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1    **Running head: Risk based prioritisation of pharmaceuticals**

2    Corresponding author: Alistair B.A. Boxall

3    Address for communication-Environment Department, University of York, Heslington, York,

4    YO10 5DD, UK. Telephone 01904 324791; email- alistair.boxall@york.ac.uk

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**Title: Toxicological and ecotoxicological risk based prioritisation of  
pharmaceuticals in the natural environment**

Jiahua Guo<sup>†</sup>, Chris J. Sinclair<sup>‡</sup>, Katherine Selby<sup>†</sup> and Alistair B.A. Boxall <sup>†</sup>

<sup>†</sup>- Environment Department, University of York, Heslington, York, United Kingdom, YO10 5DD

<sup>‡</sup>- Fera Science Ltd., Sand Hutton, York, United Kingdom, YO41 1LZ

## Abstract

Around 1500 active pharmaceutical ingredients are currently in use, however the environmental occurrence and impacts of only a small proportion of these has been investigated. Recognising that it would be impractical to monitor and assess all pharmaceuticals that are in use, a number of previous studies have proposed the use of prioritisation approaches to identify substances of most concern so that resources can be focused on these. All of these previous approaches suffer from limitations. Here, we draw on experience from previous prioritisation exercises and present a holistic approach for prioritising pharmaceuticals in the environment in terms of risks to aquatic and soil organisms, avian and mammalian wildlife and humans. The approach considers both apical ecotoxicological endpoints as well as potential non-apical effects related to the therapeutic mode of action. Application of the approach is illustrated for 146 active pharmaceuticals that are either used in the community or in hospital settings in the United Kingdom. Using the approach sixteen compounds were identified as a potential priority. These substances include compounds belonging to the antibiotic, antidepressant, anti-inflammatory, antidiabetic, antiobesity and estrogen classes as well as associated metabolites. We recommend that in the future, the prioritisation approach be applied more broadly around the different regions of the World.

**Keywords:** Active pharmaceutical ingredients (APIs); Ecotoxicity; Exposure; Hazard; Risk score

## 65    **Introduction**

66    Active pharmaceutical ingredients (APIs) have been widely detected in the natural environment  
67    across the world [1-3]. As they are biologically active compounds, designed to interact with specific  
68    pathways/processes in target humans and animals, concerns have been raised over the potential side  
69    effects of these substances in the environment and, over the past 15 years, a substantial amount of  
70    work has been done on the occurrence, fate, effects and risks of pharmaceuticals in the natural  
71    environment. There have also been regulatory developments around the monitoring of  
72    pharmaceuticals in the environment. For example, seven pharmaceuticals/hormones have been  
73    placed on the watch list under the European Environmental Quality Standards Directive [4] and Water  
74    Framework Directive [5] and it is possible that, in the future, these compounds will be included in  
75    European statutory monitoring programmes.

76    While a large amount of data has been published in the past decade on different aspects of APIs in  
77    the environment, information is still only available for a small proportion of the 1500 or so active  
78    pharmaceutical ingredients that are currently in use. It is possible, therefore, that monitoring and  
79    effects-based studies are missing substances that could be causing adverse impacts in the  
80    environment. It would be impossible to experimentally assess the hazards and risks for all the  
81    pharmaceuticals in use in a timely manner. One solution to this problem is to employ formal  
82    prioritisation approaches to identify those compounds that are likely to pose the greatest risk in a  
83    particular situation and, therefore, which need further attention. A number of prioritisation methods  
84    have already been proposed, and applied to, human and veterinary APIs [6-10]. Prioritisation  
85    approaches are also available for other classes of emerging contaminant such as pesticide  
86    metabolites [11]. Many of these approaches use exposure and toxicological predictions or information  
87    on API potency in humans so they can be readily applied to large numbers of compounds. Until now,  
88    prioritisation methods for APIs have tended to focus on risks of parent compounds in surface waters  
89    to aquatic organisms and risks to humans *via* drinking water consumption and tended to focus on  
90    single use categories (e.g. prescription or hospital use). Less emphasis has been placed on risks to  
91    other environmental compartments such as soils, sediments and ground waters, risks to top predators  
92    or on the risks of metabolites of APIs.

In the present study, we describe a holistic risk-based prioritisation approach for identifying APIs of concern in aquatic and terrestrial systems. The use of the prioritisation approach is illustrated using a subset of APIs used in primary and secondary care in the United Kingdom as well as those distributed by pharmacists 'over the counter' and major metabolites of these. The approach considers aquatic and terrestrial exposure routes and acute and chronic effects on algae, invertebrates, fish, birds and mammals, including humans. Effects relating to the therapeutic mode of action are also considered. The approach is illustrated using 146 active ingredients that were either high usage in the UK or where experts indicated that they might be of environmental concern. While the approach has been applied to the UK situation, there is no reason why it cannot be applied to prioritise APIs in use in other regions of the World.

## **Methods**

The prioritisation approach used risk scores (RS) as the primary parameter to rank the APIs in terms of their potential environmental risk (Figure 1 A, B). Risk score values were calculated by comparing predictions of exposure of APIs in different environmental compartments to measures of potential hazard towards different organisms from different trophic levels. The prioritisation process considered aquatic and terrestrial organisms as well as humans, acute and chronic apical ecotoxicological effects and potential effects related to the mode of action of an API (Figure 1 A, B). In the next sections we describe how the exposure concentrations and hazard parameters were derived. Specific equations are provided in the Supplemental Data.

### *Identification of substances for prioritisation*

In the United Kingdom (UK), the main ways that pharmaceuticals are made available to patients are through the fulfilment of primary care prescriptions by pharmacies and dispensing in secondary care (including hospitals). Some can also be purchased 'over-the-counter' at retail outlets. It would be a mammoth task to determine the usage of all compounds in the UK. We therefore, developed a substance list for prioritisation that included the top usage compounds in these different categories. To ensure that the list caught compounds of low use but very high potency, we also used expert opinion to identify potent compounds that might be of concern. Forty international experts from academia, industry and Government agencies based in North America, Europe and Asia were contacted via email. These experts were selected based on their track record in the area of

ecotoxicology and environmental risks of pharmaceuticals. Many of them had participated in the Society of Environmental Toxicology and Chemistry 'Big Questions' exercise on pharmaceuticals and personal care products in the environment [12]. Their responses were used to collate a list of substances of high perceived concern.

