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REVIEW ARTICLE

Pharmaceuticals and personal care products: A critical review of the impacts on fish reproduction

Matthew D. Overturf, Jordan C. Anderson, Zacharias Pandelides, Lindsay Beyger, and Douglas A. Holdway

Faculty of Science, University of Ontario Institute of Technology, Oshawa, ON, Canada

Abstract

Research in environmental toxicology involving pharmaceuticals and personal care products (PPCPs) has increased greatly over the last 10–15 years. Much research has been focused on the endocrine-disrupting potential of PPCPs, as they relate to negative population impacts of aquatic organisms. This review assesses the current data on the reported effects of PPCPs on fish reproduction with an emphasis on fecundity, a predictor of population effects. Studies of both individual PPCPs and PPCP mixtures are presented. As the majority of individual PPCP studies reviewed demonstrate negative effects on fish fecundity, we relate these findings to detected surface water concentrations of these compounds. Very few studies involving PPCP mixtures have been conducted; however, the need for these types of studies is warranted as fish are most likely exposed to mixtures of PPCPs in the wild. In addition, laboratory and field assessments of wastewater treatment plant (WWTP) effluents, a major source of PPCPs, are reviewed. Much of the data provided from these assessments are variable and do not generally demonstrate negative impacts on reproduction, or the studies are unable to directly associate observed effects with WWTP effluents. Finally, future research considerations are outlined to provide an avenue into understanding how wild populations of fish are affected by PPCPs. These considerations are aimed at determining the adaptation potential of fish exposed to mixtures of PPCPs over multiple generations. As global use of PPCPs continually rises, the need to discern the effects of chronic exposure to PPCPs is greatly increased.

Keywords

aquatic environment, ecotoxicology, fecundity, mixtures, wastewater

History

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Address for correspondence: Matthew D. Overturf, Faculty of Science, University of Ontario Institute of Technology, 2000 Simcoe Street North, Oshawa, ON L1H 7K4, Canada. Tel: +1 (905) 721-8668 ext. 2941. E-mail: Matthew.Overturf@uoit.ca

Introduction

Over the last 10–15 years, a significant amount of research has been conducted to investigate the risks posed by pharmaceuticals and personal care products (PPCPs) to the environment. PPCPs include numerous chemical classes with very unique physiochemical properties and biological activities. Pharmaceuticals are used to treat and prevent diseases in humans and animals, and are usually classified according to their therapeutic function. Personal care products are commonly used in applications to improve the quality of daily life and include cosmetics, shampoos, soaps, deodorants, sunscreens, and toothpastes. An unavoidable consequence of the increased use of PPCPs is their detection in the environment, although the concentrations of PPCPs reported in most aquatic environments are generally low (ng/L to low μg/L range).

There are many routes by which PPCPs enter the environment (Figure 1). The most common routes are through human consumption, elimination, and disposal via wastewater

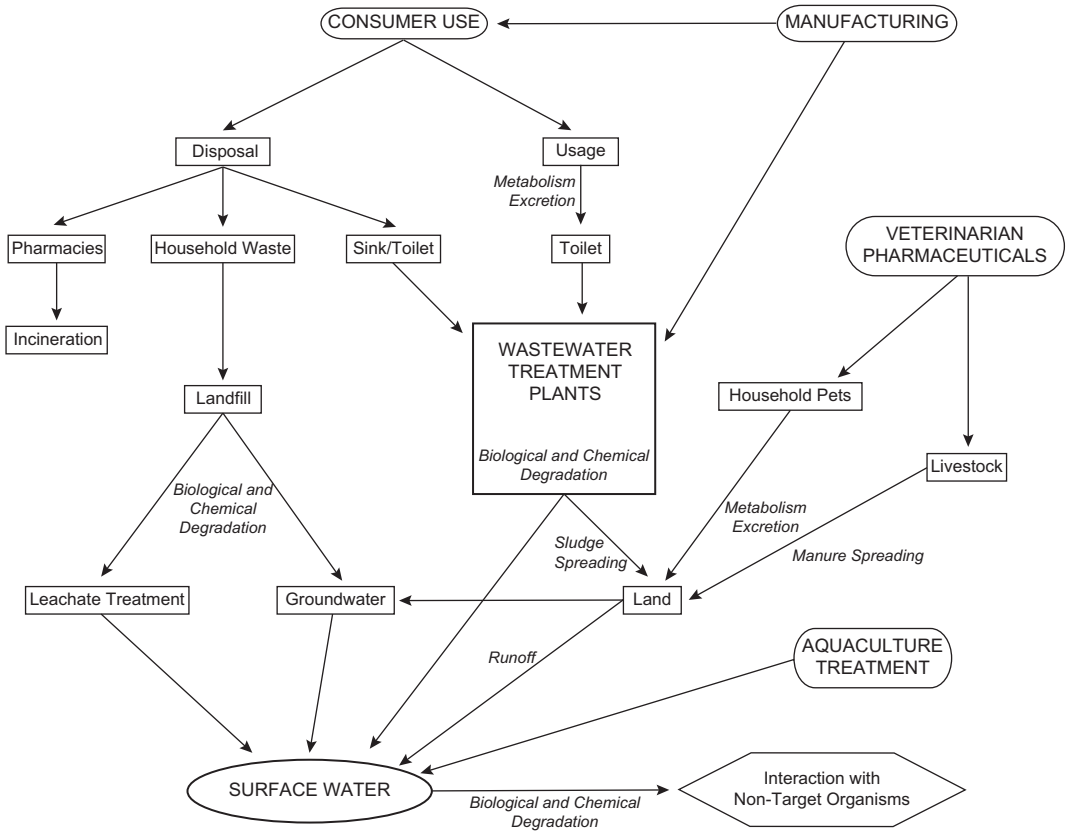

Figure 1. Major routes of entry of pharmaceuticals and personal care products (PPCPs) into the environment.

systems (Nikolaou et al. 2007). Following wastewater treatment, PPCPs are released into surface waters in treated effluent as well as onto terrestrial systems from sewage sludge when it is applied as fertilizer (Kinney et al. 2006, Ternes et al. 2004). PPCPs are also released into the environment through direct release into wastewater systems or into the environment from manufacturing sites (Bound and Voulvoulis 2005, Fick et al. 2009). Veterinary medicines enter the environment directly, through the application to household pets, livestock, and aquaculture, or indirectly, through spreading of livestock manure as fertilizer for agricultural fields and subsequent agricultural runoff (Boxall et al. 2003, Martinez-Carballo et al. 2007).

Pharmaceuticals, as well as many chemicals in personal care products, are designed to interact with specific processes to alter physiological function and produce a therapeutic response in target species. Many of the processes and mechanisms through which PPCPs interact are conserved across vertebrates and share high homology with target species (Gunnarsson et al. 2008, Huggett et al. 2003a). Therefore, there is much interest regarding the potential impacts of PPCPs on non-target aquatic species. Of major concern are the potential impacts on fish reproduction and the maintenance of healthy populations of fish in waters receiving PPCPs.

In this paper, we assess the impacts of PPCPs on fish reproduction, with an emphasis on fecundity effects. Initially, we focus on laboratory studies that investigated reproductive impairments caused by individual compounds, followed by studies conducted with mixtures of PPCPs. Next, a thorough comparison of the reproductive effects of wastewater effluents on fish in both laboratory and field studies is presented. Finally, we provide some future research directions that will aid in furthering the knowledge of the impacts of PPCPs on fish reproduction. All studies presented in this paper were obtained using PubMed, Web of Science, and Google Scholar under combinations of the following search terms: "fish reproduction", "pharmaceuticals", "personal care products", and "wastewater effluents". Papers were limited to those that dealt with water-borne exposures and included direct impacts on fish fecundity or the number of offspring produced.

Pharmaceuticals

Endocrine-active pharmaceuticals

Steroid hormones

Reproductive steroids are an important component of the endocrine system. Often mediated via environmental cues, reproductive steroids are vital in sexual differentiation, behavior, and the development of secondary sex characteristics and gonads in fish (Kime 1999). These factors can influence the overall reproductive fitness of a given organism. Therefore, xenobiotic steroids have the ability to alter the reproductive capabilities of non-target organisms, specifically fish, at environmentally relevant concentrations. The following sections outline the reproductive impairments associated with estrogens, progestins, and androgens.

Estrogens. Estrogens are commonly used pharmaceuticals in both hormone therapy and oral contraceptives. Depending on their origin, estrogens can be classified as natural, such

as 17β-estradiol (E2) and estrone, or synthetic, such as 17α-ethinylestradiol (EE_2). Estrone and 17β-estradiol (E2) are the most common estrogens produced by mammals and fish. Regarding reproduction, EE_2 and estrone function along the hypothalamus–pituitary–gonadal (HPG) axis, primarily influencing behavior, gonad differentiation, and in fish, the production of vitellogenin (VTG) in the liver (Arukwe and Goksøyr 2003, Kime 1999). In excess, estrogens can also activate a negative feedback loop along the HPG axis (Kime 1999), suppressing the release of gonadotropin releasing hormone (GnRH) from the hypothalamus, and luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary (Kime 1999). LH and FSH are responsible for activating G-protein mediated signal transduction within the gonads, initiating the production of E2 (Villeneuve et al. 2007). Activating the negative feedback loop along the HPG axis is the generalized mode of action of EE_2 in combined oral contraceptive pills in humans. Given the level of conservation among estrogen receptors in vertebrates (Brown et al. 2014), a similar mode of action is expected in fish. Estrogens and their metabolites have been known to enter the environment through wastewater treatment plant (WWTP) effluent, resulting in surface water concentrations <5 ng/L (Ternes et al. 1999, Zhou et al. 2007).

