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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>

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

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

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40 **Abstract**

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

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

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65 **Introduction**

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

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

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

103 **Methods**

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

112 *Identification of substances for prioritisation*

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

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

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

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

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

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

156 *Environmental exposure estimation*

157 Predicted environmental concentrations of selected pharmaceuticals in surface waters (PEC_{sw}) and
158 terrestrial systems (PEC_{soil}) were estimated using standard algorithms that are described in existing
159 regulatory guidance documents (Supplemental Data, Equations 1-7) [17, 18]. The algorithms assume
160 that pharmaceutical usage by the population is distributed evenly both temporally and spatially. The
161 property data for APIs, collated to aid the determination of environmental exposure, included the acid
162 dissociation constant (pKa); octanol-water partition coefficient (K_{ow}); solid-water distribution coefficient
163 (K_d) and organic carbon partition coefficient (K_{oc}). These data were collated from a number of sources
164 including the peer-reviewed literature, grey literature and available online databases (e.g. drugbank
165 [19]). Where experimentally determined data were unavailable, estimation tools, such as Quantitative
166 Structure-Property Relationships [17, 20, 21] were used to fill the data gaps. For example, K_{oc} was
167 predicted using an estimation model developed for ionisable organic chemicals (Supplemental Data,
168 Equations 8-11). Default values of pH of soil recommended by the model developers [20] were used
169 in the K_{oc} estimation (i.e. 5.8 for acids and pH 4.5 for bases).

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

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

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

183 *Hazard characterisation*

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

188 Chronic and acute aquatic and terrestrial ecotoxicity data for standard test taxa (e.g. earthworm,
189 green algae, daphnia and fish), together with non-standard taxa and end-points, were collated for the
190 146 pharmaceuticals (and relevant metabolites) under consideration (e.g. from the Fass [24] and
191 ECOTOX [25] databases). A number of the compounds under consideration had no available
192 experimentally derived ecotoxicological aquatic data. Therefore, for these compounds estimation
193 techniques were used to fill the data gaps. A read-across approach using the OECD QSAR Toolbox
194 was used for pharmaceuticals, and the estimation approach of Escher et al. [26] was used for
195 metabolites. The database present in the OECD QSAR Toolbox was used to identify experimental
196 data for molecules deemed 'similar' to each of the individual pharmaceutical with no data. Then within
197 the software a relationship was built to allow an estimation of the ecotoxicological endpoint for the
198 query molecule. The approach adopted for the identification of similar compounds was to combine the
199 protein-binding profile with endpoint specific ones, as suggested by the Toolbox instruction manual
200 [27]. The main procedures in the software were as follows: protein binding profile was selected as a
201 group method to define the category. Subcategories were then established based on the
202 classification system used by ECOSAR (US EPA). The results were then followed by a refinement for
203 structural similarity (70 - 90% similar). The identified chemicals were then used to read across and
204 estimate ecotoxicity data for the query pharmaceutical. Metabolite aquatic ecotoxicity data gaps were
205 filled using the estimation approach for pharmaceutical metabolites proposed by Escher et al. [26]
206 which uses the principle of the toxic ratio and parent ecotoxicological data to estimate the toxic range
207 for the metabolite. For compounds with no experimentally determined earthworm ecotoxicity data, the

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

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

219 *Ranking scenarios*

220 To prioritise substances a risk score was calculated for the different exposure pathway/toxicity
221 endpoint combinations by dividing the relevant exposure concentration by the relevant hazard
222 concentration (Figure 1 A, B). For example, to calculate the risk score for subtle effects on fish the
223 $F_{ss}PC$ was divided by the H_tPC . Compounds were then ranked based on their risk score with
224 substances towards the top of the ranking deemed to be of most interest for that particular pathway
225 and endpoint.

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

232 **Results**

233 *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

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

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

273 **Discussion**

274 *Results comparisons*

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

282 The ranking results for parent compounds agree with some of the previous prioritisation studies.
283 Amitriptyline, atorvastatin, carbamazepine, diclofenac, estradiol, mesalazine and orlistat were
284 identified as priority substances in use in the Swedish market by Roos et al. [32], with the ranking at
285 12th, 22nd, 16th, 5th, 4th, 10th and 11th, respectively. The risk score of diclofenac [33] was also reported
286 with a low RS value of 0.01 in a UK stream case study. Amoxicillin has been ranked the top in several
287 veterinary medicine prioritisation studies, where it was classified as a substance with high hazard to
288 aquatic environments in the UK [6, 7], Korea [34], US [35] and China [36]. Azithromycin and
289 metformin were identified in a US surface water exercise, being ranked 12th and 5th, respectively [35].
290 Clarithromycin has been identified in a prioritisation study in Germany and ranked 34th [37].

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

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

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

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

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

322 The watch list has been further developed in the European Environmental Quality Standards Directive
323 [4], where four antibiotics including erythromycin, clarithromycin, azithromycin and ciprofloxacin have
324 been added. The inclusion of antibiotics in the watch list is mainly due to their potential toxic effects to
325 algal species. Three of these antibiotics (clarithromycin, azithromycin and ciprofloxacin) were
326 identified as top priority in the current study. The 72/96 h acute EC50 values with growth as the
327 endpoint for these free antibiotics are 0.002 mg/L (*Pseudokirchneriella subcapitata*) [45], 0.001 ug/L
328 (unreported blue-green algae) [24] and 0.005 mg/L (*Microcystis aeruginosa*) [46], respectively.

