
Article:
Guo, Jiahua, Sinclair, Chris J., Selby, Katherine orcid.org/0000-0002-3055-2872 et al. (1 more author) (2016) Toxicological and ecotoxicological risk-based prioritization of pharmaceuticals in the natural environment. Environmental Toxicology and Chemistry. 1550–1559. ISSN 1552-8618

https://doi.org/10.1002/etc.3319

Running head: Risk based prioritisation of pharmaceuticals

Corresponding author: Alistair B.A. Boxall

Address for communication-Environment Department, University of York, Heslington, York, YO10 5DD, UK. Telephone 01904 324791; email- alistair.boxall@york.ac.uk

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

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

†- Environment Department, University of York, Heslington, York, United Kingdom, YO10 5DD

‡- 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 exercisea 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: Activated pharmaceutical ingredients (APIs); Ecotoxicity; Exposure; Hazard; Risk score

Introduction

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

While a large amount of data has been published in the past decade on different aspects of APIs in the environment, information is still only available for a small proportion of the 1500 or so active pharmaceutical ingredients that are currently in use. It is possible, therefore, that monitoring and effects-based studies are missing substances that could be causing adverse impacts in the environment. It would be impossible to experimentally assess the hazards and risks for all the pharmaceuticals in use in a timely manner. One solution to this problem is to employ formal prioritisation approaches to identity those compounds that are likely to pose the greatest risk in a particular situation and, therefore, which need further attention. A number of prioritisation methods have already been proposed, and applied to, human and veterinary APIs [6-10]. Prioritisation approaches are also available for other classes of emerging contaminant such as pesticide metabolites [11]. Many of these approaches use exposure and toxicological predictions or information on API potency in humans so they can be readily applied to large numbers of compounds. Until now, prioritisation methods for APIs have tended to focus on risks of parent compounds in surface waters to aquatic organisms and risks to humans *via* drinking water consumption and tended to focus on single use categories (e.g. prescription or hospital use). Less emphasis has been placed on risks to other environmental compartments such as soils, sediments and ground waters, risks to top predators 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 paramaters 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 (pKa); 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 ($PEC_{earthworm}$), the concentration in the earthworms on a wet weight basis ($C_{earthworm}$) was calculated using an estimate of the concentration in porewater ($C_{porewater}$) 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 where 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 ecotoxicty 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

234 Overall 146 compounds were identified for further quantitative prioritisation, these were distributed as
235 follows: 88 were used in primary care; 20 were used in secondary care; 12 were identified as 'high
236 hazard' concern, based on expert opinion; 25 major metabolites; and 4 from the previous Chemical
237 Investigation Program (CIP1; Table 2). Twenty three compounds, sold as OTC medicines, were also
238 identified in addition to the 146 compounds for quantitative prioritisation – these underwent a
239 qualitative assessment. A summary of the available experimental toxicological data for 146 study
240 compounds is provided in Table 2. Some high profile compounds had excellent multi-species/multi-
241 endpoint datasets. However, the majority of the compounds under consideration had limited
242 ecotoxicological data available. For the standard aquatic endpoints, 82 compounds had at least one
243 experimentally derived acute or chronic ecotoxicity endpoint available. In terms of data on mammalian
244 safety, data were available on the toxicity of 65 compounds, 139 had an acceptable daily intake and
245 113 had a human therapeutic plasma concentration (H_tPC) (Table 2). Toxicological data were not
246 available for any of the identified metabolites.

