Pharmacology beyond the patient – The environmental risks of human drugs

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(article starts on next page)

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Pharmacology beyond the patient – The environmental risks of human drugs

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ABSTRACT

Background: The presence of pharmaceuticals in the environment is a growing global concern and although environmental risk assessment is required for approval of new drugs in Europe and the USA, the adequacy of the current triggers and the effects-based assessments has been questioned.

Objective: To provide a comprehensive analysis of all regulatory compliant aquatic ecotoxicity data and evaluate the current triggers and effects-based environmental assessments to facilitate the development of more efficient approaches for pharmaceuticals toxicity testing.

Methods: Publicly-available regulatory compliant ecotoxicity data for drugs targeting human proteins was compiled together with pharmacological information including drug targets, Cmax and lipophilicity. Possible links between these factors and the ecotoxicity data for effects on, growth, mortality and/or reproduction, were evaluated. The environmental risks were then assessed based on a combined analysis of drug toxicity and predicted environmental concentrations based on European patient consumption data.

Results: For most (88%) of the of 975 approved small molecule drugs targeting human proteins a complete set of regulatory compliant ecotoxicity data in the public domain was lacking, highlighting the need for both intelligent approaches to prioritize legacy human drugs for a tailored environmental risk assessment and a transparent database that captures environmental data. We show that presence/absence of drug-target orthologues are predictive of susceptible species for the more potent drugs. Drugs that target the endocrine system represent the highest potency and greatest risk. However, for most drugs (> 80%) with a full set of ecotoxicity data, risk quotients assuming worst-case exposure assessments were below one in all European countries indicating low environmental risks for the endpoints assessed.

Conclusion: We believe that the presented analysis can guide improvements to current testing procedures, and provide valuable approaches for prioritising legacy drugs (i.e. those registered before 2006) for further ecotoxicity testing. For drugs where effects of possible concern (e.g. behaviour) are not captured in regulatory tests, additional mechanistic testing may be required to provide the highest confidence for avoiding environmental impacts.

1. Introduction

Pharmaceuticals are present in the environment as a consequence of patient use, drug production and formulation, and improper disposal (Boxall et al., 2012). They predominantly enter the aquatic environment and are typically found in concentrations from sub-ng/l to a few μg/l (Weber et al., 2015). Extremely high pharmaceutical concentrations, in the order of mg/l, however, have been detected in some industrial effluents and recipient streams, for example in India, China, USA, Korea and Israel (Larsson, 2014). Drugs are also found in the terrestrial environment through the application of sewage sludge to land, leaching from landfills, or irrigation of arable land with treated or untreated wastewaters (Kümmerer, 2008). There is precedence for adverse effects of drugs in wildlife. In particular, the non-steroidal anti-

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inflammatory drug diclofenac has been shown to cause dramatic population declines (> 99%) in *Gyps* vulture species in India and Pakistan, resulting in localised extinctions (Oaks et al., 2004). The vultures suffered from renal failure after feeding on dead cattle that had been treated with diclofenac. The contraceptive, ethinylestradiol (EE_2), together with a range of other natural and synthetic estrogens, has been shown to cause feminisation in male fish in rivers (Desbrow et al., 1998; Jobling et al., 1998; Lange et al., 2009). The purpose of conducting regulatory environmental risk assessments is to avoid similar ecological situations in the future; to protect the most vulnerable wildlife populations, ecosystem services and, by association, the wider human population.

In the European Union (EU) and the United States (US) an environmental risk assessment is required for approval of new drugs, and this is usually conducted alongside Phase III clinicals just prior to submission of the marketing application. Chronic ecotoxicity testing has however only been required since 2006 in the EU and is not necessarily required in the US (European Medicines Agency, 2006; Food and Drug Administration, 1998). Most of the legacy drugs (i.e. those registered before 2006) are therefore lacking chronic ecotoxicity data but the extent of the issue is not known as most regulatory compliant data are not accessible.

In the EU aquatic effects studies are triggered if the Predicted Environmental Concentration (PEC) exceeds 0.01 μg/l (European Medicines Agency, 2006) and in the US if the Expected Environmental Concentration exceeds 0.1 μg/l (Food and Drug Administration Guidance for industry, 1998). Effects studies are also required if the drug is highly lipophilic (logD ≥ 4.5) and potential endocrine disruptors affecting reproduction trigger a tailored assessment in both the EU and US, independent of any exposure information (European Medicines Agency, 2006, 2010; Food and Drug Administration 1998; 2016). The ecological protection goal is directed at population, biodiversity and ecosystem services levels. In the case of threatened and endangered species, the individual level of biological organisation is the protection goal but this is not considered in the current testing approach for chemicals and as such is a recognised limitation. Endpoints in the required toxicity testing are growth, mortality and reproduction. Potential effects on molecular, cell, or tissue level responses or on developmental or behavioural effects are typically not considered. There is growing concern and debate regarding the need to include for example behavioural effects, especially for psychoactive drugs, and other non-standard endpoints such tissue level damage (e.g. as assessed via histopathology). Better understanding of how tissue, developmental and behavioural level effects may be extrapolated to possible impact on wild populations and ecosystem services, is however needed to determine their significance. Equally, however, this also applies to all other chemicals tested in regulatory risk assessment frameworks and this debate is beyond the scope of the analyses conducted here for pharmaceuticals.

The adequacy of the current regulatory test triggers and the effects-based assessments are also questioned because they rely on tests developed to determine the acute toxicity of industrial chemicals and do not consider the considerable safety and pharmacology information that is available for pharmaceuticals (Ågerstrand et al., 2015; European Medicines Agency, 2016). During the development of a new medical product, safety assessments of the active drug ingredient and the product are performed to show that the medicine is effective and that its benefits outweigh any potential adverse side-effects. It is therefore perhaps unlikely that drugs would be highly toxic to wildlife unless therapy-related effects lead to toxicity. The potential use of preclinical and clinical data to improve the environmental risk assessment has been suggested previously based on theoretical reasoning and applied to data for a few case studies only (Gunnarsson et al., 2012; Winter et al., 2010). Furthermore, the suggested approaches have not been validated against a comprehensive dataset of comparable chronic endpoints across a wide range of drugs and species.

In the environmental risk assessment required for approval in the EU, a Predicted No Effect Concentration (PNEC) is established by the application of an assessment factor of 10 to the lowest No Observed Effect Concentration (NOEC) for the endpoints of growth, development and/or reproduction from species representing three trophic levels (European Medicines Agency, 2006) and the PNEC is compared to a predicted environmental concentration (PEC). The PEC is usually calculated assuming the maximum dose is taken daily and that 1% of the population takes that drug (unless robust epidemiology data exist showing otherwise). The PEC also assumes 100% patient use, no wastage, no patient metabolism or sewage treatment removal, 200 l of wastewater per person per day and a dilution factor of 10 in the aquatic environment. A risk quotient (PEC/PNEC) above 1 in the EU (or below a safety margin of 10–1000, depending on the assessment tier in the USA) triggers further evaluations and risk refinement, for example on the use, fate and behaviour of the drug. When the possibility for environmental risks cannot be excluded an evaluation of precautionary and safety measures such as proposals for additional labelling of package leaflets should be presented. Under current regulatory frameworks, human drugs are approved for patient use irrespective of their potential environmental hazard and risk. This is a unique regulatory position for any class of chemical where the benefit to society, here as medicine, is automatically assumed to outweigh potential environmental effect; it is also an exception that is subject to greater scrutiny and is currently under challenge (Ågerstrand et al., 2015; BIO Intelligence Service, 2013).

We have compiled all publicly available regulatory compliant ecotoxicity data-set for human drugs, based on the results reported in regulatory submissions or presented voluntarily by the pharmaceutical industry. The aim was to create a transparent database that captures the regulatory compliant ecotoxicity data and to facilitate the development of more efficient approaches for prioritisation of environmental testing efforts for human drugs, whether new molecular entities or legacy drugs. The majority of drugs are developed to specifically affect human proteins and we have focused our analysis on these drugs. Anti-infectives and anti-parasitic drugs were excluded as a different approach for their evaluation would be needed and assessments of anti-infectives are covered elsewhere (Brandt et al., 2015; Le Page et al., 2017).