Annual pharmaceutical usage data for the top most prescribed pharmaceuticals in primary care (by active ingredient mass) in the UK were collated from Prescription Cost Analysis (PCA) data available for England [13], Scotland [14] and Wales [15]. The available PCA data obtained from Northern Ireland was not sufficient to calculate pharmaceutical usage. To reduce the time required to collate the data, the usage of all pharmaceuticals present on the PCA data for Wales was calculated (approximately 1000 active ingredients). Usage data were then obtained for England and Scotland for the top 300 compounds in use in Wales. These data were then used to generate a list of the top 100 pharmaceuticals by mass for Great Britain. Twelve substances with high usage but considered by the project team to fall outside the scope of this project were excluded from further prioritisation. These compounds were aliginic acid compound preparations, calcium carbonate, co-magaldrox (magnesium/aluminium hydroxide), ergocalciferol, ferrous fumarate, ferrous sulphate, glucose, lithium carbonate, omega-3 marine triglycerides, potassium chloride, sodium bicarbonate and sodium valproate.

Data on pharmaceutical usage in secondary care in 2012 was provided to the project team by the British Generic Manufacturers Association (BGMA). Data were provided on the usage, by mass, of the top twenty most used pharmaceuticals in secondary care. Three compounds (paracetamol, amoxicillin and codeine) that were also present on the primary usage lists had their primary and secondary care usage combined. The identity of pharmaceutical active ingredients present in pharmaceutical products available over-the-counter were obtained from information available on online retailer websites (e.g. the Boots Company website)

As some compounds will be extensively metabolised in the body, for these substances, the environment will be exposed to the metabolite and not the parent compound. Data were therefore also obtained on the extent of metabolism of the high use compounds and on the identity of the major metabolites. The recent Chemical Investigation Program (CIP) in the UK has monitored 12 pharmaceuticals in wastewater treatment plant (WWTP) effluent [16]. Compounds monitored in CIP

but which were not in the top usage compound list or which were not identified by the experts were also added to the list for prioritisation. Overall, 146 compounds were identified for further quantitative prioritisation. An additional 23 compounds were identified that are available over-the-counter which were ranked using a more simple chemical classification approach due to the absence of quantitative usage data.

#### *Environmental exposure estimation*

Predicted environmental concentrations of selected pharmaceuticals in surface waters ( $PEC_{sw}$ ) and terrestrial systems ( $PEC_{soil}$ ) were estimated using standard algorithms that are described in existing regulatory guidance documents (Supplemental Data, Equations 1-7) [17, 18]. The algorithms assume that pharmaceutical usage by the population is distributed evenly both temporally and spatially. The property data for APIs, collated to aid the determination of environmental exposure, included the acid dissociation constant ( $pK_a$ ); octanol-water partition coefficient ( $K_{ow}$ ); solid-water distribution coefficient ( $K_d$ ) and organic carbon partition coefficient ( $K_{oc}$ ). These data were collated from a number of sources including the peer-reviewed literature, grey literature and available online databases (e.g. drugbank [19]). Where experimentally determined data were unavailable, estimation tools, such as Quantitative Structure-Property Relationships [17, 20, 21] were used to fill the data gaps. For example,  $K_{oc}$  was predicted using an estimation model developed for ionisable organic chemicals (Supplemental Data, Equations 8-11). Default values of pH of soil recommended by the model developers [20] were used in the  $K_{oc}$  estimation (i.e. 5.8 for acids and pH 4.5 for bases).

The fish steady state plasma concentration ( $F_{ss}PC$ ) resulting from exposure via surface water was predicted based on estimates of the partitioning of an API between the aqueous phase and arterial blood in the fish ( $P_{blood:water}$ ) [22]. This partition coefficient was initially estimated based on the Log  $K_{ow}$  of the API, and this was subsequently combined with the  $PEC_{sw}$  to estimate the  $F_{ss}PC$  (Supplemental Data, Equations 12-15).

To estimate concentrations in fish, the Bioconcentration factor for fish ( $BCF_{fish}$ ) was estimated according to the approach of Fu *et al.* [23] assuming a pH of surface water of 7.0. The predicted environmental concentration in fish as food ( $PEC_{fish}$ ) was then calculated from the BCF and the predicted surface water concentration (Supplemental Data, Equations 16-20). To estimate the



concentration of an API in earthworms ( $PEC_{\text{earthworm}}$ ), the concentration in the earthworms on a wet weight basis ( $C_{\text{earthworm}}$ ) was calculated using an estimate of the concentration in porewater ( $C_{\text{porewater}}$ ) and the BCF for earthworms calculated according to the approach in the Technical guideline Document (TGD; Supplemental Data, Equations 21-23) [17].

### *Hazard characterisation*

Predicted no effect concentrations (PNEC) of pharmaceuticals were derived based on either experimental or estimated ecotoxicity data, using appropriate safety factors from the Technical Guideline Document (TGD) [17] (Supplemental Data, Equations 24). Where multiple ecotoxicological values were available, the most sensitive end-point was used for the generation of the PNEC.