247 *Ranking list development*

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

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

259 In the prioritisation based on potential of secondary poisoning in the aquatic environment (i.e. fish-
260 eating birds and mammals), diazepam was ranked the highest (RS between 0.1-1), while in terrestrial
261 environments (i.e. earthworm-eating birds and mammals) the highest ranked API was orlistat (RS 0.1-
262 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 $1x10^{-5}$. 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 e 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^{th}, 22^{nd}, 16^{th}, 5^{th}, 4^{th}, 10^{th} and 11^{th}, 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^{th} and 5^{th}, respectively [35]. Clarithromycin has been identified in a prioritisation study in Germany and ranked 34^{th} [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 feminzation 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 x 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 ($F_{ss}PC$) 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, evaulation 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

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

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

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

423

424 **Acknowledgment**

425 The authors would like to thank UK Water Industry Research Limited for funding this project (WW17C)
426 and China Scholarship Council (CSC) for funding the Ph.D. work.

427

428

429

430

431

432

433

434 **References**

435 1. Hirsch R, Ternes T, Haberer K, and Kratz KL. 1999. Occurrence of antibiotics in the aquatic
436 environment. *Sci Total Environ* 225:109-118.

437 2. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, and Buxton HT.
438 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-
439 2000: A national reconnaissance. *Environ Sci Technol* 36:1202-1211.

440 3. Ramirez AJ, Brain RA, Usenko S, Mottaleb MA, O'Donnell JG, Stahl LL, Wathen JB, Snyder
441 BD, Pitt JL, Perez-Hurtado P, Dobbins LL, Brooks BW, and Chambliss CK. 2009. Occurrence of
442 pharmaceuticals and personal care products in fish: results of a national pilot study in the United
443 States. *Environ Toxicol Chem* 28:2587-2597.

444 4. JRC. 2015. Development of the first Watch List under the Environmental Quality Standards
445 Directive. Luxembourg: Publications Office of the European Union [cited 2015 August 10]. Available
446 from: http://publications.jrc.ec.europa.eu/repository/bitstream/JRC95018/lbna27142enn.pdf.

447 5. WFD. 2012. Directive 2013/39/EU of the European parliament and of the council. Italy. [cited
448 2015 August 10]. Available from: http://eur-
449 lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:EN:PDF.

450 6. Boxall ABA, Fogg LA, Kay P, Blackwell PA, Pemberton EJ, and Croxford A. 2003.
451 Prioritisation of veterinary medicines in the UK environment. *Toxicol Lett* 142:207-218.

452 7. Capleton AC, Courage C, Rumsby P, Holmes P, Stutt E, Boxall ABA, and Levy LS. 2006.
453 Prioritising veterinary medicines according to their potential indirect human exposure and toxicity
454 profile. *Toxicol Lett* 163:213-223.

455 8. Roberts PH and Thomas KV. 2006. The occurrence of selected pharmaceuticals in
456 wastewater effluent and surface waters of the lower Tyne catchment. *Sci Total Environ* 356:143-153.

457 9. Kostich MS, Batt AL, Glassmeyer ST, and Lazorchak JM. 2010. Predicting variability of
458 aquatic concentrations of human pharmaceuticals. *Sci Total Environ* 408:4504-4510.

459 10. Sanderson H, Johnson DJ, Reitsma T, Brain RA, Wilson CJ, and Solomon KR. 2004. Ranking
460 and prioritization of environmental risks of pharmaceuticals in surface waters. *Regul Toxicol Pharm*
461 39:158-183.

462 11. Sinclair CJ, Boxall ABA, Parsons SA, and Thomas MR. 2006. Prioritization of pesticide
463 environmental transformation products in drinking water supplies. *Environ Sc Technol* 40:7283-7289.

464 12. Boxall ABA, Rudd MA, et al. 2012. Pharmaceuticals and Personal Care Products in the
465 Environment: What Are the Big Questions? *Environ Health Persp* 120:1221-1229.

466 13. NHS. 2012. Prescription Cost Analysis - England. UK. [cited 2013 December16]. Available
467 from: http://www.ic.nhs.uk/home.

468 14. Scotland. 2012. Prescription Cost Analysis - Scotland. UK. [cited 2013 December15].
469 Available from: http://www.isd.scot.nhs.uk/isd/1.html.

470 15. Welsh. 2011. Prescriptions Dispensed in the Community. UK. [cited 2013 December15].
471 Available from: http://wales.gov.uk.

