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TOXICOLOGICAL AND ECOTOXICOLOGICAL RISK-BASED PRIORITIZATION OF PHARMACEUTICALS IN THE NATURAL ENVIRONMENT

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Abstract: Approximately 1500 active pharmaceutical ingredients are currently in use; however, the environmental occurrence and impacts of only a small proportion of these have been investigated. Recognizing that it would be impractical to monitor and assess all pharmaceuticals that are in use, several previous studies have proposed the use of prioritization approaches to identify substances of most concern so that resources can be focused on these. All of these previous approaches suffer from limitations. In the present study, the authors draw on experience from previous prioritization exercises and present a holistic approach for prioritizing 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 nonapical effects related to the therapeutic mode of action. Application of the approach is illustrated for 146 active pharmaceuticals that are used either in the community or in hospital settings in the United Kingdom. Using the approach, 16 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. In the future, the prioritization approach should be applied more broadly around the different regions of the world. *Environ Toxicol Chem* 2016;35:1550–1559. © 2016 SETAC

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INTRODUCTION

Active pharmaceutical ingredients (APIs) have been detected widely in the natural environment across the world [1–3]. Because APIs are biologically active compounds, designed to interact with specific pathways/processes in target humans and other animals, concerns have been raised over the potential side effects of these substances in the environment. Over the past 15 yr, 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, 7 pharmaceuticals/hormones have been placed on the watch list under the European Environmental Quality Standards Directive [4] and the Water Framework Directive [5], and it is possible that, in the future, these compounds will be included in European statutory monitoring programs.

Although a large amount of data has been published in the past decade on different aspects of APIs in the environment, information is still available only for a small proportion of the 1500 or so APIs 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 prioritization approaches to identify those compounds that are likely to pose the greatest risk in a particular situation and, therefore, need further attention. A number of prioritization methods have already been proposed for, and applied to, human and veterinary APIs [6–10]. Prioritization approaches are also available for other classes of emerging contaminants, 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, prioritization 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 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 risks of metabolites of APIs.

In the present study, we describe a holistic risk-based prioritization approach for identifying APIs of concern in aquatic and terrestrial systems. The prioritization approach is illustrated with 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 either that were highly used in the United Kingdom or that experts indicated might be of environmental concern. Although the approach has been applied to the UK situation, there is no reason why it cannot be applied to prioritize APIs in use in other regions of the world.

METHODS

The prioritization approach used risk scores as the primary parameter to rank the APIs in terms of their potential environmental risk (Figure 1). Risk score values were calculated

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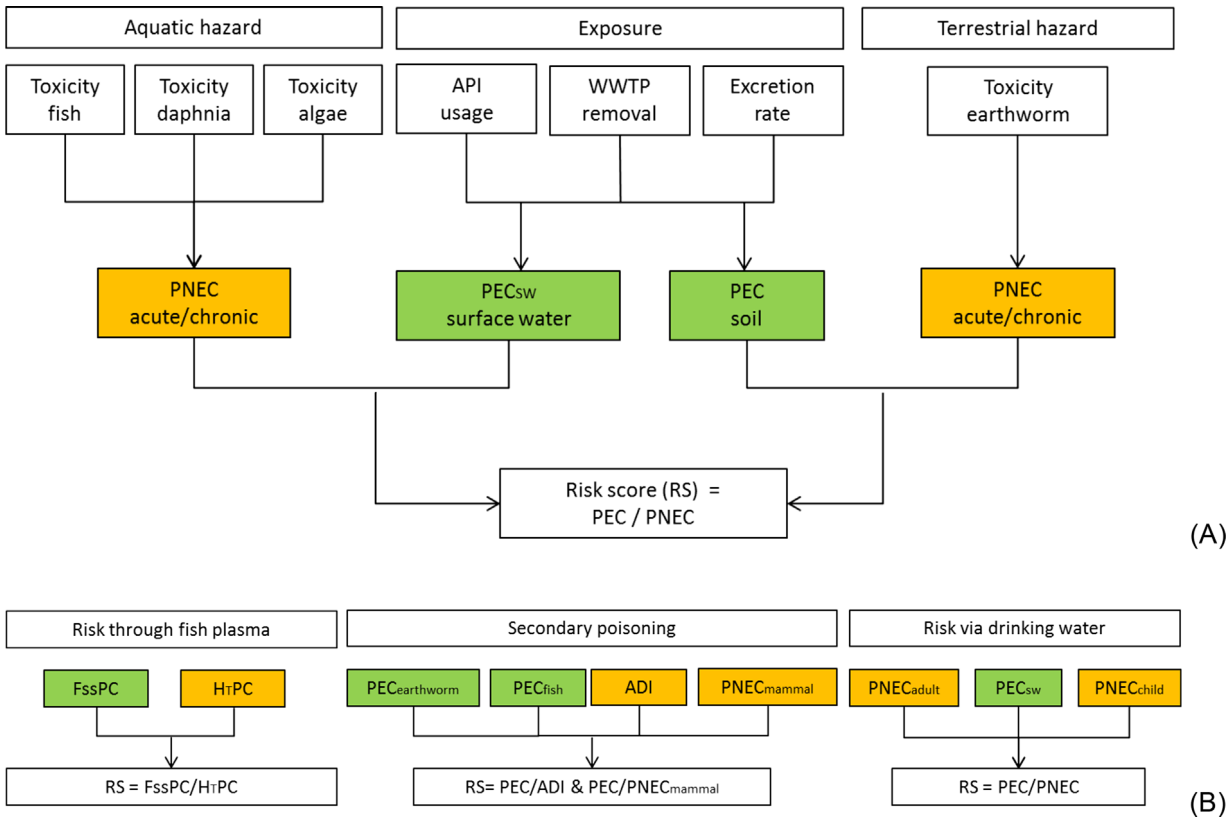

