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A comprehensive aquatic risk assessment of the beta-blocker propranolol, based on the results of over 600 research papers

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HIGHLIGHTS

- Comprehensive environmental risk assessment of the pharmaceutical propranolol
- Median river concentration of propranolol 7.1 ng/L
- Toxicity of propranolol very low (median EC50 6.64 mg/L) to all species in validated tests
- Reports of very low concentrations causing effects must be shown to be repeatable.
- Propranolol is concluded to be of very low risk to aquatic organisms.

GRAPHICAL ABSTRACT

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ABSTRACT

A comprehensive aquatic environmental risk assessment (ERA) of the human pharmaceutical propranolol was conducted, based on all available scientific literature. Over 200 papers provided information on environmental concentrations (77 of which provided river concentrations) and 98 dealt with potential environmental effects. The median concentration of propranolol in rivers was 7.1 ng/L (range of median values of individual studies 0.07 to 89 ng/L), and the highest individual value was 590 ng/L. Sixty-eight EC50 values for 35 species were available. The lowest EC50 value was 0.084 mg/L. A species sensitivity distribution (SSD) provided an HC50 value of 6.64 mg/L and an HC5 value of 0.22 mg/L. Thus, there was a difference of nearly 6 orders of magnitude between the median river concentration and the HC50 value, and over 4 orders of magnitude between the median river concentration and the HC5 value. Even if an assessment factor of 100 was applied to the HC5 value, to provide considerable protection to all species, the safety margin is over 100-fold. However, nearly half of all papers reporting effects of propranolol did not provide an EC50 value. Some reported that very low concentrations of propranolol caused effects. The lowest concentration reported to cause an effect - in fact, a range of biochemical and physiological effects on mussels - was 0.3 ng/L. In none of these 'low concentration' papers was a sigmoidal concentration-response relationship obtained. Although inclusion of data from these papers in the ERA cause a change in the conclusion reached, we are sceptical of the repeatability of these 'low concentration' results. We conclude that concentrations of propranolol present currently in rivers throughout the world do not constitute a risk to aquatic organisms. We discuss the need to improve the quality of ecotoxicology research so that more robust ERAs acceptable to all stakeholders can be completed.

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1. Introduction

For the last two decades there has been significant concern about the impact of pharmaceuticals present in the environment on both invertebrate and vertebrate wildlife (Fent et al., 2006; Arnold et al., 2014). The greatest concern exist for aquatic species, because the aquatic environment probably contains the highest concentrations of human pharmaceuticals, as a consequence of their incomplete removal in wastewater treatment plants (Gardner et al., 2012; Falås et al., 2016) and hence presence in effluents that are discharged into rivers. In general, concentrations of individual pharmaceuticals in rivers are reported to be low: low ng/L concentrations are typical (Hughes et al., 2013; Weber et al., 2015), although in a few unique situations they might reach higher concentrations (e.g. Larsson, 2014). Intuitively these low concentrations might suggest that there is little, if anything, to be concerned about. However, although it is probably true that relatively few pharmaceuticals do pose a significant risk to aquatic organisms, some of those that bioconcentrate in organisms might reach internal concentrations high enough to cause effects. This seems to be the case with some of the synthetic sex steroid hormones, which can adversely affect reproduction of fish when present in the surrounding water at extremely low concentrations, even concentrations below 1 ng/L (e.g. Caldwell et al., 2012; Runnalls et al., 2013; Zeilinger et al., 2009). This realisation has led to some of the synthetic steroidal pharmaceuticals being considered the pharmaceuticals of highest risk to aquatic organisms (Runnalls et al., 2010; Caldwell et al., 2012; Gunnarsson et al., 2019).

One pharmaceutical that has received considerable attention from both environmental chemists and environmental toxicologists is the beta-blocker propranolol. This drug often features relatively high up in lists of pharmaceuticals considered to represent a significant risk to the environment (e.g. Roos et al., 2012). In fact, Donnachie et al. (2016) reported that propranolol features very often in prioritization articles in the literature. This conclusion has been reached on the basis that propranolol has been detected very often and geographically very widely in effluents of wastewater treatment works (WWTWs), and hence also in receiving rivers, and that some papers have reported adverse effects of extremely low concentrations of the drug on a number of species of aquatic organisms (see later for specific references).

Propranolol is used mainly to treat high blood pressure (hypertension) and angina, although it is also prescribed for a wide variety of other adverse health conditions, such as migraine and some psychiatric conditions. In addition to the formally approved therapies, propranolol is known to be prescribed 'off-label' by doctors; that is, for a range of conditions that the doctor considers it may be appropriate and provide benefit. It is also used by musicians, actors and public speakers for its ability to treat anxiety symptoms, and as a performance-enhancing drug in sport where high accuracy is required. British scientist Sir James W. Black developed propranolol in the 1960s. It was patented in 1962 and approved for medical use in 1964. It was the first beta-blocker used effectively in the treatment of coronary artery disease and hypertension. It is on the World Health Organisation's List of Essential Medicines. Although it is steadily being replaced by newer, more cardio-selective beta-blockers, such as bisoprolol and metoprolol, it is still used extensively. In 2017 it was the 41st most commonly prescribed medicine in the United States, with more than 17 million prescriptions.

The pharmacodynamics of any drug are highly relevant to any effects they might cause to organisms when they are present in the environment (Rand-Weaver et al., 2013). Propranolol is a non-selective β-adrenergic receptor antagonist, or beta-blocker; that is, it blocks the action of adrenaline (epinephrine) and noradrenaline (norepinephrine) at both β1 and β2-adrenergic receptors. It is also a weak, indirect α1-adrenoreceptor agonist, in addition to its potent β-adrenoreceptor antagonism. These molecular targets of propranolol - the α and β-adrenergic receptors - are highly conserved (Gunnarsson et al., 2008), being present throughout the animal kingdom, including in unicellular organisms. However the distribution and functionality of these receptors varies (Owen et al., 2007). This has been recognised for decades by comparative physiologists who have, for example, used propranolol to block β-adrenergic activity when investigating the control of the heart of lower vertebrates, particularly fish (e.g. Keen et al., 1995; Tirri and Lehto, 1984). Given the presence of the drug's target in a wide spectrum of organisms, it was perhaps understandable that there was concern about the presence of propranolol in the environment causing adverse effects on exposed organisms.

Based on the current continuing interests, from both scientists and regulators, on pharmaceuticals in the environment, and the fact that propranolol has been one of the most widely studied drugs by both environmental chemists and environmental toxicologists, we considered that it would be instructive to conduct an environmental risk assessment on propranolol, utilising all available literature. Regulatory environmental risk assessments are now conducted as medicines are registered in many regions of the world. However, those utilise predicted environmental concentrations based on the epidemiology and potential use and compare those concentrations to effects generated in standard tests. In contrast, our risk assessment presented here uses the open scientific literature. The originality of our approach lies in the fact that we utilised the entire open scientific literature on the environmental presence and effects of propranolol: over 640 papers were examined. In contrast, other environmental risk assessments of pharmaceuticals, or any chemical for that matter, have utilised very much smaller datasets (e.g. Straub, 2009, 2017), usually because the author(s) have followed regulatory guidelines on how to conduct environmental risk assessments of chemicals. However, the open scientific literature is very much more variable and expansive. To provide just one example of why, research scientists will often use species that they are interested in, and familiar with, in toxicity tests, rather than use the limited range of species recommended by regulatory authorities, such as the OECD. Thus, the uniqueness of this paper lies in the fact that it utilises all relevant scientific literature, making it currently probably the most thorough and comprehensive risk assessment of any human pharmaceutical. It highlights many of the problems associated with regulatory decision making when it comes to deciding whether or not any particular pharmaceutical poses an unacceptable risk to the environment.

2. Materials and methods

2.1. Literature searching

The main source of relevant literature was the Web of Knowledge (WoK) Core Collection of journals. This was searched using a wide variety of search terms in late February and early March 2017. The search terms used are listed in Supplementary Information Table S1. The WoK searches for specified key search words (e.g. propranolol) in the title or abstract or keywords of scientific papers. In order not to limit our searching, we conducted multiple searches, based mostly on pairs of key words (e.g. propranolol and fish) rather than conduct one large search. This produced a lot of duplication, as expected, yet almost every individual search provided one or more references that were not included in any of the other searches. The US EPA Ecotox database (www.epa.gov/chemical-research/ecotoxicology-database) was also searched using the key word propranolol. This produced 44 references, most of which had been captured in the WoK searches.

Based on these searches, an initial list of 1100 papers was compiled. It was obvious from the titles of these papers that some were not relevant to an environmental risk assessment of propranolol. Based on the titles of the papers, a master list of 596 papers that could be relevant was compiled. To assess if it was appropriate to remove just over 500 papers from the initial list, a random sample of 25 of those papers was read; all proved to be irrelevant, thus justifying their exclusion.

One author (JPS) then searched his personal library for relevant papers, finding over 200. The majority of those papers were in the list created from the WoK searches, but some 45 were not. Those that were not often had phrases such as "selected pharmaceuticals" or "multi-residue analysis of pharmaceuticals" in their titles, but propranolol was not specifically mentioned until the Results sections of the papers. These additional 45 references were added to the master list, making it a list of 641 papers likely to be of relevance.

All 641 papers were then read in full, enabling their relevance to be determined. Only papers containing novel data on either measured concentrations of propranolol in the aquatic environment (but including at this stage influent and effluent from WWTWs) or concentrations causing effects on aquatic organisms (including all organisms and all endpoints) were considered relevant to the risk assessment.

Limited, selective, literature searching was conducted to determine if river concentrations had changed since the end of the main literature search conducted in early 2017. Details can be found in Section 2.8.

