Leveraging existing data for prioritization of the ecological risks of human and veterinary pharmaceuticals to aquatic organisms

Carlie A. LaLone1,2, Jason P. Berninger3, Daniel L. Villeneuve2 and Gerald T. Ankley2

1Water Resources Center, College of Food, Agricultural and Natural Resource Sciences, University of Minnesota, 1985 Buford Avenue, St Paul, MN 55108, USA
2Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, US Environmental Protection Agency, and 3National Research Council, 6201 Congdon Boulevard, Duluth, MN 55804, USA

Medicinal innovation has led to the discovery and use of thousands of human and veterinary drugs. With this comes the potential for unintended effects on non-target organisms exposed to pharmaceuticals inevitably entering the environment. The impracticality of generating whole-organism chronic toxicity data representative of all species in the environment has necessitated prioritization of drugs for focused empirical testing as well as field monitoring. Current prioritization strategies typically emphasize likelihood for exposure (i.e. predicted/measured environmental concentrations), while incorporating only rather limited consideration of potential effects of the drug to non-target organisms. However, substantial mammalian pharmacokinetic and mechanism/mode of action (MOA) data are produced during drug development to understand drug target specificity and efficacy for intended consumers. An integrated prioritization strategy for assessing risks of human and veterinary drugs would leverage available pharmacokinetic and toxicokinetic data for evaluation of the potential for adverse effects to non-target organisms. In this review, we demonstrate the utility of read-across approaches to leverage mammalian absorption, distribution, metabolism and elimination data; analyse cross-species molecular target conservation and translate therapeutic MOA to an adverse outcome pathway(s) relevant to aquatic organisms as a means to inform prioritization of drugs for focused toxicity testing and environmental monitoring.

1. Introduction

Active pharmaceutical ingredients are increasingly detected in the environment due to several factors, including advances in human and veterinary medicinal practices, the ageing human population and improved sensitivity of analytical instrumentation. Sources such as wastewater treatment plant effluent and run-off associated with animal feeding operations have been implicated as important contributors of pharmaceuticals to aquatic environments [1]. Owing to the continuous introduction of some of these chemicals into waterbodies, they have been termed pseudo-persistent, a characteristic that increases the possibility of chronic exposures of non-target organisms. Unintended exposures of aquatic species to pharmaceuticals are inevitable and have been documented [2–4]. Unfortunately, only limited publically available ecotoxicity data exist for most drugs, making informed, transparent, assessments of their possible ecological risks problematic [5,6]. Further, much of the ecotoxicity data that do exist for pharmaceuticals focus on short-term exposure tests or acute lethality, which is not always suitable for predicting effects of pseudo-persistent chemicals specifically designed to produce sublethal biological effects [5,6]. That is, many pharmaceuticals are designed to target specific pathways, often at
relatively low doses [7,8]. Certain of these pathways are critical to the long-term maintenance of physiological functions, and can be highly conserved across taxa, including non-target aquatic animal species. As such, it is reasonable to expect that some pharmaceuticals will elicit adverse sublethal responses in chronic exposures [5,6].

Generation of chronic ecotoxicity data for the large number of pharmaceuticals that may (or do) enter aquatic environments would be prohibitively costly, as well as requiring numerous test animals, which contradicts the growing international desire to decrease animal use. Given the number of drugs in use or development (5000±; [9]), whole-organism chronic toxicity studies for assessing all possible risks are impractical, so techniques for the prioritization of those chemicals most likely to be problematic are needed. In recognition of the challenges associated with testing pharmaceuticals for their effects to human and ecological health and the need to better focus research efforts, international experts recently gathered at a workshop to develop a list of the most critical questions to guide future studies, including questions pertaining to best practices for prioritization and effects characterization [10]. Consistent with these recommendations, development of several prioritization approaches that effectively and efficiently use available pharmaceutical knowledge is ongoing. Currently, the prominent focal point for this type of activity has been based on exposure (e.g. related to production volume, use patterns, potential for bioconcentration, etc.), occasionally with some consideration of potential for effects (e.g. predicted no effect concentration) [11,12].