Chronic and acute aquatic and terrestrial ecotoxicity data for standard test taxa (e.g. earthworm, green algae, daphnia and fish), together with non-standard taxa and end-points, were collated for the 146 pharmaceuticals (and relevant metabolites) under consideration (e.g. from the Fass [24] and ECOTOX [25] databases). A number of the compounds under consideration had no available experimentally derived ecotoxicological aquatic data. Therefore, for these compounds estimation techniques were used to fill the data gaps. A read-across approach using the OECD QSAR Toolbox was used for pharmaceuticals, and the estimation approach of Escher et al. [26] was used for metabolites. The database present in the OECD QSAR Toolbox was used to identify experimental data for molecules deemed 'similar' to each of the individual pharmaceutical with no data. Then within the software a relationship was built to allow an estimation of the ecotoxicological endpoint for the query molecule. The approach adopted for the identification of similar compounds was to combine the protein-binding profile with endpoint specific ones, as suggested by the Toolbox instruction manual [27]. The main procedures in the software were as follows: protein binding profile was selected as a group method to define the category. Subcategories were then established based on the classification system used by ECOSAR (US EPA). The results were then followed by a refinement for structural similarity (70 - 90% similar). The identified chemicals were then used to read across and estimate ecotoxicity data for the query pharmaceutical. Metabolite aquatic ecotoxicity data gaps were filled using the estimation approach for pharmaceutical metabolites proposed by Escher et al. [26] which uses the principle of the toxic ratio and parent ecotoxicological data to estimate the toxic range for the metabolite. For compounds with no experimentally determined earthworm ecotoxicity data, the

terrestrial toxicity (14 day LC50 in mM/kg dry soil) was predicted using the Quantitative structure-activity relationship (QSAR) available in ECOSAR (US EPA; Supplemental Data, Equations 25).

All human plasma therapeutic concentrations ( $H_tPC$ ) were obtained from published work. Limited data are available on the toxicology of APIs to birds. Therefore, acceptable daily intakes (ADI) for humans and mammalian toxicity data (rat/mouse) were collated as surrogates to determine the potential hazards of APIs for top predators (obtained from several databases e.g. MEDSAFE [28]), Drugs [29]). A PNEC for mammalian data ( $PNEC_{mammal}$ ) was generated from the median lethal dose (LD50) for rat/mouse, by dividing by an assessment factor of 100. The potential hazard from drinking water was quantified by calculating the predicted no effect concentration of APIs for an adult ( $PNEC_{adult}$ ) and a child ( $PNEC_{child}$ ) based on ADIs for each API using the model of Schwab *et al* [30] (Supplemental Data, Equations 26).

#### *Ranking scenarios*

To prioritise substances a risk score was calculated for the different exposure pathway/toxicity endpoint combinations by dividing the relevant exposure concentration by the relevant hazard concentration (Figure 1 A, B). For example, to calculate the risk score for subtle effects on fish the  $F_{ss}PC$  was divided by the  $H_tPC$ . Compounds were then ranked based on their risk score with substances towards the top of the ranking deemed to be of most interest for that particular pathway and endpoint.

Due to a lack of quantitative usage data, the over-the-counter (OTC) pharmaceuticals were classified based on their hazards to the aquatic environment using a classification system proposed by European Chemicals Agency (ECHA) [31]. Following these criterion, substances without adequate chronic toxicity data were categorised as either chronic 1, chronic 2 and chronic 3, on the basis of the lowest acute aquatic toxicity data from 96 h half maximal lethal concentration (LC50) for fish, 48 h half maximal effective concentration (EC50) for crustacean or 72/ 96 h EC50 for algae (Table 1).

## **Results**

### *Target APIs and collation of pharmaceutical effect data*

Overall 146 compounds were identified for further quantitative prioritisation, these were distributed as follows: 88 were used in primary care; 20 were used in secondary care; 12 were identified as 'high hazard' concern, based on expert opinion; 25 major metabolites; and 4 from the previous Chemical Investigation Program (CIP1; Table 2). Twenty three compounds, sold as OTC medicines, were also identified in addition to the 146 compounds for quantitative prioritisation – these underwent a qualitative assessment. A summary of the available experimental toxicological data for 146 study compounds is provided in Table 2. Some high profile compounds had excellent multi-species/multi-endpoint datasets. However, the majority of the compounds under consideration had limited ecotoxicological data available. For the standard aquatic endpoints, 82 compounds had at least one experimentally derived acute or chronic ecotoxicity endpoint available. In terms of data on mammalian safety, data were available on the toxicity of 65 compounds, 139 had an acceptable daily intake and 113 had a human therapeutic plasma concentration ( $H_tPC$ ) (Table 2). Toxicological data were not available for any of the identified metabolites.

#### *Ranking list development*

The top 20 compounds derived from the different prioritisations for the aquatic and terrestrial environments are provided in Tables 3 and 4. The prioritisation based on apical acute aquatic effects at lower trophic levels indicated that amoxicillin, clarithromycin, ciprofloxacin, azithromycin and mesalazine had the highest risk scores ( $RS > 1$ ). For the aquatic apical chronic prioritisation process, diclofenac, atorvastatin, estradiol, mesalazine and omeprazole demonstrated the greatest risk score ( $RS > 1$ ). The highest ranked compounds based on apical acute effects in soil organisms were orlistat, carbamazepine and the carbamazepine metabolite, 10,11-epoxycarbamazepine ( $RS$  1-10; Table 4).

When the potential impact of subtle pharmacological effects were considered by comparing the human therapeutic concentration in plasma to estimated levels in fish, the atorvastatin metabolites ortho-hydroxyatorvastatin and para-hydroxyatorvastatin were ranked highest ( $RS > 10$ ) with atorvastatin, estradiol and amitriptyline just below these substances ( $RS$  1-10; Table 3).

In the prioritisation based on potential of secondary poisoning in the aquatic environment (i.e. fish-eating birds and mammals), diazepam was ranked the highest ( $RS$  between 0.1-1), while in terrestrial environments (i.e. earthworm-eating birds and mammals) the highest ranked API was orlistat ( $RS$  0.1-1). All other pharmaceuticals had a  $RS < 0.1$  (Table 4). The risk scores of APIs prioritised according to

human consumption in drinking water for all compounds were less than  $1 \times 10^{-5}$ . The top ranked compounds were phenytoin, metformin and simvastatin (Table 3).

For over-the-counter (OTC) pharmaceuticals, amorolfine, benzalkonium chloride, cetylpyridinium chloride, dextromethorphan, dimethicone, loratadine and xylometazoline hydrochloride were assigned to category chronic 1. The category chronic 2 included cetrimide, chlorphenamine maleate, guaifenesin, hexylresorcinol and mepyramine maleate, phenylephrine and pseudoephedrine. Beclometasone dipropionate, cetirizine hydrochloride, clotrimazole, dexpanthenol, fluticasone propionate, loperamide hydrochloride and pholcodine were assigned to category chronic 3 (Table 5). Acrivastine and sodium cromoglicate were not classified as no toxicity data was available and the estimation approaches did not work for these substances.

