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The apparently very variable potency of the anti-depressant fluoxetine☆

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ABSTRACT

The anti-depressant fluoxetine is widely present in the aquatic environment. Typical river concentrations are in the low ng/L range. Many ecotoxicity studies have assessed the effects of this pharmaceutical on a range of aquatic species. Some studies report that ng, or even pg, per litre concentrations cause effects, whereas other studies report that effects only occur when the water concentration is in the μg/L range. It seems unlikely that all reported effects will be repeatable. Many of the studies have considerable limitations. Currently it is impossible to ascertain what environmental concentrations of fluoxetine pose a risk to aquatic organisms. The key question can be answered only by high quality, reproducible research.

1. Introduction

Fluoxetine (also known by a number of trade names including Prozac) is an antidepressant and one member of a class of drugs known as selective serotonin reuptake inhibitors (SSRI). It has been used for over 25 years to treat major depression and other psychiatric disorders. Its widespread use in many countries accounts for its presence in the aquatic environment. Fluoxetine is present in rivers at concentrations in the low to very low ng/L range. An extremely comprehensive report by Gardner et al. (2012) provides information on fluoxetine concentrations in the effluents of 162 wastewater treatment works in the United Kingdom. The median concentration was 23 ng/L and the 95 percentile was 69 ng/L. Hydrological modelling can then be used to predict river concentrations. Predicted fluoxetine concentrations in most locations and under most circumstances would be expected to be a few ng/L, but in highly impacted rivers under low flow conditions could reach tens of ng/L. The relatively few measured river concentrations of fluoxetine in Europe support the predicted concentrations (Alonso et al., 2010; Vystavna et al., 2012). Hence, a legitimate question to ask is whether or not aquatic organisms are affected by such concentrations. This question was first addressed seriously ten years ago (Brooks et al., 2003), in a wide range of aquatic species, but without utilising any specific mode-of-action endpoints. Since then, approximately 30 studies on the effects of fluoxetine on fish have been published, together with about a dozen studies covering effects of fluoxetine on various species of invertebrates. Thus, in the last ten years a reasonable amount of information has accumulated that ought to enable us to reach a robust conclusion regarding the possible threat that fluoxetine poses to aquatic species. However, the current body of information does not enable any such conclusion to be reached; the reasons for that are discussed below.

1.1. Effects of fluoxetine on fish

Most of the 30 papers that have reported on the effects of fluoxetine on fish have studied the effect of the drug on the behaviour of the fish. This seems a very sensible endpoint to concentrate on, because the drug affects behaviour in humans, and hence based on the read-across hypothesis (Hugget et al., 2003; Rand-Weaver et al., 2013), effects on behaviour are likely to be the primary effect of the drug on fish. Although there are many differences in the studies that have reported on the behavioural effects of fluoxetine on fish – such as differences in exposure scenario, concentration, duration, species, and age of fish as well as methodology to quantify behaviour – it might have been expected that the effective concentration of the drug would have been approximately the same in all the studies, but this is not the case. The majority of studies report that concentrations between 30 and 100 μg fluoxetine/L affected behaviour (e.g. Pittman and Ichikawa, 2013; Wong et al., 2013), as did higher concentrations (e.g. Kohlert et al., 2012; Airhart et al., 2007). However, some studies report that very much lower concentrations affected the behaviour of fish. For example,

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Barry (2013) reported that 0.3 μg fluoxetine/L affected swimming speed, schooling behaviour and response to a predator alarm, and Dzieweczynski and Hebert (2012) reported that 0.54 μg fluoxetine/L reduced aggression of male Siamese fighting fish.

Although possible effects on behaviour have understandably been the main focus of studies investigating the effects of fluoxetine on fish, possible effects on other physiological processes have been investigated. A number of studies have utilised various reproductive endpoints. For example, Schultz et al. (2011) reported that 28 ng fluoxetine/L had reproductive effects on male fathead minnows: the plasma vitellogenin concentration was increased, and the architecture of their testes affected. Mennigen et al. (2010a) studied the effects of fluoxetine on a wide range of reproductive parameters of the goldfish, and found that many of them were affected by both 0.54 and 54 μg fluoxetine/L, with the higher concentration generally producing the larger effects. The same group of authors (Mennigen et al., 2010b) also reported that both these concentrations also disrupted feeding and energy metabolism in goldfish.