The need for a more integrated exposure- and effects-based approach to pharmaceutical prioritization can be illustrated when considering the contraceptive ingredient ethinyl oestriadiol (EE2), a synthetic oestrogen known to cause endocrine-disrupting effects in fish at low ng L\(^{-1}\) concentrations [13]. Predicted (based on production volume) or measured concentrations of EE2 in aquatic systems and demonstrated potential for acute lethality in a number of species would rank EE2 as a very low priority [5]. However, empirical data from chronic toxicity studies, as well as an understanding of cross-species pathway conservation would most certainly result in a high priority ranking of EE2 when considering potential for effects [12,14,15]. Caffeine, in contrast, is abundantly and consistently found in aquatic samples, but poses negligible potential for effects to exposed species [1], thus indicating a much lower priority ranking. These relatively simple examples emphasize the need for an integrated understanding of both the potential for exposure, and the plausible effects of the drug on non-target organisms for robust pharmaceutical prioritization.

Unlike most other classes of chemicals of possible ecological concern, insights as to possible environmental exposure and effects of both human and veterinary drugs can be gleaned from a priori knowledge. For example, for many pharmaceuticals, efficacy and safety data are available concerning adsorption, distribution, metabolism and elimination (ADME), and biological pathways affected in target species (i.e. humans, livestock). From this, it should be possible to employ systematic approaches to prioritize pharmaceuticals for monitoring and testing in two ways: (i) identification of chemicals with the most potential to elicit adverse effects and (ii) identification of which species/endpoints should be used for this testing or monitoring. In this review, we describe specific techniques we are applying to this challenge.

2. Focus on likelihood for adverse effects

The fact that a drug must be present in the environment for it to cause adverse effects to non-target organisms is irrefutable. However, considerations of pharmacokinetic measures, biological pathway interactions (both with potential for specific and non-specific interactions) and knowledge of primary drug-metabolizing enzymes and conservation of molecular targets, can provide enhanced potential to rank and prioritize pharmaceuticals for their potential to cause unintended effects to wildlife (figure 1).

(a) Utility of read-across from mammalian pharmacokinetic data

Approximately 40–60% of new drug candidates for humans fail owing to poor ADME profiles [16]. Therefore, accurately measuring or predicting mammalian pharmacokinetic parameters is fundamental to drug discovery and development, and typically, data for existing drugs are publically available. A number of online databases house this type of information, including Drugbank (6825 drug entries [9]), PK/DB database for pharmacokinetic properties (ca 1400 chemicals [17]) and the PharmacoKinetics Knowledge Base (PKKB; 1685 drugs [16]). However, due to the inconsistent nature of information in these databases relative to, for example, pharmacokinetic parameters measured (including method descriptions), reporting units, linkage to primary literature, etc., we deemed these sources unsuitable for transparent prioritization efforts. Therefore, we have developed a database of the most commonly prescribed drugs, over-the-counter drugs and veterinary medicines, which is representative of nearly all therapeutic classes. Our database is populated with information from selected review articles [18,19], the Physicians’ Desk Reference [20], manufacturers/government agency monographs and the primary literature, and provides consistently referenced material with common units. This curated evaluation of the available literature includes data for 1200 drugs across 100 drug classes (defined by source description of mode of action (MOA)), and includes approximately 7000 data points related to ADME parameters. With this readily accessible mammalian pharmacokinetic data, read-across approaches can be employed to inform or hypothesize the potential pharmacodynamics of both specific and representative classes of drugs in non-target species.

Biological read-across as it relates to ecotoxicology has been described as the ability for a drug to have an effect on a non-target organism owing to molecular target conservation and similar pharmacology as the target species [21]. Pharmacokinetic parameters selected as a means to inform cross-species read-across include clearance rate, volume of distribution, therapeutic plasma concentration and half-life of elimination. Although a thorough understanding of pharmacokinetic nuances between species is lacking, currently available data can be used based on qualitative understandings of species similarities, particularly within vertebrates. Simply put, in the absence of evidence to the contrary, it is reasonable to assume that if a drug is readily absorbed, widely distributed, poorly metabolized and/or slowly eliminated in the mammalian target species, it has greater potential for hazard in non-target vertebrate species.