We analysed possible links between taxa-specific environmental toxicity, as measured by effects on growth, mortality or reproduction, and the evolutionary conservation of drug targets and looked to identify patterns of environmental toxicity across different therapeutic mode of actions. Furthermore, potential links to lipophilicity were assessed and we evaluated a previously suggested pharmacological-based approach for prioritisation of drugs with increased risk of affecting growth, mortality or reproduction in fish. Finally, we estimated the environmental risks of the drugs by a combined analysis of drug toxicity and predictions of environmental concentrations based on European patient consumption data. The results presented provide a comprehensive assessment of what is known from the limited number of drugs and therapeutic mode of actions with regulatory compliant environmental effect data. The analysis highlights some classes of therapeutic mode of actions that are more likely to affect growth, lethality or reproduction and identifies the susceptible trophic levels. Drugs and therapeutic classes lacking ecotoxicity data are identified and the analysis provides a data-driven basis for improved approaches for prioritising legacy drugs (i.e. those registered before 2006) for further ecotoxicity testing.

2. Methods

2.1. The ecotoxicity and n-octanol/water distribution coefficient data set

Publicly available aquatic ecotoxicity data from European Public Assessment Reports (EPAR) for human medicines published by the European Medicines Agency (EMA) or data voluntarily reported by the pharmaceutical industry (fass.se (Vestel et al., 2015) or https://www.

astrazeneca.com/sustainability/environmental-protection.html) were collected in March 2016 and updated in September 2018. Since 2006, the EU ecotoxicity assessment for drugs includes aquatic species representing three trophic levels; primary producer, plant eating invertebrate and a vertebrate. The recommended chronic-effects studies are 72 h growth-inhibition test in green algae (OECD, 2011) with the endpoints of growth rate, yield or biomass, 21 day reproduction test in daphnia (OECD, 2012) including the endpoints growth, immobilisation and reproductive output and fish early-life stage test, typically 32 days (OECD, 2013) assessing the endpoints of hatching, growth and lethality. Life-cycle tests in fish (approximately 300 days), which include the endpoints of growth, lethality and reproductive output, can be requested if the drug is known to affect reproduction of vertebrates. Lowest observed effect concentrations (LOEC) were not accessible for the vast majority of *Daphnia* and fish studies and the analyses therefore focused on NOECs. The LOECs from the recommended chronic-effects studies can, however, at most be 3.2 times higher than the reported NOECs, if Organisation for Economic Cooperation and Development (OECD) guidance has been followed. The lowest NOEC for each drug presented in any of the sources (e.g. EPAR and Fass.se) were used in the analysis. Specific OECD test guidelines are recommended (European Medicines Agency, 2006) and results from such tests were given precedence. Growth rate-based endpoints were given precedence when both yield- and growth rate-based endpoints in the green algae toxicity were reported. Yield-based endpoints were considered only if no growth rate-based value were available. Growth-rate based on cyanobacteria was only considered when green algal data was absent. A proportion of the NOECs were reported as the highest concentration tested (25, 19 and 26% for algae, daphnia and fish respectively). This uncertainty was considered in the analyses. For example, only drugs for which it could be concluded that one species was more sensitive than the other (i.e. the lowest NOEC could not be reported as the highest concentration tested) was included in the analysis of potential links between taxa-specific difference in toxicity and presence/absence of drug- target orthologues. The complete list of the drugs, their associated ecotoxicity data, with links to the sources are presented as Supplementary Dataset 1.

The n-octanol/water distribution coefficient (K_{ow} or D at a specified pH) represents the measured coefficient observed in an octanol:water partitioning study. For non-ionisable compounds, the logD does not vary with pH but the majority of APIs are partially ionised at environmental pHs and the $logK_{ow}$ can differ substantially from $logD_7$ for some ionisable drugs. The pharmaceutical industry typically report measured or predicted logD at pH 5, 7, and 9, to represent environmentally relevant conditions. In this study, reported $logD_7$ was given precedence and the used lipophilicity for each drug is presented in Supplementary Dataset 2.

2.2. Drug targets and predictions of orthologues

Assignments of human drug targets and the protein-target classifications (according to ChEMBL 21) were collected from Santos et al. (2017) and DrugBank.ca (accessed February 2017). The presence and absence of drug target orthologues was predicted in three model species, zebrafish (*Danio rerio*), the water flea (*Daphnia pulex*) and the green algae (*Chlamydomonas reinhardtii*) (Supplementary Dataset 1). The adopted approach for orthology assignment was recently described by Verbruggen et al. (2018) and ortholog predictions for all human drug targets are available through ecodrug.org.

2.3. Calculation of a theoretical, therapeutic water concentrations for fish

A model that utilises information on mammalian pharmacology to prioritize drugs with increased environmental hazard to fish has been suggested (Huggett et al., 2003). This approach was used to predict the surface water concentrations needed to reach theoretical therapeutic plasma concentrations in fish (which we here call the "Therapeutic Water Concentration", TWC). In short, a partitioning coefficient was predicted using a fish-blood:water partitioning model (Eq. (1)) that is applicable for compounds with a $logK_{ow}$ 1–8 (Fitzsimmons et al., 2001).

$$logK_{blood:water} = 0.73 \times logK_{ow} - 0.88 \quad (1)$$

The partitioning coefficient was calculated using both the reported log K_{ow}/$logD_{7–7.4}$ and predicted logP and log $D_{7.4}$ that were collected from chemspider.com (ACD/labs, accessed April 2017). The fish-blood:water partitioning model was originally developed for neutral chemicals, using log K_{ow} as input (Fitzsimmons et al., 2001). The relationship between logD and partitioning in fish, under environmentally relevant pH conditions, is less understood but the limited number studies that have measured the blood:water partitioning of ionisable APIs show that substitution of log K_{ow} for logD improves prediction of the measured plasma concentrations (Berninger et al., 2011; Margiotta-Casaluci et al., 2014; Nallani et al., 2016). The predictions presented in Fig. 5 are, therefore, based on reported logD, when available.

The TWCs was then calculated by relating the partitioning coefficient to the human Cmax level for each drug (Eq. (2)) (Huggett et al., 2003).

$$TWC = C_{max} / K_{blood:water} \quad (2)$$

Information regarding human therapeutic plasma concentrations (C_{max}) were retrieved from (Berninger et al., 2016; Schulz et al., 2012) and for each drug a low and a high Cmax are presented. All TWCs, human C_{max} levels and lipophilicities are presented in Supplementary Dataset 2.

2.4. Predicted environmental concentrations and risk quotients

European consumption data (retail, prescription and hospital data, in kg) for 2015, were obtained from IMS Health for up to 22 European countries. Country-specific total substance PECs were calculated assuming: even use across the whole population for each country (calculated as mass per person per day), 100% patient use and no wastage, no patient metabolism or sewage treatment removal, each person generating 200 l of waste water per day and a dilution factor of 10 in the river (as defined in the for a Phase 1 PEC determination (European Medicines Agency, 2006)). Country-specific median and 5th percentile national annual dilution factors (Keller et al., 2014) were also used to estimate how protective the dilution factor of 10 in the river was within the existing guidance. Risk quotients were then calculated for each European country with consumption data as PEC/PNEC (Supplementary Dataset 2).

3. Results

3.1. General description of the data set

Chronic environmental toxicology data from at least one of the recommended aquatic test species (European Medicines Agency, 2006) were available for 19% of the approved, small molecule drugs that target human proteins according to Drugbank (208 of 975 drugs) (Santos et al., 2017; Wishart et al., 2008). In addition, ecotoxicological data were also collected for 21 drugs that were not available through Drugbank (Supplementary Dataset 1).