Estrogens are a commonly studied pharmaceuticals in aquatic toxicology due to their high biological activity, reproductive role within many organisms, and presence within the environment. The effects of E2, estrone, and EE_2 on fish fecundity are extensive (Table 1). Japanese medaka (*Oryzias latipes*) exposed to 187.2 ng/L of E2 for 21 days experienced a reduction in both fertility and fecundity (Sun et al. 2009). However, the marine Java medaka (*Oryzias javanicus*) exposed to 68 ng/L of E2 experienced an increase in fecundity and a decrease in fertility (Imai et al. 2005). A decrease in fecundity was observed on exposure to 82 ng/L of E2 in both the parental and first-generation offspring of marine sheepshead minnow (*Cyprinodon variegatus*), while a decrease in fecundity in the second-generation offspring occurred at 290 ng/L of E2 (Cripe et al. 2009). Brion et al. (2004) observed an increase in fecundity in zebrafish (*Danio rerio*) exposed to 117 ng/L of E2 from 0–21 days post-fertilization. Conversely, a decrease in fecundity occurred when zebrafish were exposed at 21–42 and 215–236 days post-fertilization to 109 and 16.5 ng/L of E2, respectively (Brion et al. 2004). Dammann et al. (2011) compared the potencies of E2 and estrone over a 21-d exposure in the fathead minnow (*Pimephales promelas*). A reduction in fecundity was observed at 26 ng/L of E2 and 54 ng/L of estrone (Dammann et al. 2011). The Java medaka also experienced a reduction in fecundity and fertilization on exposure to 1,188 and 3,701 ng/L of estrone, respectively, following a full life-cycle exposure (Imai et al. 2007).

When examining the reproductive effects of EE_2, an increase in fecundity was observed in Japanese medaka when exposed for 14 d to 0.2 ng/L of EE_2 (Tilton et al. 2005). The increase in fecundity at low concentrations was supported by Pawlowski et al. (2004b), where an increase in egg production occurred in fathead minnows exposed to 0.7 ng/L of EE_2 for 21 d. Pawlowski et al. (2004b) also observed a decrease in fecundity and fertilization in the same experiment at 7.8 ng/L EE_2. Similarly, a complete life-cycle exposure to EE_2 in fathead minnows resulted in an increase in egg production at 0.32 ng/L, coupled with a cessation in egg production at 3.5 ng/L (Parrott and Blunt 2005). Additionally, a reduction in fertilization at 0.32 ng/L of EE_2 was observed in this study (Parrott and Blunt 2005). These results suggest that EE_2 concentrations below 1 ng/L may act as a stimulant for ovulation; conversely, the reduction in fertilization may indicate reproductive impairment in males, evidenced by the apparent demasculinization of male fish exposed to EE_2 (Parrott and Blunt 2005, Pawlowski et al. 2004b).

In contrast, many studies have shown that EE_2 at low ng/L concentrations does reduce fecundity (see Table 1). Xu et al. (2008) demonstrated that zebrafish exposed to 0.4 ng/L of EE_2 from 0–90 days post-hatch followed by 90 days in clean water resulted in a reduction in fecundity during a reproduction study. Furthermore, Schäfers et al. (2007) showed that impairments in fecundity on zebrafish were reversible during a chronic exposure to ≤ 1.1 ng/L of EE_2, but irreversible at exposure to 9.3 ng/L of EE_2 after a clean water depuration. When examining the generational effects of EE_2, Nash et al. (2004), showed that F0 zebrafish experienced a reduction in fecundity at 50 ng/L of EE_2, while their offspring, had a reduction in fecundity at 5 ng/L of EE_2. Finally, a 7-year whole-lake experiment using 5–6 ng/L of EE_2 conducted in the Experimental Lakes Area in northwestern Ontario, Canada, showed VTG induction and intersex in wild male fathead minnows and altered oogenesis in females (Kidd et al. 2007). Consequently, the fathead minnow population in this lake collapsed and was nearly extirpated (Kidd et al. 2007). However, by the spring of the fourth year post-treatment, abundance of adult fathead minnows had returned to pretreatment levels, VTG concentrations had returned to baseline levels, and testicular abnormalities were absent (Blanchfield et al. 2015). Current literature has thus demonstrated that both natural and synthetic estrogen exposure can alter reproductive function in fish at environmentally relevant concentrations.

Progestins. Progestins can be broken down into two groups: natural and synthetic. Natural progestin, progesterone (P4), 17α,20β-dihydroxypregn-4-en-3-one (17,20-DHP), is responsible for the maintenance of many normal reproductive functions in humans and fish, respectively (DeQuattro et al. 2012, Erkkola and Landgren 2005, Nagahama 1997). Synthetic progestins are commonly used in oral contraceptives and hormone therapy. The general mode of action of progestins operates through the activation of progesterone receptors (PR) within the body. Progestins in oral contraceptives activate a negative feedback loop in the HPG axis, which inhibits the release of GnRH from the hypothalamus, suppressing the midcycle peaks of LH and FSH from the pituitary gland, leading to cessation of ovulation in mammals (Erkkola and Landgren 2005). The relative binding affinity (RBA) to the human PR varies greatly depending on the progestin in question (Sitruk-Ware 2004). Currently, progestins are derived from three different parent compounds—19-nortestosterone, 19-norprogesterone, or spironolactone (Schindler et al. 2003). Although 15 synthetic progestins are currently used in pharmaceuticals, this review discusses only those that have undergone reproductive testing on fish. Importantly, natural and synthetic progestins are present in the environment with reported surface water concentrations up to 375 ng/L (reviewed by Orlando and Ellestad 2014).

Table 1. The effects of pharmaceuticals and personal care products on fish fecundity.

Chemical	Fish species	Life stage	Exposure length	LOEC (ng/L)	Effect on fecundity	References
Estrogens						
17β-estradiol	Zebrafish (*D. rerio*)	Embryo to larval	21 days	117	↑	Brion et al. (2004)
		Juvenile	21 days	109	↓	
		Mature adult	75 days	16.5	↓	
	Japanese medaka (*O. latipes*)	Embryo-larval	21 days	1660	↓	Nimrod and Benson (1998)
		Reproductive males	14 days	820	↓	Shioda and Wakabayashi (2000)
		Mature adult	21 days	463	↓	Kang et al. (2002)
		Life-cycle	100 days	27.9	↓	Seki et al. (2005)
		Mature adult	14 days	2528.3	↓	Jukosky et al. (2008)
		Mature adult	21 days	187.2	↓	Sun et al. (2009)
	Fathead minnow (*P. promelas*)	Mature adult	21 days	26	↓	Dammann et al. (2011)
	Eastern mosquitofish (*G. holbrooki*)	Adult male	12 weeks	100	↓	Doyle and Lim (2005)
	Sheepshead minnow (*C. variegatus*)	Life-cycle	F_0 generation	290	↓	Cripe et al. (2009)
			F_1 generation	82	↓	
			F_2 generation	82	↓	
	Java medaka (*O. javanicus*)	Life-cycle	6 months	68	↑	Imai et al. (2005)
				159	↓	
	Sand goby (*P. minutus*)	Life-cycle	8 months	669	↓	Robinson et al. (2007)
Estrone	Fathead minnow	Mature adult	21 days	54	↓	Dammann et al. (2011)
	Japanese medaka	Mature adult	28 days	1000	↓	Nakamura et al. (2015)
		Life-cycle	F_0	>91.4	n/a	
			F_1	91.4	↓	
	Java medaka	Life-cycle	8 months	1188	↓	Imai et al. (2007)
17α-ethinylestradiol	Zebrafish	Egg to juvenile	90 days	10†	↓	Van den Belt et al. (2003)
		Juvenile (15–42 dpf)	28 days	>10†	n/a	Maack and Segner (2004)
		Juvenile (43–71 dpf)	28 days	3†	↓	
		Juvenile (72–99 dpf)	21 days	>10†	n/a	
		Life-cycle	F_0 generation	50†	↓	Nash et al. (2004)
			F_1 generation	4.5	↓	
		Egg to juvenile	42 days	>3†	n/a	Fenske et al. (2005)
		Egg to adult	118 days	3†	↓	
		Post-hatch to juvenile	60 days	1†	↓	Lin and Janz (2006)
		Partial life cycle	75 days	>9.3	n/a	Schäfers et al. (2007)
		Life-cycle	F_1 generation	1.1	↓	
			F_2 generation	2.0	↓	
		Mature adult	17 days	>10.6	n/a	Coe et al. (2008)
		Post-hatch to adult	3 months	0.4†	↓	Xu et al. (2008)
		Reproductive males	14 days	25†	↓	Reyhanian et al. (2011)
		Mature adults	14 days	>2.2*	n/a	Söffker et al. (2012)
	Fathead minnow	Life-cycle	~10 months	3.2	↓	Länge et al. (2001)
		Mature adult	21 days	1	↑	Pawlowski et al. (2004b)
				100	↓	

(*Continued*)

Table 1. (Continued)

