage steroid data for the select catchment, the modelling approach developed was modified to apply English population values to the results of the case study excretion predictions, in order to allow comparison with measured sewerage influent data for England as a whole. For the values based on prescription data (HRT users and EE2 excretion), this modification is predicated on the assumption that prescribing practices for physicians in the case study catchment mimic those of physicians in England as a whole. Outputs from this modification and a description of the process are presented in Table 3.

| | | | Total excretion (natural + synthetic) in mg day$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **CASE STUDY CATCHMENT** | | | **ENGLAND** | | |
| Population Group | % of catchment Population | % of England's Population | E1 | E2 | EE2 | E1 | E2 | EE2 |
| A. Pregnant | 0.78 | 0.99 | 95.15 | 67.99 | 0 | 267020 | 190798 | 0 |
| B. Menstrual Females (not pregnant) | 20.60 | 24.37 | 53.40 | 14.60 | 9.37 | 140121 | 38324 | 24587 |
| C. HRT users | 3.16 | 2.38 | 11.34 | 14.45 | 0 | 18881 | 24059 | 0 |
| D. Menopausal Females (non-medicated) | 20.80 | 15.64 | 8.31 | 4.62 | 0 | 13835 | 7686 | 0 |
| E. Males | 47.70 | 48.68 | 27.55 | 19.08 | 0 | 62194 | 43057 | 0 |
| Average for total population of area (μg / day / capita) | | | 8.82 | 5.44 | 0.42 | 10.22 | 6.19 | 0.50 |

**Table S3: Comparison between demographics and modelled estrogen excretion rates for the select catchment and England as a whole**

Population percentages are calculated from 2001 census data [6] by completing the following calculations, followed by conversion to a percentage of total population: A = population x birth rate x multiplier accounting for pregnancy duration; B = female population aged 13-49 minus the number of pregnant females; C = female population aged 50+ x HRT usage rate (13.2%); D = female population aged 50+ - number of HRT users; E = obtained from census data. Average per capita excretion data is presented in reference to total population (i.e. adults and children). Total natural excretion was calculated based upon figures detailed in the manuscript (Figure 1) and census data. Total synthetic excretion for England was based upon average catchment level per capita excretion of synthetic compounds (mass excreted in catchment ÷ number of individuals in corresponding catchment population group) and English population group specific demographics. Total natural and synthetic excretion were summed and divided by the total population size to find average for total population of area.

**Predicting in-sewer transformations: a case study**

The UK water industry has recently undertaken a £30 million research programme to determine priority chemicals entering STW, impacts of primary, secondary and tertiary treatment and effluent concentrations. A total of 25 STW were sampled over the course of a year and influent concentrations and removal rates calculated for chemicals including the steroid estrogens [7]. Table S4 provide a summary of the influent data reported in an earlier predictive study, the UK Chemical Investigation Programme and this modelling approach (using a range of E1 to E2 transformation rates). For the purpose of an accurate comparison, concentrations have been calculated as µg capita$^{-1}$ day$^{-1}$.

| | E1 | E2 | EE2 |
|---|---|---|---|
| Predicted influent (µg capita$^{-1}$ day$^{-1}$) – this work, select catchment; 50% E2 to E1 conversion | 11.5 | 2.72 | 0.42 |
| Predicted influent (µg capita$^{-1}$ day$^{-1}$) – this work, select catchment; 28.75% E2 to E1 conversion | 10.4 | 3.88 | 0.42 |
| Predicted influent (µg capita$^{-1}$ day$^{-1}$) – this work, select catchment; 6% E2 to E1 conversion | 9.1 | 5.11 | 0.42 |
| Predicted influent (µg capita$^{-1}$ day$^{-1}$) – this work, England as a whole; 50% E2 to E1 conversion | 13.3 | 3.09 | 0.5 |
| Predicted influent (µg capita$^{-1}$ day$^{-1}$) – this work, England as a whole; 28.75% E2 to E1 conversion | 12.0 | 4.41 | 0.5 |
| Predicted influent (µg capita$^{-1}$ day$^{-1}$) – this work, England as a whole; 6% E2 to E1 conversion | 10.6 | 5.81 | 0.5 |
| Predicted influent (µg capita$^{-1}$ day$^{-1}$) – [8] | 13.8 | 3.30 | 0.89 |
| Mean measure influent (µg capita$^{-1}$ day$^{-1}$) - [7] | 16.1 | 5.90 | 0.31 |
| Median influent (µg capita$^{-1}$ day$^{-1}$) - [7] | 16.7 | 5.90 | 0.23 |
| Standard Deviation (µg capita$^{-1}$ day$^{-1}$) - [7] | 3.9 | 1.4 | 0.21 |
| Range (µg capita$^{-1}$ day$^{-1}$) - [7] | 7.6-25.9 | 3.1-9.6 | 0.12-1.12 |