Twelve percent of the drugs available through Drugbank had environmental toxicology data derived from species representing three trophic levels and together their drug targets represent 42% of all human protein-based drug targets (Fig. 1). Most of the common pharmacological modes of actions were represented in the dataset. For example, more than half of the targets classified as kinases, reductases, or G-protein coupled receptors (GPCRs) were associated with environmental effects data. The coverage of other target classes such as ion

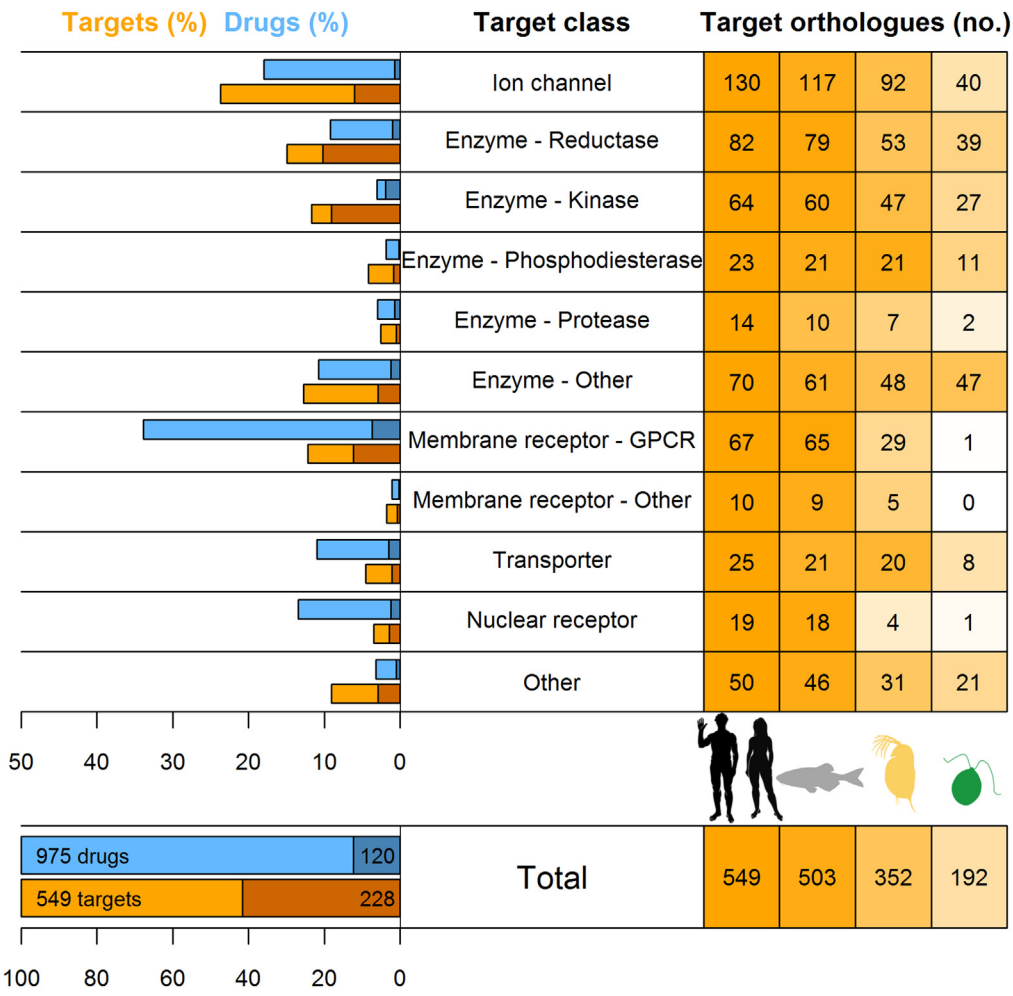

Fig. 1. Distribution of the environmental toxicology data across drugs and drug-target classes, and the evolutionary conservation of the drug targets. The number of small molecule drugs (blue) and their corresponding human protein targets (orange) are represented by the bars on the left-hand side of the figure. The coverage of drugs available in DrugBank.ca with a full set of aquatic environmental effects data (120) are indicated by the darker colours inlaid within these bars. The number of human drug targets with orthologues in zebrafish (*Danio rerio*), the water flea (*Daphnia pulex*) and the green algae (*Chlamydomonas reinhardtii*) for each protein class are shown on the right-hand side of the figure. The shade of orange corresponds to the proportion of drug targets for which orthologues were present. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

channels and "other enzyme targets" were significantly less. Cardiovascular drugs inhibiting the angiotensin-converting enzyme (encoded by *ACE*), antiepileptic's blocking voltage-gated T-type calcium channels (encoded by *CACNA1G, CACNA1H* and *CACNA1I*) and muscle relaxants inhibiting muscle-type nicotinic acetylcholine receptors (encoded by e.g. *CHRNA1* and *CHRNB2*) are examples of drug targets for which there is no chronic fish toxicity data available. The thyroid hormone receptors (encoded by *THRA* and *THRB*) or the retinoic acid receptors (encoded by for example *RXRA* and *RXRB*) are examples of nuclear receptors that also lack chronic ecotoxicity information from fish.

The comparison of NOECs for taxa-specific toxicology showed that fish generally represented the most susceptible trophic level and green algae were the least susceptible (Fig. 2a and Supplementary Fig. 3). The PNEC for twelve drugs were lower than the EMA Phase I PEC action trigger of 0.01 µg/l. Fish were particularly susceptible to seven drugs targeting sex-hormone receptors or sex-steroid synthesis. Levonorgestrel, EE_2, oestradiol, etonogestrel and fulvestrant had NOECs below 0.01 µg/l, supporting that fecundity or life-cycle tests in fish should be triggered for drugs affecting sex-steroid signalling, independent of the regulatory exposure trigger, as per the existing guidance (European Medicines Agency, 2006; Food and Drug Administration Guidance for Industry, 1998; Food and Drug Administration, 2016; Murray-Smith et al., 2012). Three antineoplastic agents, regorafenib, everolimus and ridaforolimus, also caused toxicity at very low concentrations (NOECs of 0.007, 0.014 and 0.031 µg/l, respectively) and four additional drugs (dalcetrapib, vortioxetine, mometasone and sorafenib in descending order of toxicity) had PNECs below 0.1 µg/l (Fig. 2b).

3.2. Ecotoxicity and drug-target orthologues

Information on the evolutionary conservation (i.e. inference of orthology) could potentially help identify susceptible environmental species (Caldwell et al., 2014; Gunnarsson et al., 2008; Verbruggen et al., 2018). Here we used robust orthology predictions of drug targets obtained from a recently published database (Ecodrug.com). > 90% of all human drug targets had orthologues in zebrafish (*D. rerio*), 64% of the targets had orthologues in the water flea (*D. pulex*) and 34% in the green algae (*C. reinhardtii*). The number of conserved drug targets in each species is visualized to the right in Fig. 1.

The ratio between NOECs from two species exposed to the same drug represented a feasible approach to compare species susceptibility across a wide range of drugs with different physiochemical properties and mode of toxicities. Adopting this approach, fish was shown to be the most susceptible taxa to > 70% of the drugs where conserved targets were lacking in *Daphnia* and green algae (Fig. 3a,c). However, if orthologues were also present in daphnia and green algae, then their frequency of being the most susceptible taxa were equal to fish (Fig. 3b,d). The NOEC ratios for drugs with targets conserved only in fish was also significantly lower compared with the same ratio for drugs with targets conserved in both taxa, *p*-value: 0.010 for $NOEC_{fish}/NOEC_{daphnia}$ and p-value: 0.007 $NOEC_{fish}/NOEC_{algae}$, Wilcoxon rank sum test. The link between taxa-specific differences in toxicity and the presence/absence of target orthologues was driven predominantly by the more harmful drugs (e.g. with NOECs < 10 µg/l) and for these drugs a linkage between the toxicity mechanism in wildlife and the therapeutic mode of action is likely. The presence/absence of drug target orthologues was less successful in predicting susceptible species