472 16. Gardner M. 2013. Pharmaceuticals in wastewater treatment works' effluents (CIP program),
473 UK Water Industry Research Limited, London, UK.

474 17. TGD. 2003. Technical Guidance Document on Risk Assessment. Italy. [cited 2013 December
475 16]. Available from: http://ihcp.jrc.ec.europa.eu/our_activities/public-
476 health/risk_assessment_of_Biocides/doc/tgd.

477 18. CHMP. 2006. Guideline on the Environmental Risk Assessment of Medicinal Products for
478 Human Use. London. [cited 2013 December 16]. Available from:
479 http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000400.jsp.

480 19. Drugbank. 2013. Open Data Drug and Drug Target Database. Canada. [cited 2013 December
481 16]. Available from: http://www.drugbank.ca.

482 20. Franco A and Trapp S. 2008. Estimation of the soil-water partition coefficient normalized to
483 organic carbon for ionizable organic chemicals. *Environ Toxicol Chem* 27:1995-2004.

21. Drillia P, Stamatelatou K, and Lyberatos G. 2005. Fate and mobility of pharmaceuticals in solid matrices. *Chemosphere* 60:1034-1044.

22. Fick J, Lindberg RH, Tysklind M, and Larsson DGJ. 2010. Predicted critical environmental concentrations for 500 pharmaceuticals. *Regul Toxicol Pharm* 58:516-523.

23. Fu WJ, Franco A, and Trapp S. 2009. Methods for estimating the bioconcentration factor of ionizable organic chemicals. *Environ Toxicol Chem* 28:1372-1379.

24. Fass.se. 2011. Swedish Environmental Classification of Pharmaceuticals Database. [cited 2013 December 16]. Available from: http://www.fass.se/.

25. EPA. 2015. ECOTOX Database. US. [cited 2015 May 15. Available from: http://cfpub.epa.gov/ecotox/quick_query.htm.

26. Sinclair C and Boxall AB, 2009. Ecotoxicity of Transformation Products. In Boxall AB, eds, *Transformation Products of Synthetic Chemicals in the Environment*, 1st ed, Vol 2, Springer-Verlag Berlin Heidelberg, Berlin, Germany, pp 177-204.

27. OECD. 2013. QSAR toolbox. Available from: http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm.

28. MEDSAFE. 2013. Medicines and Medical Device Safety and Authority. New Zealand. [cited 2013 December 16]. Available from: http://www.medsafe.govt.nz/.

29. Drugs. 2014. Database for drugs. [2013 November 1]. Available from: http://www.drugs.com/.

30. Schwab BW, Hayes EP, Fiori JM, Mastrocco FJ, Roden NM, Cragin D, Meyerhoff RD, D'Aco VJ, and Anderson PD. 2005. Human pharmaceuticals in US surface waters: A human health risk assessment. *Regul Toxicol Pharm* 42:296-312.

31. ECHA, Guidance on the Application of the CLP Criteria. Finland. [cited 2015 August 10]. Available from: https://echa.europa.eu/documents/10162/13562/clp_en.pdf.

32. Roos V, Gunnarsson L, Fick J, Larsson DGJ, and Ruden C. 2012. Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. *Sci Total Environ* 421:102-110.

510 33. Ashton D, Hilton M, and Thomas KV. 2004. Investigating the environmental transport of
511 human pharmaceuticals to streams in the United Kingdom. *Sci Total Environ* 333:167-184.

512 34. Kim Y, Jung J, Kim M, Park J, Boxall ABA, and Choi K. 2008. Prioritizing veterinary
513 pharmaceuticals for aquatic environment in Korea. *Environ Toxicol Phar* 26:167-176.

514 35. Dong Z, Senn DB, Moran RE, and Shine JP. 2013. Prioritizing environmental risk of
515 prescription pharmaceuticals. *Regul Toxicol Pharm* 65:60-67.