With this basic assumption in mind, our database serves as a component of a dynamic framework for prioritizing
drugs. Specifically, each data point is ranked within the pharmacokinetic parameter using a probabilistic distribution [22]. The ranking provides a regression from which data can be scored, from 1 to 10, according to specific 10th centile thresholds, and translated into relative potential for hazard. For example, total body clearance (ml min$^{-1}$ kg$^{-1}$) data are considered important, with the assumption that the slower the clearance the greater the potential hazard (i.e. as a drug would have more time to initiate its biological action). The database includes 832 data points ranging between 0.0037 and 1070 ml min$^{-1}$ kg$^{-1}$ for total body clearance. Scores were established using the probabilistic distribution of the data, and subsequent regression threshold values for each 10th centile (figure 2). Drugs with lower clearance values are subsequently assigned higher scores based on potential hazard. When multiple pharmacokinetic parameters are assessed for each drug in this manner, scores can be summed to develop an overall prioritization metric for available ADME data as a component of a comprehensive prioritization strategy. A critical attribute of this approach is that relative prioritization rankings can be produced by grouping drugs by class or chemical structure allowing for predictions of probable ADME priorities for newly developed drugs or older pharmaceuticals falling within that class, for which significant pharmacokinetic information is lacking.

**(b) Conservation of molecular target for cross-species extrapolation**

Pharmaceuticals are generally designed to act on specific molecular targets to produce their desired therapeutic benefits and lessen the potential for undesirable off-target interactions. The biomolecular targets typically are involved in key metabolic or signal transduction pathways specific to a disease or medical condition. Conservation of these molecular targets at the protein level is likely when considering species with close phylogeny. However, some targets are well conserved across more diverse phyla. Another key component for focusing testing efforts on organisms with the greatest likelihood for susceptibility to a given pharmaceutical includes considering cross-species similarity of therapeutic molecular targets. Efforts have been made to explore the utility of protein sequence comparisons as a means to estimate sensitivity to pharmaceuticals, demonstrating cross-species conservation of many drug targets [11,23]. Recently, we developed a computational tool, Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS), that facilitates rapid and strategic examination of protein sequence similarity at the level of the primary amino acid sequence (including orthologue candidate identification), conserved functional domains and (when possible) individual amino acid residue position(s) across species, as a means to predict relative intrinsic susceptibility to chemicals with known MOA [14]. Output derived from the SeqAPASS analysis can be used to define the relevance (or lack thereof) of known protein targets across taxa (e.g. figure 3). A growing list of case studies, including examples with the human pharmaceutical E2E, the veterinary drug 17β-trenbolone (an anabolic steroid) and the pesticide permethrin, for which predictions of susceptibility compared favourably with empirical toxicity data [14,24], have demonstrated the relevance and utility of the SeqAPASS tool. Others have explored molecular docking of pharmaceuticals to drug targets in common ecotoxicological model species, further demonstrating the

**Figure 1.** Connections between key components of effects-based prioritization strategy for human and veterinary pharmaceuticals. Rectangular shapes describe the components considered for prioritization. Long dashed line represents instances where data or information derived from one of the components can be used to better understand or guide decisions within another component. Small dashed line represents parameters assessed within component (a). Solid arrows represent the information used to guide prioritization derived from each component. AOPs, adverse outcome pathways. (a–c) Correspond to §2a,b and c in the text.
utility of protein structural information, when it exists or can be modelled, for predicting potential for effects [25,26]. These types of strategies for species extrapolation use existing (and rapidly expanding) protein sequence/structural information to explore species similarities and differences meaningful to the molecular initiating event (MIE; e.g. receptor/ligand interaction, enzyme inhibition, etc.) responsible for producing adverse effects [27,28]. Exploring commonalities among species with regard to structure of key protein targets such as receptors (or primary xenobiotic-metabolizing enzymes) is a key piece of information that can guide predictions of potential cross-species susceptibility to pharmaceuticals, particularly when quantitative data regarding receptor/ligand interaction, binding affinity and potency for most non-mammalian species are lacking. This approach, in conjunction with leveraging mammalian ADME data, can serve as the basis for prioritizing testing and monitoring of pharmaceuticals.