## Discussion

### *Results comparisons*

A final list of 16 substances including 13 parent compounds (amitriptyline, amoxicillin, atorvastatin, azithromycin, carbamazepine, ciprofloxacin, clarithromycin, diclofenac, estradiol, mesalazine, metformin, omeprazole, orlistat) and 3 metabolites (ortho-hydroxyatovastatin, para-hydroxyatovastatin and 10,11-epoxycarbamazepine) were identified that had a risk score  $> 1$  for one or more of the risk comparisons. A substance with RS more than 1 indicates that the estimated exposure is higher than the predicted no effect concentration, so more attention should be paid as the hazards might occur in the different environment compartments.

The ranking results for parent compounds agree with some of the previous prioritisation studies. Amitriptyline, atorvastatin, carbamazepine, diclofenac, estradiol, mesalazine and orlistat were identified as priority substances in use in the Swedish market by Roos et al. [32], with the ranking at 12<sup>th</sup>, 22<sup>nd</sup>, 16<sup>th</sup>, 5<sup>th</sup>, 4<sup>th</sup>, 10<sup>th</sup> and 11<sup>th</sup>, respectively. The risk score of diclofenac [33] was also reported with a low RS value of 0.01 in a UK stream case study. Amoxicillin has been ranked the top in several veterinary medicine prioritisation studies, where it was classified as a substance with high hazard to aquatic environments in the UK [6, 7], Korea [34], US [35] and China [36]. Azithromycin and metformin were identified in a US surface water exercise, being ranked 12<sup>th</sup> and 5<sup>th</sup>, respectively [35]. Clarithromycin has been identified in a prioritisation study in Germany and ranked 34<sup>th</sup> [37].

Ciprofloxacin was classified as a substance with a high ranking (8th) in the aquatic environment in US [35], besides, it was assigned to categories with a high and medium toxicity in China [36] and Korea [34], respectively. Omeprazole was considered in the prioritisation studies in the US and Sweden, ranking 18th and 22nd, respectively [32, 35].

Previously published work considering the prioritisation of pharmaceuticals has only focused on parent compounds [8, 32], whereas in reality following consumption by patients, compounds may be metabolised and excreted as metabolites, partly or completely [6]. This project is the first study that considered the impact that metabolism may have on the ranking of APIs. The ranking results demonstrated that it is important to consider these compounds, particularly the metabolites of atorvastatin (ortho-hydroxyatorvastatin and para-hydroxyatorvastatin) which were highly ranked using a number of the prioritisation indices. The classification of 'over-the-counter' APIs is a novel method applied in a prioritisation exercise, and therefore, no published works are available with which to compare our findings.

#### *Potential risk of highly ranked substances in the environment*

A number of the compounds we identified as high priority are receiving increasing regulatory scrutiny. For example, as part of Directive 2013/39/EU [38] which relates to priority substances in water, three APIs: diclofenac and two hormones 17-beta-estradiol (E2) and 17-alpha-ethinylestradiol (EE2) have been added to EU's pollutant watch list, two of these (diclofenac and E2) appear in our top 16 list. While EE2 did not fall in the top 16, it was still ranked highly using the plasma therapeutic concentration approach (number 11), even though the amounts of this compound used in the UK are small. Side effects of diclofenac on the fish kidneys (histopathological damages) have been documented [39, 40]. Diclofenac is also considered to have threatened some sensitive organisms (e.g. vultures from the *Gyps* genus) through secondary poisoning [41]. E2 and EE2 are the two APIs for which the toxicity have been determined at environmental relevant concentrations. E2 is a natural estrogen with endocrine disrupting properties. Potent effects of E2 on gamete quality and maturation in two salmonid species (rainbow trout *Oncorhynchus mykiss* and grayling *Thymallus thymallus*) have been reported, even at ng/L exposure concentration levels [42]. 17-alpha-ethinylestradiol (EE2) has been ranked in the top 20 list (Table 3). There is widespread evidence that exposure of male fish to EE2 at ng/L levels can result in feminization of male fish [43] and that chronic exposure of fish (i.e.

fathead minnow *Pimephales promelas*) to EE2 could ultimately result in a the collapse of fathead minnow populations in surface waters [44].

The watch list has been further developed in the European Environmental Quality Standards Directive [4], where four antibiotics including erythromycin, clarithromycin, azithromycin and ciprofloxacin have been added. The inclusion of antibiotics in the watch list is mainly due to their potential toxic effects to algal species. Three of these antibiotics (clarithromycin, azithromycin and ciprofloxacin) were identified as top priority in the current study. The 72/96 h acute EC50 values with growth as the endpoint for these free antibiotics are 0.002 mg/L (*Pseudokirchneriella subcapitata*) [45], 0.001 ug/L (unreported blue-green algae) [24] and 0.005 mg/L (*Microcystis aeruginosa*) [46], respectively.

The occurrence of some of the highly ranked parent APIs in aquatic the environment has been reported with concentrations at ng/L in surface waters and at up to µg/L levels in WWTP effluents [47]. Amitriptyline was reported to inhibit the growth of the macrophyte *Lemna minor* with 7 d EC50 1.69 mg/L [48] and cause inhibition of crustacea *Daphnia magna* with an EC50 of 5 mg/L [49]. Atorvastatin and metformin were reported to inhibit the growth of a wide range of organisms such as macrophyte (e.g. lemna) and vertebrate (e.g. fish), where the lowest 14 d NOEC 0.013 ug/L of atorvastatin with genetic endpoint was documented for Zebrafish (*Danio rerio*) [25] and 48 h LC50 1.35 mg/L of metformin for a crustacea *Daphnia magna* [50]. While currently no experimental toxicity data were recorded for mesalazine and omeprazole, in the present study a read-cross approach was used to predict their hazards to aquatic organisms. The lowest predictive chronic toxicity data of mesalazine and omeprazole each was 0.031 mg/L and 0.009 mg/L, both of these being for crustacea *Daphnia magna*. Hazards of five classified OTC APIs to three aquatic trophic levels have been illustrated in Table 5. Of the three highly ranked metabolites, only the occurrence of 10,11-epoxycarbamazepine has been reported, with a mean value of 19.1 ng/L in the WWTP effluent [47].