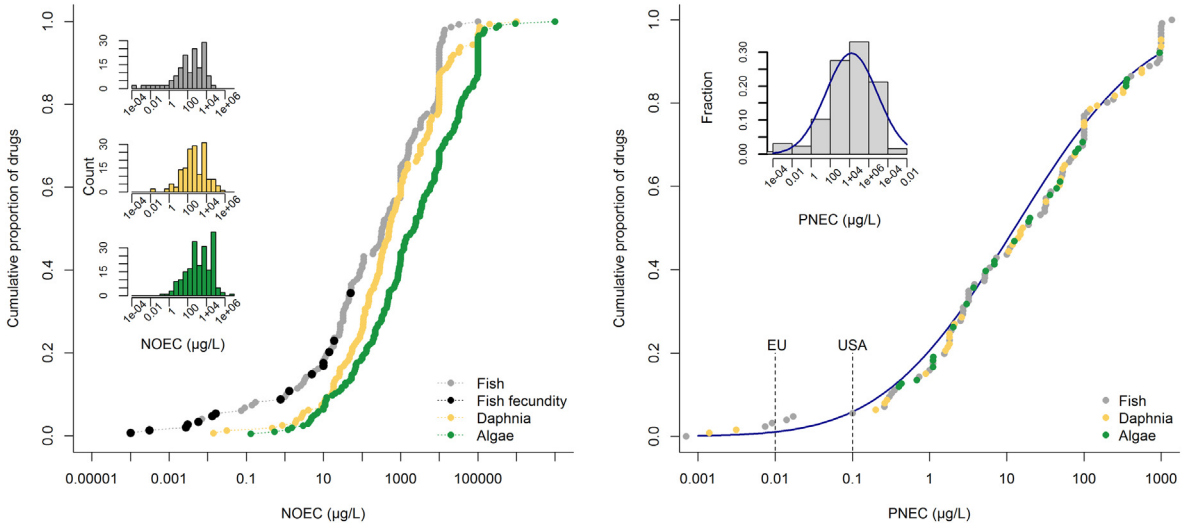

Fig. 2. Distribution of No Observed Effect Concentrations (NOECs) from tests with fish (grey), *Daphnia* (yellow) and algae (green). a| The distributions include all available NOECs (in total for 208 drugs) and data from tests where the highest concentration did not induce any observable effect (148, 160 and 204 NOECs from fish, *Daphnia* and algae tests, respectively). The NOECs derived from fecundity tests in fish with drugs expected a priori to affect vertebrate reproduction are marked in black (15 drugs). Overall, green algae represented the least sensitive trophic level with significantly higher NOECs compared to fish and *Daphnia* (*p*-values: 3.2×10^{-8} and 2.8×10^{-4}, respectively, Wilcoxon rank sum test). Fish represented the most sensitive taxa with significantly lower NOECs than *Daphnia* (p-value: 0.033). This pattern was clearer when the NOECs reported as the highest concentration tested were removed from the analysis (Supplementary Fig. 3) b| Distribution of the Predicted No Effect Concentrations (PNECs) for 127 drugs with environmental effects data from fish, *Daphnia* and algae, excluding drugs targeting sex-steroid receptors or sex-steroid synthesis. The Predicted Environmental Concentration (PEC) that triggers aquatic toxicity testing in the European Union (EU) and the United States of America (USA) are indicated in the graph. The blue curve describes a fitted log normal distribution (maximum likelihood estimation). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

for drugs with higher NOECs. Indeed, baseline toxicity, such as narcosis, or toxicity mediated via off-targets is generally more likely for drugs with high NOECs. However, the environmental risks are generally low for such drugs as environmental exposures in surfaces waters, via patient use and excretion, rarely exceeds concentrations of 1 µg/l.

A link between the therapeutic mode of action and the measured toxicity is a reasonable association for many of the drugs with NOECs below 10 µg/l. For example, the potential for drugs acting on the reproductive axis to affect reproduction in fish is well established (Kidd et al., 2007; Lange et al., 2009; Villeneuve, 2016; Zeilinger et al., 2009) and their drug targets (e.g. sex-steroid receptors) were not conserved in *Daphnia* and green algae. Accordingly, fish were at least 1000 times more susceptible than *Daphnia* and algae to five drugs targeting the estrogen- or the progesterone receptors (encoded by *ESR1* and *PGR*). Invertebrates and green algae also lacked orthologues to the glucocorticoid receptor, *NR3C1* and the NOEC for the glucocorticoids mometasone and betamethasone were 1000 and 100 times lower for fish than *Daphnia* and algae. Some antineoplastic agents such as lapatinib and axitinib also lacked conserved drug targets in *Daphnia* and/or green algae and their NOECs were around 100-fold lower in fish.

If the drug targets were conserved in green algae and/or *Daphnia* these species were occasionally substantially more susceptible to toxicological effects than fish (Fig. 3b and d). For example, the antineoplastic agents fluorouracil and vorinostat, were at least 100 times more toxic to *Daphnia* and/or green algae compared to fish. *Daphnia* were also particularly susceptible to exposure to the immunosuppressants, everolimus and ridaforolimus (150 and 35 times more sensitive compared to fish). Their common drug target, MTOR, acts as a central regulator of protein synthesis, cell proliferation and cell survival in mammals and in drosophila the orthologue (*dTOR*) is required for normal growth during larval development (Zhang et al., 2000). Thus, it is likely that chronic inhibition of MTOR in *Daphnia* could cause growth effects and/or lethality. The acute to chronic ratio of everolimus (data are lacking for ridaforolimus) was > 8000 for both fish and *Daphnia*, further suggesting a specific mode of toxicity in both taxa.

The presence/absence of drug-target orthologues was predictive of susceptible species for all but two of the drugs with NOECs below 10 µg/l (propranolol and duloxetine Fig. 3a,c). In the case of propranolol the data presented on the manufacturers website suggests that effects on growth and development of a non-standard marine echinoderm occur at the lowest exposure concentrations (Ribeiroet al., 2015) but invertebrates such as the echinoderm (*Strongylocentrotus purpuratus*) are not predicted to have orthologs to the targets (ADRB1, ADRB2 and ADRB3) (Bittner et al., 2018). This result may indicate a specific mode of toxicity mediated via off-targets. It should, however, be appreciated that tunicates such as *Ciona intestinalis* are predicted to have an ortholog to the three targets and the ortholog predictions for the deuterostome invertebrate are uncertain. It is worth emphasising also that although the beta-blocker propranolol is one of the most studied pharmaceuticals in the environmental literature, there are conflicting results regarding the effect levels in both invertebrates and fish even across relatively similar studies (e.g. Giltrow et al. 2009; Huggett et al. 2002; Parrott and Balakrishnan, 2017) highlighting the added uncertainty when trying to align data from non-standard studies reported in the literature. Transparent and improved reporting of non-standard as well as standard regulatory data is urgently needed to facilitate the assessment of the reproducibility, relevance and quality of the data from these studies, for example according to the CRED guidance (Kase et al., 2016).

Green algae were slightly more sensitive than fish to the serotonin-norepinephrine reuptake inhibitor, duloxetine, despite the fact that algae lacked conserved drug targets (Fig. 3b). Duloxetine is a weak base with a pK_a around 9 and it has been shown that weak bases, which are fully protonated, and thus cationic at a pH around 7, can exhibit comparably high toxicity in green algae (Neuwoehner and Escher, 2011). Environmental toxicology studies with fish are typically performed at a pH around 7.5 and the pH is stable throughout the experiment, whereas the pH in green-algal tests typically increases throughout the exposure due to CO_2-fixation. The increase in pH (up to 1.5 pH units are deemed acceptable (European Medicines Agency, 2006)) can lead to an increase of the neutral fraction of the weak bases which can result in ion-trapping in the algal cells (Brooks et al., 2003;