(c) Translating therapeutic mode of action into adverse outcome(s) relevant for risk assessment

Adverse outcome pathways (AOPs) have been proposed as a conceptual framework through which to link chemical–biological interactions at the molecular level (termed MIEs) with key events at multiple biological levels of organization, culminating in an adverse outcome of regulatory significance at the individual or population level [27]. The Organisation for Economic Cooperation and Development (OECD) is coordinating an internationally harmonized effort to develop a knowledgebase of AOPs relevant to both human health and ecological effects [29]. Examples of well-defined AOPs relevant to established molecular targets of pharmaceuticals include aromatase inhibition, androgen receptor (AR) activation, oestrogen receptor activation or antagonism, and steroidogenesis inhibition leading to impaired reproduction in fish [27]. Several existing AOPs, and/or studies from which putative AOPs relevant to the function(s) of fish orthologues to human drug targets are summarized in table 1. Based on ongoing efforts in the international scientific community to delineate and disseminate AOPs suitable for regulatory application (http://www.oecd.org/env/ehs/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm and www.aopwiki.org), it is expected that growing numbers of well-described AOPs associated with orthologues to human drug targets will become available. Notably, further development of AOPs relevant to ecological risk assessment can be accelerated through strategic application of the SeqAPASS tool to help define
Table 1. Putative adverse outcome pathways for fish relevant to human and veterinary drugs. Single asterisk indicates established AOPs developed in accordance with OECD guidance and submitted to the AOP wiki (www.aopwiki.org). Double asterisk indicates hypothesized molecular initiating event or adverse outcome.

<table>
<thead>
<tr>
<th>drug class</th>
<th>molecular initiating event</th>
<th>example key events</th>
<th>adverse outcome</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>anabolic steroids</td>
<td>androgen receptor activation*</td>
<td>— impaired vitellogenesis, ovulation and spawning</td>
<td>— reproductive impairment</td>
<td>[24,27,30,31]</td>
</tr>
<tr>
<td>anti-androgens</td>
<td>androgen receptor antagonism</td>
<td>— impaired ovulation and spawning</td>
<td>— reproductive impairment</td>
<td>[32–37]</td>
</tr>
<tr>
<td>hormonal contraceptive</td>
<td>oestrogen receptor activation</td>
<td>— impaired ovulation and spawning</td>
<td>— reproductive impairment</td>
<td>[27]</td>
</tr>
<tr>
<td>hormonal contraceptive</td>
<td>oestrogen receptor antagonism*</td>
<td>— impaired vitellogenesis, ovulation and spawning</td>
<td>— reproductive impairment</td>
<td>[38–42]</td>
</tr>
<tr>
<td>hormonal contraceptive</td>
<td>progesterone receptor activation</td>
<td>— reduced fecundity</td>
<td>— reproductive impairment</td>
<td>[43–46]</td>
</tr>
<tr>
<td>anti-inflammatory</td>
<td>glucocorticoid receptor activation</td>
<td>— reduced aetrateadiol synthesis and fecundity</td>
<td>— reproductive impairment</td>
<td>[47–49]</td>
</tr>
<tr>
<td>anti-depressants</td>
<td>serotonin reuptake inhibition</td>
<td>— morphological abnormalities</td>
<td>— reproductive impairment</td>
<td>[50–55]</td>
</tr>
<tr>
<td>anti-convulsant</td>
<td>gamma-aminobutyric-acid receptor opening**</td>
<td>— impaired predator avoidance</td>
<td>— reduced probability of young of year survival**</td>
<td>[56,57]</td>
</tr>
<tr>
<td>non-steroidal anti-inflammatory drugs</td>
<td>cyclooxygenase inhibition</td>
<td>— increased probability of mortality**</td>
<td>— increased probability of young of year survival**</td>
<td>[58–60]</td>
</tr>
<tr>
<td>fibrates</td>
<td>peroxisome proliferator-activated receptor activation</td>
<td>— reduced fecundity</td>
<td>— reproductive impairment</td>
<td>[61]</td>
</tr>
<tr>
<td>beta-blockers</td>
<td>beta-adrenergic receptor antagonist</td>
<td>— reduced growth</td>
<td>— reproductive impairment</td>
<td>[62–64]</td>
</tr>
<tr>
<td>conazoles(^b)</td>
<td>aromatase inhibition*</td>
<td>— impaired vitellogenesis, ovulation and spawning</td>
<td>— impaired reproduction</td>
<td>[65–69]</td>
</tr>
<tr>
<td>anti-psychotics</td>
<td>dopamine receptor antagonism</td>
<td>— increased dominance</td>
<td>— adverse consequence unknown</td>
<td>[70,71]</td>
</tr>
<tr>
<td>goitrogens</td>
<td>thyroid peroxidase inhibition</td>
<td>— delayed development</td>
<td>— reduced probability of young of year survival**</td>
<td>[72–75]</td>
</tr>
</tbody>
</table>

\(^a\)D. Martinovic-Weigelt, University of St Thomas, St Paul, MN, personal communication.