Except for the impacts of prioritised APIs on organism and population levels of non-target organisms in the environment, side effects of some targeted APIs (Table 6) on the cellular and genomic levels have also been documented. Hepatocyte cytotoxicity of the antibiotic amoxicillin has been reported in rainbow trout (*Oncorhynchus mykiss*) with a 24 h EC50 >182.7 mg/L [51]. Detrimental effects of carbamazepine on the liver and kidney cytopathology of rainbow trout (*Oncorhynchus mykiss*) has been observed with LOECs >0.1 and 0.001 mg/L, respectively [52]. Carbamazepine and diclofenac have been reported to significantly affect the genomic template stability in Zebrafish, at concentrations

of 310 ng/L and 810 ng/L, respectively [53]. Niemuth *et al.* [54] found that 4 wk metformin exposure at the concentration of 40 ng/L causes potential endocrine disruption in adult male fathead minnows (*Pimephales promelas*), through inducing significant up-regulation of messenger ribonucleic acid (mRNA) encoding the protein vitellogenin.

In terrestrial environments, the antiepileptic carbamazepine and antiobesity orlistat were the two highest ranked substances. The occurrence of carbamazepine in soil was reported at concentrations up to  $6.85 \times 10^{-3}$  mg/kg, and the QSAR based 14 d LC50 toxicity to earthworm was 1060 mg/kg. While the detection of orlistat in the terrestrial environment has not been reported, a relatively high experimental BCF of 51.1 for the orlistat treated earthworm has been documented [55] and the predictive 14 d LC50 toxicity to earthworm was 28.28 mg/kg. It should be recognised that prioritisation of several substances was based on the predicted properties and/ or toxicity data (Table 6), especially for  $K_{oc}$  values that were absent for all compounds. For some prioritised substances selected from subtle pharmacological effect scenario, exposures ( $F_{ssPC}$ ) were all estimated from  $\log K_{ow}$  on the basis of QSAR.

#### *Limitation of methods and future improvement*

Approaches for exposure estimations of APIs used in the present study rely heavily on the annual usage information for individual pharmaceutical active ingredients. However it is well recognised that as well as the primary and secondary care pharmaceutical usage, for a limited number of compounds 'over-the-counter' sales through retail outlets such as supermarkets and pharmacies may add a significant contribution to the overall usage [56]. Attempts were made to obtain quantitative usage data for OTC compounds during the present study but these were unsuccessful. A previous study has estimated that in Germany OTC usage can contribute up to 50% of the total usage of some pharmaceuticals. However, this can vary on a compound by compound basis, and usage through this route could not be included in the quantitative risk score based element of this project. An accurate quantification approach of OTC usage should be further established.

The exposure of APIs in the terrestrial environment was estimated by only considering a simple input pathway: APIs adsorbed to sludge in WWTP and a this sludge was then applied to the land [18]. Experimentally determined biodegradation data of APIs were not available. PECs and therefore, the risk scores of APIs that were susceptible to biodegradation during wastewater treatment will therefore have been significantly overestimated. Limited information on experimental physical-chemical

properties such as soil-water partition coefficients ( $K_{oc}$ ) was available for some listed APIs. To fill in the data gaps, an empirical estimation model developed by Franco and Trapp [20] was used to estimate adsorption during wastewater treatment. This model was developed for soils and its applicability to estimating sorption in sludge is not known. The model also omits selected sorption processes, such as complexation, which may be important for some pharmaceuticals [20].

In the secondary poisoning assessment of APIs in the terrestrial compartment, as very limited experimental data was available on bioconcentration factors for worms ( $BCF_{worm}$ ), this parameter was predicted using the regression equation outlined in TGD [17]. This regression can well describe uptake by worms kept in water. However, evaluation of the model against real data indicate that the estimated  $BCF_{worm}$  in the soil are usually higher than the experimental BCFs [17]. Higher  $PEC_{oral, predator(earthworm)}$  values than those that occur in reality could therefore have been obtained in the current study, and secondary poisoning effects of APIs in terrestrial environments on earthworm-eating birds may well be overestimated. Therefore, an improvement in the accuracy of  $BCF_{worm}$  estimation in soil warrants further consideration.

To target the metabolites for prioritisation, metabolic rates and metabolites of a wide range of APIs in human have been identified from the literature (e.g. Drugbank [19]). However for substances without metabolism information, we assumed that no biodegradation and biotransformation occurred in the body to implement a conservative risk score estimation [34]. In this case, the exposures of these parent compounds in aquatic and terrestrial compartments may have been overestimated, and their metabolites will have been missed in our prioritisation list. For the highly ranked compounds without available metabolism data, it is recommended that information on the properties such as the excretion rate of parent compounds and the properties and toxicities of related metabolites should be produced.

## Conclusions

A holistic methodology has been developed and implemented to prioritise pharmaceuticals of concern that are released into the environment through wastewater. Pharmaceutical usage data in the UK has been used, together with information on the physical-chemical properties, patient metabolism and wastewater treatment removal to estimate concentrations in the aquatic and terrestrial environments. To rank the APIs, these concentrations have been compared to a range of hazard end-points. A series of end-points have been considered, including traditional risk assessment PEC/PNEC ratios for



the aquatic and terrestrial compartments as well as non-standard endpoints such as the potential for subtle pharmacological effects and the impact on animals consuming fish and earthworms.

Sixteen substances, including parent compounds from the therapeutic classes of antibiotic, antidiabetic, anti-inflammatory, antidepressant, antiobesity, antisecretory, lipid modifying agents, antiepileptics, estrogens and three metabolites have been highly ranked. Due to significant data gaps, the rankings of some compounds were based on data generated from predictive methods. A targeted monitoring study for these compounds, therefore, needs to be performed at a few treatment works to identify whether or not these high priority substances do occur in wastewater effluents and sludge.