516 36. Wang N, Guo XY, Shan ZJ, Wang ZC, Jin Y, and Gao SX. 2014. Prioritization of Veterinary
517 Medicines in China's Environment. *Hum Ecol Risk Assess* 20:1313-1328.

518 37. Webb S, Ternes T, Gibert M, and Olejniczak K. 2003. Indirect human exposure to
519 pharmaceuticals via drinking water. *Toxicol Lett* 142:157-167.

520 38. EC. 2013. Amending Directives 2000/60/EC and 2008/105/EC as regards priority substances
521 in the field of water policy. [cited 2015 August 10]. Available from: http://eur-
522 lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:EN:PDF.

523 39. Schwaiger J, Ferling H, Mallow U, Wintermayr H, and Negele RD. 2004. Toxic effects of the
524 non-steroidal anti-inflammatory drug diclofenac Part 1: histopathological alterations and
525 bioaccumulation in rainbow trout. *Aquat Toxicol* 68:141-150.

526 40. Triebskorn R, Casper H, Heyd A, Eikemper R, Kohler HR, and Schwaiger J. 2004. Toxic
527 effects of the non-steroidal anti-inflammatory drug diclofenac Part II. Cytological effects in liver, kidney,
528 gills and intestine of rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 68:151-166.

529 41. SCHER. 2011. Opinion on chemical and the water framework directive: Draft environmental
530 quality standards, diclofeanac. Brussel. [cited 2015 August 10]. Available from:
531 http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_134.pdf.

532 42. Lahnsteiner F, Berger B, Kletzl A, and Weismann T. 2006. Effect of 17 beta-estradiol on
533 gamete quality and maturation in two salmonid species. *Aquat Toxicol* 79:124-131.

534 43. Zha JM, Sun LW, Spear PA, and Wang ZJ. 2008. Comparison of ethinylestradiol and
535 nonylphenol effects on reproduction of Chinese rare minnows (*Gobiocypris rarus*). *Ecotox Environ*
536 *Safe* 71:390-399.

537 44. Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, and Flick RW. 2007.
538 Collapse of a fish population after exposure to a synthetic estrogen. *P Natl Acad Sci USA* 104:8897-
539 8901.

540 45. Santos LHMLM, Araujo AN, Fachini A, Pena A, Delerue-Matos C, and Montenegro MCBSM.
541 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment.
542 *J Hazard Mater* 175:45-95.

543 46. Halling-Sorensen B. 2000. Algal toxicity of antibacterial agents used in intensive farming.
544 *Chemosphere* 40:731-739.

545 47. Monteiro SC and Boxall ABA. 2010. Occurrence and Fate of Human Pharmaceuticals in the
546 Environment. *Rev Environ Contam* T 202: 53-154.

547 48. Agerstrand M and Ruden C. 2010. Evaluation of the accuracy and consistency of the Swedish
548 Environmental Classification and Information System for pharmaceuticals. *Sci Total Environ*
549 408:2327-2339.

550 49. NCCOS. 2013. Science Servicing Coastal Communities. USA. [cited 2013 December 16].
551 Available from: http://coastalscience.noaa.gov/.

552 50. Crane M, Watts C, and Boucard T. 2006. Chronic aquatic environmental risks from exposure
553 to human pharmaceuticals. *Sci Total Environ* 367:23-41.

554 51. Laville N, Ait-Aissa S, Gomez E, Casellas C, and Porcher JM. 2004. Effects of human
555 pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish hepatocytes. *Toxicology*
556 196:41-55.

557 52. Triebskorn R, Casper H, Scheil V, and Schwaiger J. 2007. Ultrastructural effects of
558 pharmaceuticals (carbamazepine, clofibric acid, metoprolol, diclofenac) in rainbow trout
559 (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). *Anal Bioanal Chem* 387:1405-1416.