\(^b\)Aromatase inhibition is an established molecular initiating event for propiconazole, however other conazoles have been shown to inhibit other cytochrome P450 enzymes [76].
which pharmaceutical-relevant MIEs are applicable to different phyla of aquatic organisms.

3. Prioritization based on potential for effects reveals uncommonly identified drugs

To demonstrate the utility of our ADME database and SeqA-PASS tool (in conjunction with AOP knowledge) for prioritizing pharmaceuticals for toxicity testing and environmental monitoring, we provide an example that focuses on drugs that interact with the AR in target species (humans, livestock). Owing to the critical role of the AR in endocrine function, it is an important drug target in humans for treating certain cancers, testosterone deficiencies, hypogonadism, dermatological conditions, hirsutism and delayed puberty in males, and in livestock, particularly beef cattle, for increasing muscle mass [24]. Using our ADME database, DrugBank and the Veterinary Substance Database [77], we first identified human and veterinary pharmaceuticals whose therapeutic MOA involved an interaction with the AR (table 2). The majority of these chemicals, which all have hormone-type structures, have neither been tested for toxicity to non-target species nor routinely monitored in the environment.

To determine the potential for effects in non-target species via interaction with the AR, we first assessed cross-species conservation of the human and bovine (target species) AR using SeqAPASS. Data from both analyses indicate a high degree of AR conservation across vertebrates, including fish species (figure 3; note that results are similar using either the bovine or human AR as queries, so only bovine is shown). The drugs in table 2 may target the AR as either agonists or antagonists. Importantly, both AR agonists and antagonists are linked to established or putative AOPs resulting in adverse reproductive effects in fish (table 1). Therefore, as either AR agonists or antagonists, these drugs would be considered high priority owing to conservation of their molecular target across vertebrate species, and the demonstration (through linkages established via AOPs) of the potential for adverse population-relevant effects.

Table 2. Prioritization of androgen receptor modulating drugs based on potential for adverse effects. Dashes represent drugs with limited pharmacokinetic data available for scoring individually. Tick indicates empirical evidence exists, represented by a select publication in the reference column.