Chemical	Fish species	Life stage	Exposure length	LOEC (ng/L)	Effect on fecundity	References
		Life-cycle	5 months	0.32	↑	Parrott and Blunt (2005)
				3.5	↓	
	Japanese medaka	Larval	14 days	>2000	n/a	Foran et al. (2002)
		Larval and adult	14 days each	2000	↓	
		Mature adult	21 days	488	↓	Seki et al. (2002)
		Post-hatch to adult	6 months	10†	↓	Balch et al. (2004)
		Mature adult	14 days	0.2	↑	Tilton et al. (2005)
				500	↓	
		Reproductive males	21 days	>245	n/a	Hashimoto et al. (2009)
		Reproductive males	14 days	1000†	↓	Miller et al. (2012)
	Guppy (*P. reticulata*)	Post-hatch to adult males	108 days	112	↓	Kristensen et al. (2005)
	Three-spined stickleback (*G. aculeatus*)	Juvenile	28 days	27.7	↓	Maunder et al. (2007)
	Mummichog (*F. heterclitus*)	Mature adult	21 days	18.1	↓	Peters et al. (2007)
	Sheepshead minnow	Juvenile to adult	60 days	117	↓	Zillioux et al. (2001)
	Chinese rare minnow (*G. rarus*)	Mature adult	21 days	0.18	↓	Zha et al. (2008)
Progestins						
Progesterone	Fathead minnow	Mature adult	21 days	100	↓	DeQuattro et al. (2012)
	Zebrafish	Mature adult	21 days	>25	n/a	Blüthgen et al. (2013)
Norethindrone	Fathead minnow	Mature adult	21 days	1.2	↓	Paulos et al. (2010)
	Japanese medaka	Mature adult	28 days	22	↓	Paulos et al. (2010)
Levonorgestrel	Fathead minnow	Mature adult	21 days	0.8	↓	Zeilinger et al. (2009)
		Mature adult	21 days	81.2	↓	Runnalls et al. (2013)
Gestodene	Fathead minnow	Mature adult	21 days	1.1	↓	Runnalls et al. (2013)
Desogestrel	Fathead minnow	Mature adult	21 days	8380	↓	Runnalls et al. (2013)
Drospirenone	Fathead minnow	Mature adult	21 days	6500	↓	Zeilinger et al. (2009)
		Mature adult	21 days	>72.9	n/a	Runnalls et al. (2013)
Megestrol acetate	Zebrafish	Mature adult	21 days	606	↓	Han et al. (2014)
Androgens						
17β-trenbolone	Fathead minnow	Mature adult	21 days	26	↓	Ankley et al. (2003)
	Sheepshead minnow	Life-cycle	F_0 generation	870	↓	Cripe et al. (2010)
			F_1 generation	130	↓	
			F_2 generation	27	↓	
Methyltestosterone	Fathead minnow	Mature adult	14 days	200000	↓	Ankley et al. (2001)
		Mature adult	21 days	5000	↓	Pawlowski et al. (2004a)
	Japanese medaka	Life-cycle	100 days	>9.98	n/a	Seki et al. (2004)
		Mature adult	21 days	46.8	↓	Kang et al. (2008)
Steroid receptor antagonists						
Tamoxifen	Fathead minnow	Mature adult	42 days	18200	↓	Williams et al. (2007)
		Life-cycle	284 days	>4076	n/a	
Mifepristone	Zebrafish	Mature adult	21 days	5	↑	Blüthgen et al. (2013)
Flutamide	Fathead minnow	Mature adult	21 days	651000	↓	Jensen et al. (2004)
	Japanese medaka	Mature adult	21 days	1560000	↓	Kang et al. (2006)
		Mature adult	21 days	>982100	n/a	Nakamura et al. (2014)
Bicalutamide	Fathead minnow	Life-cycle	F_0 generation	>92100	n/a	Panter et al. (2012)
			F_1 generation	92100	↓	
Aromatase inhibitors						
Ketoconazole	Fathead minnow	Mature adult	21 days	25000	↓	Ankley et al. (2007)
	Japanese medaka	Mature adult	7 days	300000†	↓	Zhang et al. (2008)
Fadrozole	Fathead minnow	Mature adult	21 days	1400	↓	Ankley et al. (2002)
Letrozole	Japanese medaka	Mature adult	21 days	114500	↓	Sun et al. (2007)
Other endocrine-active pharmaceuticals						
Dexamethasone	Fathead minnow	Mature adult	21 days	469000	↓	LaLone et al. (2012)
Spironolactone	Fathead minnow	Mature adult	21 days	2600	↓	LaLone et al. (2013b)
	Japanese medaka	Mature adult	21 days	47500	↓	LaLone et al. (2013b)
Dutasteride	Fathead minnow	Mature adult	21 days	35300	↓	Margiotta-Casaluci et al. (2013)

(*Continued*)

Table 1. (Continued)

Chemical	Fish species	Life stage	Exposure length	LOEC (ng/L)	Effect on fecundity	References
Trilostane	Fathead minnow	Mature adult	21 days	1555000	↓	Villeneuve et al. (2008)
NSAIDs						
Ibuprofen	Japanese medaka	Mature adult	6 weeks	>100000†	n/a	Flippin et al. (2007)
	Zebrafish	Mature adult	21 days	1000	↓	Ji et al. (2013)
		Mature adult	7 days	>506000	n/a	Morthorst et al. (2013)
Indomethacin	Zebrafish	Mature adult	16 days	100000	↓	Lister and Van Der Kraak (2008)
SSRIs						
Fluoxetine	Zebrafish	Mature adult	7 days	32000	↓	Lister et al. (2009)
	Fathead minnow	Mature adult	4 weeks	100000‡	↓	Weinberger and Klaper (2014)
	Japanese medaka	Mature adult	4 weeks	>5000	n/a	Foran et al. (2004)
SNRIs						
Venlafaxine	Zebrafish	Mature adult	6 weeks	10000†	↓	Galus et al. (2013b)
Beta-blockers						
Propranolol	Fathead minnow	Mature adult	21 days	970000	↓	Giltrow et al. (2009)
		Mature adult	21 days	>4110	n/a	Lorenzi et al. (2012)
	Japanese medaka	Mature adult	2 weeks	>500000	n/a	Huggett et al. (2002)
		Mature adult	4 weeks	500	↓	
Atenolol	Fathead minnow	Mature adult	21 days	>10000000	n/a	Winter et al. (2008)
Fibrates						
Gemfibrozil	Fathead minnow	Mature adult	21 days	>1559000	n/a	Skolness et al. (2012)
	Zebrafish	Mature adult	6 weeks	500†	↓	Galus et al. (2013b)
Others						
Carbamazepine	Zebrafish	Mature adult	6 weeks	500†	↓	Galus et al. (2013b)
Acetaminophen	Zebrafish	Mature adult	6 weeks	10000†	↓	Galus et al. (2013b)
Haloperidol	Fathead minnow	Mature adult	21 days	>20000	n/a	Villeneuve et al. (2010)
Metformin	Fathead minnow	Mature adult	28 days	>41000	n/a	Niemuth et al. (2015)
Personal care products						
Triclosan	Japanese medaka	Mature adult	21 days	>136900	n/a	Ishibashi et al. (2004)
Musk ketone	Zebrafish	Adult females	8 weeks	10 mg/kg¶	↓	Carlsson et al. (2000)
3-benzylidene camphor	Fathead minnow	Mature adult	21 days	74000	↓	Kunz et al. (2006)
Benzophenone-2	Fathead minnow	Mature adult	15 days	1200000	↓	Weisbrod et al. (2007)
Benzophenone-3	Japanese medaka	Mature adult	21 days	620000	↓	Coronado et al. (2008)
		Mature adult	28 days	26000	↓	Kim et al. (2014)
Diethylenetriamine penta acetic acid (DTPA)	Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*)	Mature adult	28 days	>100000000	n/a	van Dam et al. (1999)

*Söffker et al. (2012) investigated the differences in sensitivity of two strains of zebrafish (wild and laboratory) and demonstrated no differences in sensitivity of each strain.
†Nominal values.
‡Increase in female mortality (66.6%) attributed to increase in aggressive mating behavior of males (Weinberger and Klaper 2014).
¶Only study presented using a food-borne exposure. Due to the high lipophilicity of synthetic musks, these compounds warrant further research to assess their impacts on fecundity.

The natural progestin P4 in humans is understood to have a 100% RBA for the PR, however the affinity for the fish PR is currently unknown (Krattenmacher 2000). P4 was tested over a 21-d reproductive study on fathead minnows, and a decrease in fecundity was observed at 100 ng/L (DeQuattro et al. 2012). Furthermore, no effect on zebrafish egg production at 25 ng/L of P4 was observed in a 21-d reproductive study (Blüthgen et al. 2013).

Norethindrone (NET), a first generation progestin derived from 19-nortestosterone, has been shown to have strong affinity for the human PR (134% RBA) (Philibert et al. 1999). A 28-d reproductive study exposing Japanese medaka to NET demonstrated a decrease in egg production at 22 ng/L (Paulos et al. 2010). A similar reduction in egg production was observed in fathead minnows over a 21-d exposure to 1.2 ng/L of NET (Paulos et al. 2010). These results demonstrate that NET can impair fecundity in fish and indicates that the fathead minnow may be more sensitive to NET than the Japanese medaka.

A second-generation progestin derived from 19-nortestosterone—levonorgestrel (LNG)—has greater affinity for the PR, with 323% RBA, compared to that of NET (Philibert et al. 1999). In two separate 21-d reproductive studies using fathead minnows, Zeilinger et al. (2009) and Runnalls et al. (2013), observed a decrease at 0.8 and 81.2 ng/L of LNG respectively. Although LNG has greater affinity for the PR, there does not appear to be a great difference in effects on fecundity in fish, compared to NET.

Gestodene (GES) and desogestrel (DSG) are both third-generation progestins derived from 19-nortestostrerone. GES

has very high bioavailability and affinity for PR (864% RBA) in humans (Philibert et al. 1999, Schindler et al. 2003). The reproductive effects observed in the fathead minnow over a 21-d exposure support high bioavailability and affinity, with a reduction in fecundity observed at 1.1 ng/L in fathead minnows (Runnalls et al. 2013). DSG, on the other hand, has very low affinity for the PR (1% RBA) in humans (Schindler et al. 2003). A 21-d reproductive exposure to DSG using fathead minnows found a reduction in egg production at 8,380 ng/L, a concentration much higher than most other progestins as well as expected surface water concentrations (Runnalls et al. 2013). Reduced affinity for the PR in humans can be attributed to the fact that DSG is a prodrug in humans and is metabolized into a much more biologically active compound, 3-keto-desogestrel, which has a PR RBA of 220% (Schindler et al. 2003). DSG may not act as a prodrug in fish, which could be a potential explanation for the high effect concentration observed in fathead minnows.

The only progestin derived from 19-norprogesterone that has undergone reproductive testing in fish is megestrol acetate (MTA). The RBA of MTA to the PR is 121% in humans (Shields-Botella et al. 2003). A 21-d reproductive study with zebrafish found a decrease in fecundity at 606 ng/L of MTA (Han et al. 2014). However, when MTA is compared to NET, a progestin with similar RBA for PR, a much higher effective concentration is observed. This could be due to species differences (i.e., zebrafish vs fathead minnow), or other factors including differences in uptake and metabolism in fish.

Drospirenone (DRO) is a fourth-generation progestin with a unique characteristic in that it is the only progestin that is an analog of the anti-mineralocorticoid spironolactone (Krattenmacher 2000, Sitruk-Ware 2004). DRO has reduced RBA for the PR (19 %) compared to most other progestins (Krattenmacher 2000). Fathead minnows experienced a decrease in egg production at 6,500 ng/L of DRO over a 21-d reproductive study (Zeilinger et al. 2009). This high effective concentration was supported by Runnalls et al. (2013), in which they determined that DRO caused no effect on fecundity up to 72.8 ng/L in fathead minnows over a 21-d reproductive study. Given that DRO has lower affinity for the PR when compared to other progestins in humans, it is plausible that this is the case in fish as well, thereby minimizing the impact on fecundity.