In summary, although many authors report effects of fluoxetine on fish occurring when the concentration in the water is in the tens to hundreds of μgs/L, some authors report significant effects when the water concentration is in the ng/L range.

1.2. Effects of fluoxetine on invertebrates

It is not only vertebrates that utilise serotonin as a neurotransmitter: invertebrates do as well. Therefore, following the concept of read-across (Rand-Weaver et al., 2013), which states that as long as the target is present, pharmaceuticals will have the same mode-of-action in all species (see later for a more detailed description of this hypothesis), it is possible that fluoxetine could also have specific mode-of-action effects on a variety of different species of invertebrates. The concentrations of fluoxetine reported to cause effects are highly variable, covering as they do a range of more than one million-fold. Currently the lowest effective concentration is 0.3 ng/L; this concentration was reported to cause a number of biochemical and molecular effects on a species of mussel (Franzellitti et al., 2013). Concentrations in the ng/L range have also been reported to adversely affect learning and memory retention in a cuttlefish (Di Poi et al., 2013), to trigger gamete release from mussels (Lazzara et al., 2012), and to affect swimming behaviour of an amphipod (Guler and Ford, 2010). In contrast, the lowest concentration of fluoxetine required to induce spawning and parturition in other species of bivalves was 30.9 μg/L (Fong and Molnar, 2008), and Bringolf et al. (2010) found that only the two highest concentrations they tested, namely 300 and 3000 μg/L, had reproductive effects on various species of freshwater mussels.

In summary, much of the published data can be interpreted as meaning that, in general, invertebrates are more sensitive to fluoxetine than fish are. However, different species appear to demonstrate very different sensitivities, making it impossible to reach any general conclusions.

1.3. Limitations of the published studies

Perhaps understandably, the first studies in any new field – here 'Pharmaceuticals in the environment', or PIE – have limitations, because there is little, if any, existing information on which to build. Most of the currently published studies on the possible effects of fluoxetine on aquatic organisms suffer from one or more limitations, the most important of which are briefly discussed below:

(a) Lack of a concentration–response relationship.

Although many scientists appear to believe in 'low-dose' effects, meaning that low doses (or concentrations) can cause effects that higher doses/concentrations do not (Vanderberg et al., 2012), we believe that most 'low dose' effects are artefacts, and are unlikely to be repeatable. Many studies of the possible effects of fluoxetine on aquatic organisms involved the use of only one concentration of the drug (e.g. Dzieweczynski and Hebert, 2012; Egan et al., 2009; Franzellitti et al., 2013), or at most two (Wong et al., 2013; Kohlert et al., 2012; Mennigen et al., 2010; Di Poi et al., 2013), making it very difficult, if not impossible, to determine if any effects were concentration-related. When a wide range of concentrations has been used, it has usually been reported that only the highest concentration(s) causes significant effects (e.g. Winder et al., 2012; Bringolf et al., 2010; Fong and Molnar, 2013). However, sometimes a sigmoidal dose–response relationship has not been found when a wide range of concentrations have been tested, and instead a non-monotonic relationship has been reported, where low concentrations appeared to cause effects that higher concentrations did not (e.g. Guler and Ford, 2010). In such cases, it is unknown whether or not the results are repeatable (see below).

(b) No replication.

As far as we can judge, very few of the studies reporting apparent effects of fluoxetine on aquatic organisms have involved more than one experiment. If practical, scientists should always assess the repeatability of their results before publishing them. This is especially true if the results are surprising, or even startling, and likely to elicit interest and possibly also considerable concern. Hence, results suggesting that concentrations of fluoxetine in the ng/L range, which are therefore similar to environmental concentrations, cause adverse effects to aquatic organisms need to be demonstrated to be repeatable. Currently it is unclear if the apparent effects of ng or even sub-ng/L concentrations of fluoxetine in, for example, fish behaviour (Barry, 2013), reproduction and metabolism of fish (Mennigen et al., 2010a,b), behaviour of cuttlefish (Di Poi et al., 2013), and various processes in mussels (Franzellitti et al., 2013; Lazzara et al., 2012) will be reproducible, because in all cases the results of only a single experiment were reported. However, other authors have taken a more robust approach and, for example, conducted one or more range-finding studies prior to their definitive studies (e.g. Wong et al., 2013).

