Accepted Manuscript

Title: From gender benders to brain benders (and beyond!)

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PII: S0166-445X(14)00055-1

DOI: <http://dx.doi.org/doi:10.1016/j.aquatox.2014.02.005>

Reference: AQTOX 3762

To appear in: *Aquatic Toxicology*

Please cite this article as: Alex T Ford From gender benders to brain benders (and beyond!) (2014), <http://dx.doi.org/10.1016/j.aquatox.2014.02.005>

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From gender benders to brain benders (and beyond!)

Editorial

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The impact of pharmaceuticals in the environment has been a concern for several decades (Cleavers, 2003; Ferrari et al, 2003; Fent et al, 2006, Santos et al, 2010). Following on from the field of endocrine disrupting chemicals (EDCs) in the early 1990s, where awareness to population level effects of sub-lethal chemicals were raised, the scientific community was alerted to the potential impact of antidepressants over a decade ago (Fong, 1998; Fong, 2001, Brooks et al 2003). A considerable amount of research on EDCs was based around synthetic estrogens and estrogen mimics/agonists (Tyler et al, 1998; Lange et al 2001; Sumpter and Johnson, 2005) resulting in a focussed international research effort that culminated in a vast body of research. Interestingly, and despite the fact that in some countries the prescriptions for contraceptive pills and those for antidepressants are broadly similar (~8-9% of the population; US DHHS report, 2012); and both compounds are found at low ng/L concentration in the environment, the body of ecotoxicological work covering effects of synthetic estrogens hugely grosses that of the antidepressants. Why there hasn't been more recent into the antidepressants is not entirely clear but may have occurred for a variety of reasons. Firstly, the numbers of studies recording these compounds in aquatic systems were previously limited and secondly the effects of antidepressants that were recorded were at relatively high concentrations arguably due to the endpoints available at the time and thus perhaps did not stimulate the field. In addition, the field of behavioural ecotoxicology, despite coming a long way is still developing. Therefore, it is arguably more difficult to extrapolate aberrant behavioural of aquatic organisms into risk assessment compared to impacts observed on reproductive systems, growth and mortality. And finally, the research funding for the field of ecotoxicology is ever transient and antidepressants are one small group of pharmaceuticals amongst many thousands needing to be fully evaluated for their impact on the environment.

32 Recently, an increasing number of studies have raised the concerns over the impact of antidepressants
33 in the aquatic environment due to effects on aquatic organisms (both invertebrates and vertebrates)
34 being observed at concentrations at very low and environmentally relevant concentrations (Shultz et al,
35 2011; Di Poi et al 2013; Franselitti et al 2003; Guler and Ford, 2010; De Lang et al, 2006; Fong and Hoy,
36 2012) even down into the pg/L range. For this reason it was decided to put together this special edition
37 with the view to consolidating the most up to date research and views on the topic, to generate debate
38 and aid researchers and regulators in evaluating the risk posed by these special biologically active
39 pharmaceuticals. Having decided to entitle this editorial from gender benders to brain benders it is
40 worth pointing out one of the major shortcomings in this title. A large focus on the effects of
41 antidepressants, as one expect from their therapeutic effect in humans, has been on behavioural
42 endpoints observed across a range of Phyla. However, it is also important to note that the
43 antidepressants were designed to target neurohormones (e.g. serotonin, dopamine and epinephrine)
44 which are involved in a wide variety of biological processes “beyond” just behaviour.

45
46 The special edition starts with a review whereby we provide an overview of the neuroendocrine systems
47 of the Mollusca and Crustacea (Fong and Ford, 2014). Based on the biology and reviewing the current
48 literature on the effects of antidepressants in these groups, this paper notes gaps in our current
49 knowledge and highlight the increasing number of effects demonstrated at environmentally relevant
50 concentrations and non-monotonic concentration curves. Interestingly, antidepressants look like strong
51 candidates for model compounds for studying non-monotonic concentrations effects (Vandenberg et al,
52 2012), especially given their neuronal and hormonal activity. Following this, Franzellitti et al (2014)
53 highlight that other adverse outcome pathways (AOPs) need to be considered beyond those solely
54 related to the mammalian mode of action (MOA) for which the drug was designed. Through
55 investigating a wide variety of biomarkers the authors report significant effects of the SSRI fluoxetine on
56 a marine mussel at sub-ng/L concentrations. Similarly, Bringoff et al (2014) studying the effects of
57 fluoxetine on a freshwater mussel following a 67-d exposure report altered behavioural and metabolic
58 endpoints at higher concentrations. Providing further evidence of environmentally relevant and novel
59 adverse outcome pathways, Di Poi et al (2014) report on the effects of the fluoxetine on crypsis in a
60 cuttlefish. Completing the section on invertebrates, Bossus et al (2014) report on the effects of
61 fluoxetine and sertraline on behaviour and gene expression in a marine amphipod. The authors report
62 effects as low as 1ng/L and interestingly in some instances behavioural alterations were seen within 1hr

of exposure. Investigations into the transcriptional changes highlight plausible links between SSRI exposure and altered phototransduction pathways.