**Table S4: Comparative findings for the case study catchment: influent loads**

**Description of Ratio-Approach used to ascertain In-Sewer Transformation rates**

Based upon Johnson and Williams assumption of 50% degradation of E2 to E1, the modelling approach developed herein and modified with English demographics data, predicts E1 and E2 influent loads of 13.3 and 3.09 μg capita$^{-1}$ day$^{-1}$ respectively, which is in line with Johnson and Williams predictions (E1 = 13.8, E2 = 3.30 μg capita$^{-1}$ day$^{-1}$), but is lower than measured means (E1 = 16.1, E2 = 5.9 μg capita$^{-1}$ day$^{-1}$). Reduction of the degradation rate within the sewerage system will decrease E1 influent predictions, but increase E2 predictions. However, as predicted E1 + E2 equates to ~16 μg capita$^{-1}$ day$^{-1}$ in total, modification of the sewer removal rate will not result in achieving the higher values seen in measured data for both compounds simultaneously. Therefore sewer removal rates were optimised to achieve the same ratio of E1:E2 observed in the measured data. An identical E1:E2 ratio of 2.72 is achieved with a 28.75% removal rate; resulting in E1 and E2 predictions of 12.0 and 4.41 μg capita$^{-1}$ day$^{-1}$ respectively, with an 'optimised value' of 75% of the observed influent values, for the England prediction. Lowering the removal rate further, to the 6% indicated in the findings of M.E. Jarvie and D.W. Hand [9], changes the E1:E2 ratio to 1.82, resulting in substantially higher proportions of E2 relative to E1 than is found in observed data, with 98% and 66% of observed values for E2 and E1 being predicted, respectively. Based on this empirical data it may therefore be concluded that using a value of 28.75% loss of E2, with subsequent conversion to E1, within the sewer system, is the most accurate assumption for the purpose of this risk assessment for achieving realistic predictions of E1 and E2 simultaneously. Application of this 28.75% removal rate to the catchment scale model, results in E1 and E2 predictions of 10.4 and 3.88 μg capita$^{-1}$ day$^{-1}$ respectively.

**Predicting losses during sewage treatment: a case study**

The data (Figure S1) show that measured E1 and E2 removal rates are similar to those used in previous risk assessments and are within the 95% confidence intervals. For EE2, however, a very different result is observed, with previous estimates using a removal rate of 85%. The recent UK data however, generated widely varying removal rates, of between 0 and 92% with a mean of only 27% and median of 52% [7], greater removal rates were achieved with increased biological treatment, including nitrifying processes for ammonia removal (Figure S1). One explanation for this is analytical errors and measurements below the limit of detection which lead to ranges much more extreme than those calculated for E1 and E2, which are present at an order of magnitude or higher.

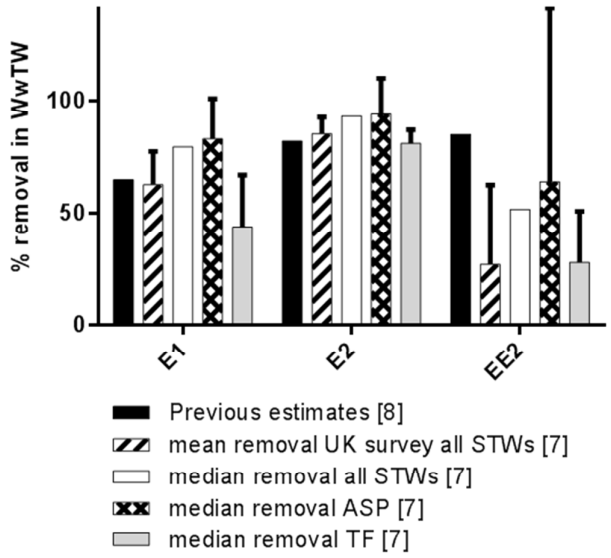


**Figure S1: Reported STW removal rates for estrogens previously measured or estimated**

Error bars represent 95% confidence intervals for overall mean removal and variations in median removal rates between activated sludge processes (ASP) and biological filter works (trickling filter: TF)

| | | E1 | E2 | EE2 |
|---|---|---|---|---|
| Predicted influent (ng $L^{-1}$) | | 48.0 | 17.6 | 2.0 |
| Measured Effluent (ng $L^{-1}$) [& % observed removal] | Mean all STW | 17.8 [63] | 2.6 [85] | 0.56 [27] |
| | Median all STW | 10.9 [80] | 1.3 [93] | 0.36 [28] |
| | Range for all STW (95% conf) | 10.9 – 24.7 | 1.3 – 3.9 | 0.33 – 0.78 |
| | Median TF only | 27.1 [44] | 2.7 [81] | 0.58 [53] |
| | Median ASP only | 10.3 [83] | 0.88 [94] | 0.36 [64] |
| Required predicted: measured ratio | | 0.75 | 0.75 | 1.61 |
| Predicted Effluent (ng $L^{-1}$; using optimised % removal) | Mean all STW | 13.3 | 1.9 | 0.9 |
| | Median all STW | 8.1 | 1.0 | 0.6 |
| | Median TF only | 20.2 | 2.0 | 0.9 |
| | Median ASP only | 7.7 | 0.7 | 0.6 |
| Optimised % removal | Mean all STW | 72.3 | 89.0 | 54.9 |
| | Median all STW | 83.1 | 94.5 | 71.0 |
| | Median TF only | 57.9 | 88.6 | 53.3 |
| | Median ASP only | 84.0 | 96.3 | 71.0 |
| | Range | 58 – 84 | 89 – 96 | 53 - 71 |

**Table S5: Optimised STW removal rates (based upon a ratio approach) for calculation of predicted effluents**

Predicted data presented above are for the case study presented herein, for England's population demographics. Predicted influent concentrations are calculated on a basis of 28.8% sewer transformation of E2 to E1. Required predicted:measured ratio is calculated from influent data (Table S4).

Optimised removal rates for E1 and E2 correlate well with observed rates (i.e. mean & median across all STW; median TF and median ASP; Table S5). Of more interest are the optimised removal rates

generated for EE2. For specific STW types EE2 optimised removal rates (ASP 71%; TF 53%) and measured removal rates (ASP 64%; TF 53%) are alike. However, when the specific STW processes are not considered, and average or median values across different types of STW are used, optimised removal rates are found to be double those observed. These findings therefore suggest that use of predictive models would achieve more accurate effluent predictions if the specific treatment processes at a given works is taken into consideration. However, in the absence of process data for the works within the case study catchment, the optimised removal rates (calculated upon England's demographic data and presented above) were applied, resulting in effluent concentrations as summarised (Table S5).

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