Progestins have been shown to cause significant impairments in fecundity in the low ng/L range, potentially making them an important environmental pollutant. Also, the RBA of progestins to the human PR could provide insight into the effects of these compounds on fecundity. There is an inverse relationship between human PR RBA of progestins and their lowest observed effect concentrations (LOECs) for fish fecundity (Figure 2). Further research is needed to determine the reproductive effects of the remaining unstudied progestins. Furthermore, there is currently not enough reproductive data to understand species differences in the response to progestins.

Androgens. Synthetic androgens are used in human hormone therapy, as well as in some agricultural and aquacultural practices (Ankley et al. 2001, 2003, Cripe et al. 2010, Durhan et al. 2006). In mammals, the main testicular androgen is testosterone; while in male fish, although they still produce testosterone, 11-ketotestosterone functions as the main androgenic sex hormone (Kime 1999, Yamaguchi et al. 2005). Regulated through the HPG axis, the main role of androgens within fish is to promote spermatogenesis in the male gonads, stimulate

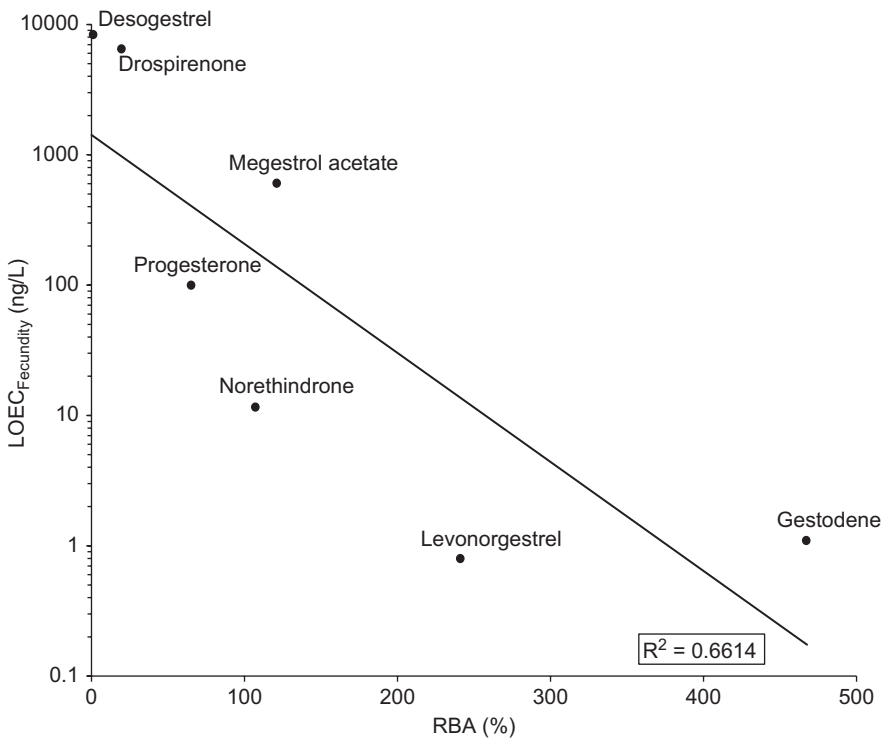

Figure 2. Relationship between the relative binding affinity (RBA %) of progestins to the human progesterone receptor (PR) and the lowest observed effect concentration (LOEC) for fecundity reported in fish. Binding affinities are depicted as the average RBA reported for each progestin (reviewed in Kumar et al. 2015).

secondary sexual characteristics, and promote muscle growth (Cripe et al. 2010, Villeneuve et al. 2007, Yamaguchi et al. 2005). Testosterone produced in female gonads can be aromatized to E_2 (Arukwe and Goksøyr 2003).

Androgens have become important in the agricultural industry as a method of increasing muscle yields in cattle (Cripe et al. 2010, Durhan et al. 2006). Trenbolone acetate is a commonly used synthetic androgen in the cattle industry, where once administered, it is readily metabolized to 17β-trenbolone, the active form (Ankley et al. 2003, Cripe et al. 2010, Durhan et al. 2006). 17β-trenbolone stimulates growth while reducing aggressiveness in bovines, making it a highly desirable steroid in the cattle industry (Ankley et al. 2003, Cripe et al. 2010). High usage has raised concerns regarding potential run-off into nearby receiving waters (Ankley et al. 2003, Cripe et al. 2010, Schiffer et al. 2001). Another commonly used synthetic androgen is methyltestosterone, primarily utilized in the aquaculture industry as a masculinizing agent in fish to increase yields (Ankley et al. 2001, Kang et al. 2008, Rivero-Wendt et al. 2014, Schiffer et al. 2001). Both 17β-trenbolone and methyltestosterone have been detected in the environment at concentrations $\leq$ 162 ng/L and $\leq$ 1.5 ng/L, respectively (Gall et al. 2011, Liu et al. 2011a).

Non-target organisms, including fish, residing in waters surrounding agricultural regions, may be susceptible to the androgenic effects of 17β-trenbolone. Ankley et al. (2003) demonstrated a reduction in cumulative egg production by fathead minnows exposed to 26 ng/L of 17β-trenbolone over 21 d. Furthermore, generational reproductive effects of 17β-trenbolone were observed in sheepshead minnow, with significant reductions in fecundity at 870, 130, and 27 ng/L for the F_0, F_1, and F_2 generations, respectively (Cripe et al. 2010). These results indicate that the reproductive toxicity of 17β-trenbolone may increase with multi-generational exposures.

Similarly, the proximity of many aquaculture facilities to aquatic environments has made methyltestosterone an environmental concern (Rivero-Wendt et al. 2014). As such, multiple studies have been conducted to uncover the effect of methyltestosterone on fish fecundity. A reduction in cumulative egg production occurred in fathead minnows exposed to 200 μg/L of methyltestosterone for 14 d (Ankley et al. 2001) Additionally, Pawlowski et al. (2004a) observed a reduction in total egg production in fathead minnows exposed to 5 μg/L of methyltestosterone for 21 d. A study examining the effects of methyltestosterone on Japanese medaka showed no effect on fecundity on exposure to a concentration of 9.98 ng/L; however, an all-male population was observed when exposed to 27.75 ng/L (Seki et al. 2004). Total egg production was reduced when Japanese medaka were exposed to 46.8 ng/L of methyltestosterone (Kang et al. 2008).

Overall, reported results indicate that fish fecundity can be significantly disrupted by methyltestosterone at environmentally relevant concentrations, and that Japanese medaka may be more sensitive than fathead minnows to such effects. Thus, synthetic androgens may alter the reproductive function of fish, at or near environmentally relevant concentrations.

Steroid hormone receptor antagonists

Receptor antagonists are chemicals that bind to their respective receptors and block or do not activate the receptor's action. Steroid hormone receptor antagonists are commonly used as pharmaceuticals in the treatment of hormonally active cancers. Therefore, their use has increased in recent years. Evidence of the potential for reproductive impairments of steroid hormone receptor antagonists in fish is presented below.

Tamoxifen is an antiestrogen used in the treatment of hormone receptor-positive breast cancer. Reported surface water concentrations of tamoxifen range from 0.3–to 212 ng/L (Loos et al. 2012, Roberts and Thomas 2006). In a partial life-cycle study using fathead minnows, fecundity was reduced at exposures of 1.65 and 18.2 μg/L, while 5.97 μg/L had no effect. However, no impairments on fish reproduction were observed during a full life-cycle exposure to concentrations ranging from 0.007–4.076 μg/L (Williams et al. 2007).

Mifepristone is an antiprogestin compound used for medical termination of pregnancy (Spitz 2003). In zebrafish, mifepristone increased fish reproduction at concentrations of 4.5 and 76.9 ng/L, while 39.4 ng/L resulted in no changes in fecundity (Blüthgen et al. 2013). Mifepristone thus may be an environmental risk, since concentrations as high as 195 ng/L have been detected in wastewater effluent (Liu et al. 2011b). However, additional research is needed to confirm these effects with zebrafish as well as to assess species sensitivity to mifepristone.

The antiandrogens, flutamide and bicalutamide, have been the most widely studied of the receptor antagonists. These compounds are commonly used in the treatment of prostate cancer. Flutamide has been detected in surface waters at concentrations up to 5.8 ng/L (Loos et al. 2012). In fathead minnows, exposure to 651 μg/L of flutamide decreased egg production and also reduced hatching success of resultant offspring (Jensen et al. 2004). A reduction in both fecundity and egg fertility occurred in Japanese medaka exposed to 1.56 mg/L of flutamide (Kang et al. 2006). However, at concentrations ranging from 118.4–982.1 μg/L, no changes in fecundity or fertility in Japanese medaka were observed (Nakamura et al. 2014). Panter et al. (2012) conducted a two-generational study using fathead minnows exposed to bicalutamide. No reproductive impairments were observed in the parental generation; however, egg production was reduced in the F_1 generation exposed to 92.1 μg/L of bicalutamide (Panter et al. 2012). These compounds thus have the ability to impair reproduction in fish, albeit at concentrations much higher than those generally found in the environment.

Aromatase inhibitors

Antimycotics are pharmaceuticals commonly used in human and veterinary medicine for the treatment of fungal infections, and consist of imidazoles and triazoles. These antifungal compounds are general cytochrome P450 (CYP) inhibitors with the ability to inhibit CYP families 1–3 (Hasselberg et al. 2008, Hegelund et al. 2004). In addition to these CYP families, azole antifungals are also implicated in the inhibition of aromatase (Trösken et al. 2004); therefore, azole antifungals may adversely affect fish reproduction. Ketoconazole, a common imidazole antifungal, reportedly reduced egg production in fathead minnows exposed to 25 and 357 μg/L (Ankley et al. 2007) and Japanese medaka exposed to 300 μg/L (Zhang et al. 2008), exposure concentrations much higher than those that

are found in the environment. Concentrations of ketoconazole have been reported in surface waters at < 5 ng/L and in wastewater effluent at ≤ 21.2 ng/L (Lindberg et al. 2010, Peng et al. 2012, Van De Steene et al. 2010). Although environmental concentrations of ketoconazole may not impair reproduction of teleost species, ketoconazole effects may be helpful in the understanding of how endocrine-active compounds interact in the HPG axis (Ankley et al. 2012, Villeneuve et al. 2007).