Figure 1. The overall approach for prioritization of activated pharmaceutical ingredients (APIs): risk scores on (**A**) standard endpoint effect and (**B**) non-standard endpoint effects. Green boxes represent exposure concentrations for each compartment, and orange boxes represent effects concentrations. WWTP = wastewater treatment plant; PEC_{sw}/$PEC_{earthworm}$/PEC_{fish} = predicted exposure concentration in surface water, earthworm, and fish, respectively; FssPC = steady state concentration in fish plasma; $PNEC_{mammal}$/$PNEC_{adult}$/$PNEC_{child}$ = predicted no-effect concentration in mammals, adults, and children, respectively; H_TPC = human plasma therapeutic concentration; ADI = acceptable daily intake.

by comparing predictions of exposure of APIs in different environmental compartments to measures of potential hazard toward different organisms from different trophic levels. The prioritization 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). 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 prioritization

In the United Kingdom, the main ways that pharmaceuticals are made available to patients are through the fulfillment 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 United Kingdom. We therefore developed a substance list for prioritization 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 e-mail. 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 perceived to be of high concern.

Annual pharmaceutical usage data for the most prescribed pharmaceuticals in primary care (by active ingredient mass) in the United Kingdom were collated from prescription cost analysis data available for England [13], Scotland [14], and Wales [15]. The available prescription cost analysis data obtained from Northern Ireland were not sufficient to calculate pharmaceutical usage. To reduce the time required to collate the data, the usage of all pharmaceuticals present in the prescription cost analysis 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 the present project were excluded from further prioritization. These compounds were alginic acid compound preparations, calcium carbonate, co-magaldrox (magnesium/aluminium hydroxide), ergocalciferol, ferrous fumarate, ferrous sulfate, glucose, lithium carbonate, omega-3 marine triglycerides, potassium chloride, sodium bicarbonate, and sodium valproate.

Data on pharmaceutical usage in secondary care in 2012 were provided to the project team by the British Generic Manufacturers Association. Data were provided on the usage, by mass, of the 20 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 was obtained from information available on online retailer websites.

Because some compounds will be extensively metabolized in the body, the environment will be exposed to the metabolite of these substances and not to the parent compound. Therefore, data were 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 in the United Kingdom has monitored 12 pharmaceuticals in wastewater treatment plant (WWTP) effluent [16]. Compounds that were monitored in the Chemical Investigation Program but not in the top usage compound list or not identified by the experts were also added to the list for prioritization. Overall, 146 compounds were identified for further quantitative prioritization. An additional 23 compounds were identified that are available over the counter, which were ranked using a more simple chemical classification approach because of the absence of quantitative usage data.