2.2. Environmental concentrations

There were 211 papers dealing with environmental concentrations (exposure) amongst the 641 papers considered to be of potential relevance. About half of these papers provided river concentrations; the rest provided only WWTW influent and/or effluent concentrations (about 30% of the papers), hospital wastewater concentrations (10%), estuary concentrations (5%), groundwater (5%) or marine concentrations (5%). Marine concentrations were excluded from subsequent analyses on the basis that they were likely to be lower than river concentrations. Estuary concentrations were also excluded because they originated anywhere from the mouth of the estuary (i.e. were almost 100% seawater) to 20 Km upstream (almost, but not quite, 100% freshwater). Data from hospital wastewater was also excluded on the basis that this wastewater usually goes to a conventional municipal WWTW before the latter discharges its effluent into rivers. In contrast, data from freshwater lakes was included in all analyses. These freshwater lakes were fed by rivers; often rivers receiving WWTW effluent. Some received WWTW effluent directly. The lakes varied in size; some were very large, especially some in China. These lakes all contained populations of aquatic organisms (e.g. fish), and thus were considered relevant from an environmental risk assessment perspective. Despite not utilising reported propranolol concentrations in the marine environment, estuaries, and groundwater, for completeness these are reported in the Supplementary Information (Table S2).

Seventy-seven (77) papers provided novel, quantifiable river concentrations of propranolol (i.e. data not reported in any other paper); these papers are listed in Table S3. Some of these papers provided only one single concentration (because only one sample was collected and analysed), whereas other papers provided information on tens, and very occasionally over one hundred, river water samples. A few papers did not state how many samples were analysed. About three quarters of the papers provided numerical concentrations (i.e. individual numbers, usually in tables), but one quarter provided data in figures only. In the latter case we estimated the numerical concentrations from the figures.

2.3. Dealing with the limit of quantification (LOQ) issue

Most papers provided limits of quantification (LOQ), but some did not do so. There were 12 papers in which river concentrations were measured but all samples had concentrations below the LOQ; these papers are listed in Table S4. However, in the majority of papers some samples had concentrations below the LOQ, whereas other samples had measurable concentrations. When the latter situation occurred, it was often not specified how many samples had concentrations below the LOQ; only the data for samples with measurable concentrations were provided. It seems that most authors excluded the samples with concentrations below their LOQ when calculating mean/median concentrations. Thus, they determined and reported the mean and/or median concentration of only those samples that had quantifiable propranolol concentrations. Doing so introduces a positive bias, of course, but we could not correct that bias because the papers usually did not contain the information needed to do so.

We are sympathetic to the situation many authors found themselves in, because it is very difficult to know how to deal with the 'non-detects': those samples with propranolol concentrations below the LOQ. A variety of approaches are possible, including ignoring them, treating them as zeros when determining means or medians, assigning to all samples with concentrations below the LOQ a value half way between zero and the LOQ (i.e. if the LOQ was 4, assign a value of 2), or assigning to all samples with concentrations below the LOQ the LOQ value (i.e. 4 in the example above). As far as we can judge, all approaches had been adopted in the literature from which we extracted environmental propranolol concentrations. As recently discussed (Hites, 2019), there is no generally accepted way of dealing with the limit of detection (LOD/LOQ) issue. We chose to use an approach that utilised all the data available to us, rather than omit any of them from our analyses.

2.4. Dealing with the chirality issue

Propranolol is chiral; it consists of a 50/50 racemic mixture of R(+) and S(−) enantiomers, and the S enantiomer is considered the beta-blocker (100× potency of the R). Six papers separately measured concentrations of these two propranolol enantiomers, and hence provided the concentrations of each one. In two of these papers the sum of the two enantiomers was also provided. When this was not done by authors, we added together the concentrations of the two enantiomers to obtain a 'propranolol concentration'.

2.5. Calculating a representative concentration

The number of samples analysed by the 77 studies providing quantifiable concentrations of propranolol in the freshwater environment ranged from 1 to 154 (Table S3). It is not possible to provide the exact number of samples in this category for a number of reasons, including that some studies did not provide the number of samples analysed and other studies provided the number of samples analysed but not the number that had quantifiable concentrations above the LOQ. With these caveats in mind, we estimate that the number of samples with measurable concentrations was around 1700.

Most studies provided median concentrations of the samples analysed, although some provided mean concentrations. To obtain a representative freshwater propranolol concentration we aggregated the medians by determining the median of the individual medians: this is an acceptable meta-analysis approach (McGrath et al., 2019). Hence we did not incorporate the study-specific, and very variable, sample size into our analysis. Doing so would have introduced a strong bias towards those studies that analysed high numbers of samples. Instead, each study was equally weighted in our calculation of a representative freshwater propranolol concentration.

2.6. Effect concentrations

Out of the master set of 641 papers, 98 were categorised as dealing with a potential environmental effect of propranolol on any aquatic organism. In addition, over 60 papers dealt with the role of adrenaline/noradrenaline in a wide range of physiological processes in a variety of species. In those studies propranolol was used to block the normal physiological responses to endogenous adrenaline/noradrenaline, thereby determining the roles of these catecholamines. The physiological processes studied in these laboratory studies included control of the cardiovascular system, reproduction, colour change, release of hormones from endocrine glands, and stress. Usually only one, very high,

concentration of propranolol was used in these comparative physiology studies, because the aim was to block completely the effects of endogenous adrenaline/noradrenaline. Thus these studies were not of use as far as determining the potential effects of propranolol on freshwater species present in the natural environment, and as a consequence they were excluded from this environmental risk assessment.

Out of the 98 potentially useful papers, 8 were based solely on the use of various cell lines or primary cells; these were excluded from subsequent analyses. A few of the 98 papers repeated results that had previously been reported. Forty-two (42) papers contained EC50 values, and hence were used in the construction of a species sensitivity distribution (SSD; see below). However, some papers duplicated results already published; 35 papers provided unique EC50 values. A further 26 papers reported statistically significant effects of propranolol, but from which an EC50 value could not be obtained (Table S5). There were a number of reasons for his, the major two being that only one concentration of propranolol was tested and that a non-sigmoidal concentration-response relationship was reported. Results from those 26 papers could not be incorporated into a SSD, but were used and displayed by us here in other types of presentation of the effect concentrations. Thirteen (13) of the papers covering environmental effects of propranolol reported that no effects occurred, and in two papers it was unclear whether or not any effects had occurred. We have included all effect data obtained from laboratory studies utilising marine species (e.g. mussels) because there is a considerable amount of them and because much of those data appear to suggest that effects can occur at very low concentrations of propranolol, and hence excluding those data might introduce bias into our analysis.

2.7. Presentation of the data

Two approaches to displaying the data were adopted. The most widely utilised methodology is to create a Species Sensitivity Distribution (SSD). A SSD is a probability distribution describing the sensitivity of multiple species to a hazardous compound, in this case the human pharmaceutical propranolol. To create the SSD we used the executable tool ETX, access to which was kindly provided by Professor Leo Posthuma of RIVA, The Netherlands (see also Posthuma and De Zwart, 2014). The second approach was developed recently by Donnachie and her co-workers. It allows both environmental concentrations and effect concentrations of a chemical to be displayed in a single figure (see, for example, Donnachie et al., 2016). Whereas SSDs are based solely on EC50 values, the methodology developed by Donnachie and colleagues is more flexible, and can also incorporate all reported effect concentrations, not only EC50 values.

2.8. Recent information on environmental concentrations of propranolol

The main literature searches were conducted in early 2017. To determine whether or not the results derived from that literature are still valid today, reported concentrations of propranolol in rivers were obtained from recently published papers. A comprehensive literature search for relevant papers published between 2017 and 2020 was not conducted; instead, ten recently published papers providing information on concentrations of propranolol in rivers, each based on large sampling programmes, were randomly selected (Table S6) and the relevant information extracted from them.

3. Results

3.1. River concentrations of propranolol

Concentrations of propranolol in rivers were available from 20 countries, with Spain, in particular, and France being the most represented. Well over half of all data came from Western European countries. No information was available from Africa (but see below for recent information) or the Middle East, and very little was available from South American countries (one study only).

The median concentration of propranolol in rivers was 7.1 ng/L. The range of the 77 median/mean values of individual studies was 0.07 to 89 ng/L. Only 9 papers reported any individual river water samples with concentrations above 100 ng/L. The highest single sample concentration was 590 ng/L.

3.2. Concentrations of propranolol causing effects

A significant proportion of the available data did not come from standardised toxicity tests, such as those recommended by the OECD. Ideally, acute and chronic toxicity data would have been separated, and analysed separately. However, doing so was problematic for two reasons. One was that relatively few authors stated whether the tests they conducted were considered acute or chronic. The other was that there is no agreed position on what constitutes acute toxicity and what constitutes chronic toxicity. This problem is illustrated in the generally excellent paper of Ferrari et al. (2004). For example, they consider a two-day fish embryo test as providing acute toxicity data, whereas a ten-day fish embryo test provides chronic toxicity data. In both cases the same endpoint, namely mortality, was utilised. But the same paper also demonstrates that the duration of the test cannot necessarily be used as the differentiator, because a 96-hour alga test can be considered both acute (if the EC50 value is the parameter obtained) or chronic (if the NOEC value is the parameter obtained). Because we did not want to make the decisions on whether a test was acute or chronic, we have not separated the toxicity data along those lines.

Another potential strategy would have been to utilise NOEC values in our assessment of toxicity. However, only 8 out of the 98 papers dealing with effects of propranolol provided NOECs. In total, those 8 papers provided 17 NOECs, 6 of which came from just one paper (Ferrari et al., 2004). In contrast, 68 EC50 values were available, thus providing a much larger dataset, and hence we chose to use that dataset. There were 13 examples where both an EC50 and a NOEC were provided from the same test. Based on this limited dataset, the median EC50: NOEC ratio was 6.4. That is, the NOEC was lower than the EC50 by 6.4-fold.

Given that by far the largest dataset was based on EC50s, and our strong desire not to bias our analysis by us conducting further analysis on the published information (for example, by us trying to determine NOECs based on the published information), we chose to use EC50 values in our analysis. The limitations of doing so are discussed later in the paper.