Potent aromatase inhibitors, fadrozole and letrozole, used in the treatment of hormonally responsive breast cancer, are chemicals of emerging concern (Hamilton and Piccart 1999, Schieweck et al. 1988). Ankley et al. (2002) demonstrated reduced egg production in fathead minnows exposed to fadrozole concentrations ranging from 1.4–57 μg/L. Similar effects were shown in Japanese medaka exposed to letrozole. Letrozole exposure reduced egg production by Japanese medaka at concentrations ranging from 50–625 μg/L (Liao et al. 2014, Sun et al. 2007). Also, fertilization and hatching success were reduced at concentrations as low as 23.51 μg/L of letrozole (Sun et al. 2007). Although these pharmaceuticals have not been detected in environmental samples, aromatase inhibitors have the ability to impair reproduction of teleost species.

Other endocrine-active pharmaceuticals

Several other endocrine-active pharmaceutical compounds outside of the main classes mentioned above have been detected in the environment and assessed for their impacts on fish reproduction.

Dexamethasone is a corticosteroid with a wide range of uses including the treatment of inflammation, autoimmune diseases, and adrenal insufficiencies, and in chemotherapy (Parker and Schimmer 2001). Measured surface water concentrations of dexamethasone have been reported to range from 0.02–0.31 ng/L (Chang et al. 2007). Reproductive impairments by dexamethasone have only been assessed in fathead minnows, with a decrease in egg production observed following a 21-d exposure to 500 μg/L of dexamethasone (LaLone et al. 2012). Since these effects occurred at a concentration several orders of magnitude higher than that reported in the environment, dexamethasone appears to pose little or no risk to fish reproduction in the wild.

Spironolactone is a mineralocorticoid antagonist with antiandrogen and progestin properties. It is commonly used as a diuretic and to treat hypertension, but may also be used to lower unwanted androgen levels (Garthwaite and McMahon 2004). In fathead minnows, spironolactone exposure to concentrations of 43.7 and 2.6 μg/L resulted in cessation of egg laying at days 0 and 2, respectively (LaLone et al. 2013b). Exposure of medaka to 47.5 μg/L of spironolactone also halted reproduction within 8 days (LaLone et al. 2013b). Spironolactone has yet to be detected in the environment; however, additional studies would be warranted to understand the potential endocrine-disrupting effects of this compound.

Dutasteride is a 5α-reductase inhibitor used in the treatment of benign prostatic hyperplasia (enlarged prostate). It works by inhibiting the conversion of testosterone to dihydrotestosterone. Margiotta-Casaluci et al. (2013) demonstrated that exposure of fathead minnows to 35.3 and 104.3 μg/L of dutasteride caused decreased egg production. Trilostane, a 3β-hydroxysteroid dehydrogenase inhibitor, is commonly used in the treatment of Cushing's syndrome. Exposure of fathead minnows to 1555 μg/L of trilostane for 21 d reduced fecundity (Villeneuve et al. 2008). As these pharmaceuticals are not commonly used, their presence in the environment has not yet been detected. With the effective concentrations of both compounds being high, there is likely little to no risk posed by these compounds to wild fish reproduction.

Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) including diclofenac, naproxen, ibuprofen, and indomethacin are pharmaceuticals that function by inhibiting one or both of the two isoforms of the cyclooxygenase enzymes (COX-1 and COX-2) (Vane and Botting 1998). Due to their prevalent use, NSAIDs are found in surface waters at higher concentrations compared to other human pharmaceuticals, with reported surface water concentrations ranging from 0.018–6 μg/L (Fent 2008, Kolpin et al. 2002).

COX enzymes are generally conserved across vertebrate species and normally catalyze the production of prostaglandins (Grosser et al. 2002). Prostaglandins are involved in a large range of physiological processes including thermoregulation, water balance, glomerular filtration, homeostasis, and the control of ovulation (Fujimori et al. 2011). Prostaglandin E2 is involved in fish ovulation and it may be regulated by COX-2 (Fujimori et al. 2011). Cultured zebrafish follicles are able to produce prostaglandin E2 in response to arachidonic acid (AA), and AA stimulates the *in vitro* production of E_2 (Lister and Van Der Kraak 2008). NSAIDs may cause reproductive impairment by disrupting the production of prostaglandin E2 by inhibiting COX-2 (Fujimori et al. 2011, Lister and Van Der Kraak 2008).

Exposure of medaka to ibuprofen resulted in no significant changes in total egg production; however, at concentrations as low as 10 μg/L, a decrease in frequency of spawning and an increase in the number of eggs per spawning event were noted (Flippin et al. 2007, Han et al. 2010). Moreover, a delay in hatching time of embryos exposed to concentrations as low as 0.1 μg/L was observed (Han et al. 2010). Morthorst et al. (2013) exposed zebrafish for 7 d to 21–506 ug/L of ibuprofen and found a reduction in prostaglandin E2, but did not observe any effects on any of the measured reproductive parameters such as egg production. In contrast, zebrafish exposed to ibuprofen for 21 d showed a significant decrease in egg production, reduced hatchability and increased embryo mortality at 1–10 μg/L (Ji et al. 2013). These studies suggest that zebrafish are more sensitive to ibuprofen exposure than medaka, but only for long-term exposures.

In addition to ibuprofen, other NSAIDs have also been shown to alter reproduction in fish. Diclofenac (10 mg/L) significantly reduced fertility and hatchability of embryos produced by second-generation exposed Japanese medaka (Lee et al. 2011). Furthermore, indomethacin caused a reduction of spawning rate and clutch size in zebrafish at 100 μg/L (Lister and Van Der Kraak 2008).

The reproductive effects of NSAIDs tend to vary between experiments and species. NSAIDs may inhibit prostaglandin E2, but what effect this has on the overall reproductive success

of fish is unclear. Based on the evidence reported, NSAIDs may affect fish reproduction, but such effects would likely only occur chronically and likely only at high environmental concentrations.

Selective serotonin reuptake inhibitors

Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, paroxetine, sertraline, and citalopram, are commonly prescribed as antidepressants. SSRIs exert therapeutic effects in mammals by inhibiting the reuptake of the neurotransmitter serotonin at presynaptic membranes, thus elevating the concentration of serotonin in the synaptic cleft (Hiemke and Hartter 2000). These compounds are routinely detected in surface waters at concentrations ranging from 6–205 ng/L (Metcalfe et al. 2010), reflecting their widespread and heavy use.

In fish, several physiological processes including behavior, endocrine signaling, and reproduction are influenced by serotonin. Reproductive phases in female rainbow trout (*Oncorhyncus mykiss*) are correlated to changes in serotonin levels (Hernandez-Rauda et al. 1999); thus, SSRIs are implicated in alterations to reproduction in fish. Fluoxetine reduced egg production in zebrafish following a 7-d exposure to a concentration of 32 μg/L and in fathead minnows following a 4-week exposure to 94.85 μg/L, concentrations much higher than normally found in surface waters (Lister et al. 2009, Weinberger and Klaper 2014). The reduction in fecundity of fathead minnows observed by Weinberger and Klaper (2014) was attributed to an increase in female mortalities (66.7%) during the exposure period, which was attributed to an increase in aggressive mating behavior (nipping or attacking) of male fathead minnows. Foran et al. (2004) found no significant reproductive impairments (fecundity, fertilization, and hatching success) in Japanese medaka following exposures to fluoxetine between 0.1 and 5 ug/L.

Since SSRIs exert therapeutic effects in the central nervous system of mammals, it is reasonable to assume that similar effects occur in fish. Therefore, several studies have assessed reproductive behavioral changes in fish following exposure to SSRIs. Male mating behaviors of fathead minnows (nest building and defending) were affected at concentrations as low as 1 ug/L of fluoxetine; however, these behavioral changes did not result in any alterations to fecundity (Weinberger and Klaper 2014). In contrast, courtship behavior of Siamese fighting fish (*Betta splendens*) exposed to 0.54 μg/L of fluoxetine was not affected (Dzieweczynski and Hebert 2012). Citalopram exposures (1–100 μg/L) also resulted in no significant behavioral changes in two species of guppy (*Poecilia reticulata and P. wingei*; Holmberg et al. 2011, Olsén et al. 2014). While adverse reproductive effects of SSRIs occur at concentrations greater than those found in surface waters, additional research is warranted to assess changes in fecundity and behavior following longer duration studies such as full life-cycle or multigenerational experiments.

A newer class of pharmaceuticals related to SSRIs was introduced in the early 1990s. Serotonin–norepinephrine reuptake inhibitors (SNRIs) have an additional mechanism of action compared to SSRIs by blocking the reuptake of norepinephrine at the presynaptic neuron, resulting in a rapid therapeutic effect (Beique et al. 1998). Surface water concentrations of venlafaxine, a common SNRI, range from 1.7–1003 ng/L (Giebułtowicz and Nałęcz-Jawecki 2014, Metcalfe et al. 2010, Schultz et al. 2010, Valcárcel et al. 2011). A 6-week exposure of zebrafish to 10 μg/L of venlafaxine (nominal; average measured concentration $< 50\%$) resulted in decreased egg production (Galus et al. 2013b). With the increased incidence of venlafaxine in surface waters, SNRIs warrant further research to assess the risk posed by this class of compounds on wild fish reproduction.

Beta-blockers

With the high incidence of cardiovascular disease, β-blockers are one of the most prescribed classes of pharmaceuticals. β-blockers are used to treat a number of cardiovascular conditions, including angina, hypertension, and myocardial infarction, by competitively binding to β-adrenergic receptors, resulting in decreased heart rate and contractility (Mehvar and Brocks 2001). These pharmaceuticals have been detected in surface waters at concentrations ranging from 3–2900 ng/L (reviewed in Owen et al. 2007). Therefore, with high homology of mammalian β-adrenergic receptors in fish (Huggett et al. 2003a), and the potential to interfere with the endocrine system (Massarsky et al. 2011), β-blockers likely pose an environmental risk to aquatic organisms.