<table>
<thead>
<tr>
<th>active ingredient</th>
<th>primary CAS no.</th>
<th>average ADME score</th>
<th>empirical evidence of adverse effect in fish</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>bicalutamide</td>
<td>90357-06-5</td>
<td>6.8</td>
<td>√</td>
<td>[78]</td>
</tr>
<tr>
<td>boldenone</td>
<td>846-48-0</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>calusterone</td>
<td>17021-26-0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyproterone</td>
<td>2098-66-0</td>
<td></td>
<td>√</td>
<td>[32]</td>
</tr>
<tr>
<td>danazol</td>
<td>17230-88-5</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dihydrotestosterone</td>
<td>521-18-6</td>
<td></td>
<td>√</td>
<td>[79]</td>
</tr>
<tr>
<td>drospirenone</td>
<td>67392-87-4</td>
<td></td>
<td>√</td>
<td>[80]</td>
</tr>
<tr>
<td>drostanolone</td>
<td>58-19-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>enzalutamide</td>
<td>915087-33-1</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fludrocortisone</td>
<td>127-31-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fluoxymesterone</td>
<td>76-43-7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flutamide</td>
<td>13311-84-7</td>
<td>5.0</td>
<td>√</td>
<td>[33]</td>
</tr>
<tr>
<td>levonorgestrel</td>
<td>17489-40-6</td>
<td>7.3</td>
<td>√</td>
<td>[81]</td>
</tr>
<tr>
<td>methylone testosterone, 7α, 19-</td>
<td>3764-87-2</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>methyltestosterone</td>
<td>58-18-4</td>
<td></td>
<td>√</td>
<td>[82]</td>
</tr>
<tr>
<td>methyltrienolone</td>
<td>965-93-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nandrolone</td>
<td>434-22-0</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nilutamide</td>
<td>63612-50-0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>norgestimate</td>
<td>35189-28-7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxandrolone</td>
<td>53-39-4</td>
<td>7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spironolactone</td>
<td>52-01-7</td>
<td>5.3</td>
<td>√</td>
<td>[24]</td>
</tr>
<tr>
<td>testolactone</td>
<td>968-93-4</td>
<td></td>
<td>√</td>
<td>[83]</td>
</tr>
<tr>
<td>testosterone</td>
<td>57-85-2</td>
<td></td>
<td>√</td>
<td>[30]</td>
</tr>
<tr>
<td>trenbolone</td>
<td>10161-34-9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aAverage ADME score: calculated by averaging hazard score across pharmacokinetic parameters (clearance rate, volume of distribution, therapeutic plasma concentration and half-life of elimination).
drugs classified as hormones in our ADME database (53 compounds) to derive a class-level priority ranking. Accordingly, probabilistic distributions were derived for each pharmacokinetic parameter individually to assess hormone-like drugs only. Within each parameter, we identified the value representing the 90th or 10th percentile (depending on hazard assumption) and used those values to represent hormones as a class. Subsequently, probabilistic distributions for all drugs in our database were derived for each pharmacokinetic parameter (e.g. figure 2). The hormone class assigned values were then scored based on the centiles from the distributions of all drugs. The final hazard score of 9.3 of 10 was then calculated by averaging hormone class scores across all parameters, ranking this drug class as high priority compared with other classes (e.g. narcotics score: 8.3; non-steroidal anti-inflammatory drugs score: 7; and antibiotics score: 6.8).

Relevant mammalian pharmacokinetic information (i.e. volume of distribution, clearance rate and/or half-life of elimination) was available in our ADME database for 10 of the 24 AR-active drugs identified. Based on this, individual AR-active drugs can be evaluated using distributions derived from all drugs for each pharmacokinetic parameter, scores assigned, and then averaged across each parameter (table 2). The resultant average ADME score allows for ranking an individual drug against all others evaluated, thus further helping assign priority based on the potential for hazard. For example, based on ADME properties, boldenone (an anabolic steroid illegally used to enhance athletic performance in canine, equine and human athletes, and improve food conversion in cattle [84]) would rank as a higher priority than 7α, 19-methylnortestosterone (under study in humans to treat hypogonadism in males and as a male contraceptive [85]) for testing or monitoring.

For some AR-active chemicals, empirical chronic toxicity data in non-target species (predominantly fish) are available, providing additional input for priority ranking and also illustrating where test data are needed (table 2). Significantly, a majority of the drugs listed in table 2 have not been identified by other prioritization strategies focused primarily on the potential for exposure in the environment. Analogous prioritizations have been and are being employed in our laboratory to guide our toxicity testing and field monitoring, which, as an example, recently led to the identification of spironolactone as a pharmaceutical of potential environmental concern to fish species [24]. In this case, the knowledge of a well-defined AOP for AR activation leading to reproductive impairment in small fish helped to focus toxicity testing with spironolactone on endpoints previously recognized as being impacted by perturbation of the AR. Additionally, SeqAPASS data directed the selection of test organisms likely to be susceptible to spironolactone, with predictions indicating fish would be more susceptible than invertebrates such as daphnids, which we subsequently confirmed experimentally [24]. In this manner, AOP knowledge in combination with SeqAPASS evaluation of cross-species susceptibility offers a powerful means to guide toxicity testing, with the potential to reduce the investment of time, resources and animal testing.

4. Summary and vision for prioritization
The read-across components of our effects-based prioritization strategy for human and veterinary drugs, combined with application of the AOP framework and approaches for defining taxonomic relevance of key events (e.g. MIE), and evaluation of available empirical toxicity data, provide insights that complement analyses based on exposure. Although potential for exposure is undeniably important, biological considerations are critical to a comprehensive approach for drug prioritization in terms of assessing potential for adverse effects in non-target organisms. Central to this strategy is evaluation of biological pathways and processes, and their conservation across taxa, a central theme to the broader issue of cross-species extrapolation of chemical effects in the discipline of toxicology [86].