The danger of not repeating a study before publishing the results from a single experiment is well illustrated by the following two examples, both involving effects of pharmaceuticals on fish. Owen et al. (2010) investigated the effects of clofibric acid, the main metabolite of two representatives of one class of lipid lowering (hypolipidemic) drugs known as fibrates. In their first experiment, all six concentrations (0.1 μg/L to 10 mg/L) of clofibric acid reduced the growth rate of the fish, which was an unexpected result. However, a second experiment, which was more powerful in many respects (many more fish per concentration, replication of each concentration, etc.), failed to reproduce the results of the first experiment. The authors (Owen et al., 2010) concluded that the second experiment is more likely to have provided the 'right' (i.e. reproducible) result. The second example involves diclofenac, a non-steroidal anti-inflammatory drug (NSAID). A number of individual studies suggested that relatively low μg/L concentrations of diclofenac had major adverse effects on fish (Schwaiger et al., 2004; Triebskorn et al., 2004; Hoeger et al., 2005; Mehinto et al., 2010), but these results were not reproduced in a series of very robust studies (Memmert et al., 2013). Examples such as these should caution authors not to publish results of single experiments, or if they do, to be open and honest about any possible limitations of their studies in their papers.

(c) Non-standard endpoints.

Although fish behaviour has been used as an endpoint for quite some time, in studies on stress and anxiety for example,

it is not especially easy to quantify. Many different aspects of fish behaviour in response to exposure to fluoxetine have been investigated, including the position of the fish in a tank, their exploratory behaviour when placed in a new tank, response to an alarm pheromone, response to a predator, swimming speed and aggression. Few of these potential effects have been studied enough to establish the baseline (of non-exposed fish) or the degree of variability of the behavioural endpoint between individual fish.

In the case of invertebrates, the endpoints selected, although often understandable because they are thought to be under the control of serotonin, are rarely, if ever, well studied. This is partly a consequence of the fact that only a small proportion of environmental biologists work with invertebrates, and partly because there are so many different species of invertebrate to work with.

Using non-standard endpoints brings a number of major problems. One is that the baseline is not well established: that is, what is normal when the organism is not exposed to fluoxetine? The inherent variability around the baseline also needs to be established. Without knowing the baseline, and how much it varies, it is impossible to conclude whether or not fluoxetine has had an effect. Another major problem with utilising non-standard endpoints is that the factors that alter that baseline are usually unknown. For example, what environmental conditions affect the endpoint of interest, in what direction, and to what degree?

Often quantifying non-standard endpoints involves the use of assays that have not been fully validated for the use they are put to. This introduces yet another uncertainty.

(d) Can clinical data help us?

One huge advantage of studying the environmental impacts of pharmaceuticals, as opposed to any other group of chemicals, is that a very large amount of data is available on the chemicals, particularly on their pharmacokinetics and mode of action. A number of authors have emphasised the importance of using all available and appropriate clinical data to guide ecotoxicological studies (e.g. Kreke and Dietrich, 2008; Winter et al., 2010; Berninger and Brooks, 2010). Most of this thinking is encapsulated in the read-across hypothesis (Hugget et al., 2003), which states that the mode-of-action of a drug will 'read-across' from humans to other organisms, leading to similar effects in the different organisms (e.g. in humans and fish) and, further, that these similar effects will occur at similar blood concentrations. The current level of support for this hypothesis is discussed in Rand-Weaver et al. (2013). If this hypothesis was applied to fluoxetine, it would mean that the main effects of fluoxetine on aquatic organisms would be behavioural ones, and that these would occur only when the concentration of fluoxetine in the blood of the aquatic organism, such as a fish, was similar to the human therapeutic concentration (which is between 50 and 300 μg/L). Unfortunately, to date nobody has reported plasma concentrations of fluoxetine in fish exposed to the drug. There seems to be an assumption, as discussed above, that effects of fluoxetine on aquatic organisms will be mode-of-action related. If that proves to be correct, then one might expect concentrations of fluoxetine in water would need to be in the same range (tens of hundreds of μg's/L) before effects occur, because the drug does not bioconcentrate to any significant extent, as a consequence of fluoxetine not being particularly hydrophobic, with a Log *D* at pH 7.4 of between 1.5 and 2 (Nakamura et al., 2008). Such reasoning would support the results of the many studies showing behavioural effects of fluoxetine when water concentrations are in the tens or hundreds of μgs/L, but question those reporting behavioural effects at much lower concentrations of fluoxetine (see earlier discussion).