While, the approach has been illustrated for the UK, there is no reason why the concept cannot be applied to identify APIs of priority in other regions of the World. In doing this, the risk ranking algorithms may need to be refined to reflect regionally relevant pathways of exposure. We believe that the broader application of the approach would be highly beneficial in focusing monitoring and testing on substances that really matter which should ultimately result in better protection of the natural environment and of human health.

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580 Table 1 Classification categories for chemicals without adequate available chronic aquatic toxicity  
581 data

Category	Concentration range (mg/L)
Chronic 1	<=1
Chronic 2	>1 to <=10
Chronic 3	>10 to <=100

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597 Table 2 Summary of the numbers of compounds selected for prioritisation from each compound  
 598 identification method and availability of experimental ecotoxicological data collated for the 146  
 599 compounds under consideration

Prioritisation type	Compound identification methodology	Number of compounds	Parameter	Number of compounds
Quantitative prioritisation	Primary care usage <sup>a</sup>	88 <sup>a</sup>	Acute Fish LC50	89
	Secondary care usage <sup>a</sup>	20 <sup>a</sup>	Daphnia EC50	76
	High hazard concern	12	Algae EC50	74
	Metabolites	25		
	CIP1	4	Chronic Fish LC50	13
	TOTAL	146	Daphnia EC50	40
Qualitative prioritisation	Over-the-counter	23		
			Bioconcentration factor in fish	3
			Therapeutic plasma concentration	113
			Acceptable daily intake	139
			Mammalian toxicity	65

<sup>a</sup> – three compounds, paracetamol, codeine and amoxicillin, identified as high usage in primary and secondary care

606 Table 3 Top 20 compounds from each prioritisation approach for exposure via water.

Risk Score	Low trophic levels		Higher trophic levels				F <sub>ss</sub> PC: H <sub>i</sub> PC ratio
	Acute aquatic (PEC <sub>sw</sub> / acute PNEC <sub>aquatic</sub> )	Chronic aquatic (PEC <sub>sw</sub> / chronic PNEC <sub>aquatic</sub> )	Mammalian predator		Human (uptake from drinking water)		
			PEC <sub>fish</sub> : PNEC <sub>mammal</sub>	PEC <sub>fish</sub> : ADI	Adult (PEC <sub>sw</sub> : PNEC <sub>adult</sub> )	Child (PEC <sub>sw</sub> : PNEC <sub>child</sub> )	
>10	1 amoxicillin	1 diclofenac					1 ortho-hydroxy atorvastatin
			n.d.	n.d.	n.d.	n.d.	2 para-hydroxy Atorvastatin
1 – 10	2 clarithromycin	2 atorvastatin					3 atorvastatin
	3 ciprofloxacin	3 estradiol					4 estradiol
	4 azithromycin	4 mesalazine	n.d.	n.d.	n.d.	n.d.	5 amitriptyline
	5 metformin	5 omeprazole					
	6 mesalazine						
0.1 – 1	7 paracetamol	6 paracetamol	1 diazepam				6 tamoxifen
	8 phenytoin	7 mebeverine					7 propranolol
	9 n-acetyl-5-aminosalicylic acid	8 sulfasalazine					8 norsertraline
	10 omeprazole						9 terbinafine
	11 iminoquinone						
	12 mycophenolic acid			n.d.	n.d.	n.d.	
	13 norsertraline						
	14 sulfasalazine						
	15 ranitidine						
	16 oxytetracycline						
	17 homovanillic acid						
	18 carbocisteine						
	19 mebeverine						
	20 propranolol						
<0.1		9 codeine	2 miconazole	1 miconazole	1 phenytoin	1 phenytoin	10 simvastatin
		10 fluoxetine	3 paracetamol	2 phenytoin	2 metformin	2 metformin	11
		11 azithromycin	4 propranolol	3 ortho-hydroxyatorvastatin	3 simvastatin	3 simvastatin	ethinylestradiol
		12 diltiazem	5 tramadol	4 estradiol	4 estradiol	4 estradiol	12 amlodipine
		13 mefenamic acid	6 naproxen	4 estradiol	5 codeine	5 codeine	13 diltiazem
		14 ranitidine	7 quinine	5 para-hydroxyatorvastatin	6 omeprazole sulfone	6 omeprazole sulfone <sup>d</sup>	14 fenofibrate
	n.d.	15 clarithromycin	8 trazodone	hydroxyatorvastatin	7 lisinopril	7 lisinopril	15 quetiapine
		16 terbinafine	9 diltiazem	6 simvastatin	8 paracetamol	8 paracetamol	16 miconazole
		17 metformin	10 ibuprofen	7 omeprazole sulfone	9 para-hydroxyatorvastatin	9 para-hydroxyatorvastatin	17 ibuprofen
		18 etodolac	11 ranitidine	8 2-oxoclopidogrel	10 citalopram	10 citalopram	18 azithromycin
		19 carbocisteine	12	9 omeprazole	11 ortho-hydroxyatorvastatin	11 ortho-hydroxyatorvastatin	19 tramadol
		20 atenolol	cyclophosphamide	10 propranolol	12 5'-o-desmethylatorvastatin	12 5'-o-desmethylatorvastatin	20 donepezil
			13 carbamazepine-o-quinone	11 diltiazem			
				12 norsertraline			

	14 iminoquinone	13 tramadol	omeprazole	12 5'-o-desmethyl
	15 phenytoin	14 irbesartan	13 naproxen	omeprazole
	16 2-oxoclopidogrel	15 terbinafine	14 gliclazide	13 naproxen
	17 lidocaine	16 quetiapine	15 3-hydroxy	14 gliclazide
	18 2-	17 tamoxifen	omeprazole	15 3-hydroxy
	hydroxyiminostilbene	18 citalopram	16 5-hydroxy	omeprazole
	19 mycophenolic	19 5'-o-desmethyl	omeprazole	16 5-hydroxy
	acid	omeprazole	17 2-oxoclopidogrel	omeprazole
	20 carbamazepine	20 codeine	18 omeprazole	17 2-oxoclopidogrel
	diol		19 pancreatin	18 omeprazole
			20 diltiazem	19 pancreatin
				20 diltiazem