A number of recent publications have presented various methods for drug prioritization. Briefly, these papers included consideration of conserved receptors and enzymes important as drug targets across species [11, 14, 23, 24, 87]; calculated effects ratios using human and fish plasma concentration data [87]; acute to therapeutic ratios [22, 88]; identification of high production volume drugs, identification of those that are persistent, bioaccumulative and toxic [89]; maximum observed (environmental) concentrations, predicted effect concentrations and predicted no-effect concentrations [11, 87]; prescription volume and sales data, evaluation of days of water consumption required to ingest equivalent of a single minimum daily therapeutic dose for a given pharmaceutical MOA [11]; and consideration of AOPs [24, 88]. From these prioritization methods, it is clear that an integrated approach is on the horizon when the tools become available for rapidly and strategically disseminating available pharmaceutical data. Our strategy builds on some of the concepts described previously and specifically introduces a novel tool (i.e. SeqAPASS) and database (ADME database) being developed for use by the scientific community which will be publically released in the near future for others to use for drug prioritization.

Importantly, the prioritization strategy and tools described herein are intended to guide toxicological research and inform lists of chemicals to monitor in the environment. We recognize that the quality of the assumptions used for the read-across approaches described are based on current knowledge of the science and therefore must be applied with an understanding that uncertainties exist. Although research is ongoing, empirical studies are limited relative to the assumption that mammalian ADME data translates well to fish. For example, a recent study assessing metabolism of common human pharmaceuticals in fish provided evidence that, in rainbow trout liver 59 fractions, little substantial biotransformation occurred for several known substrates of human cytochrome P450 (CYP) 2D6, CYP2C9 or CYP3A4 [90]. These results suggest that further research is necessary to understand the challenges associated with the ADME read-across approach [91]. However, in the absence of fish-specific data, mammalian pharmacokinetic knowledge currently presents the most logical starting point for read-across approaches owing to its abundance and availability. Further, uncertainties related to cross-species extrapolation using protein sequence/structure comparisons have been identified and reviewed elsewhere [14, 23]; for example, it is recognized that other factors play a role in determining susceptibility to a chemical beyond the presence of a molecular target (e.g. metabolism, life stage, life history) [92]. Off-target molecular effects can also impact predictions of susceptibility. Finally, fully developed AOPs, and even putative AOPs, for pharmaceuticals are sparse and though the AOP framework has strong support from the international scientific community, it will take time for new AOP
constructs to be developed, accepted as having adequate supporting evidence, and be available for use in a prioritization strategy for the majority of pharmaceutical therapeutic classes. Nonetheless, important advances in hazard prediction relevant to drug prioritization for toxicity testing and monitoring can be obtained from making use of available pharmaceutical information with strategic and thoughtful approaches, such as those presented here. Specifically, the presence of a drug on a prioritized list indicates that sufficient predictive and/or empirical support ranks it as having an increased potential for adverse effects in non-target species, and therefore evidence to proceed with further exploration.

Acknowledgements. The authors thank A. Schroeder for providing thoughtful review comments on an earlier version of the paper. We also acknowledge J. Tietge and C. Russom for their insightful discussions and input regarding development of our methods for drug prioritization. This manuscript has been reviewed in accordance with the requirements of the US EPA Office of Research and Development; however, the recommendations made herein do not represent US EPA policy. Mention of products or trade names does not indicate endorsement by the US EPA.

References


57. Ankley GT et al. 2005 Effects of two fungicides with multiple modes of action on reproductive endocrine function in the fathead minnow (Pimephales


72. Sharma P, Patino R. 2013 Regulation of gonadal sex ratios and pubertal development by the thyroid hormone *Pimephales promelas* (DHT) and 5α-dihydrotestosterone (DHT). Environ. Health Perspect. 121, 1002 – 1010. (doi:10.1289/ehp.1306638)


83. Connors KA, Du B, Fitzsimmons PN, Hoffman AD, LaLone CA, Johnson MS, Tietge JE, Villeneuve DL. 2012.08.036)