Sixty-eight independent EC50 values for 35 species, covering a wide range of endpoints, were available (Table S7), and these were used to construct an SSD (Fig. 1). The species represented in the SSD include algae (both freshwater and marine), higher plants, bacteria, a variety of invertebrates (including ciliates, rotifers, crustaceans, echinoderms, insects and planarians) and fish. For some species more than one, independently-derived, EC50 value was available. For example, four EC50 values were available for the green algae *Raphidocelis subcapitata*, five for the duckweed *Lemna minor*, and eleven for the crustacean *Daphnia magna*, enabling intra-species sensitivity values to be obtained for some species. In the case of *Daphnia magna*, the EC50 values for immobilisation ranged from 0.46 to 18.1 mg/L, a 39-fold difference.

Most EC50 values, irrespective of the species, were in the low mg/L range. No particular species, or group of species, were significantly more sensitive than any others. For example, algae were of comparable sensitivity to Daphnia and other invertebrates. The lowest EC50 value was 0.084 mg/L, which was for a marine periphyton community. The SSD provided an HC50 value of 6.64 mg/L (95% confidence intervals 4.38 and 10.08 mg/L) and an HC5 value of 0.22 mg/L (95% confidence intervals 0.1 and 0.39 mg/L).

The spread of EC50 values can be more easily visualized in Fig. 2. They cover a range greater than 50,000-fold.

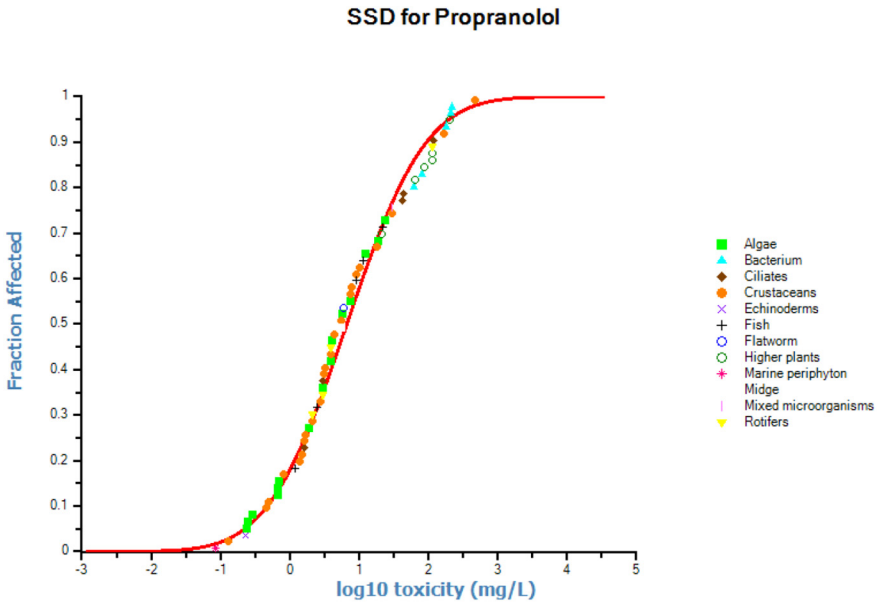

Fig. 1. A species sensitivity distribution (SSD) in mg/L for propranolol based on all acute and chronic EC50 values identified in this study.

Fig. 2 also displays the measured river concentrations of propranolol, and hence the degree of overlap - if any, of course - between concentrations in rivers and those causing effects can be readily observed. In this case it is clear that there is no overlap between environmental concentrations and effect concentrations (here represented by the EC50 values). In fact there is an almost 1000-fold difference between the highest median river concentration (89 ng/L) and the lowest EC50 concentration (0.084 mg/L). This very significant gap is most clearly displayed in Fig. 3, in which cumulative frequency distributions of both river concentrations and EC50 effect concentrations are plotted. Even if an assessment factor of 1000 was applied by a regulator, thus producing a very small degree of overlap between effect concentrations and river concentrations (just one EC50 value out of 68 would be lower than the highest median river concentration), this still does not imply risk.

As stated in the Methods and Materials, nearly half of the papers reporting effects of propranolol on aquatic organisms did not provide an EC50 value, nor could one be calculated or estimated from the published information. In many cases this was because only one, or two, concentrations were tested, rather than a full concentration-response curve being obtained. When these data are added to the EC50 effect data, to obtain a complete set of effect data, a different picture emerges. Fig. 4 shows that the spread of the non-EC50 values was extremely large, ranging from 0.3 ng/L (3 independent studies, albeit all from the same research group) to over 100 mg/L. The most noticeable factor, however, is that a considerable number of non-EC50 values are lower than any of the EC50 values, often very markedly lower. Eleven separate studies report effect concentrations below 1 μg/L. The species reported to respond to concentrations of propranolol in the ng/L range include

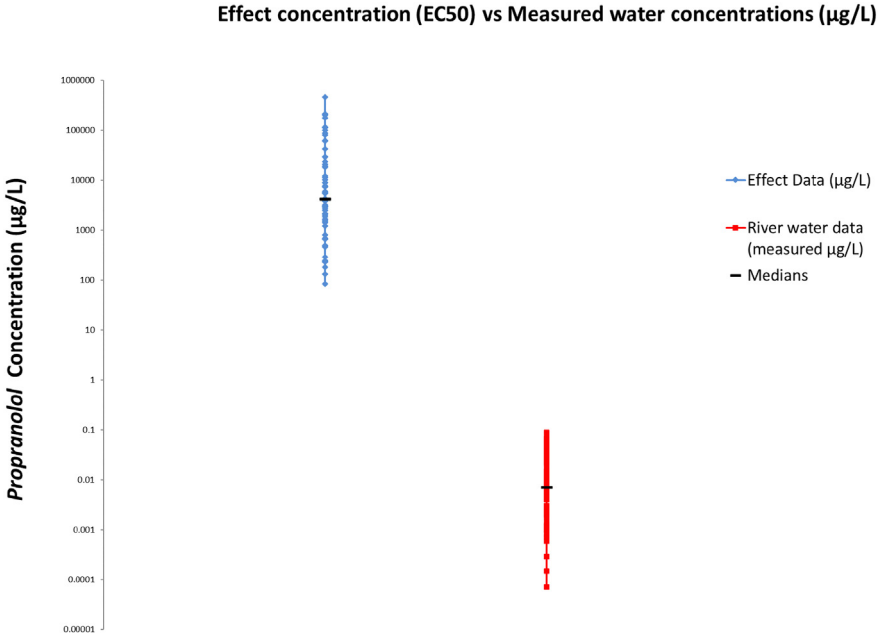

Fig. 2. A comparison of literature EC50 concentrations for propranolol (blue diamonds; left-hand column) with measured river concentrations of propranolol (red squares; right-hand column). The median values are plotted as black dashes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

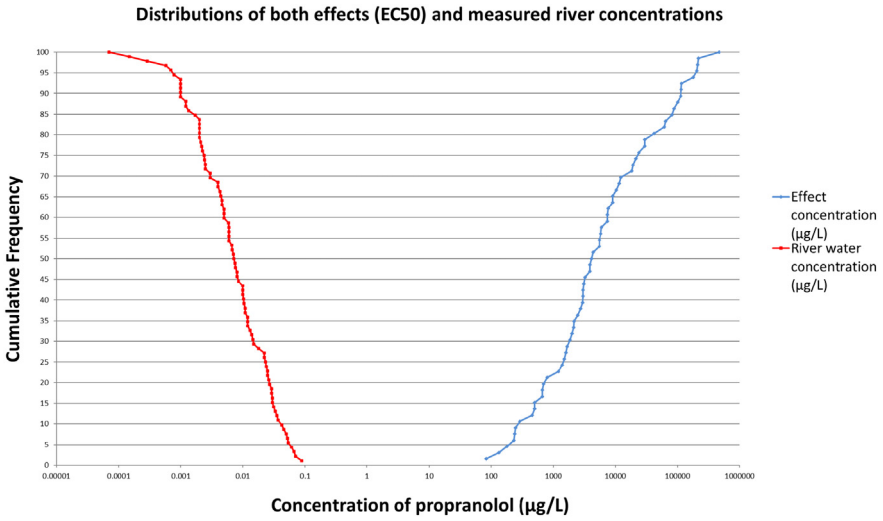

Fig. 3. Cumulative frequency distributions of the EC50 concentrations for propranolol (blue diamonds) and measured river concentrations of propranolol (red squares). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microalgae, Daphnia, marine mussels and zebrafish, with an equally wide range of endpoints being reported.

The inclusion of the non-EC50 data leads to an overlap of the river concentrations and the effect concentrations (Fig. 5A). Put another way, effects of propranolol on some species have been reported to occur at concentrations of propranolol present in some rivers. This consequence of including all effect data (EC50 and non-EC50) is most clearly observed in Fig. 5B, in which separate cumulative frequency distributions for the EC50 values and the non-EC50 values are shown. Only the non-EC50 effect concentrations provide any overlap with the median river concentrations. Around 20% of the non-EC50 values overlap with propranolol concentrations in rivers.

3.3. River concentrations of propranolol reported in recent publications

In general, recent studies reported lower LOQ levels than older studies. They also often involved larger sampling campaigns and report more 'raw' data, often in Supplementary Information. Data from many countries (Egypt, Sri Lanka, China, Spain, Wales, Mexico, Slovenia, and England) were collected. Most papers reported significant proportions of samples having non-detectable propranolol concentrations, even studies in which the LOQ was lower than 0.5 ng/L. In a comprehensive study of water quality in small, rural and effluent-dominated streams and rivers in northeast Spain, it was reported that all samples taken upstream of any wastewater entry point had non-detectable propranolol concentrations (<LOQ of 0.26 ng/L), whereas downstream water samples had concentrations ranging from <LOQ to 5.44 ng/L (Mandaric et al., 2018). The median concentration of the samples containing detectable propranolol concentrations was 3.77 ng/L. However, a much more representative median concentration would be below the LOQ value of 0.26 ng/L, because 24 of the 33 water samples collected downstream of wastewater entry points had concentrations at or below the LOQ.