Environmental exposure estimation

Predicted environmental concentrations (PECs) of selected pharmaceuticals in surface waters (PEC_{SW}) and terrestrial systems 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), the octanol–water partition coefficient (K_{OW}), the solid-water distribution coefficient (K_d), and the organic carbon partition coefficient (K_{OC}). These data were collated from a number of sources, including peer-reviewed literature, gray 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 ionizable 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 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 [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 (BCF) for 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 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, the concentration in the earthworms on a wet weight basis was calculated using an estimate of the concentration in porewater and the BCF for earthworms calculated according to the approach in the technical guidance document (Supplemental Data, Equations 21–23) [17].

Hazard characterization

Predicted no-effect concentrations (PNECs) of pharmaceuticals were derived based on either experimental or estimated ecotoxicity data, using appropriate safety factors from the technical guidance document [17] (Supplemental Data, Equation 24). Where multiple ecotoxicological values were available, the most sensitive endpoint 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 nonstandard taxa and endpoints, 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. For these compounds, therefore estimation techniques were used to fill the data gaps. A read-across approach using the Organisation for Economic Co-operation and Development's (OECD's) 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 pharmaceuticals with no data. Then, a relationship was built within the software 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: The 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 Environmental Protection Agency [USEPA]). 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-d 50% lethal concentration [LC50] in mM/kg dry soil) was predicted using the quantitative structure–activity relationship (QSAR) available in ECOSAR (USEPA; Supplemental Data, Equation 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 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 was generated from the median lethal dose 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 and a child based on acceptable daily intakes for each API using the model of Schwab et al. [30] (Supplemental Data, Equation 26).

Ranking scenarios

To prioritize substances, a risk score was calculated for the different exposure pathway/toxicity endpoint combinations by

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

dividing the relevant exposure concentration by the relevant hazard concentration (Figure 1). 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 toward the top of the ranking deemed to be of most interest for that particular pathway and endpoint.

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

RESULTS

Target APIs and collation of pharmaceutical effect data

Overall, 146 compounds were identified for further quantitative prioritization, 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 were major metabolites; and 4 were from the previous Chemical Investigation Program (Table 2). Twenty-three compounds, sold as over-the-counter medicines, were also identified in addition to the 146 compounds for quantitative prioritization; 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 multispecies/multi-endpoint data sets. However, the majority of the compounds under consideration had limited ecotoxicological data available. For the standard aquatic endpoints, 82 compounds had at least 1 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 an 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 prioritizations for the aquatic and terrestrial environments are provided in Tables 3 and 4. The prioritization based on apical acute aquatic effects at lower trophic levels indicated that amoxicillin, clarithromycin, ciprofloxacin, azithromycin, and mesalazine had the highest risk scores (risk score >1). For the aquatic apical chronic prioritization process, diclofenac, atorvastatin, estradiol, mesalazine, and omeprazole demonstrated the greatest risk score (>1). The highest-ranked compounds based on apical acute effects in soil organisms were orlistat, carbamazepine, and the carbamazepine metabolite 10,11-epoxycarbamazepine (risk score 1–10; Table 4).

When the potential impact of subtle pharmacological effects was considered by comparing the H_TPC to estimated levels in fish, the atorvastatin metabolites ortho-hydroxyatorvastatin and para-hydroxyatorvastatin were ranked highest (risk score >10), with atorvastatin, estradiol, and amitriptyline just below these substances (risk score 1–10; Table 3).

In the prioritization based on potential of secondary poisoning in the aquatic environment (i.e., fish-eating birds and mammals), diazepam was ranked the highest (risk score 0.1–1), whereas in terrestrial environments (i.e., earthworm-eating birds and mammals), the highest-ranked API was orlistat (risk score 0.1–1). All other pharmaceuticals had a risk score <0.1 (Table 4). The risk scores of APIs prioritized according to human consumption in drinking water for all compounds were less than 1×10^{-5}. The top-ranked compounds were phenytoin, metformin, and simvastatin (Table 3).