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

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

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

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

364 *Limitation of methods and future improvement*

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

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

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

385 In the secondary poisoning assessment of APIs in the terrestrial compartment, as very limited
386 experimental data was available on bioconcentration factors for worms (BCF_{worm}), this parameter was
387 predicted using the regression equation outlined in TGD [17]. This regression can well describe
388 uptake by worms kept in water. However, evaluation of the model against real data indicate that the
389 estimated BCF_{worm} in the soil are usually higher than the experimental BCFs [17]. Higher $PEC_{oral,}$
390 $predator(earthworm)$ values than those that occur in reality could therefore have been obtained in the current
391 study, and secondary poisoning effects of APIs in terrestrial environments on earthworm-eating birds
392 may well be overestimated. Therefore, an improvement in the accuracy of BCF_{worm} estimation in soil
393 warrants further consideration.

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

402 **Conclusions**

403 A holistic methodology has been developed and implemented to prioritise pharmaceuticals of concern
404 that are released into the environment through wastewater. Pharmaceutical usage data in the UK has
405 been used, together with information on the physical-chemical properties, patient metabolism and
406 wastewater treatment removal to estimate concentrations in the aquatic and terrestrial environments.
407 To rank the APIs, these concentrations have been compared to a range of hazard end-points. A
408 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.

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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.

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

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

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

Prioritisation type	Compound identification methodology	Number of compounds	Parameter	Number of compounds
Quantitative prioritisation	Primary care usage ^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

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

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

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

607 n.d. no data

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

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

627 n.d. no data

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

Pharmaceutical	Acute aquatic ecotoxicity			Chronic ecotoxicity		Classification category
	(mg/L)			(mg/L)		
	Algae	Daphnia	Fish	Daphnia	Fish	
Acrivastine	n.a.	n.a.	n.a.	n.a.	n.a.	Not classified
Amorolfine	0.69 ^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

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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

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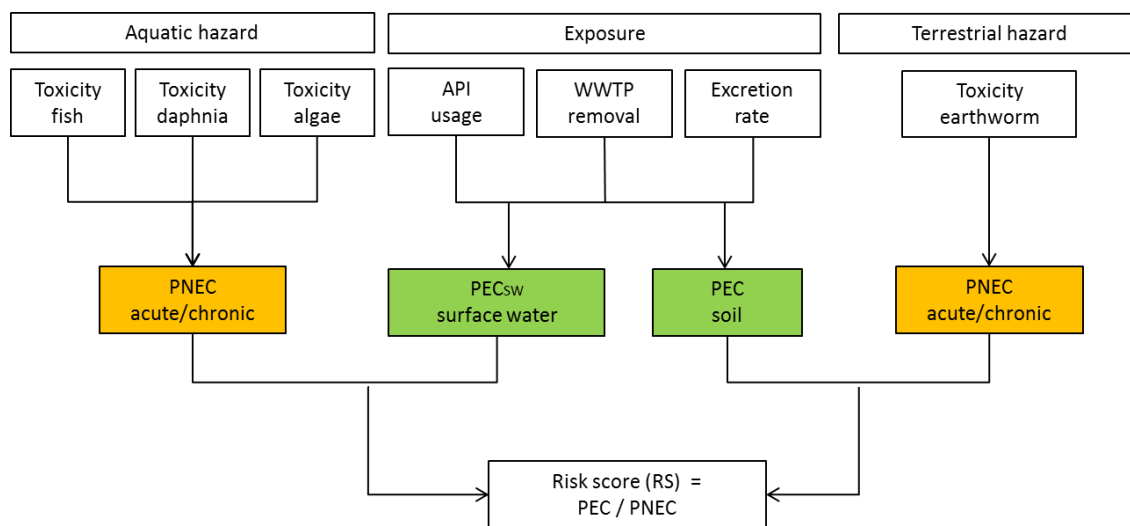
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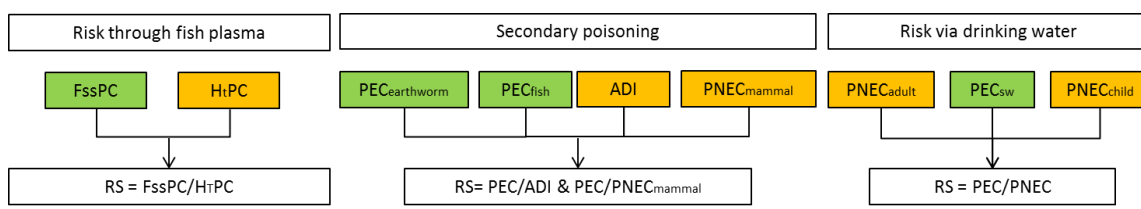
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662 (A)



663 (B)

664 Figure 1: The overall approach for prioritisation of activated pharmaceutical ingredients (APIs). Risk
 665 scores on (A) standard end-point effect; (B) non-standard end-point effects. Green: estimated
 666 exposure; Orange: estimated effect. $PNEC_{aquatic}$: predicted no effect concentration for aquatic
 667 organisms, including fish, daphnia and algae; PEC_{sw} : predicted environmental concentration in
 668 surface water; PEC_{soil} : predicted environmental concentration in soil; $PNEC_{earthworm}$: predicted no effect
 669 concentration in earthworm; $F_{ss}PC$: fish steady state plasma concentration; H_tPC : human therapeutic
 670 plasma concentration; $PEC_{earthworm}$: predicted environmental concentration in earthworm; PEC_{fish} :
 671 predicted environmental concentration in fish; ADI: acceptable daily intake for human; $PNEC_{mammal}$:
 672 predicted no effect concentration in mammal; $PNEC_{adult}$: predicted no effect concentration for adult;
 673 $PNEC_{child}$: predicted no effect concentration for child.

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