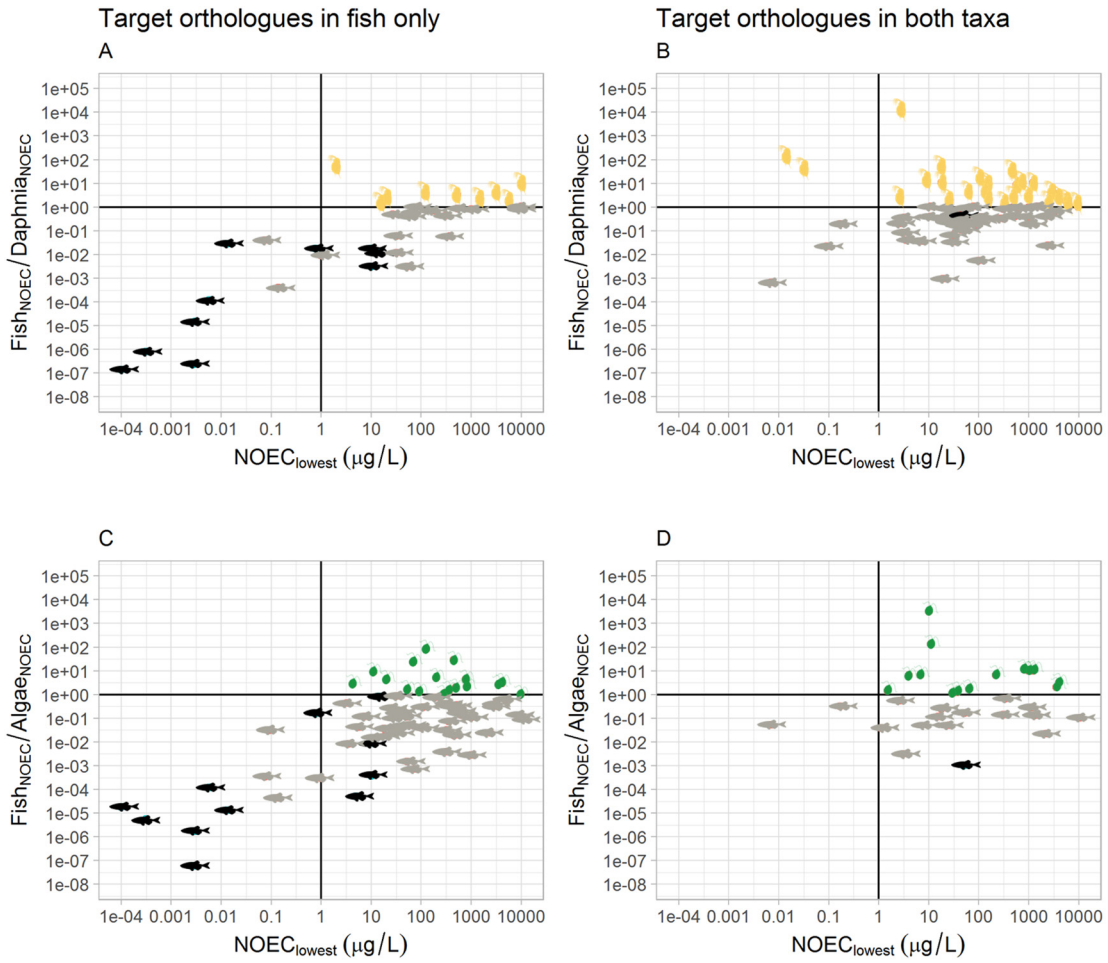

Fig. 3. Analysis of potential links between taxa-specific difference in toxicity and presence/absence of drug- target orthologues. The fold-change in susceptibility between the two compared taxa is presented on the y-axis and the silhouettes indicate which of the taxa that is the most susceptible. The lowest No Observed Effect Concentration (NOEC) of the two taxa is presented on the x-axis. The NOECs from fecundity tests in fish with drugs expected a priori to affect vertebrate reproduction are marked in black. Comparison between toxicity in fish and daphnia, a| 36 drugs with drug-target orthologues only in fish and b| 72 drugs with orthologues in both taxa. Comparison between toxicity in fish and green algae, c| 74 drugs with drug-target orthologues only in fish and d| 30 drugs with drug targets in both taxa. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Neuwoehner and Escher, 2011).

3.3. Environmental toxicology and drug lipophilicity

Lipophilicity is usually measured as the octanol-water partitioning coefficient and is used to rationalise the ability of drugs to cross biological membranes both in drug discovery (Lipinski et al., 1997) and in environmental toxicology (Veith et al., 1983). The lipophilicity of industrial chemicals is generally well correlated with the environmental toxicity for aquatic animals (Veith et al., 1983) but this relationship is much weaker for pharmaceuticals (Vestel et al., 2015). Industrial chemicals are to a large extent neutral, non-polar compounds while many drugs are ionisable and the relationship between logD to partitioning, bioaccumulation, and toxicity in aquatic species, under environmentally relevant pH conditions, is less well understood. In addition, if the drug exerts a specific mode of toxicity, for example mediated via evolutionary conserved drug targets, a weaker correlation would be expected compared with that driven by a general promiscuity of the drug (e.g. narcosis). Overall, the compiled dataset reaffirms that lipophilicity is an important factor affecting the uptake and thus the environmental hazard for pharmaceuticals. All the drugs with NOECs below 1 µg/l (i.e. PNECs < 0.1 µg/l) had reported $logK_{ow}$ or $logD_{7-7.4}$ above 3 (Fig. 4). The PNECs of drugs with a lipophilicity above 3 were significantly lower compared to the PNECs of the other drugs (*p*-value: 4.9×10^{-6} including all drugs and p-value: 1.6×10^{-4} when PNECs from fecundity tests in fish were excluded, Supplementary Fig. 4).

3.4. Prioritisation of legacy drugs

Fish generally represented the most susceptible trophic level and 95% of all legacy drugs had drug target orthologues in zebrafish. Results from fish early life stage tests were lacking for > 80% of drugs targeting human proteins and methods to support their prioritisation are needed. A model based on lipophilicity and therapeutic potency has been suggested for prioritisation of drugs with increased environmental hazard in fish (Brooks, 2014; Caldwell et al., 2014; Fick et al., 2010; Huggett et al., 2003). The model assumes that fish plasma concentrations can be predicted by the lipophilicity of the drug, that targets are conserved and a specific interaction between the drug and its target(s) in fish will result in a comparable target mediated response at a plasma concentration similar to that necessary to elicit a therapeutic effect in humans. Concentrations above those levels will increase the probability of off-target effects and if high enough induce narcosis. Conversely, it is assumed that toxicity in fish is unlikely to occur if water concentrations are not high enough to produce pharmacologically active levels of drug in the blood. The model is commonly used within academia to assess relative environmental potency of drugs and for estimations of environmental risks when measured environmental concentrations are

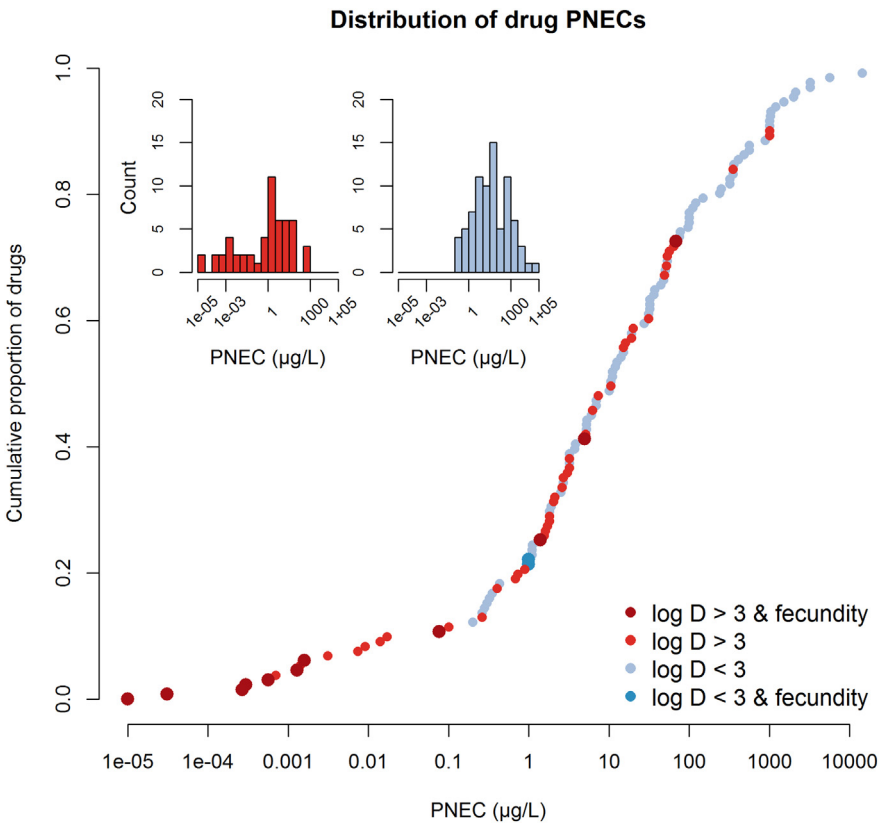

Fig. 4. Distribution of definitive PNECs for 133 drugs with environmental effects data from three taxa (drugs with the NOEC reported from a limit test for the most sensitive taxa, were removed from this analysis). Red indicates NOECs for drugs with reported lipophilicity above 3 and darker spots indicate NOECs based on fish fecundity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

available but pharmacologically related or chronic toxicity data for fish (e.g. life cycle test or early-life stage test) are lacking (Brooks, 2014; Du et al., 2014; Kristofco and Brooks, 2017).