Similarly, a comprehensive nationwide study of pharmaceuticals in streams in China reported a median propranolol concentration below the LOQ of 0.2 ng/L (Yao et al., 2018). In that study the mean concentration was reported to be 0.25 ng/L and the maximum concentration was 3.1 ng/L.

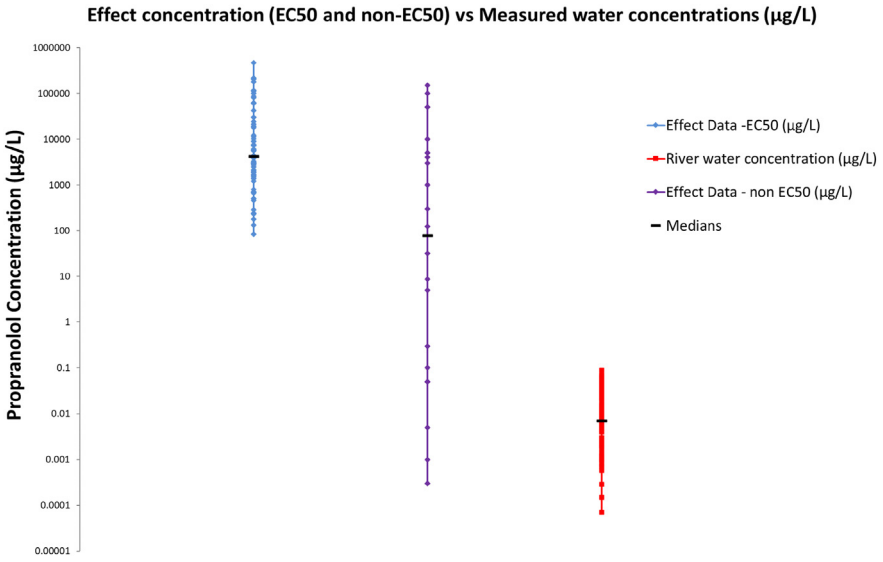

Fig. 4. A comparison of all concentrations of propranolol reported to cause an effect (left-hand columns; EC50 values in blue diamonds, non-EC values in purple diamonds) with measured river concentrations of propranolol (right-hand column; red squares). The median values are plotted as black dashes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

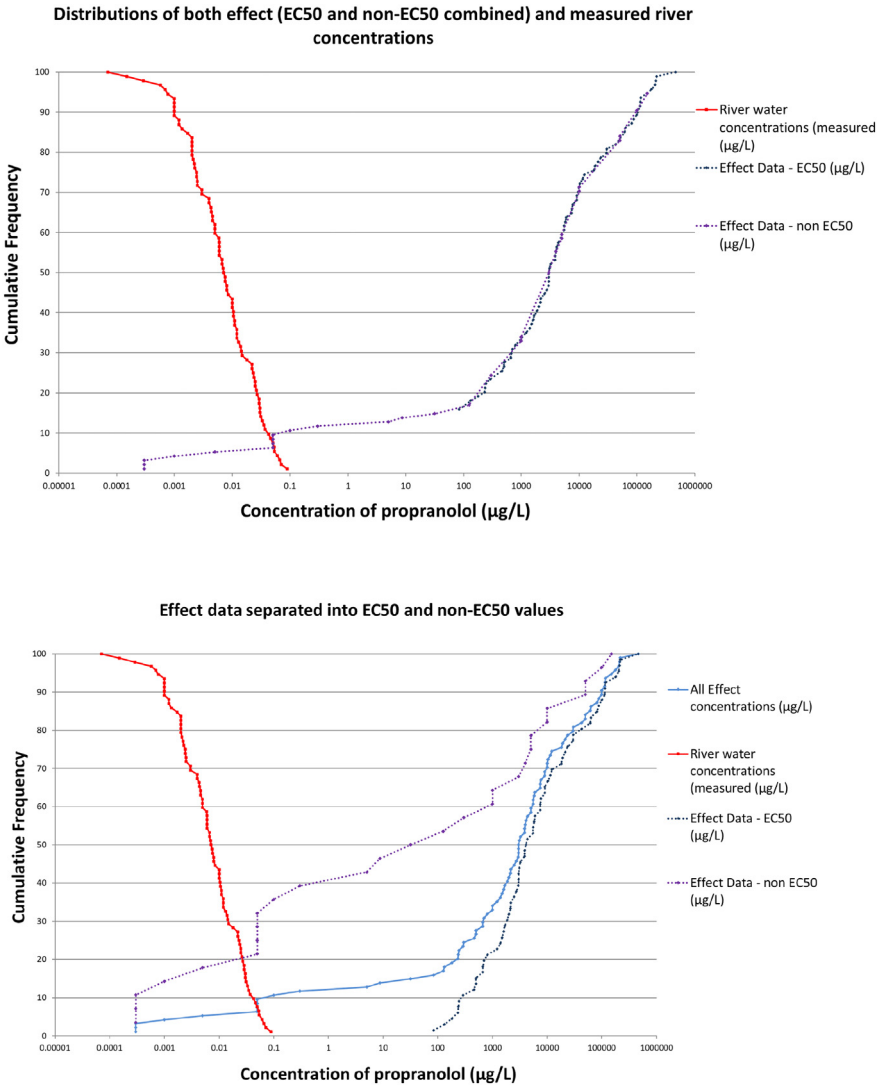

Fig. 5. Cumulative frequency distributions of all concentrations of propranolol reported to cause an effect (right-hand distributions) and measured river concentrations of propranolol (red squares). Panel A shows all effect data (EC50 values in blue diamonds, non-EC50 values in purple diamonds) combined into a single frequency distribution, whereas Panel B shows separately the EC50 values (black diamonds) and non-EC50 values (purple diamonds) as two separate frequency distributions, as well as the frequency distribution based on the combined dataset. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The highest propranolol concentrations reported recently were from the UK. In Wales, an average concentration of 20.6 +/− 3.5 ng/L was reported (Proctor et al., 2019) and in England the range was reported to be <5 to 67 ng/L (White et al., 2019).

4. Discussion

4.1. Collection of data

Large amounts of data for both concentrations of propranolol in rivers and lakes and concentrations of propranolol reported to cause effects to aquatic organisms were available. Despite the fact that it is very likely that some of the source papers contained errors, and equally likely that we introduced errors when we extracted the data required and entered them onto spreadsheets, the quantity of data available probably largely negated any problems, allowing us to reach reliable, robust conclusions free of significant bias. To provide just one example of the errors we found in published papers, the otherwise excellent review by Fent and colleagues (Fent et al., 2006) contains an error in the reported concentrations of propranolol. Whereas Roberts and Thomas (2006) report both influent and effluent propranolol concentrations to be in the ng/L range, their values are reported in Table 2 of Fent et al. (2006) to be in µgs/L. Hence a concentration of 304 ng/L became a concentration of 304 µg/L. Fortunately, Fent et al. (2006) did not repeat this error in their figures, or in their calculations of the median propranolol concentration. Thus a very comprehensive, thoughtful and important review was not adversely affected by this simple, easy-to-make error.

It is inevitable that our literature searches failed to locate some relevant papers, although we consider that the number of relevant papers unintentionally omitted is likely to be low, and hence their inclusion would probably have had little, if any, significant impact on our conclusions.

4.2. Concentrations of propranolol in rivers

In general, there was a high degree of consistency of reported propranolol concentrations in rivers, which provides a great deal of confidence in the data. Measured concentrations of propranolol in rivers were in the low ng/L range, or even lower, irrespective of the river (large or small), the country, or the analytical methodology employed to measure those concentrations. In fact the median concentration we obtained of 7.1 ng/L is very likely to be an over-estimate of the true representative concentration, mainly as a consequence of the issues outlined in the Materials and Methods. The most important issue was

how the LOQ problem was dealt with in the individual papers: often it appeared that the median (or mean) concentration provided in a paper was the median only of the river water samples that had quantifiable propranolol concentrations. If all samples that had limit of detection (LOD) or LOQ propranolol concentrations had been included when authors reported their data, then it is probable that our calculated overall median concentration of 7.1 ng/L would have been lower, possibly by an appreciable amount. However, we sympathise with authors presenting concentration data because there is no generally accepted way of arriving at a median (or mean) concentration when some samples have either no detectable target analyte or concentrations that cannot be quantified accurately (Hites, 2019). We applaud some authors who carefully described how their median propranolol concentrations were derived (see, for example, Guruge et al., 2019).

Drug manufacturing can lead to pollution 'hot spots' where environmental concentrations of pharmaceuticals can be very much higher than typical representative concentrations (Larsson, 2014). However, we are not aware that any propranolol 'hot spot' has been identified, leading us to conclude that it seems unlikely, based on current knowledge, that any aquatic environment has a propranolol concentration above the ng/L range identified here as representative.

Although we did not conduct a thorough review of the literature published after we conducted our extensive literature searches in early 2017, the recent literature that we did select provided a tentative indication that reported propranolol concentrations in rivers may be lower now than they were in earlier years. There are at least three possible explanations for why this might be the case. One is that steady improvements in analytical techniques often lead to lower concentrations being determined, as problems (e.g. matrix effects) are better understood and minimised or even eliminated. Another is that the medical use of propranolol might be decreasing as it is replaced by more specific beta-blockers, such as metoprolol and atenolol. The third is that steady improvements in wastewater treatment will be leading to higher removal rates of micropollutants such as propranolol, hence there should be lower concentrations in effluents discharged into rivers.