607 n.d. no data

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Table 4 Top 20 compounds from each prioritisation approach considered, according to the predicted concentrations in soil ( $PEC_{soil}$ )

Risk score	Low trophic levels	Higher trophic levels	
		Mammalian predator	
	$PEC_{soil} : PNEC_{earthworm}$	$PEC_{earthworm} : PNEC_{mammal}$	$PEC_{earthworm} : ADI$
>10	n.d.	n.d.	n.d.
1 – 10	1 orlistat		
	2 10,11-epoxycarbamazepine	n.d.	n.d.
	3 carbamazepine		
	4 venlafaxine	n.d.	1 orlistat
	5 dipyridamole		
0.1 – 1	6 progesterone		
	7 3-hydroxyquinine		
	8 2-hydroxyiminostilbene		
	9 norsertraline		
	10 terbinafine		
	11 cyproterone	1 phenytoin	2 atorvastatin
	12 norethromycin	2 bisoprolol	3 ortho-hydroxyatorvastatin
	13 3-hydroxycarbamazepine	3 progesterone	4 tamoxifen
	14 2-hydroxycarbamazepine	4 3-hydroxyquinine	5 estradiol
	15 metoprolol	5 diazepam	5 terbinafine
	16 atorvastatin	6 10,11-epoxycarbamazepine	6 para-hydroxyatorvastatin
	17 levetiracetam	7 carbamazepine	7 bisoprolol
	18 methocarbamol	8 quinine	8 phenytoin
	19 bisoprolol	9 normorphine	9 norsertraline
	20 amitriptyline	10 fluoxetine	10 10,11-epoxycarbamazepine
<0.1		11 isosorbide	11 dipyridamole
		12 amitriptyline	12 fenofibrate
		13 miconazole	13 venlafaxine
		14 ranitidine	14 miconazole
		15 dipyridamole	15 carbamazepine
		16 3-hydroxyomeprazole	16 isosorbide
		17 5-hydroxyomeprazole	17 progesterone
		18 5'-O-desmethyl omeprazole	18 aripiprazole
		19 2-hydroxyiminostilbene	19 3-hydroxyomeprazole
		20 ibuprofen	20 5-hydroxyomeprazole

n.d. no data

630 Table 5 Classification of over the counter pharmaceuticals based on potential hazard to the aquatic  
631 environment

Pharmaceutical	Acute aquatic ecotoxicity			Chronic ecotoxicity		Classification category
	(mg/L)			(mg/L)		
	Algae	Daphnia	Fish	Daphnia	Fish	
Acrivastine	n.a.	n.a.	n.a.	n.a.	n.a.	Not classified
Amorolfine	0.69 <sup>a</sup>	0.68 <sup>a</sup>	>500 <sup>b</sup>	n.a.	n.a.	Chronic 1
Beclometasone dipropionate	n.a.	n.a.	23.7 <sup>a</sup>	n.a.	n.a.	Chronic 3
Benzalkonium chloride	0.056 <sup>b</sup>	0.037 <sup>b</sup>	0.28 <sup>b</sup>	0.04 <sup>b</sup>	0.032 <sup>b</sup>	Chronic 1
Cetirizine hydrochloride	102 <sup>a</sup>	29.6 <sup>a</sup>	n.a.	15.2 <sup>a</sup>	n.a.	Chronic 3
Cetrimide	1.03 <sup>a</sup>	1.38 <sup>a</sup>	4.63 <sup>a</sup>	n.a.	n.a.	Chronic 2
Cetylpyridinium chloride	1.26 <sup>a</sup>	0.0032 <sup>b</sup>	0.11 <sup>b</sup>	0.44 <sup>a</sup>	n.a.	Chronic 1
Chlorphenamine maleate	5.05 <sup>a</sup>	n.a.	n.a.	n.a.	n.a.	Chronic 2
Clotrimazole	n.a.	n.a.	30 <sup>b</sup>	n.a.	n.a.	Chronic 3
Dexpanthenol	n.a.	76.5 <sup>a</sup>	1220 <sup>a</sup>	n.a.	n.a.	Chronic 3
Dextromethorphan	2.6 <sup>a</sup>	0.95 <sup>a</sup>	5.81 <sup>a</sup>	2.04 <sup>a</sup>	n.a.	Chronic 1
Dimethicone	n.a.	0.36 <sup>a</sup>	5.83 <sup>a</sup>	0.096 <sup>a</sup>	n.a.	Chronic 1
Fluticasone propionate	n.a.	n.a.	39.4 <sup>a</sup>	n.a.	n.a.	Chronic 3
Guaifenesin	9.26 <sup>a</sup>	292 <sup>a</sup>	n.a.	6.08 <sup>a</sup>	n.a.	Chronic 2
Hexylresorcinol	2.19 <sup>a</sup>	11.7 <sup>a</sup>	2.89 <sup>a</sup>	3.6 <sup>a</sup>	n.a.	Chronic 2
Loperamide hydrochloride	>54 <sup>c</sup>	>56 <sup>c</sup>	>52.3 <sup>c</sup>	n.a.	n.a.	Chronic 3
Loratadine	0.7 <sup>c</sup>	0.83 <sup>c</sup>	0.38 <sup>c</sup>	n.a.	n.a.	Chronic 1
Mepyramine maleate	8.12 <sup>a</sup>	181 <sup>a</sup>	20.4 <sup>a</sup>	10.7 <sup>a</sup>	n.a.	Chronic 2