560 53. Rocco L, Valentino IV, Peluso C, and Stingo V. 2013. Genomic Template Stability Variation in
561 Zebrafish Exposed to Pharmacological Agents. *IJEP* 3:6.

562 54. Niemuth NJ, Jordan R, Crago J, Blanksma C, Johnson R, and Klaper RD. 2015. Metformin
563 exposure at environmentally relevant concentrations causes potential endocrine disruption in adult
564 male fish. *Environ Toxicol Chem* 34:291-296.

565 55. Carter LJ, Garman CD, Ryan J, Dowle A, Bergstroem E, Thomas-Oates J, and Boxall ABA.
566 2014. Fate and Uptake of Pharmaceuticals in Soil-Earthworm Systems. *Environ Sci Technol* 48:5955-
567 5963.

568 56. Cooper RJ. 2013. Over-the-counter medicine abuse - a review of the literature. *J Subst Use*
569 18:82-107.

570 57. Statistics. 2012. Office for National Statistics. [cited 2014 May 10]. Available from:
571 http://www.statistics.gov.uk/hub/index.html.

572 58. Struijs J, Stoltenkamp J, and Vandemeent D. 1991. A spreadsheet-based box model to
573 predict the fate of xenobiotics in a municipal waste-water treatment-plant. *Water Res* 25:891-900.

574 59. Fitzsimmons PN, Fernandez JD, Hoffman AD, Butterworth BC, and Nichols JW. 2001.
575 Branchial elimination of superhydrophobic organic compounds by rainbow trout (*Oncorhynchus*
576 *mykiss*). *Aquat Toxicol* 55:23-34.

577

578

579

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

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

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 [a]	88[a]	Acute	Fish LC50	89
	Secondary care usage [a]	20[a]		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

600 [a] – three compounds, paracetamol, codeine and amoxicillin, identified as high usage in primary and secondary care
601
602
603
604
605

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

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

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

n.d. no data

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	n.d.	n.d.
	2 10,11-epoxycarbamazepine		
	3 carbamazepine		
	4 venlafaxine	n.d.	1 orlistat
	5 dipyridamole		
	6 progesterone		
0.1 – 1	7 3-hydroxyquinine		
	8 2-hydroxyiminostilbene		
	9 norsertraline		
	10 terbinafine		
	11 cyproterone	1 phenytoin	2 atorvastatin
	12 norerythromycin	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	18 aripiprazole
		omeprazole	19 3-hydroxyomeprazole
		19 2-hydroxyiminostilbene	20 5-hydroxyomeprazole
		20 ibuprofen	

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

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

632 [a] estimated by QSAR toolbox; [b] EPA ecotox; [c] FASS; [d]

633

634

635

636

637

638

639

640

641

642

643

644

645

646

647

648

649 Table 6 Data gaps for the highly ranked substances

Compound	Priority scheme	Comments
Amitriptyline,	Subtle pharmacological effect	Predicted $F_{ss}PC$
Amoxicillin,	Acute aquatic low trophic level	Predicted K_{oc},
Atorvastatin,	Chronic aquatic low trophic level	Predicted K_{oc}
	Subtle pharmacological effect	Predicted $F_{ss}PC$
Azithromycin,	Acute aquatic low trophic level	Predicted K_{oc}
Carbamazepine,	Terrestrial low trophic level	Predicted K_{oc}, LC50 earthworm
Ciprofloxacin,	Acute aquatic low trophic level	Predicted K_{oc}
Clarithromycin,	Acute aquatic low trophic level	Predicted K_{oc}
Diclofenac,	Chronic aquatic low trophic level	Predicted $K_{oc,}$
Estradiol	Subtle pharmacological effect	Predicted $F_{ss}PC$
Metformin,	Acute aquatic low trophic level	Predicted $K_{oc,}$
Mesalazine	Acute aquatic low trophic level	Predicted $K_{oc,}$ acute daphnia LC50
	Chronic aquatic low trophic level	Predicted $K_{oc,}$ chronic daphnia NOEC
Omeprazole,	Chronic aquatic low trophic level	Predicted $K_{oc,}$ chronic daphnia NOEC
Orlistat	Terrestrial low trophic level	Predicted K_{oc}, LC50 earthworm