4.3. Concentrations of propranolol reported to cause effects to aquatic organisms

If only EC50 values obtained from usually standardised tests protocols in which full concentration-response relationships were considered, then there was a fairly high degree of consistency in the concentrations reported to cause effects on aquatic organisms. Most EC50 values were in the low to medium mg/L range with, perhaps surprisingly, no evidence that chronic values were lower than acute values, as might have been expected. This relative consistency in EC50 values led to the SSD being reasonably steep, and providing no convincing evidence that one group of organisms was significantly more sensitive to propranolol than other groups of organisms. This is true for both acute and chronic toxicity data. A similar conclusion was reported recently by Damasceno de Oliveira et al. (2018), who provided an SSD for propranolol based on a smaller, albeit similar, dataset to ours (they used 20 EC50 values from 18 species). In both cases the median effect concentration is in the low mg/L range (our SSD provided an HC50 of 6.64 mg/L). Little apparent difference between acute and chronic effect concentrations provides an interesting outcome of this global comparison of reported effects, and not one we would might have predicted for a compound with a wide safety range in humans. Perhaps this is a reflection of the homogenisation of data points where we combine the EC50s without differentiating the endpoints, as is the accepted method for generating an SSD. Ferrari et al. (2004) did separate their toxicity data for propranolol into acute and chronic. They showed that the chronic data were about 25-fold lower (our estimate from their figure) than the acute data. This difference would not appreciably affect our conclusion that propranolol poses little or no risk to aquatic organisms, because we report that there was nearly a million-fold difference between the HC50 obtained from our SSD and the median river concentration. A detailed discussion of the use of both acute and chronic toxicity data in the environmental risk assessment of pharmaceuticals can be found in Vestel et al. (2016). Based on a reasonably large dataset, they show that, once the recommended safety assessment factors are applied (1000 for acute toxicity data; 10 or 50 for chronic toxicity data), PNECs derived from acute data "were lower than the PNECs derived from chronic data". Thus, they argue that acute toxicity data can be used with confidence in environmental risk assessment.

The mg/L range of most EC50s also points towards non-specific toxicity (narcosis) rather than specific mode of action driven effects that might be expected for a compound designed to induce specific biological effect in humans. Crucially, we see no particular pattern of species sensitivity in the distribution (Fig. 1). Previously, our focus has been on the concentration within an organism causing the effect rather than the concentration in the water. We worked on the hypothesis of better predicting environmental toxicity of pharmaceuticals on the basis that internal concentration within an aquatic organism is the key for comparison with the pharmacological effect in humans. That is, we might reasonably expect effects at the same concentrations inside a fish as those in circulating blood plasma of a human (Rand-Weaver et al., 2013). That hypothesis provides a framework to make a reasonable worst case estimate of what effects one might predict if aquatic animals have the physiology and receptor systems comparable to humans. But to date we have little evidence for the assumption of equivalent sensitivity in aquatic life as occurs in mammals. Gunnarsson and workers (2019) examined all the available regulatory data for effects of pharmaceuticals in aquatic life (fish, invertebrates and algae). They concluded that when the targets are conserved only in fish, then they are likely the most sensitive species, but when pharmacological targets are conserved across phyla we should continue to test the three trophic levels of fish, invertebrates and algae. We can look at propranolol pharmacological targets across a range of phyla using a tool developed by that same group (ECOdrug; www.ecodrug.org; Verbruggen et al., 2017). Currently the annotation of adrenergic receptors suggests only chordates likely have the adrenergic receptors -beta-1, beta-2, beta-3 (ADRB1/ADRB2/ADRB3) and we would expect these to be the most sensitive. This is not what we see from the present study. Indeed it is the invertebrates and algae that appear to be some of the more sensitive species, despite apparent lack of target. We suggest that at mg/L concentrations in the water, these literature studies are effectively reporting a generalised narcosis response, and this toxicity is not differentiated by presence or absence of receptors, and thus EC50 data tells us little about the relative sensitivities in adrenergic blockade in wildlife.

However, the non-EC50 data tell a very different story. The range of concentrations reported to cause effects - any significant effect in any aquatic organism - is now very wide, varying from 0.3 ng/L to over 100 mg/L, with many papers reporting effects occurring at concentrations below any EC50 values. Of course, the toxicity tests providing EC50 values also demonstrate that effects occurred at lower concentrations than those EC50 values. For example, the EC5 value is often around 10% of the EC50 value, so if an EC50 value was 1 mg/L, then an effect equivalent to 5% of the maximum – but still an effect – would occur at 0.1 mg/L. It is perhaps not surprising that the range of concentrations causing effects is fairly wide when all molecular, biochemical, physiological and apical endpoints are included in the analysis. The apical endpoints that are the basis for most OECD ecotoxicity tests are likely to be somewhat less sensitive than molecular and biochemical endpoints in particular. Inclusion of these data has a dramatic effect on any conclusions regarding the environmental risk posed by propranolol (see later). There were many different reasons why these papers did not report an EC50 value, including the following:

1: Only one concentration was tested.
2: Many concentrations were tested, but only the highest one produced an effect, allowing a LOEC, but not an EC50 value, to be determined.

3: Many concentrations were tested, but they all caused the same magnitude of effect. That is, there was no concentration-response.
4: Many concentrations were tested, but no sigmoidal concentration-related response relationship occurred. Instead, the response relationship varied in shape; for example, being an inverted U-shape (intermediate concentrations only caused effects).
5: An EC50 value probably could have been obtained from the data, but one was not reported (and could not be calculated by us).

Eleven papers reported effects occurring in the ng/L range, with the lowest effect concentration being 0.3 ng/L. Two research groups contributed four papers each to this collection of eleven papers. In none of these eleven papers was a sigmoidal concentration-response reported. In two of them only one concentration of propranolol was tested (Franzellitti et al., 2013, 2015), hence no concentration-response information was provided, and in the other nine papers, all of which involved testing 3 or more concentrations of propranolol, non-sigmoidal concentration-response relationships were reported (e.g. Finn et al., 2012; Maranho et al., 2015; Maranho et al., 2014), varying from U-shaped and inverted U-shaped concentration responses (e.g. Finn et al., 2012; Franzellitti et al., 2011; Maranho et al., 2015) to all concentrations causing similar degrees of apparent effect that were significantly different from the controls but not from each other (e.g. Finn et al., 2012; Franzellitti et al., 2011; Maranho et al., 2015).

It is impossible to know how repeatable these 'low concentration' effects are. It is extremely unusual for published ecotoxicology results, even surprising ones, to be repeated, although Franzellitti and colleagues do appear to have shown that some of their 'low concentration' effects are repeatable (compare, for example, the results in Franzellitti et al., 2011 and Franzellitti et al., 2013). It is even more unusual for ecotoxicologists to demonstrate that surprising results, such as effects occurring at very low concentrations, cannot be repeated. Even when authors describe their own published results as surprising, they appear not to have assessed their repeatability. However, Owen et al. (2010) did test the issue of repeatability of surprising results in the case of a different pharmaceutical, clofibric acid. They showed that in one experiment all tested concentrations, including very low ones, apparently caused significant effects, whereas in a repeat experiment, utilising a more robust experimental design, only the very highest concentration caused any effects. A very vigorous scientific debate has been ongoing for more than two decades on the repeatability, or otherwise, of so-called 'low dose' effects, with some scientists believing strongly in such effects (see the review by Vandenberg et al., 2012), whereas other scientists equally strongly do not (e.g. Rhomberg and Goodman, 2012). Our personal opinion is that we remain sceptical of the repeatability of the reported 'low concentration' effects of propranolol on various aquatic organisms, and will remain so until they are shown to be repeatable based on robust experiments conducted by independent research groups. To their credit, and importantly, Franzellitti and colleagues state that the effects they observed did not appear to result in any obvious detrimental effects on their experimental animals (e.g. Franzellitti et al., 2011).

A number of authors have argued that the ecotoxicology of propranolol, or any pharmaceutical for that matter, should be based on the mode-of-action (MoA) of the drug (e.g. Franzellitti et al., 2013; Gunnarsson et al., 2008; Owen et al., 2007; Rand-Weaver et al., 2013). It is therefore somewhat ironic that relatively few ecotoxicological studies appear to have utilised the clinically important effects of propranolol, which are reduced heart rate and lower arterial blood pressure, as endpoints. Out of all the papers we reviewed reporting effects of propranolol, only five assessed heart rate as an endpoint and none assessed arterial blood pressure. Our literature search found only one specific ecotoxicological paper (as opposed to the many comparative physiology papers) that investigated the effects of propranolol on the heart rate of adult fish. In that paper (Larsson et al., 2006) the authors concluded "During a 48h exposure .... to a very high concentration of propranolol (70.9 μg/L) no effects on heart rate were found". Investigating potential effects on the heart rate of fish embryos/larvae is technically easier, and this strategy has been employed in a few studies, although consistent results have not been obtained. Fraysse et al. (2006) obtained a clear concentration-response relationship when they exposed 4 day-old zebrafish larvae to propranolol. The lowest concentration having a significant effect was 27 μM (about 7 mg/L). In contrast, no consistent effect on heart rate was obtained by Mitchell and Moon (2016) when they tested concentrations up to 20 mg/L. Much lower concentrations, in the μg/L range, were tested by Finn et al. (2012), who reported no effect on the heart rate of either Japanese Medaka or Zebrafish embryos when they were directly exposed, but reduced heart rate of embryos of both species if their parents had also been exposed to the drug. A concentration as low as 0.09 μg/L appeared to cause a significant reduction. However, all 3 concentrations tested were reported to cause the same degree of decrease in heart rate. It would be extremely helpful to the environmental risk assessment of propranolol, or any other beta-blocker, if consistent, reproducible, concentration-related MoA effects, such as those on the cardiovascular system, were available. However, this is apparently equally difficult to do even within the mammalian experimental literature. Margiotta-Casaluci et al. (2019) conducted a meta-analysis of the effects of propranolol (and two other cardiovascular drugs) in the literature and found that both the degree of pharmacological effect and the direction of that effect (increase or decrease) varied widely across human, dog, rat, mouse and zebrafish studies. They also demonstrated an apparent dose-response reduction in heart rate from 16 to 125 μM over an hour exposure and similarly depressed heart rate and reduced blood flow at 16 μM after 48 h exposure. Since fish have a low pressure circulatory system, further reduced pressure is difficult to measure and may be one reason this endpoint was not measured in fish physiological studies using propranolol, but perhaps blood flow is a surrogate? There is little doubt that propranolol can cause the anticipated MoA effects, because the drug has been widely used for decades by comparative physiologists studying the role of the adrenergic nervous system in regulating the heart of fish in particular, but also other species. For example, Steele et al. (2011) used 10^{-4} Molar propranolol (about 26 mg/L) to produce a 25% decrease in heart rate of larval zebrafish. It would be extremely useful if a few well-designed, well-conducted studies with fish, or fish tissue or even fish cells, could be conducted, incorporating appropriate MoA endpoints, in order to establish if those chronic studies do, or do not, reveal that they are the most sensitive toxicity tests. The results of such studies could then be used to assess the suitability of the SSD methodology. The results of such studies would also test our conclusion that current concentrations of propranolol in rivers pose no risk to aquatic organisms.