Most fish reproductive studies with β-blockers have focused on propranolol, a very lipophilic β-blocker (Massarsky et al. 2011). Giltrow et al. (2009) demonstrated a decrease in egg production of fathead minnows exposed to 970 μg/L of propranolol for 21 d. However, at environmental concentrations of propranolol (0.05–4.11 μg/L), no changes in reproductive parameters or behavior were observed in fathead minnows following a 21-d exposure (Lorenzi et al. 2012). In contrast, Japanese medaka exposed for 4 weeks to propranolol concentrations similar to those found in WWTP effluents (0.5 and 1 μg/L; Huggett et al. 2003b) resulted in reduced egg production and hatching success (Huggett et al. 2002). These studies suggest species differences in sensitivity to propranolol. Fathead minnows exposed to atenolol, a hydrophilic β-blocker (Massarsky et al. 2011), at concentrations ranging from 0.1–10 mg/L, resulted in no changes in measured reproductive parameters (Winter et al. 2008). Overall, the reproductive toxicity associated with β-blockers may be highly modified by the physiochemical properties of these compounds.

Blood lipid regulators (fibrates)

Lipid regulators, particularly fibrate drugs, are one the most common classes of pharmaceuticals detected in the environment, with reported surface water concentrations up to 847 ng/L (Brun et al. 2006, Gros et al. 2006, Quintana et al. 2004). Fibrates exert their therapeutic effects of lowering blood plasma lipid levels by binding and activating the peroxisomal proliferator-activated receptor, alpha transcription factor (Gervois et al. 2000), which in turn activates acyl-coenzyme A oxidase, an enzyme responsible for peroxisomal β oxidation of fatty acids (Desvergne and Wahli 1999). One side effect associated with fibrates is increased production of hydrogen peroxide, which may lead to oxidative stress (Gonzalez et al. 1998). Oxidative stress has been identified as playing a key role in adverse reproductive functions in mammals (Agarwal

et al. 2012); however, these mechanisms are unclear in teleost species.

The impact of fibrates on fish reproduction has only been assessed for gemfibrozil. In fathead minnows, water-borne exposures to gemfibrozil for 21 d had no effects on egg production or hatching success at concentrations ranging from 1.65–1558 μg/L (Skolness et al. 2012). In contrast, egg production by zebrafish was significantly reduced in fish exposed to 0.5 and 10 μg/L of gemfibrozil for 6 weeks (Galus et al. 2013b). Furthermore, Galus et al. (2014) demonstrated that parental exposure of zebrafish to 10 μg/L of gemfibrozil reduced breeding success and fecundity, altered courtship behaviors, and affected sperm morphology and velocity in their offspring. These studies indicate the potential for fibrates to interfere with fish reproduction; however, additional research is needed to assess species differences in toxicity among the fibrate class.

Others

Carbamazepine, a commonly prescribed anticonvulsant and mood stabilizer, exerts its therapeutic action by binding to sodium channels, subsequently reducing action potential transduction (Brodie 2010). Due to its high use, surface water concentrations of carbamazepine range from 0–1.1 μg/L (Ternes 1998). In zebrafish, exposure to carbamazepine at 0.5 and 10 μg/L for 6 weeks resulted in decreased egg production (Galus et al. 2013b). Furthermore, Galus et al. (2014) demonstrated that parental exposure to 10 μg/L of carbamazepine reduced breeding success and fecundity, altered courtship behaviors, and affected sperm morphology and velocity in their offspring. These studies demonstrate the potential for carbamazepine to interfere with reproductive processes of zebrafish; however, more detailed studies are needed to assess reproductive impairments in other species, as well as at reduced concentrations.

Acetaminophen, a common over-the-counter analgesic and antipyretic, is found in surface waters at concentrations ranging from <0.005–10 μg/L (Kolpin et al. 2002, Osorio et al. 2012, Tran et al. 2014). Acetaminophen has a mechanism of action very similar to that of NSAIDs, by inhibiting COX enzymes (Hinz et al. 2008); therefore, there is a potential for acetaminophen to interfere with reproduction. Galus et al. (2013b) demonstrated a reduction in egg production by zebrafish following a 21-d exposure to 10 μg/L of acetaminophen (nominal concentration). Also, acetaminophen exposure resulted in decreased hatchability of Japanese medaka eggs exposed to 9.5 μg/L of acetaminophen (Kim et al. 2012). Although these limited studies suggest that acetaminophen could disrupt reproduction in fish, further research is warranted to fully understand the impacts of this compound in teleost species.

Antibiotics are a commonly used and important group of pharmaceuticals used to treat bacterial infections. Due to their high use, many classes of antibiotics are detected in surface waters worldwide, at concentrations ranging from ng/L to low μg/L (Calamari et al. 2003, Kolpin et al. 2002, Lissemore et al. 2006). However, lincomycin, a lincosamide antibiotic, is the only antibiotic that has been assessed for reproductive impacts on fish (Kim et al. 2012). While lincomycin is detected in surface waters at concentrations ranging from 0.012–0.73 μg/L (Calamari et al. 2003, Kolpin et al. 2002, Lissemore et al. 2006), hatching success of Japanese medaka offspring was not affected by parental exposure to 0.42–4200 μg/L of lincomycin (Kim et al. 2012). Likewise, there are infrequent studies in the literature reporting toxic effects of antibiotics in fish. However, it appears unlikely that antibiotics pose a significant risk to fish reproduction, despite their ubiquitous occurrence in the environment.

Dopamine antagonists are commonly prescribed as antipsychotics to treat a myriad of diseases including schizophrenia, bipolar disorder, nausea, and vomiting (Kapur and Mamo 2003, Smith et al. 2012). Many side effects are associated with dopamine antagonists, including sexual dysfunction in human subjects (Park et al. 2012). This side effect is related to increased prolactin levels associated with dopamine blockade in the tuberoinfundibular pathway (Holt and Peveler 2011). Although prolactin's role in fish reproduction is not fully understood (reviewed in Whittington and Wilson 2013), there is potential for dopamine antagonists to interfere with fish reproduction. However, haloperidol, an antipsychotic, had no effect on reproduction of fathead minnows exposed to water-borne concentrations ranging from 0.017–22 μg/L (Villeneuve et al. 2010). Dopamine antagonists in surface waters appear unlikely to affect fish reproduction; however, additional research is warranted to fully understand the impacts of these compounds on fecundity as it relates to alterations in prolactin levels in fish.

Metformin is a widely prescribed antidiabetic drug that is also indicated in the treatment of various cancers as well as polycystic ovary syndrome, an endocrine disorder in reproductive women (Ben Sahra et al. 2010, Tang et al. 2012). Metformin acts on metabolic pathways to promote catabolism and glucose uptake, thus affecting pathways regulated by insulin signaling, such as the steroidogenic pathway (Viollet et al. 2012). Therefore, metformin may act as an endocrine disruptor in fish. Recently, metformin has been found in surface waters at concentrations ranging from 0.06–3 μg/L (Blair et al. 2013, Ghoshdastidar et al. 2015, Oosterhuis et al. 2013, Scheurer et al. 2012). However, fathead minnow fecundity was not affected following a 28-d exposure to 41 μg/L of metformin (Niemuth et al. 2015). While this study does not provide compelling evidence of the potential negative impacts of metformin, additional research is warranted to fully understand the effects of metformin on wild populations at environmentally relevant concentrations.

Personal care products

Disinfectants

The use of disinfectants in personal care products has increased over the last several years. Triclosan (TCS) is the most common disinfectant used and is found in antimicrobial soaps, toothpastes, mouthwashes, deodorants, and household items such as garden hoses, toys, cutting boards, and furniture (Chalew and Halden 2009). Given its wide use, TCS enters the environment through WWTP effluents, with worldwide surface water values ranging from 0.2–1023 ng/L (reviewed in Bedoux et al. 2012). Ishibashi et al. (2004) observed no reproductive effects in Japanese medaka exposed for 21 d to 20–200 μg/L of TCS. Many other disinfectants, such as

triclocarban, biphenylol, chlorophene, bromophene, etc., are also often found in personal care products (Daughton and Ternes 1999). However, these disinfectants are lacking in ecotoxicological data, especially concerning the reproductive impairments in fish.

Fragrances

Synthetic musks are used in a broad spectrum of consumer products as fragrances or fixatives. This class of chemicals include synthetic nitro musks (e.g., musk ketone and musk xylene) and polycyclic musks (e.g., Galaxolide and Tonalide). Both nitro and polycyclic musks are deemed to be ubiquitous and persistent in nature, with concentrations ranging from 0.62–390 ng/L and 0.09–12,470 ng/L in surface waters, respectively (reviewed in Rimkus 1999). Much of the research conducted to date has focused on the bioconcentration and bioaccumulation potential of these compounds due to their high lipophilicity. Carlsson et al. (2000) conducted the only reported study assessing the impact of musks on fish reproduction. Following a food-borne exposure of adult female zebrafish to 10 mg/kg of musk ketone for 8 weeks, egg production was found significantly reduced, as was offspring survival time (Carlsson et al. 2000). Synthetic musks warrant more research to understand the impacts these compounds have on fish reproduction, especially due to their high bioconcentration and bioaccumulation potential.

Sunscreen agents (UV-filters)

Chemical UV-filters are found in a number of personal care products to protect consumers from UV radiation (Fent et al. 2010, Kaiser et al. 2012). These compounds enter surface waters either directly by washing-off from the skin during recreational activities, or indirectly, through wastewater effluents (Fent et al. 2010). Sunscreen agents have been detected in surface waters at concentrations as high as 4 μg/L (Rodil et al. 2009).

Water-borne exposure of fathead minnows to 3-benzylidene camphor for 21 days reduced fecundity at 74 and 285 μg/L (Kunz et al. 2006). Similar effects were observed in fathead minnows exposed to $\geq$ 1.2 mg/L of benzophenone-2 for 15 days (Weisbrod et al. 2007). In Japanese medaka, exposure to 620 μg/L of benzophenone-3 decreased egg production at week 1, but egg production returned to control values by week 3 (Coronado et al. 2008). Decreased hatching success was also noted with exposure to benzophenone-3 (Coronado et al. 2008). However, decreased fecundity was observed in medaka exposed to 26 μg/L of benzophenone-3 for 28 days (Kim et al. 2014). Sunscreen agents have the ability to impair reproduction in fish, and warrant further research to assess their full environmental risk.