For over-the-counter 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, because no toxicity data were

Table 2. Summary of the numbers of compounds selected for prioritization from each compound identification method and availability of experimental ecotoxicological data collated for the 146 compounds under consideration

Prioritization type	Compound identification methodology	No. of compounds	Parameter	No. of compounds
Quantitative prioritization	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		
	Chemical Investigation Program 1	4	Chronic fish LC50	13
	Total	146	*Daphnia* EC50	40
Qualitative prioritization	Over-the-counter	23		
			Bioconcentration factor in fish	3
			Therapeutic plasma concentration	113
			Acceptable daily intake	139
			Mammalian toxicity	65

[a]Three compounds—paracetamol, codeine, and amoxicillin—identified as high usage in primary and secondary care.
EC50 = 50% effective concentration; LC50 = 50% lethal concentration.

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

	Low trophic levels		Higher trophic levels: Mammalian predator		Higher trophic levels: Human (uptake from drinking water)		
Risk score	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-hydroxya torvastatin
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 14. Iminoquinone 15. Phenytoin 16. 2-oxoclopidogrel 17. Lidocaine 18. 2-hydroxyiminostilbene 19. Mycophenolic acid 20. Carbamazepine diol	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 13. Tramadol 14. Irbesartan 15. Terbinafine 16. Quetiapine 17. Tamoxifen 18. Citalopram 19. 5'-o-desmethyl omeprazole 20. Codeine	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 omeprazole 13. Naproxen 14. Gliclazide 15. 3-hydroxy omeprazole 16. 5-hydroxy omeprazole 17. 2-oxoclopidogrel 18. Omeprazole 19. Pancreatin 20. Diltiazem	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 omeprazole 13. Naproxen 14. Gliclazide 15. 3-hydroxy omeprazole 16. 5-hydroxy omeprazole 17. 2-oxoclopidogrel 18. Omeprazole 19. Pancreatin 20. Diltiazem	10. Ssimvastatin 11. Ethinylestradiol 12. Amlodipine 13. Diltiazem 14. Fenofibrate 15. Quetiapine 16. Miconazole 17. Ibuprofen 18. Azithromycin 19. Tramadol 20. Donepezil

ADI = acceptable daily intake; $F_{SS}PC$ = fish steady-state plasma concentration; H_TPC = human plasma therapeutic concentration; n.d. = no data; PEC_{FISH} = predicted environmental concentration in fish; PEC_{SW} = predicted environmental concentration in surface water; $PNEC_{ADULT}$/$PNEC_{CHILD}$ = predicted no-effect concentrations in adults and children; $PNEC_{AQUATIC}$/$PNEC_{MAMMAL}$ = predicted no-effect concentrations in aquatic and mammalian organisms.

Table 4. Top 20 compounds from each prioritization approach considered, according to the predicted concentrations in soil

	Low trophic levels	Higher trophic levels	
		Mammalian predator	
Risk score	PEC_{SOIL}:$PNEC_{WORM}$	$PEC_{EARTHWORM}$:$PNEC_{MAMMAL}$	$PEC_{EARTHWORM}$:ADI
>10	n.d.	n.d.	n.d.
1–10	1. Orlistat 2. 10,11-epoxycarbamazepine 3. Carbamazepine	n.d.	n.d.
0.1–1	4. Venlafaxine 5. Dipyridamole 6. Progesterone 7. 3-hydroxyquinine 8. 2-hydroxyiminostilbene 9. Norsertraline10. Terbinafine	n.d.	1. Orlistat
<0.1	11. Cyproterone 12. Norerythromycin 13. 3-hydroxycarbamazepine 14. 2-hydroxycarbamazepine 15. Metoprolol 16. Atorvastatin 17. Levetiracetam 18. Methocarbamol 19. Bisoprolol 20. Amitriptyline	1. Phenytoin 2. Bisoprolol 3. Progesterone 4. 3-hydroxyquinine 5. Diazepam 6. 10,11-epoxycarbamazepine 7. Carbamazepine 8. Quinine 9. Normorphine 10. Fluoxetine 11. Isosorbide 12. Amitriptyline 13. Miconazole 14. Ranitidine 15. Dipyridamole 16. 3-hydroxyomeprazole 17. 5-hydroxyomeprazole 18. 5'-o-desmethyl omeprazole 19. 2-hydroxyiminostilbene 20. Ibuprofen	2. Atorvastatin 3. Ortho-hydroxyatorvastatin 4. Tamoxifen 5. Estradiol 6. Terbinafine 7. Para-hydroxyatorvastatin 8. Bisoprolol 9. Phenytoin 10. Norsertraline 11. 10,11-epoxycarbamazepine 12. Dipyridamole 13. Fenofibrate 14. Venlafaxine 15. Miconazole 16. Carbamazepine 17. Isosorbide 18. Progesterone 19. Aripiprazole 20. 3-hydroxyomeprazole 21. 5-hydroxyomeprazole