In a paper specifically focussed on MoA effects of propranolol on an invertebrate, the crustacean *Daphnia magna*, Jeong et al. (2018) report that the drug affected a number of behavioural and physiological endpoints, but no concentration-related responses were obtained; instead, all concentrations apparently caused effects of similar magnitude. Despite this, the authors concluded that the MoA of propranolol "was revealed to be not baseline toxicity but heart specific". Determining whether or not all the effects of propranolol on both vertebrates and invertebrates are MoA driven, and hence could be predicted if the physiological roles of the adrenergic system are known, should be a high priority if predictive ecotoxicology is to become a reality (as also suggested by Zhang et al., 2020).

4.4. Comparison of river concentrations of propranolol with concentrations reported to cause effects to aquatic organisms

Based on a comparison of all reported concentrations of propranolol in the freshwater environment (median concentration of 7.1 ng/L; highest individual sample concentration 590 ng/L) and all reported EC50 values (lowest value 0.084 mg/L in a non-standard test based on marine periphyton communities; lowest with a freshwater species 0.132 mg/L; Table S7), there is a very large gap between the two. The

difference between the median river concentration of 7.1 ng/L and the HC50 value from a SSD of 6.64 mg/L is nearly six orders of magnitude (i.e., nearly one million-fold). However, there are very important caveats relevant to this comparison. One is that the endpoint in well over half of the toxicity tests that provided EC50 values was death (or immobilisation), which is probably a relatively insensitive endpoint. As stated above, reliance solely on EC50 values obscures the fact that the same tests that provided those values also demonstrate that some test organisms died when exposed to lower concentrations. The other is that non-lethal effects (e.g., molecular, biochemical) are very likely to occur at lower concentrations than the EC50 values. But even if robust, repeatable, non-lethal effects could be demonstrated to occur at concentrations one thousand-fold lower than the median EC50 value, there would still be a difference of nearly 1000-fold between the two concentrations.

Only when the non-EC50 effect data are included is there any overlap between propranolol concentrations in rivers and those reported to cause effects on aquatic organisms. Thus, it is possible that environmental concentrations of propranolol are high enough to cause effects to at least some aquatic organisms. However, this conclusion depends completely on whether or not the 'low effect' data are repeatable: data that we are sceptical about. In our opinion, the vast majority of the available data demonstrate that existing concentrations of propranolol in the freshwater environment present no risk to aquatic organisms. A similar conclusion was reached by Huggett et al. (2002) nearly two decades ago, although they based their opinion on results from tests using invertebrates only. In contrast, Zhang et al. (2020) concluded that propranolol "may pose an environmental risk". They reached their conclusion, which they admirably term 'tentative', because they accepted and incorporated data into their risk assessment, in particular some of the low-concentration effect data from mussels (e.g. Franzellitti et al., 2011), that we consider potentially problematic.

Regarding other beta-blockers in worldwide use, such as atenolol and metoprolol, significantly less has been reported in the scientific literature about their environmental presence and potential impact on aquatic organisms compared to propranolol. Nevertheless, it seems clear that they pose an even lower threat to the environment than propranolol does. This is a consequence both of their lower toxicity and greater ease of degradation (e.g. Aydin et al., 2017; Zhang et al., 2020).

4.5. General, important issues

4.5.1. Why was propranolol considered a significant environmental risk?

One of the first challenges for environmental scientists ought to be to think hard about which chemical, or group of chemicals, to focus their research on: that should be the chemicals that pose the greatest risk (Johnson et al., 2017). Yet surprisingly, no authors stated explicitly why they focussed on propranolol. No authors developed a logical argument as to why propranolol might present an environmental risk to aquatic organisms, and hence merited study. In contrast, logical arguments were developed to suggest that steroidal pharmaceuticals might pose a risk to fish in particular (Runnalls et al., 2010); arguments that proved to be correct (Donnachie et al., 2016; Gunnarsson et al., 2019).

In a thoughtful paper, Roos et al. (2012) compared nine different strategies for prioritising pharmaceuticals for the degree of environmental risk they might pose. Propranolol featured in the top ten pharmaceuticals in three of these strategies, including being number 1 in the ranking based on measured environmental concentrations divided by the predicted no effect concentration (MEC/PNEC). Donnachie et al. (2016) explicitly questioned the rationale for selecting certain pharmaceuticals for study, as well as questioning the need to focus on pharmaceuticals as opposed to other chemicals, such as metals. They identified 22 research papers providing prioritization schemes for pharmaceuticals, and used the frequency of pharmaceuticals appearing in these lists to select a 'top 12' pharmaceuticals. Propranolol was one of the most cited pharmaceuticals. Yet when Donnachie et al. (2016) compared reported environmental concentrations with concentrations reported to cause effects on aquatic organisms, they found that the two median values were more than 100,000-fold apart; that is, the median environmental concentration was over 5 orders of magnitude lower than the median concentration reported to cause an effect on an aquatic species. In this paper we very significantly expand the database used by Donnachie and her co-workers (Donnachie et al., 2016), but come to the same conclusion, although with the caveat that inclusion of the 'low concentration' effect results discussed above raises uncertainties about the robustness of this conclusion. We consider it likely that the perceptions of many scientists that propranolol posed a significant degree of risk to aquatic organisms was driven largely by the popularity of the chemical to environmental scientists (both chemists and ecotoxicologists), and thus is an example of the so-called Matthew Effect (Daughton, 2014).

We have reached a very different conclusion to that reached by Ferrari et al. (2004), who concluded that propranolol did pose a risk to the aquatic environment. In their generally excellent paper, they used the European Medicines Agency (EMEA) guidelines on how to conduct environmental risk assessments of human pharmaceuticals to assess the risks posed by six pharmaceuticals, including propranolol. They report that the PEC:PNEC ratios varied between 0.49 (if acute toxicity data were used) and 104 (if chronic toxicity data were used). The main reasons for their conclusion that propranolol probably posed a risk to the aquatic environment were (1) they used only the highest reported surface water concentration of propranolol, which as we stated earlier is 590 ng/L, for their exposure concentration, and (2) they applied an assessment factor of between 10 and 1000, depending on data availability, to determine the risk ratio. The novelty, and robustness, of our approach is that we used all published surface water concentration data, and we did not apply any arbitrary assessment factors because a large toxicity database was available to us.

4.5.2. Was so much research on propranolol required?

The answer to this question must be no. A much smaller amount of research, selected intelligently and done to high standards (see below), would have demonstrated that propranolol constituted a very small threat, if any threat at all, to aquatic organisms. The highest environmental concentrations of propranolol are likely to occur in countries where the drug is widely prescribed, where population density is high, and where rivers receive high volumes of wastewater effluents (Keller et al., 2014). Hence, determining propranolol concentrations in southern England, The Netherlands, and north-east Spain should provide some of the highest concentrations of propranolol, worldwide, in rivers.

In the case of effects of propranolol on aquatic organisms, conducting OECD-validated tests on a range of organisms would probably have provided all the necessary effect concentrations required for an ERA. In fact some authors did exactly that (e.g. Calleja et al., 1994; Ferrari et al., 2004; Huggett et al., 2002; Yamamoto et al., 2007), and they produced very reproducible, consistent results. Hence, no more than half a dozen papers provide all the effect data required for a robust risk assessment, based on widely accepted methodology as used by regulators across the world, to be conducted. However, as these OECD-validated tests do not have as an endpoint a specific effect based on the MoA of propranolol (e.g. reduced heart rate), it could be reasonably argued that a few ecotoxicity studies needed to be conducted based on the read-across hypothesis (Rand-Weaver et al., 2013; Zhang et al., 2020). As discussed above, some authors did conduct appropriate studies, and found no obviously increased risk of propranolol.

4.5.3. How can things be done better in the future?

There is now fairly wide acceptance that the quality of research generally could, and probably should, be better than it currently is. Ecotoxicology research is no exception (Harris and Sumpter, 2015; Mebane et al., 2019). Various suggestions have been put forward covering how

the quality of ecotoxicology research could be improved (e.g. Harris et al., 2014; Hanson et al., 2017). Although we did not attempt to assess all the papers we utilised in order to rate their credibility (this would have been a monumental task), it was obvious to us that a high proportion of those papers, particularly many of those covering potential effects of propranolol, would not have rated highly. Many did not abide by the 'Principles of Sound Ecotoxicology' (Harris et al., 2014) and many would have scored poorly based on the CRED reporting criteria (Moermond et al., 2016) and the similar but wider quality criteria outlined by Hanson et al. (2017). This finding is, of course, not relevant only to the risk assessment of propranolol; it is equally relevant to all ecotoxicology research, irrespective of which chemical, or mixture of chemicals, is under investigation. The section below provides guidance on how the relevance (=usefulness) of ecotoxicology research can be increased.