Attempts to evaluate how well the model predicts the relative environmental toxicity of drugs as measured by effects on growth, lethality or fecundity have been hampered by the limited number of drugs with available fish toxicology data (Roos et al., 2012). Here, the correlations between the fish NOECs in this dataset and the modelled 'therapeutic water concentration' (TWC) showed some promise for first tier prioritisation (Pearson correlation coefficient ranging from 0.51 to 0.61, Supplementary Table 5). However, the approach substantially underestimated the toxicity for the synthetic progestin levonorgestrel and the protein-kinase inhibitor regorafenib (> 100-fold) and to a lesser extent for about two dozen other drugs (Fig. 5 and Supplementary Dataset 2). On the other hand, for the majority of drugs (60%), the model over-estimated environmental potency by > 10-fold. Thus, the model needs further development from its current form to enable it to be applicable for first-tier prioritisation of drugs in a regulatory framework. A better understanding of the accuracy of the partitioning model(s) is needed as well as the potential links between the therapeutic potency and mechanism of actions in humans and the mechanism of toxicity in fish. Here we hypothesise that in fish therapeutic modes of actions that consistently have NOECs that approach, or are lower than, the modelled TWC, could suggest a linkage between the effects on lethality, growth or fecundity and the therapeutic mode of action of the drug. We suggest that testing of legacy drugs with these therapeutic modes of actions should be prioritised.

Many of the drugs affecting sex-steroid receptors and synthesis in humans (e.g. levonorgestrel, etonogestrel, EE_2, letrozole, and anastrozole) had NOECs in fish at, or below, the modelled TWCs (Fig. 5a). However, the selective estrogen receptor modulators ospemifene and bazedoxifene, both used for treatment of undesired post-menopausal symptoms, were at least 100-fold less toxic to fish than predicted. This suggests that considerations of the degree and severity of effects associated with the human Cmax, could improve the exposure outcome predictions in fish.

The NOECs for the serotonin re-uptake inhibitors (SRIs, vortioxetine, fluoxetine, duloxetine and dapoxetine) in fish were all close to the modelled TWC. While the potential for an adverse outcome as a consequence of inhibition of the 5-hydroxytryptamine (serotonin) transporter in fish has been suggested, experimental mechanistic information is lacking (Brooks, 2014; Groh et al., 2015; McDonald, 2017). The high level and increasing usage of antidepressants, however, warrants further assessments on their environmental risks that should include an assessment of the potential effects of environmentally relevant mixtures.

The toxicity of some antineoplastic agents was substantially underestimated by the model. As examples of this, the toxicities of two kinase inhibitors (regorafenib and sorafenib) were both underestimated by > 60-fold (Fig. 5b) and may be a reflection of the multi-kinase polypharmacology of these two anti-VEGFR drugs. Potential links between the therapeutic mode of action and adverse effects has been suggested for antineoplastic agents (Kümmerer et al., 2016). The relationship between the measured toxicity and the modelled effect concentration, however, varied considerably. Kinase inhibitors often exhibit polypharmacology and both on-target and off-target mediated toxicity is relatively common (Hopkins et al., 2014; Zhang et al., 2016) which makes it challenging to identify specific targets that could be associated with higher environmental risk. It should also be noted that *Daphnia* and green algae were the most susceptible species to many of the antineoplastic agents that have conserved drug targets in these species. Testing of this drug class should therefore be prioritised for all species having drug-target orthologues, independent of the modelled potency in fish.