Phenylephrine	78.1 <sup>a</sup>	40.8 <sup>a</sup>	210 <sup>a</sup>	8.19 <sup>a</sup>	n.a	Chronic 2
Pholcodine	83.4 <sup>a</sup>	401 <sup>a</sup>	855 <sup>a</sup>	54.2 <sup>a</sup>	n.a	Chronic 3
Pseudoephedrine	15.7 <sup>a</sup>	95.7 <sup>a</sup>	331 <sup>a</sup>	7.23 <sup>a</sup>	n.a	Chronic 2.
Sodium cromoglicate	n.a	n.a	n.a	n.a	n.a	Not classified
Xylometazoline hydrochloride	2.17 <sup>a</sup>	n.a	0.66 <sup>a</sup>	0.49 <sup>a</sup>	n.a	Chronic 1

<sup>a</sup> estimated by QSAR toolbox; <sup>b</sup> EPA ecotox; <sup>c</sup> FASS; <sup>d</sup>

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649 Table 6 Data gaps for the highly ranked substances

Compound	Priority scheme	Comments
Amitriptyline,	Subtle pharmacological effect	Predicted F <sub>ss</sub> PC
Amoxicillin,	Acute aquatic low trophic level	Predicted K <sub>oc</sub> ,
Atorvastatin,	Chronic aquatic low trophic level	Predicted K <sub>oc</sub>
	Subtle pharmacological effect	Predicted F <sub>ss</sub> PC
Azithromycin,	Acute aquatic low trophic level	Predicted K <sub>oc</sub>
Carbamazepine,	Terrestrial low trophic level	Predicted K <sub>oc</sub> , LC50 earthworm
Ciprofloxacin,	Acute aquatic low trophic level	Predicted K <sub>oc</sub>
Clarithromycin,	Acute aquatic low trophic level	Predicted K <sub>oc</sub>
Diclofenac,	Chronic aquatic low trophic level	Predicted K <sub>oc</sub> ,
Estradiol	Subtle pharmacological effect	Predicted F <sub>ss</sub> PC
Metformin,	Acute aquatic low trophic level	Predicted K <sub>oc</sub> ,
Mesalazine	Acute aquatic low trophic level	Predicted K <sub>oc</sub> , acute daphnia LC50
	Chronic aquatic low trophic level	Predicted K <sub>oc</sub> , chronic daphnia NOEC
Omeprazole,	Chronic aquatic low trophic level	Predicted K <sub>oc</sub> , chronic daphnia NOEC
Orlistat	Terrestrial low trophic level	Predicted K <sub>oc</sub> , LC50 earthworm

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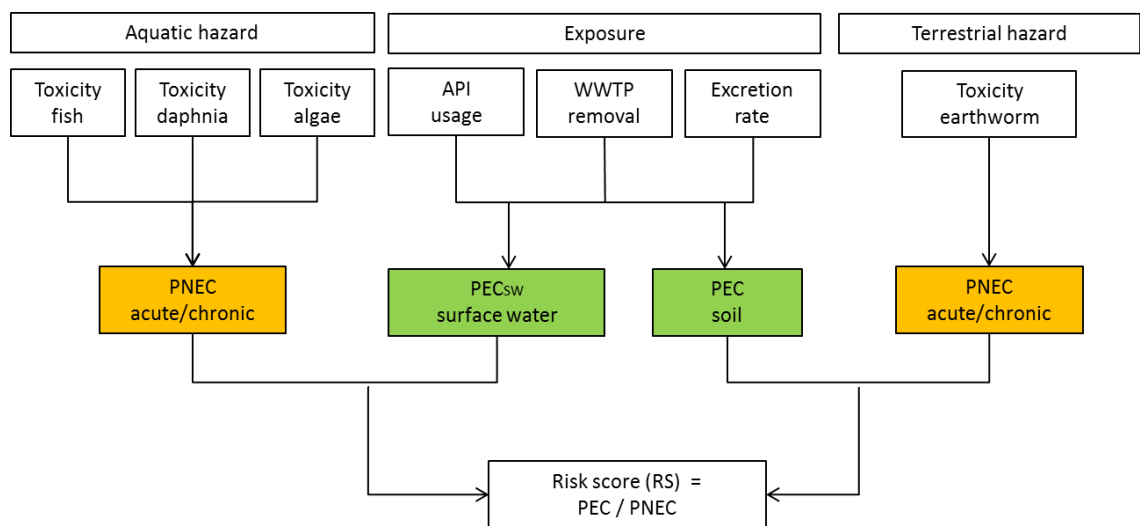
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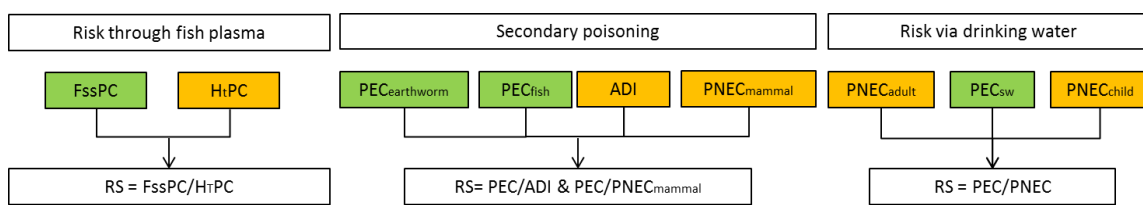
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(A)



(B)

Figure 1: The overall approach for prioritisation of activated pharmaceutical ingredients (APIs). Risk scores on (A) standard end-point effect; (B) non-standard end-point effects. Green: estimated exposure; Orange: estimated effect. PNEC<sub>aquatic</sub>: predicted no effect concentration for aquatic organisms, including fish, daphnia and algae; PEC<sub>sw</sub>: predicted environmental concentration in surface water; PEC<sub>soil</sub>: predicted environmental concentration in soil; PNEC<sub>earthworm</sub>: predicted no effect concentration in earthworm; F<sub>ss</sub>PC: fish steady state plasma concentration; H<sub>t</sub>PC: human therapeutic plasma concentration; PEC<sub>earthworm</sub>: predicted environmental concentration in earthworm; PEC<sub>fish</sub>: predicted environmental concentration in fish; ADI: acceptable daily intake for human; PNEC<sub>mammal</sub>: predicted no effect concentration in mammal; PNEC<sub>adult</sub>: predicted no effect concentration for adult; PNEC<sub>child</sub>: predicted no effect concentration for child.