Most of the research that we based this meta-analysis on was, presumably, conducted in order to protect the environment from chemicals released into it. If research is not conducted to high standards it is of minimal, or no, use to regulators. If regulators cannot use it to help them protect the environment, then it is questionable whether that research should have been conducted in the first place. This is particularly true if that research involved the use of animals. An identical conclusion was reached independently recently by Constantine et al. (2020), based on their inability to reproduce some reported 'low concentration' effects of the pharmaceutical ibuprofen: they wrote "Applying Harris, et al (2014) and Hanson, (2017) recommendations would lead to more reliable and relevant ecotoxicity data, thereby increasing stakeholder confidence …etc".

In order to improve the current situation, scientists need to be trained better (Harris et al., 2017; Mebane et al., 2019). Much more thought needs to be devoted to deciding what research to do, then how to do the research considered the most important and relevant to protecting the environment from chemicals, including propranolol. Many of the factors that need addressing in order to improve the quality of both scientists and the research they do are covered in the book 'How to be a better scientist' (Johnson and Sumpter, 2019).

5. Conclusions

Based on the results of many hundreds of research papers, the median concentration of propranolol in rivers was found to be 7.1 ng/L. The median effect concentration of propranolol based on ecotoxicity tests from which EC50 values were provided was 6.64 mg/L. Even if robust, non-lethal molecular or biochemical effects occur at lower concentrations, nevertheless we conclude that propranolol does not constitute any risk to freshwater organisms. However, some non-standard ecotoxicity studies have suggested that very low (ng/L or even sub-ng/L) concentrations of propranolol can cause a range of effects to some aquatic species. Often these apparent effects were not concentration-related, raising concerns about the repeatability of those 'low concentration' effects. Until they are demonstrated to be repeatable, we consider that propranolol concentrations in rivers across the world currently pose no risk to aquatic species.

CRediT authorship contribution statement

John Sumpter: Conceptualization, Data Curation, Formal Analysis, Methodology, Project Administration, Writing - original draft and subsequent editing.

Tamsin Runnalls: Data Curation, Project Administration, Visualization, Writing - review and editing.

Rachel Donnachie: Data curation, Methodology, Visualization.

Stewart Owen: Conceptualization, Funding Acquisition, Visualization, Writing - review and editing.

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Declaration of competing interest

This study was partially funded by the pharmaceutical company AstraZeneca, and one of the authors (SFO) is an employee of that company. However, the literature searches, identification of relevant research, extraction of information, and writing the complete first draft of the paper were all conducted entirely by the university-based authors, with no interference from any employee of AstraZeneca. SFO and other employees of AstraZeneca subsequently improved that initial draft, but none of the data, or their interpretation, were changed during that process.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148617.

References

Arnold, K.E., Brown, A.R., Brown, A.R., Ankley, G.T., Sumpter, J.P., 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Philos. Trans. R. Soc. B Biol. Sci. 369 (1656). https://doi.org/10.1098/rstb.2013.0569.

Aydin, S., Aydin, M.E., Tekinay, A., Kilic, H., 2017. Occurrence and environmental risk assessment of β-blockers in urban wastewater. Fresenius Environ. Bull. 1800–1805.

Caldwell, D.J., Mastrocco, F., Anderson, P.D., Länge, R., Sumpter, J.P., 2012. Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol. Environ. Toxicol. Chem. 31 (6), 1396–1406. https://doi.org/10.1002/etc.1825.

Calleja, M.C., Persoone, G., Geladi, P., 1994. Comparative acute toxicity of the first 50 multicentre evaluation of in vitro cytotoxicity chemicals to aquatic non-vertebrates. Arch. Environ. Contam. Toxicol. 26, 69–78.

Constantine, L.A., Green, J.W., Schneider, S.Z., 2020. Ibuprofen: fish short-term reproduction assay with zebrafish (Danio rerio) based on an extended OECD 229 protocol. Environ. Toxicol. Chem. https://doi.org/10.1002/etc.4742.

Damasceno de Oliveira, L.L., Nunes, B., Antunes, S.C., Campitelli-Ramos, R., Rocha, O., 2018. Acute and chronic effects of three pharmaceuticals on the tropical freshwater Cladoceran Ceriodaphnia silvestrii. Water Air Soil Pollut. 229, 116.

Daughton, C.G., 2014. The Matthew Effect and widely prescribed pharmaceuticals lacking environmental monitoring: case study of an exposure-assessment vulnerability. Sci. Total Environ. 466, 315–325.

Donnachie, R.L., Johnson, A.C., Sumpter, J.P., 2016. A rational approach to selecting and ranking some pharmaceuticals of concern for the aquatic environment and their relative importance compared with other chemicals. Environ. Toxicol. Chem. 35 (4), 1021–1027. https://doi.org/10.1002/etc.3165.

Falås, P., Wick, A., Castronovo, S., Habermacher, J., Ternes, T.A., Joss, A., 2016. Tracing the limits of organic micropollutant removal in biological wastewater treatment. Water Res. 95, 240–249. https://doi.org/10.1016/j.watres.2016.03.009.

Fent, K., Weston, Anna, Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. Aquatic Toxicology. Vol. 76, Issue 2. Elsevier, pp. 122–159. https://doi.org/10.1016/j.aquatox.2005.09.009.

Ferrari, B., Mons, R., Vollat, B., Fraysse, B., Paxeus, N., Giudice, R.L., Pollio, A., Garric, J., 2004. Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environ. Toxicol. Chem. 23, 1344–1354.

Finn, J., Hui, M., Li, V., Lorenzi, V., de la Paz, N., Cheng, S.H., Lai-Chan, L., Schlenk, D., 2012. Effects of propranolol on heart rate and development in Japanese medaka (Oryzias latipes) and zebrafish (Danio rerio). Aquat. Toxicol. 122–123, 214–221.

Franzellitti, S., Buratti, S., Valbonesi, P., Capuzzo, A., Fabbri, E., 2011. The B-blocker prpranolol affects cAMP-dependent signaling and induces the stress response in Mediterranean mussels, Mytilis galloprovincialis. Aquat. Toxicol. 101, 299–308.

Franzellitti, S., Buratti, S., Valbonesi, P., Capuzzo, A., Fabbri, E., 2013. The mode of action (MOA) approach reveals interactive effects of environmental pharmaceuticals on Mytilis galloprovincialis. Aquat. Toxicol. 140–141, 249–256.

Franzellitti, S., Buratti, S., Du, B., Haddad, S.P., Chambliss, C.K., Brooks, B.W., Fabbri, E., 2015. A multibiomarker approach to explore interactive effects of propranolol and fluoxetine in marine mussels. Environ. Pollut. 205, 60–69.

Fraysse, B., Mons, R., Garric, J., 2006. Development of a zebrafish 4-day embryo-larval bioassay to assess toxicity of chemicals. Ecotoxicol. Environ. Saf. 63, 253–267.

Gardner, M., Comber, S., Scrimshaw, M.D., Cartmell, E., Lester, J., Ellor, B., 2012. The significance of hazardous chemicals in wastewater treatment works effluents. Sci. Total Environ. 437, 363–372. https://doi.org/10.1016/j.scitotenv.2012.07.086.

Gunnarsson, L., Jauhiainen, A., Kristiansson, E., Nerman, O., Larsson, D.G.J., 2008. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environ. Sci. Technol. 42 (15), 5807–5813. https://doi.org/10.1021/es8005173.

Gunnarsson, L., Snape, J.R., Verbruggen, B., Owen, S.F., Kristiansson, E., Margiotta-Casaluci, L., Österlund, T., Hutchinson, K., Leverett, D., Marks, B., Tyler, C.R., 2019. Pharmacology beyond the patient – the environmental risks of human drugs. Environ. Int. 129 (May), 320–332. https://doi.org/10.1016/j.envint.2019.04.075.

Guruge, K.S., Goswami, P., Tanoue, R., Nomiyama, K., Wijesekara, R.G.S., Dharmaratne, T.S., 2019. First nationwide investigation and environmental risk assessment of 72 pharmaceuticals and personal care products from Sri Lankan surface waterways. Sci. Total Environ. 690, 683–695.

Hanson, M.L., Wolff, B.A., Green, J.W., Kivi, M., Panter, G.H., Warne, M.S.J., Agerstrand, M., Sumpter, J.P., 2017. How we can make ecotoxicology more valuable to environmental protection. Sci. Total Environ. 578, 228–235.

Harris, C.A., Sumpter, J.P., 2015. Could the quality of published ecotoxicological research be better? Environ. Sci. Technol. 49, 9495–9496.

Harris, C.A., Scott, A.P., Johnson, A.C., Panter, G.H., Sheahan, D., Roberts, M., Sumpter, J.P., 2014. Principles of sound ecotoxicology. Environ. Sci. Technol. 48 (6), 3100–3111. https://doi.org/10.1021/es4047507.

Harris, M.J., Huggett, D.B., Staveley, J.P., Sumpter, J.P., 2017. What training and skills will the ecotoxicologists of the future require? Integr. Environ. Assess. Manag. 13, 580–584.

Hites, R.A., 2019. Correcting for censored environmental measurements. Environ. Sci. Technol. 53 (19), 11059–11060. https://doi.org/10.1021/acs.est.9b05042.

Huggett, D.B., Brooks, B.W., Peterson, B., Foran, C.M., Schlenk, D., 2002. Toxicity of select beta adrenergic receptor-blocking pharmaceuticals (B-blockers) on aquatic organisms. Arch. Environ. Contam. Toxicol. 43 (2), 229–235. https://doi.org/10.1007/s00244-002-1182-7.

Hughes, S.R., Kay, P., Brown, L.E., 2013. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol. 47 (2), 661–677. https://doi.org/10.1021/es3030148.

Jeong, T.-Y., Yoon, D., Kim, S., Kim, H.Y., Kim, S.D., 2018. Mode of action characterization for adverse effect of propranolol in Daphnia magna based on behaviour and physiology monitoring and metabolic profiling. Environ. Pollut. 233, 99–108.