3.5. Estimation of the environmental risks

Environmental risk is a reflection of both environmental hazard and

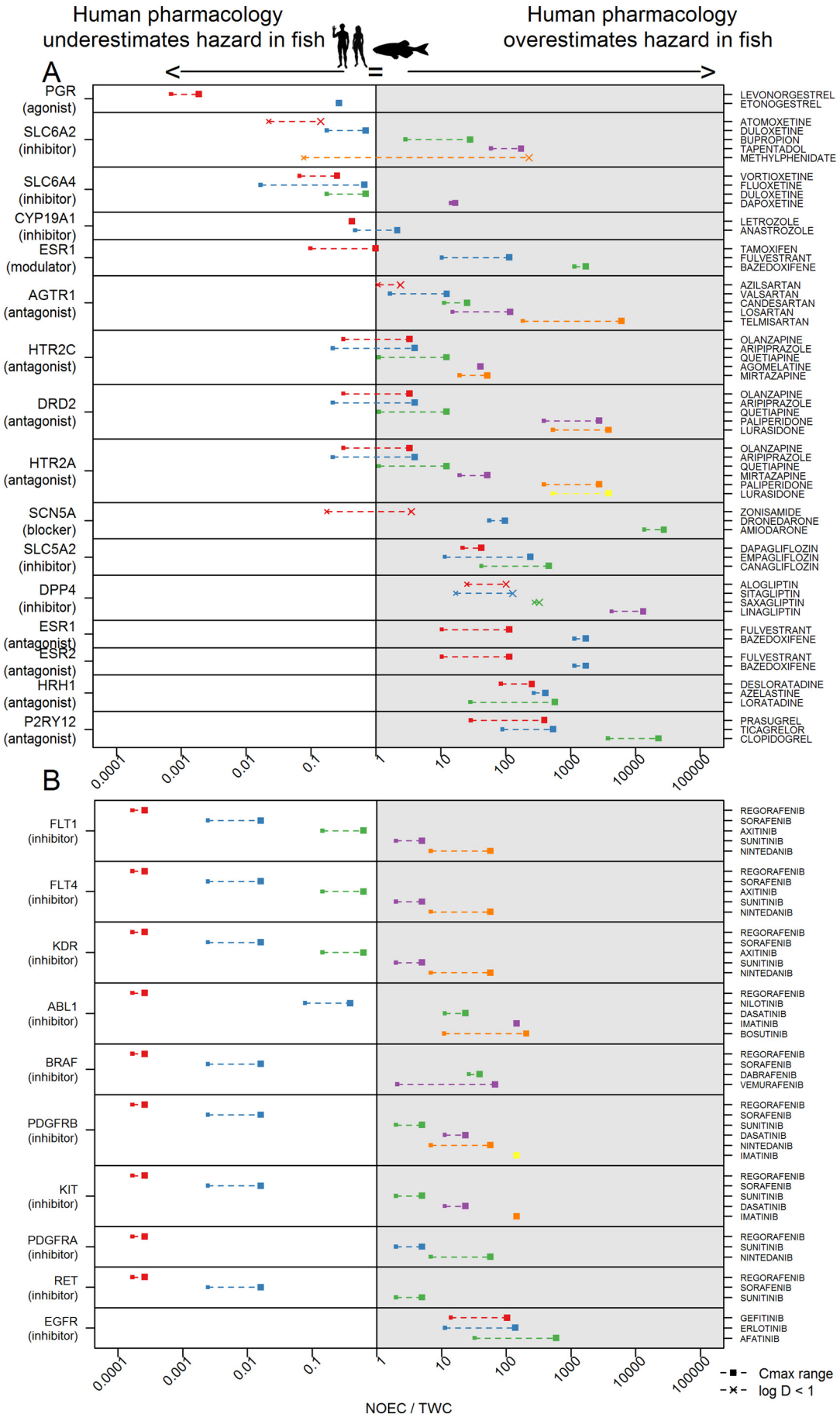

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Fig. 5. Comparison of measured environmental effects in fish and a theoretical "therapeutic water concentration". A ratio between the No Observed Effect Concentration (NOEC) and the modelled "therapeutic water concentration" (TWC) below 1 suggest that the model underestimated the environmental effect whereas a ratio above 1 suggest an overestimation. Drug targets are presented on the left-hand side in the figure and the associated drugs to the right. The TWC was calculated using both a high and a low human therapeutic plasma concentration (the lower Cmax is indicated by the bigger symbol). A fixed partitioning coefficient of 0.7 was applied to drugs with reported logD < 1. a| Drug targets associated with fish NOECs from at least three drugs. The targets PGR, CYP191A, ESR1 and ESR2 were included despite only being associated with fish NOECs from two drugs. b| Drug-target kinases associated with fish NOECs from at least three antineoplastic agents.

exposure. In general, the level of exposure depends on the usage and the environmental fate of the drug. The environmental risk assessments for a new marketing authorisation estimate the PEC on a product-by-product basis rather than a substance-by-substance basis (European Medicines Agency, 2006). In the cases where a substance is used to treat multiple clinical diseases, there is the potential to under-estimate the environmental risks for substances used to treat diseases with a high prevalence since different formulations and applications are treated separately and may not address a total PEC. Here, we calculated PECs using a substance-based approach, using EU wide consumption data, and the worst-case exposure assumptions (European Medicines Agency, 2006).

Seven drugs (levonorgestrel, EE_2, estradiol, abiraterone, propranolol, fulvestrant and fluoxetine) had PECs that exceeded their corresponding PNEC, in at least one European country (Fig. 6). There were, however, substantial differences between countries (often two orders of magnitude), which reflects different preferences for drugs to treat specific diseases, differences in disease epidemiology (as prevalence can vary between countries), and/or differences in product approval at the national level. Four drugs affecting sex-steroid signalling had median risk quotients of above 10 suggesting the potential for high environmental risks in many of the European countries. As a consequence of the placement of EE2 and oestradiol on the European Water Framework Directive Watch List (Commission of the European Union, 2015) the current EU-wide monitoring of these sex steroids will support exposure refinements to better validate their PECs.

The PECs used to calculate environmental risk within our analysis assumed the EU-wide regulatory default dilution factor of 10 (i.e. domestic sewage effluents are diluted ten-fold to estimate the surface water concentration of the drug), which is deemed to be conservative given that median estimated dilution is 18 and higher in the assessed European countries (Keller et al., 2014). However, 5% of catchments in Europe, including in Belgium (lowest annual median flows in Europe), Hungary, Netherlands, Romania, Spain and UK can have lower dilutions, especially during low flow conditions, and this generic dilution factor could underestimate environmental drug concentrations in some receiving environments (Link et al., 2017). The number of drugs with a risk quotient above one in any of the European countries was reduced to five when country specific dilution factors (median) were used (Supplementary Fig. 7). However, if the 5th percentile dilution factors were used 15 drugs had a risk quotient above one (Supplementary 8). For example, the risk quotient were above 1 also for ibuprofen (Spain), sorafenib (Spain and Romania), celecoxib (Spain), naproxen (UK), dronedarone (Spain), carbamazepine (Hungary, UK and Romania), metformin (UK, Romania and Spain) and duloxetine (Belgium and Spain).

4. Discussion

Here we present an analysis of the publicly-available and regulatory-compliant ecotoxicity data for human drugs to provide a much needed information database for the development of more intelligent approaches for environmental drug testing. We show that presence/absence of drug-target orthologues is predictive of susceptible species based on standardized model organisms and endpoints. The risk assessment confirmed that drugs targeting the endocrine system represented the highest potency and greatest risk. The assessment also showed that only a small number of the drugs were predicted to pose significant environmental risks (PEC/PNEC > 1) based on European sales statistics, worst-case exposure assumptions and the endpoints included in regulatory testing (effects on, growth, mortality and/or reproduction). Importantly, the compiled data set also suggest that chronic ecotoxicity data is lacking for 88% of the drugs targeting human proteins. We suggest the results presented can guide improvements to current testing procedures, and provides a valuable approach for prioritising legacy drugs (i.e. those registered before 2006) for further ecotoxicity testing.

The presence/absence of drug-target orthologues is a key factor that predicts species susceptibility for drug exposure at low concentrations. The substantial difference in toxicity across species highlights that the European transition to chronic ecotoxicological testing in 2006 (European Medicines Agency, 2006) was justified. Most medicinal products approved before 2006 lack chronic testing in fish and their associated PNEC may therefore not be protective for fish if it is based on short-term acute tests or on species that lack drug-target orthologues. On the other hand, our results question the current need to always include tests with *Daphnia* and green algae in the environmental risk assessment of new or legacy drugs where the drug target is conserved only in fish. This highlights the potential for development of appropriate science-based assessment factors to extrapolate ecotoxicity data from species with drug-target orthologues to protect species lacking targets.

The European exposure (PEC) trigger (0.01 μg/l) for toxicity testing is based on historical acute environmental toxicology data only (European Medicines Agency, 2006). Our analyses suggest that this trigger is conservative for hydrophilic drugs. All the drugs with NOECs below 1 μg/l (i.e. PNECs < 0.1 μg/l) had reported $\log K_{ow}$ or $\log D_{7\text{–}7.4}$ above 3. However, the hydrophilic drugs propranolol (logD: 1.6) and fluorouracil (logD: −0.89) had relatively low NOECs (2 and 2.8 μg/l, respectively) but relatively high potencies. A pragmatic way forward to develop a first screen prioritisation for legacy APIs would be to base it on consumption data. If the consumption-based PEC in any EU country is > 100 ng/l then it should be prioritised, whereas if the consumption-based PEC for all EU countries is < 10 ng/l and data already exist for that MoA then is should be considered as low priority. If the PEC for the EU country with highest use is 10–100 ng/l and the API has a log Kow > 3 then this would be considered as a high priority and for APIs with a log Kow < 3 for the same PECs of 10–100 ng/l, a medium priority. Such an approach however should be subject to re-iterative review and potentially modifications as more data becomes available.

The conducted risk assessment, based on the regulatory compliant assessment test endpoints, European consumption statistics, and country specific annual dilution factors (5th percentile), predicted that > 80% of the drugs in the dataset have a risk quotient below 1 for all of the individual 22 European countries analysed. The predictions assumed 100% patient use and no wastage, no patient metabolism or sewage treatment removal. However there are some uncertainties that could lead to higher environmental concentrations or 'hot spots', for example, higher spatial concentrations due to population demographics, seasonal differences in dilution factor, or poorly managed wastewaters from manufacturing (Larsson, 2014; Weber et al., 2015). Nevertheless, most of the measured concentrations in European surface waters were substantially lower than the presented PECs. It is important to note also that the amount of data for water measurements varies across the different drugs. As an example, concentrations of levonorgestrel have been analysed in only 14 samples from European