Providing a cautionary tone to these studies, a commentary from Sumpter et al (2014) urges scientists and the scientific community to replicate some of these studies, given the low pg-ng/L being reported. They report this is especially important given the lack of concentration-response relationships, the use of none-standard endpoints and considerable differences in observed effects between species given the 'read-across' hypothesis (see Hugget et al. 2003; Rand-Weaver et al. 2013; and further commentary by Sumpter and Margiotta-Casaluci, 2013).

Brooks (2014) "Fish on Prozac (and Zoloft): Ten Years Later" provides a historical overview of the work undertaken and lessons learnt by Brooks and colleagues and in the area of antidepressant ecotoxicology since the publication of their early research ten years ago. In addition, Brooks (2014) highlights the use of probabilistic hazard assessment and fish plasma modelling approaches with SSRIs and tricyclic antidepressants and suggests that these drugs are predicted to result in therapeutic hazard to fish (internal fish plasma level equalling mammalian therapeutic dose) when exposed to water (inhalational) at or below 1 µg/L.

Beginning the section on fish, Yang et al (2014) studied the effects of the tricyclic antidepressant, amitriptyline (1ng-1mg/L), on growth and a range of physiological and biochemical biomarkers in zebrafish embryos. The authors report significant effects as low as 10ng/L and highlight a range of U and inverted-U shaped concentration-response curves. Next follows a group of studies looking at behavioural endpoints in fish exposed to variety of antidepressants. Firstly, Weinberger and Klaper (2014) using fathead minnows, reported a variety of altered mating behaviours including nest building and defending in male fish with exposure to 1µg/L fluoxetine. Higher doses altered predator avoidance behaviour and feeding and interestingly 100µg/L exposures resulted in increased mortality in females caused by male aggression. Secondly, Hedgespath et al (2014) studied the effects of the SSRI sertraline on feeding in the Eurasian perch and found that the ability to prey on *Daphnia magna* was impaired at 83 and 300 µg/L at two different stocking densities but not at lower concentrations (0.12 µg/L). In another feeding study, Bisesi et al (2014) investigated the ability of hybrid striped bass to prey upon fathead minnows following exposure to the SNRI venlafaxine (0-500 µg/L) and demonstrated increased time to capture prey at higher concentrations. The authors also reported significant reductions in brain

serotonin levels in exposed fish, however, no effects on brain dopamine or epinephrine were observed. Thirdly, Olsén et al (2014) investigated the effects of the SSRI citalopram on reproductive and non-reproductive behaviour in the Endler guppy and report a variety of anxiolytic behaviours, including reduced freezing behaviour in both males and females and greater exploration of upper parts of the aquaria in male specimens. Finally, Connors et al (2014) highlight that although the primary pharmaceutical site for the action of SSRIs is serotonin transporters (SERTs), others are known to target 5-HT_{1A} receptors as well as SERT. With this in mind Connors et al (2014) investigated the 5-HT and cannabinoid (CB) receptors in zebrafish and used an anxiolytic behaviour tests against 5-HT and CB agonists.

Two studies close the special edition whereby two different fish species were exposed to the antiepileptic and mood stabilising drug, Carbamazepine (CBZ). Hampel et al (2014) looked at the transcriptomic responses in salmon exposed to CBZ using a 17K cDNA microarray. The study observed enrichment and down-regulation effects across a range of biological processes including focal adhesion, extracellular matrix – receptor interaction pathways, apoptotic process, extracellular matrix organization and heme biosynthesis. Moving from molecular to population level effects, Wilson et al (2014) using the zebrafish model, observed generational effects in fish exposed to the mood stabilizer Carbamazepine (CBZ) and plasma lipid reducing drug Gemfibrozil (GEM) at 10µg/L. Interestingly, reciprocal crosses between F₁ group controls with CBZ or GEM exposed F₁ individuals indicated male exposure induced greater effects. These male specific effects were also observed in courtship behaviour and sperm morphology although these effects were drug specific.

It is hoped this special edition will spark some debate within the field and assist in moving the science forward. In addition, we hope the international community take on the challenge to test the repeatability of these surprisingly low dose effects and address some of the concerns raised in these studies. We would strongly encourage any opinions the scientific community may have on the science presented in this edition to submit their views under the “letters to the editor” section.

Acknowledgements:

One sincerely would like to thank all the authors and reviewers who have contributed to this special edition. In addition, I would personally like to thank the Editors in Chief at Aquatic Toxicology and the supporting staff at Elsevier.

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