ADI = acceptable daily intake; n.d. = no data; $PEC_{EARTHWORM}$/PEC_{SOIL} = predicted environmental concentrations in earthworm and in soil; $PNEC_{MAMMAL}$/$PNEC_{WORM}$ = predicted no-effect concentrations in mammal and in worm.

available and the estimation approaches did not work for these substances.

DISCUSSION

Results comparisons

Sixteen 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, 10,11-epoxycarbamazepine), were identified that had a risk score >1 for 1 or more of the risk comparisons. A substance with a risk score >1 indicates that the estimated exposure is higher than the PNEC, so more attention should be paid because the hazards might occur in the different environmental compartments.

The ranking results for parent compounds agree with some of the previous prioritization 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 rankings at 12th, 22nd, 16th, 5th, 4th, 10th, and 11th, respectively. The risk score of diclofenac [33] was also reported with a low risk score of 0.01 in a United Kingdom stream case study. Amoxicillin has been ranked the top in several veterinary medicine prioritization studies, where it was classified as a substance with high hazard to aquatic environments in the United Kingdom [6,7], Korea [34], the United States [35], and China [36]. Azithromycin and metformin were identified in a US surface water exercise, being ranked 12th and 5th, respectively [35]. Clarithromycin was identified in a prioritization study in Germany and ranked 34th [37]. Ciprofloxacin was classified as a substance with a high ranking (8th) in the aquatic environment in the United States [35]; it also was assigned to categories with a high and medium toxicity in China [36] and Korea [34], respectively. Omeprazole was considered in the prioritization studies in the United States and Sweden, ranking 18th and 22nd, respectively [32,35].

Previously published work considering the prioritization of pharmaceuticals has focused only on parent compounds [8,32]; in reality, however, following consumption by patients, compounds may be metabolized and excreted as metabolites, partly or completely [6]. The present study is the first study to consider 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 prioritization indices. The classification of over-the-counter APIs is a novel method applied in a prioritization exercise, and therefore, no published works are available with which to compare our findings.

Potential risk of highly ranked substances in the environment

Several 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

Table 5. Classification of over-the-counter pharmaceuticals based on potential hazard to the aquatic environment

	Acute aquatic ecotoxicity (mg/L)			Chronic ecotoxicity (mg/L)		
Pharmaceutical	Algae	*Daphnia*	Fish	*Daphnia*	Fish	Classification category
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

[a]Estimated by quantitative structure–activity relationship (QSAR) toolbox [27].
[b]US Environmental Protection Agency's ECOTOX [25].
[c]FASS database [24].
n.a. = not applicable.

in water, 3 APIs—diclofenac and the 2 hormones 17β-estradiol (E2) and 17α-ethinylestradiol (EE2)—have been added to the European Union's pollutant watch list; 2 of these (diclofenac and E2) appear in our top 16 list. Although EE2 did not fall in the top 16, it was still ranked highly using the plasma therapeutic concentration approach (11th), even though the amounts of this compound used in the United Kingdom are small. Side effects of diclofenac on fish kidneys (histopathological damage) 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]. The hormones E2 and EE2 are the 2 APIs for which toxicity has been determined at environmentally relevant concentrations. The former is a natural estrogen with endocrine-disrupting properties. Potent effects of E2 on gamete quality and maturation in 2 salmonid species (rainbow trout [*Oncorhynchus mykiss*] and grayling [*Thymallus thymallus*]) have been reported, even at nanograms-per-liter exposure concentration levels [42]. The hormone EE2 has been ranked in the top 20 list (Table 3). There is widespread evidence that exposure of male fish to EE2 at nanograms-per-liter levels can result in feminization [43] and that chronic exposure of fish (i.e., fathead minnow [*Pimephales promelas*]) to EE2 could ultimately result in 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 the 4 antibiotics erythromycin, clarithromycin, azithromycin, and ciprofloxacin have been added. The inclusion of antibiotics in the watch list is mainly because of 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-h/96-h acute EC50 values with growth as the endpoint for these 3 antibiotics are 0.002 mg/L (*Pseudokirchneriella subcapitata*) [45], 0.001 μg/L (unreported blue-green algae) [24], and 0.005 mg/L (*Microcystis aeruginosa*) [46], for clarithromycin, azithromycin, and ciprofloxacin, respectively.