650

651

652

653

654

655

656

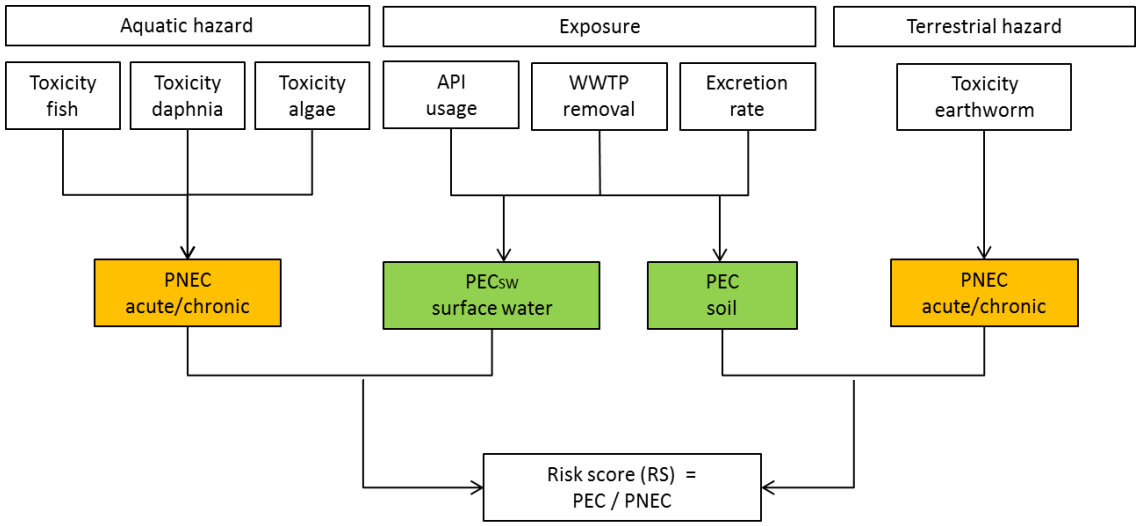
657

658

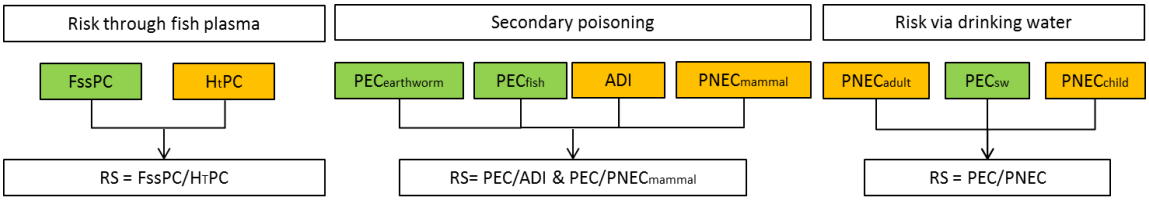
659

660

661



(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. $PNEC_{aquatic}$: predicted no effect concentration for aquatic organisms, including fish, daphnia and algae; PEC_{sw}: predicted environmental concentration in surface water; PEC_{soil}: predicted environmental concentration in soil; $PNEC_{earthworm}$: predicted no effect concentration in earthworm; $F_{ss}PC$: fish steady state plasma concentration; H_tPC: human therapeutic plasma concentration; $PEC_{earthworm}$: predicted environmental concentration in earthworm; PEC_{fish}: predicted environmental concentration in fish; ADI: acceptable daily intake for human; $PNEC_{mammal}$: predicted no effect concentration in mammal; $PNEC_{adult}$: predicted no effect concentration for adult; $PNEC_{child}$: predicted no effect concentration for child.