Others

As the number of compounds in the class of personal care products is high, aside from the limited number of chemicals described above, very little research has been conducted to assess the impacts of these compounds on fish reproduction. Diethylenetriamine penta acetic acid (DTPA) is commonly used in cosmetic products for its ability to chelate calcium and magnesium ions (Benes and Burnett 2008). No reproductive impairments were observed in Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) when exposed to 1–100 mg/L of DTPA (van Dam et al. 1999). With the limited published data, additional research is warranted to investigate the impacts of personal care products on fish reproduction.

Mixtures

While most studies on the effects of PPCPs have focused on single compounds, PPCPs are present in the environment in complex mixtures. Chemical mixtures can be tested directly either as an environmental sample or as a mixture prepared in the laboratory. The results of whole-mixture tests have limitations as they are only applicable to an environment under the same concentration range and component ratio (Backhaus et al. 2008). Whole-mixture results are challenging to apply to environmental conditions, as the number and concentration of pollutants vary greatly both spatially and temporally.

The mixture toxicity of pharmaceuticals can produce a variety of interactions such as synergistic (less-than-additive, additive, or potentiated), or antagonistic effects (Barata et al. 2006). The toxicity of pharmaceutical mixtures can be at concentrations where a single substance shows no or very little effects, which is important for environmental risk assessment (Cleuvers 2004). EE_2 acts additively with E_2 at environmentally relevant concentrations of 0.6 ng/L of EE_2 and 14.4 ng/L of E_2 in juvenile rainbow trout (Thorpe et al. 2003). However, in WWTP effluents, there are many different types of endocrine-disrupting chemicals. It could be hypothesized that the presence of antiestrogenic compounds may cancel out the effects of the estrogenic compounds. However, this hypothesis is not supported since a co-exposure of medaka to E_2 and antiestrogens reduced some of the effects of E_2 alone (such as VTG induction), but did not prevent reproductive impairment, and in some cases, made it more severe (Sun et al. 2009).

Pharmaceutical mixtures often involve compounds with unrelated modes of action, and there are relatively few studies that look at such mixtures. One such study was a life-cycle exposure of fathead minnows to a mixture of water-borne PPCPs at exposure concentrations ranging from 10–1000 ng/L (nominal), of six common PPCPs including naproxen, gemfibrozil, diclofenac, ibuprofen, salicylic acid, acetaminophen, and triclosan (Parrott and Bennie 2009). This study is unique in that the chemicals tested have very different modes of action and are ubiquitous in surface water. Impairments of reproduction were not observed at the concentrations tested; however, an increase in larval deformities was detected in the F_1 generation (Parrott and Bennie 2009). A shorter 6-week exposure of zebrafish to a mixture of 0.5 and 10 μg/L of acetaminophen, carbamazepine, gemfibrozil and venlafaxine significantly reduced embryo production and increased the incidence of developmental abnormalities (Galus et al. 2013a).

Mixture studies are of particular importance since they are relevant to environmental exposure conditions. However, chronic mixture studies on fish are relatively few, and multigenerational mixture studies do not currently exist. Understanding reproductive effects of mixtures of pharmaceuticals is needed, as fish are likely exposed to multiple compounds over extended periods of time with, the potential to disrupt their endocrine system at very low levels.

Wastewater treatment plants

Emerging contaminants are released into the environment in a number of ways (Figure 1). As with traditional pollutants, many of these chemicals are released as by-products of industrial activity such as agriculture and manufacturing. Unlike conventional pollutants, many emerging contaminants—in particular PPCPs—are released into the environment as a direct result of household and consumer use, mainly through WWTP-treated effluents and sludge (Nikolaou et al. 2007). A recent life-cycle analysis suggested that emerging contaminants (primarily PPCPs) contributed more to the toxicity of wastewater than traditional pollutants; however, limitations such as PPCP data quality and availability, exclusion of relevant substances such as important metabolites, and the inability of the analysis to take into account mixture effects, could have affected these results (Munoz et al. 2008).

Presently, many unique emerging contaminants have been detected in environmental samples (Kolpin et al. 2002, Vasskog et al. 2006, Weigel et al. 2004). The most frequently detected compounds are non-prescription and prescription pharmaceuticals, estrogen-based hormones, and human and veterinary antibiotics (Kolpin et al. 2002, Miege et al. 2009, Vasskog et al. 2006, Weigel et al. 2004). Such contaminants are nearly ubiquitous in WWTP effluents, where concentrations can range from low ng/L to hundreds of μg/L (Kolpin et al. 2002, Miege et al. 2009). PPCPs are also frequently detected in surface waters, particularly waters that receive WWTP effluents, indicating that such compounds are not completely removed by WWTP processes, which were not designed to treat PPCPs.

The reproductive effects of WWTP effluents, specifically their effects on fecundity have been studied in the laboratory. Thorpe et al. (2009) assessed three WWTP effluents [estradiol-equivalent concentrations (E_2EQ) of 21.2 ± 2.9, 2.0 ± 0.4, and 18.0 ± 4.0 ng/L for effluents I, II, and III, respectively] for their effects on fecundity in fathead minnows. A decrease in egg production occurred with 50 and 100% for effluent I and 100% for effluent III, while no changes were noted for effluent II (Thorpe et al. 2009). Similarly, a decrease in fecundity with exposure to 50% and 100% effluent was observed in fathead minnows following a 21-d exposure (Filby et al. 2010). Japanese medaka exposed for 28 d to varying treated effluent concentrations also demonstrated decreased fecundity with exposure to 40% and 50% effluent, subsequently leading to a complete collapse of the population exposed to 50% effluent, because female medaka stopped spawning (Ma et al. 2005). Studies performed on zebrafish exposed to WWTP effluents showed a decrease in fecundity as well (Galus et al. 2013a, Smolders et al. 2002). One study with zebrafish found a two-fold decrease in fecundity of fish exposed to 50% effluent; however, due to poor water quality (increased ammonia and nitrate) of the effluent, it could not be concluded whether it was the contaminants present in the effluent or the poor water quality that caused the decreased fecundity (Lister et al. 2009).

A few studies observed increased fecundity after exposure to WWTP effluents (Höger et al. 2006, Robinson et al. 2003). Höger et al. (2006) showed that rainbow trout exposed for 32 weeks to 15% effluent had an increase in fecundity, measured as a higher number of eggs per kilogram of body weight. Increased fecundity also occurred in the sand goby (*Pomatoschistus minutus*) following 7 months of exposure to 0.03% and 0.3% WWTP effluent (Robinson et al. 2003).

In contrast to these studies, long-term exposure to 0, 50, and 100% WWTP effluent of parental and offspring fathead minnows caused no effects on egg production after 145 days of exposure of the parent generation (Sowers et al. 2009). However, a positive relationship between the onset of reproductive activity and exposure of parents to wastewater was observed in the F1 generation (Sowers et al. 2009).

Field studies have demonstrated a wide variety of WWTP effects on wild fish with respect to fecundity and intersex. Relative fecundity (number of eggs per gram of gonad weight) was significantly reduced in wild female roach (*Rutilus rutilus*) collected from WWTP effluent-dominated streams (Jobling et al. 2002). Gamete quality was negatively correlated with the degree of feminization in intersex roach (Jobling et al. 2002). A 50% reduction in sperm motility and 75% reduction in fertilization were also observed in feminized fish (Jobling et al. 2002). Blazer et al. (2012) collected smallmouth bass (*Micropterus dolomieu*) from 3 tributaries of the Potomac River (USA) over 2 years in order to monitor the effects of temporal and spatial exposure to WWTP effluents. Bass from the south branch of the Potomac (which demonstrated a moderate to high prevalence of testicular oocytes) had fewer sperm per testes mass and lower sperm motility, when compared to bass from the Gauley River (which had a low rate of testicular oocytes) (Blazer et al. 2012). Based on these findings, an inverse relationship between sperm motility and testicular oocyte presence was described (Blazer et al. 2012). Male fathead minnows collected downstream of WWTP discharge during spawning demonstrated delayed spawning activity (Tetreault et al. 2012). Adult female lambari (*Astyanax faciatus*) sampled immediately downstream from WWTP during the reproductive season displayed reduced fecundity (Prado et al. 2014).

Recent research has focused on the efficiencies of removal of PPCPs from wastewater by different treatment processes (Roccaro et al. 2013, Verlicchi et al. 2012). While this research is ongoing, a few studies have assessed how different treatment processes have affected fish fecundity. Different treatment methods, including the use of granular activated carbon (GAC), ozone (O_3), and chlorine dioxide (ClO_2) were investigated for the removal of steroidal estrogen in WWTP effluents (Filby et al. 2010). The various treatment methods studied resulted in a 70–100% removal efficiency of E_2EQ. GAC-treated effluent (70% removal efficiency) resulted in no improvement in egg production when compared to untreated effluent, while fecundity was not assessed with the other treatment methods (Filby et al. 2010). A 21-d exposure of zebrafish to effluent from different treatment processes resulted in decreased fecundity in zebrafish exposed to effluent treated by sand filter/biofilter/after sedimentation treatments (Akande et al. 2010). Decreased spawning activity was also observed in sand filter/after sedimentation and sand filter/ozonation/post-sedimentation treatments (Akande et al. 2010). The authors also noted that these treatment processes resulted in increased concentrations of endocrine-disrupting chemicals (Akande et al. 2010).

The reproductive effects of WWTP effluents vary between studies and species. Laboratory studies suggest that there is a

negative impact of these effluents on fish reproduction; however, field studies remain inconclusive.