The occurrence of some of the highly ranked parent APIs in the aquatic environment has been reported at nanograms-per-liter concentrations in surface waters and up to micrograms-per-liter concentrations in WWTP effluents [47]. Amitriptyline was reported to inhibit the growth of the macrophyte *Lemna minor* with a 7-d EC50 of 1.69 mg/L [48] and to 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 no-observed-effect concentration (0.013 μg/L) of atorvastatin with a genetic endpoint was documented for zebrafish (*Danio rerio*) [25] and a 48-h LC50 of 1.35 mg/L for metformin for the crustacea *D. magna* [50]. Although 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 were 0.031 mg/L and 0.009 mg/L, respectively, both of these being for *D. magna*. Hazards of 5 classified over-the-counter APIs to 3 aquatic trophic levels are illustrated in Table 5. Of the 3 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 prioritized APIs on organism and population levels of nontarget 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 (*O. mykiss*) with a 24-h EC50 >182.7 mg/L [51]. Detrimental effects of carbamazepine on the liver and kidney

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

$F_{SS}PC$ = fish steady-state plasma concentration; K_{OC} = organic carbon partition coefficient; LC50 = 50% lethal concentration; NOEC = no-observed-effect concentration.

cytopathology of rainbow trout (*O. mykiss*) have been observed with lowest-observed-effect concentrations $>$ 0.1 mg/L 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 (*P. promelas*), through inducing significant upregulation of mRNA encoding the protein vitellogenin.

In terrestrial environments, the antiepileptic carbamazepine and the antiobesity agent orlistat were the 2 highest-ranked substances. The occurrence of carbamazepine in soil was reported at concentrations up to 6.85×10^{-3} mg/kg, and the QSAR-based 14-d LC50 toxicity to earthworm was 1060 mg/kg. Although 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 recognized that prioritization 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 prioritized substances selected from subtle pharmacological effect scenarios, exposures ($F_{SS}PC$) were all estimated from log K_{OW} on the basis of QSARs.

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 recognized that, in addition to 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 over-the-counter compounds during the present study, but these were unsuccessful. A previous study has estimated that in Germany over-the-counter 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 the present project. An accurate quantification approach of over-the-counter 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 this sludge was then applied to the land [18]. Experimentally determined biodegradation data of APIs were not available. Predicted environmental concentrations and, therefore, the risk scores of APIs that were susceptible to biodegradation during wastewater treatment will have been significantly overestimated. Limited information on experimental physical–chemical properties such as 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 for 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 were available on bioconcentration factors for worms (BCF_{worm}), this parameter was predicted using the regression equation outlined in the technical guidance document [17]. This regression can well describe uptake by worms kept in water. However, evaulation of the model against real data indicated that the estimated BCF_{worm} values in the soil are usually higher than the experimental BCFs [17]. Higher $PEC_{earthworm,}$ 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 prioritization, 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 the present prioritization list. For the highly ranked compounds without available metabolism data, it is recommended that information on 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 prioritize pharmaceuticals of concern that are released into the environment through wastewater. Pharmaceutical usage data in the United Kingdom have 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 endpoints. A series of endpoints was considered, including traditional risk-assessment PEC/PNEC ratios for the aquatic and terrestrial compartments, and nonstandard 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 3 metabolites have been highly ranked. Because of 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 these high-priority substances do occur in wastewater effluents and sludge.

Although the approach has been illustrated for the United Kingdom, 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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Data availability—Data, associated metadata, and calculation tools are available by contacting the author (alistair.boxall@york.ac.uk).

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