Johnson, A.C., Sumpter, J.P., 2019. How To Be a Better Scientist. Routledge, London and New York.

Johnson, A.C., Donnachie, R.L., Sumpter, J.P., Jurgens, M.D., Moeckel, C., Pereira, M.G., 2017. An alternative approach to risk rank chemicals on the threat they pose to the aquatic environment. Sci. Total Environ. 599–600, 1372–1381.

Keen, J.E., Brill, R.W., Aota, S., Farrell, A.P., Randall, D.J., 1995. Cholinergic and adrenergic regulation of heart rate and ventral aortic pressure in 2 species of tunas, Katsuwonus pelamis and Thunnus albacares. Can. J. Zool. 73, 1681–1688.

Keller, V.D., Williams, R.J., Lofthouse, C., Johnson, A.C., 2014. Worldwide estimation of river concentrations of any chemical originating from sewage-treatment plants using dilution factors. Environ. Toxicol. Chem. 33, 447–452.

Larsson, D.G.J., 2014. Pollution from drug manufacturing : review and perspectives. Philos. Trans. R. Soc. B 369, 20130571.

Larsson, D.G.J., Fredriksson, S., Sandblom, E., Paxeus, N., Axelsson, M., 2006. Is heart rate in fish a sensitive indicator to evaluate acute effects of B-blockers in surface waters? Environ. Toxicol. Pharmacol. 22, 338–340.

Mandaric, L., Mor, J.-R., Sabater, S., Petrovic, M., 2018. Impact of urban chemical pollution on water quality in small, rural and effluent dominated Mediterranean streams and rivers. Sci. Total Environ. 613–614, 763–772.

Maranho, L.A., Baena-Nogueras, R.M., Lara-Martin, P.A., DelValls, T.A., Martin-Diaz, M.L., 2014. Bioavailability, oxidative stress, neurotoxicity, and genotoxicity of pharmaceuticals bound to marine sediments. The use of the polychaete Hediste diversicolor as bioindicator species. Environ. Res. 134, 353–365.

Maranho, L.A., Andre, C., DelValls, T.A., Gagne, F., Martin-Diaz, M.L., 2015. Toxicological evaluation of sediment samples spiked with human pharmaceutical products: energy status and neuroendocrine effects in marine polychaetes Hediste diversicolor. Ecotoxicol. Environ. Saf. 118, 27–36.

Margiotta-Casaluci, L., Owen, S.F., Rand-Weaver, M., Winter, M.J., 2019. Testing the translational power of the zebrafish: an inter-species analysis of responses to cardiovascular drugs. Front. Pharmacol. 10 (JULY), 1–22. https://doi.org/10.3389/fphar.2019.00893.

McGrath, S., Zhao, X., Qin, Z.Z., Steele, R., Benedetti, A., 2019. One-sample aggregate data meta-analysis of medians. Stat. Med. 38, 969–984.

Mebane, C.A., Sumpter, J.P., Fairbrother, A., Augspurger, T.P., Canfield, T.J., Goodfellow, W.L., Guiney, P.D., LeHuray, A., Maltby, L., Mayfield, D.B., McLaughlin, M.J., Ortego, L.S., Schlekat, T., Scroggins, R.P., Verslycke, T.A., 2019. Scientific integrity issues in Environmental Toxicology and Chemistry: improving research reproducibility, credibility, and transparency. Integr. Environ. Assess. Manag. 15 (3), 320–344. https://doi.org/10.1002/ieam.4119.

Mitchell, K.M., Moon, T.W., 2016. Behavioural and biochemical adjustments of the zebrafish Danio rerio exposed to the B-blocker propranolol. Comp. Biochem. Physiol. B 199, 105–114.

Moermond, C.T.A., Kase, R., Korkaric, M., Agerstrand, M., 2016. CRED: criteria for reporting and evaluating ecotoxicity data. Environ. Toxicol. Chem. 35, 1297–1309.

Owen, S.F., Giltrow, E., Huggett, D.B., Hutchinson, T.H., Saye, J., Winter, M.J., Sumpter, J.P., 2007. Comparative physiology, pharmacology and toxicology of beta-blockers: mammals versus fish. Aquat. Toxicol. 82, 145–162.

Owen, S.F., Huggett, D.B., Hutchinson, T.H., Hetheridge, M.J., McCormack, P., Kinter, L.B., Ericson, J.F., Constantine, L.A., Sumpter, J.P., 2010. The value of repeating studies and multiple controls: replicated 28-day growth studies of rainbow trout exposed to clofibric acid. Environ. Toxicol. Chem. 29, 2831–2839.

Posthuma, L., De Zwart, D., 2014. Species sensitivity distributions. Encyclopedia of Toxicology, 3rd edition, pp. 363–368.

Proctor, K., Petrie, B., Barden, R., Arnot, T., Kasprzyk-Hordern, B., 2019. Multiresidue ultra-performance liquid chromatography coupled with tandem mass spectrometry method for comprehensive multi-class anthropogenic compounds of emerging concern analysis in a catchment-based exposure-driven study. Anal. Bioanal. Chem. 411, 7061–7086.

Rand-Weaver, M., Margiotta-Casaluci, L., Patel, A., Panter, G.H., Owen, S.F., Sumpter, J.P., 2013. The read-across hypothesis and environmental risk assessment of pharmaceuticals. Environ. Sci. Technol. 47 (20), 11384–11395. https://doi.org/10.1021/es402065a.

Rhomberg, L.R., Goodman, J.E., 2012. Low-dose and nonmonotonic dose-responses of endocrine disrupting chemicals: has the case been made? Regul. Toxicol. Pharmacol. 64, 130–133.

Roberts, P.H., Thomas, K.V., 2006. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci. Total Environ. 356, 143–153.

Roos, V., Gunnarsson, L., Fick, J., Larsson, D.G.J., Rudén, C., 2012. Prioritising pharmaceuticals for environmental risk assessment: towards adequate and feasible first-tier selection. Sci. Total Environ. 421–422, 102–110. https://doi.org/10.1016/j.scitotenv.2012.01.039.

Runnalls, T.J., Margiotta-Casaluci, L., Kugathas, S., Sumpter, J.P., 2010. Pharmaceuticals in the aquatic environment: steroids and anti-steroids as high priorities for research. Hum. Ecol. Risk. Assess. 16 (6), 1318–1338. https://doi.org/10.1080/10807039.2010.526503.

Runnalls, T.J., Beresford, N., Losty, E., Scott, A.P., Sumpter, J.P., 2013. Several synthetic progestins with different potencies adversely affect reproduction of fish. Environ. Sci. Technol. 47 (4), 2077–2084. https://doi.org/10.1021/es3048834.

Steele, S.L., Yang, X., Debias-Thibaud, M., Schwerte, T., Pelster, B., Ekker, M., Tiberi, M., Perry, S.F., 2011. In vivo and in vitro assessment of cardiac bata-adrenergic receptors in larval zebrafish (Danio rerio). J. Exp. Biol. 214, 1445–1457.

Straub, J.O., 2009. An environmental risk assessment for oseltamivir (Tamiflu(R)) for sewage works and surface waters under seasonal-influenza-and pandemic-use conditions. Ecotoxicol. Environ. Saf. 72, 1625–1634.

Straub, J.O., 2017. Combined environmental risk assessment for the antiviral pharmaceuticals ganciclovir and valganciclovir in Europe. Environ. Toxicol. Chem. 36, 2205–2216.

Tirri, R., Lehto, H., 1984. Alpha and beta adrenergic control of contraction force of perch heart (Perca fluviatilis) in vitro. Comp. Biochem. Physiol. C Comp. Physiol. Pharmacol. 77 (2), 301–304. https://doi.org/10.1016/0742-8413(84)90017-3.

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.-H., Shioda, T., Soto, A.M., vom Saal, F., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose and nonmonotonic dose responses. Endocr. Rev. 33, 378–455.

Verbruggen, B., Gunnarsson, L., Kristansson, E., Österlund, T., Owen, S.F., S., J.R., T., C.R., 2017. ECOdrug: a database connecting drugs and conservation of their targets across species. Nucleic Acids Res. 46 (D1), D930–D936.

Vestel, J., Caldwell, D.J., Constantine, L., D'Aco, V.J., Davidson, T., Dolan, D.G., Millard, S.P., Murray-Smith, R., Parke, N.J., Ryan, J.J., Straub, J.O., Wilson, P., 2016. Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. Environ. Toxicol. Chem. 35, 1201–1212.

Weber, F.A., Bergmann, A., Hickman, S., Ehert, L., Hein, A., Kuster, A., 2015. Pharmaceuticals in the environment - global occurrences and perspectives. Environ. Toxicol. Chem. 35, 823–835.

White, D., Lapworth, D.J., Civil, W., Williams, P., 2019. Tracking changes in the occurrence and source of pharmaceuticals within the River Thames, UK; from source to sea. Environ. Pollut. 249, 257–266.

Yamamoto, H., Nakamura, Y., Nakamura, Y., Kitani, C., Imari, T., Sekizawa, J., Takao, Y., Yamashita, N., Hirai, N., Oda, S., Tatarazako, N., 2007. Initial ecological risk assessment of eight selected human pharmaceuticals in Japan. Environ. Sci. 14, 177–193.

Yao, B., Yan, S., Lian, L., Yang, X., Wan, C., Dong, H., Song, W., 2018. Occurrence and indicators of pharmaceuticals in Chinese streams: a nationwide study. Environ. Pollut. 236, 889–898.

Zeilinger, J., Steger-Hartmann, T., Maser, E., Goller, S., Vonk, R., Länge, R., 2009. Effects of synthetic gestagens on fish reproduction. Environ. Toxicol. Chem. 28 (12), 2663–2670. https://doi.org/10.1897/08-485.1.

Zhang, K., Zhao, Y., Fent, K., 2020. Cardiovascular drugs and lipid regulating agents in surface waters at global scale: occurrence, ecotoxicity and risk assessment. Sci. Total Environ. 729, 138770.