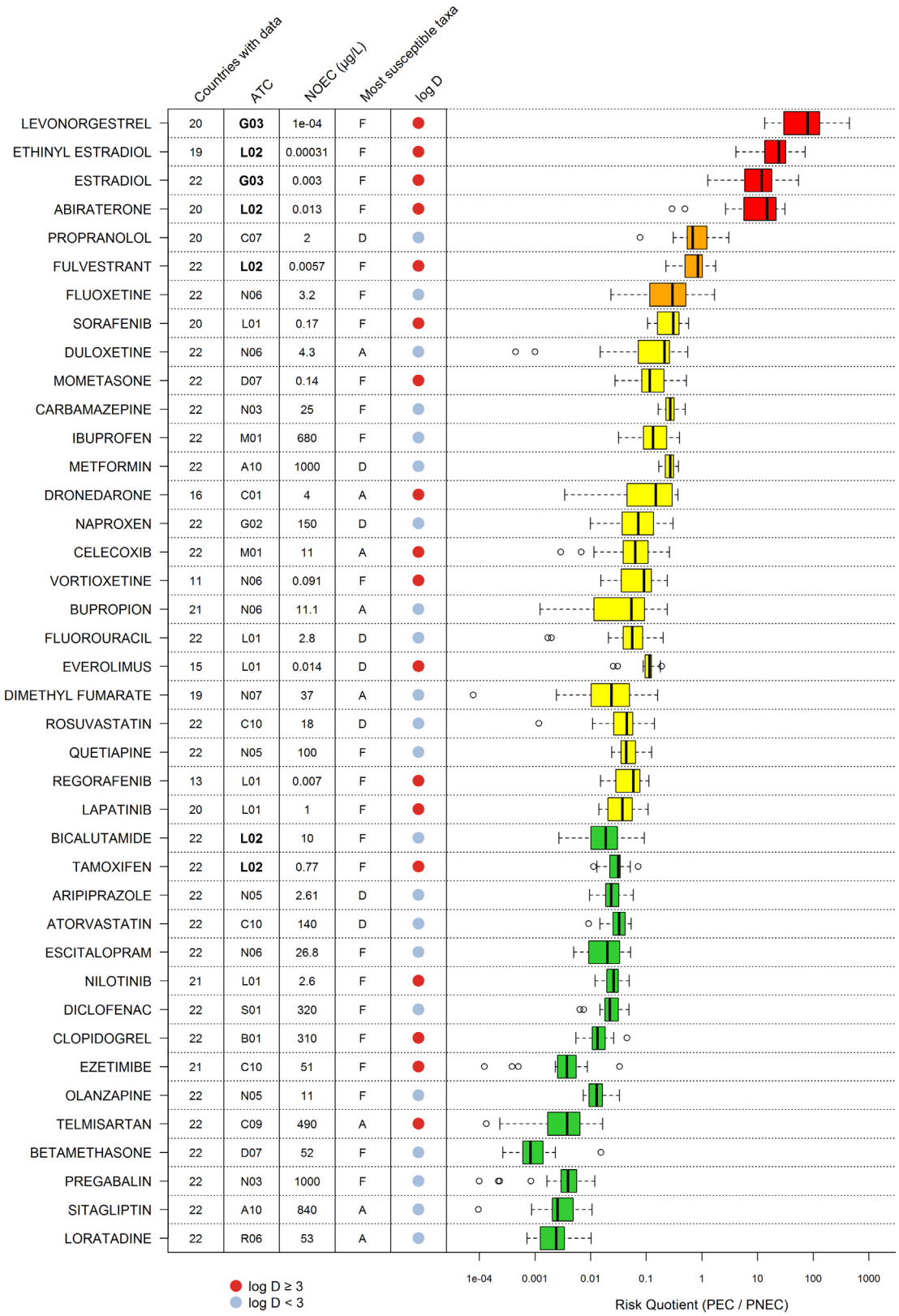

Fig. 6. Drugs with substance risk quotients with a margin of safety < 100-fold in all the assessed countries. The environmental risk is calculated by dividing the Predicted Environmental Concentration (PEC) with the Predicted No Effect Concentration (PNEC). For each drug the Anatomical Therapeutic Chemical (ATC) classification code and the No Observed Effect Concentrations (NOEC) for the most susceptible taxa are presented in the columns. Bold ATC codes indicate that the drug has triggered fecundity tests with fish. The colours of the boxes show the environmental risk, based on the country with the highest ratio, according to the fass.se scheme: high risk (PEC/PNEC > 10; red), moderate risk (PEC/PNEC > 1–10; orange), low risk (PEC/PNEC > 0.1–1; yellow) and insignificant risk (PEC/PNEC < 0.1; green). The median and the interquartile range (i.e. 50% of data in this range) are shown in the box and the whiskers indicate the min/max data point 1.5 times in distance from the first and third quartile. The complete list of risk quotients for all drugs assessed is available as S6 Figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

surface water whereas EE_2, fluoxetine and propranolol have each been analysed in at least 500 samples. The inclusion of additional drugs (e.g. levonorgestrel) in the EU-wide monitoring (Commission of the European Union Decision, 2015) warrants consideration to better establish their surface water concentrations.

The environmental risks of human medicinal products are defined in Article 8(3) of Directive 2001/83/EC, as "any risk of undesirable effects on the environment". The ecological protection goal, however, is at the population and ecosystem services level. Potential molecular, cell or tissue level effects or effects on behaviour are therefore not considered. There is a growing concern regarding behavioural alterations that may affect wildlife populations (Brodin et al., 2013; Hellström et al., 2016; Klaminder et al., 2014; Rand-Weaver et al., 2013). A better understanding of behavioural traits and effects is needed to determine the ecological relevance of these reported effects (Brodin et al., 2014; Brooks, 2014). There is also an ongoing debate regarding the potential use of non-standard endpoints, such as histological alterations that indicate tissue damage, in environmental risk assessment of human drugs. For example, the environmental risk assessment for diclofenac based on standard OECD tests provides a NOEC of 320 μg/l, which is based on effects on survival of larvae and juvenile zebrafish (Memmert et al., 2013). A slightly higher NOEC (369 μg/l) for histological alterations in gills of rainbow trout is reported in the same article. Several other studies have shown histological and molecular effects, in the kidney and other organs, in fish exposed to diclofenac at substantially lower concentrations, ranging from 1 to 25 μg/l (Bickley et al., 2017; Mehinto et al., 2010; Näslund et al., 2017; Schwaiger et al., 2004; Triebskorn et al., 2004). As with behavioural effects, there is a need to extrapolate molecular, cellular, tissue, organ and individual level effects to the ecosystem level in order to determine the significance of these variations in wild populations. There is also a need to develop approaches to assess the risks associated with long-term exposure to mixtures of pharmaceuticals and other environmental stressors (Boxall et al., 2012).

Furthermore, environmental assessments are typically conducted only once, at the point of authorisation and unlike the ongoing pharmacovigilance requirements to provide patient safety updates, environmental assessments are rarely refined in light of emerging scientific data; consequently, there is a need to update environmental assessments if robust and reliable data are published that has a material impact on the NOEC for a given drug (Holm et al., 2013). Making environmental data for human pharmaceuticals more widely available through the development of a central and searchable substance database would greatly enhance the environmental assessments of pharmaceuticals.

The growing regulatory and scientific concerns regarding pharmaceuticals in the environment have reached the point where some stakeholders are advocating the inclusion of environmental hazard and risk within the patient-benefit evaluation that underpins the marketing authorisation of a drug (Ågerstrand et al., 2014; BIO Intelligence Service, 2013). Currently the environmental risk assessment is conducted in parallel to Phase III clinical trials, i.e. after significant investment in drug discovery and development. The dataset we have collated in this study; however, indicates that most drugs pose low risk based on the regulatory test endpoints measured and the usage levels in Europe. Irrespective of the outcome of this ongoing regulatory debate it is critical that predictive in silico, in vitro and in vivo tools and models are developed and integrated earlier within drug development to identify environmental concerns much sooner than operates within the current industry model. These tools will be useful in helping to identify drugs of potential environmental concern and ensure that the right species are chosen for a tailored environmental assessment. The availability of these tools and models will also assist with the prioritisation of the > 800 legacy drugs that lack any environmental data for definitive regulatory assessments. Identifying drugs, whether new substances or legacy drugs, of potential environmental concern and deriving appropriate PNECs will help to focus monitoring efforts, ensure establishing safe discharge levels from manufacturing and municipal sewage treatment plants, and ultimately help direct mitigation efforts to minimise the environmental effect of drugs. The dataset presented in this study provides a fundamental underpinning for more intelligent approaches for drug testing for environmental protection, directing testing efforts on drugs that pose the greatest environmental risks whether new substances or legacy drugs.

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