Knowledge of the serotonin transporter (SERT), the main target for SSRI anti-depressants, of the zebrafish is very relevant in this regard (Tatsumi et al., 1997). The anti-depressant fluoxetine has a similar affinity for the zebrafish SERT as it does for the human SERT (Wang et al., 2006). This would suggest that plasma concentrations of SSRIs such as fluoxetine needed to cause effects will be similar in fish and patients (humans) taking these drugs; that is, upwards of 50 μg/L.

The lack of a vertebrate-like blood system in more 'simple' aquatic organisms makes the application of the read-across hypothesis to these organisms more problematic. However, it has been argued that the reported effects of very low (ng/L) concentrations of fluoxetine on a number of species of invertebrates are very difficult to reconcile with the read-across hypothesis (Sumpter and Margiotta-Casaluci, 2013). A water concentration of 0.3 ng/L (Franzellitti et al., 2013) seems unlikely to produce a concentration of fluoxetine in the nerve synapses of mussels that is as high as that in the nerve synapses of people taking the drug to treat their depression; as stated above, it requires a plasma concentration of between 50 and 300 μg/L to produce serotonin uptake inhibition in these people. Measuring the internal (perhaps whole body) concentrations of fluoxetine in the species of invertebrates that seems exquisitely sensitive to fluoxetine would provide very useful information.

If the target for fluoxetine, the SERT, had a greater affinity for fluoxetine in some, or all, invertebrates than the vertebrate SERT does, then lower concentrations of the drug would likely be required to cause effects. Such a situation could explain why some invertebrates appear more sensitive to fluoxetine than vertebrates do. However, this remains only a theoretical possibility until the affinity of the SERT in a range of invertebrates is established; Sumpter and Margiotta-Casaluci (2013) have suggested that researchers working with species apparently extremely sensitive to fluoxetine should conduct the appropriate work.

Other explanations for the apparent much greater sensitivity of at least some invertebrates to fluoxetine are also possible. For example, exposure during development may affect the number and/or affinity of SERT, which in turn could affect the sensitivity of an organism to fluoxetine. This remains no more than a theoretical possibility until the idea is tested experimentally. As stated above, if authors are to claim that their particular test animal is exquisitely sensitive to fluoxetine, we consider it incumbent on them to offer an explanation for the increased sensitivity, preferably one that they have made an effort to address experimentally.

(e) What can be concluded?

The answer to this question at the present time must be "not a great deal". The apparent variability in the sensitivity of different aquatic species to fluoxetine prevents any consensus being reached. Does it require concentrations of fluoxetine in the μg/L range to cause effects, or can ng/L, or even sub-ng/L, concentrations cause effects to at least some species? From an environmental standpoint, the answer to this question is extremely important. If the former answer is correct, then environmental concentrations of fluoxetine probably do not pose a threat to aquatic wildlife, but if the latter answer is correct, then they do. It will probably take many years, and a lot more research, before a consensus is reached, if it ever is.

Despite what is said above, there is a consensus that fluoxetine affects a wide range of aquatic organisms, both vertebrate and invertebrate. This is perhaps not surprising, because serotonin seems to be a ubiquitous neurotransmitter, and therefore serotonin transporters (the target for fluoxetine) are likely to be equally ubiquitous. Thus effects are to be expected; the only uncertainty is the external (water) concentration required to

elicit those effects. The best situation would be not to have fluoxetine in the environment; then no effects would occur however sensitive some organisms were.

We have concentrated on fluoxetine because it is the most studied anti-depressant from an ecotoxicological perspective. However, a number of other anti-depressants are in widespread use clinically, and as a consequence are present in the aquatic environment. Our conclusions probably apply to anti-depressants in general.

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