Interpretation of PPCP reproduction studies

Environmental risk assessments (ERAs) benefit greatly from the growing body of literature assessing the fecundity effects of PPCPs. However, ERAs are based on studies using model fish species (fathead minnow, Japanese medaka, and zebrafish). As fish comprise about half of all vertebrate species, the use of three small fish models likely does not encompass the large diversity in physiology, reproductive biology, and population ecology of fish species. With over 5,000 human and veterinary pharmaceuticals in use or development targeting specific physiological functions (Knox et al. 2011), many of these drug targets are relatively conserved across animal phyla (Brown et al. 2014, Gunnarsson et al. 2008, Huggett et al. 2003a, LaLone et al. 2013a, McRobb et al. 2014). Brown et al. (2014) demonstrated that 65–86% of 459 human drug targets are conserved across 12 diverse fish species. In addition, the majority of the fish species have at least one drug target to $>90\%$ of pharmaceuticals analyzed (Brown et al. 2014). When assessing drug targets associated with pharmaceuticals with therapeutic classification codes suggesting direct effects on reproduction, ligand-binding domains of nuclear steroid receptors had higher sequence similarities compared to the full-protein sequence (Brown et al. 2014). These data suggest that fish should be impacted by individual pharmaceuticals in a similar manner across species. However, based on the literature reviewed in this paper, differences in species sensitivity exist among the model fish species with similar durations of exposure (Table 2). This very limited data suggests that zebrafish may be more sensitive to pharmaceutical exposure compared to fathead minnows and Japanese medaka, while the latter species vary in sensitivity to pharmaceutical compounds.

One method to assess species sensitivities and to extrapolate to community-level effects utilizes species sensitivity distributions (SSDs). Reviewed in detail in Posthuma et al. (2002), SSDs are "a statistical distribution describing the variation among a set of species in toxicity of a certain compound." The use of SSDs in ERA has increased over the past couple of decades (Posthuma et al. 2002). With very limited data concerning reproductive effects of individual pharmaceuticals, SSDs are not produced frequently. However, Figure 3 demonstrates a SSD for EE_2 using both mature adult and life-cycle studies, by fitting a log–probit distribution to NOEC values derived from studies in Table 1 using USEPA's CADDIS SSD Generator (www.epa.gov/caddis/downloads/SSD_Generator_V1.xlt). With the limited data for fecundity of EE_2, the calculated HC_5—hazardous concentration for 5% of species or 95% protection limit—was determined to be 0.35 ng/L.

Similarly, Caldwell et al. (2008) calculated a similar HC_5 for EE_2 using 39 papers utilizing varying endpoints in 26 species. As the HC_5 values can be used in deriving a predicted no-effect concentration (PNEC), one must be careful in the extrapolation of said results to wild populations as these data are derived from laboratory studies. Laboratory conditions

Table 2. Species sensitivities in effects on fecundity of select pharmaceuticals*.

Pharmaceutical	Fathead minnow	Japanese medaka	Zebrafish	Most sensitive FHM	JM	ZEB
17α-ethinylestradiol	100	488	14†			✓
Fluoxetine	100‡		32¶			✓
Flutamide	651	1560		✓		
Methyltestosterone	5	0.0468			✓	
Norethindrone	0.0012	0.022		✓		
Propranolol	970	0.5			✓	
Spironolactone	2.6	47.5		✓		
Total				3	2	2
Percentage of studies most sensitive				42.9	33.3	100

*All values are lowest observed effect concentrations (LOECs) reported in µg/L; data and references can be found in Table 1.
†14-day study.
‡28-day study.
¶7-day study.

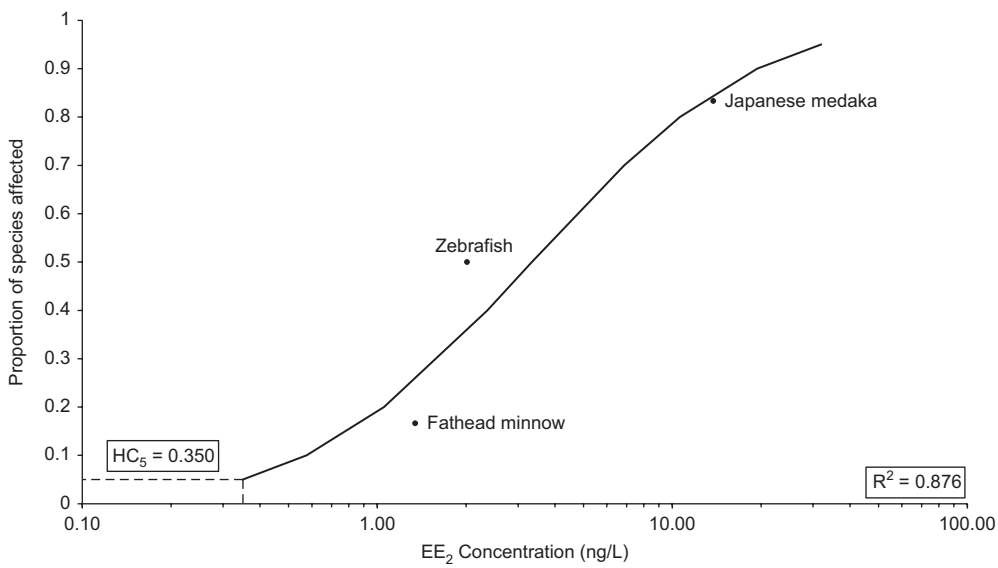

Figure 3. Species sensitivity distribution for 17α-ethinylestradiol based on mature adult and life-cycle reproductive no observed effect concentrations (NOECs). The value for HC_5 (hazardous concentration for 5% of species or 95% protection limit) was calculated to be 0.350 ng/L.

are often very different from natural environments, as many confounding factors, both abiotic (i.e., temperature, light, pH, hardness) and biotic (i.e., food availability, life-stage, fish species), influence pharmaceutical bioavailability and fish reproduction. As SSDs may provide greater insight into species sensitivity, further research is warranted to accurately produce these distributions to aid in ERA.

A challenge for risk assessment is in trying to use the effects of short-term studies (days, weeks, months) to predict long-term environmental exposures. Generally, it is more cost effective to run shorter-term studies, but these results may underestimate the exposure concentrations equivalent to those found in the environment of the compound in question (Table 3). Both the OECD and USEPA have provided guidelines for conducting a Fish Short-Term Reproductive Assay (FSTRA) in which reproductively mature fish are exposed for 21 days to a test chemical (OECD 2012b, USEPA 2009c). The FSTRA is particularly useful in providing information on adverse effects on fecundity that could be used for ERA. However, these assays have the following inherent concerns.

The FSTRAs were designed as screening tools for detecting *in vivo* endocrine-disrupting activity in fish. Fecundity serves as an apical endpoint, with additional endpoints such as secondary sexual characteristics, plasma VTG and sex steroid hormone concentrations, and gonad size and morphology as indicators of HPG axis disruption. The OECD Guidance Document 150 (2012a) warns that if only the fecundity endpoint responds, the chemical of interest is a reproductive or general systemic toxicant with a low probability of being an endocrine disruptor. However, USEPA OPPTS 890.1350 (2009c) states that reductions in fecundity are considered positive endocrine-disrupting effects as this may be indicative of disruption of the HPG axis in fish, although fecundity may also be impacted through non-endocrine stresses. Of all studies cited in this review, only 27 papers demonstrated an adverse impact on fecundity within the framework of the FSTRA. Of those 27 studies, only one (Kang et al. 2006) demonstrated reduced fecundity to flutamide, although no other additional endpoints were impacted. Therefore, flutamide may act as a systemic toxicant in Japanese medaka in contrast to fathead minnows, where flutamide not only reduced fecundity but also affected gonad histology, sex steroid concentrations, and VTG responses.

The fecundity endpoint of the FSTRA is relatively variable. The coefficients of variation for reproduction studies conducted under the FSTRA framework range from 8.9%–52% (Coady et al. 2014). The increased coefficient of variation impacts the ability to detect significant decreases in egg production smaller than 70% as the coefficient of variation approaches 50% or higher, leading to an increased probability of committing a type II error (Bosker et al. 2009, OECD 2012b). Two approaches are recommended to decrease the coefficient of variation among egg production data. First, both regulatory tests recommend pre-exposure periods to confirm that breeding harems are reproductively active and that egg production is similar among all harems to be used during chemical exposure. The USEPA guideline suggests that spawning occurs at least two times in the preceding 7 days and that a minimum of 15 eggs/female/day/tank need to be produced for that replicate to be included. Contrarily, the OECD guideline does not provide specific guidance on egg production, but states that fish should be spawning and that it is common to observe at least 10 eggs/female/day. Bosker et al. (2009) provides a statistical method for determining which pre-exposure tanks should be included for chemical exposure. Applying this method reduced the coefficient of variation from 40% in 54 tanks to 19% in 39 tanks, however, biological causes of egg production were not taken into consideration (Bosker et al. 2009). When selecting breeding harems based on biological criteria, the coefficient of variation was reduced from 40% in 54 tanks to 29% in 37 tanks (Bosker et al. 2009). Both biological criteria and statistical methods should be utilized in selecting pre-exposure tanks for inclusion into the chemical exposure portion of the FSTRA.

A second approach to reduce the coefficient of variation associated with fecundity data is to increase the sample size of the FSTRA. While both the OECD and USEPA guidelines suggest using 4 replicates for fathead minnow, only the OECD guideline recommends using additional species with a different number of replicates—4 replicates for Japanese medaka and 2 replicates for zebrafish. Using mummichog (*Fundulus*

Table 3. Impacts on fecundity as a function of testing duration*.

	Fathead minnow		Japanese medaka		Zebrafish	
Pharmaceutical	Duration†	LOEC‡	Duration†	LOEC‡	Duration†	LOEC‡
17α-ethinylestradiol	21	100	14	500	17	>10.6
	140	3.5	21	488	75	>9.3
	280	3.2	168	10	118	3
17β-estradiol			14	2528.3		
			21	187–463		
			100	27.9		
Ibuprofen					7	>50600
					21	1000
Methyltestosterone	14	200000				
	21	5000				
Propranolol			14	>500000		
			28	500		

*Referenced from Table 1.
†Duration = days.
‡ Lowest observed effect concentration (ng/L).

heteroclitus), Bosker et al. (2009) demonstrated that both the range around the mean and the coefficient of variation stabilized with a minimum of 8 replicate tanks. By using both approaches, the coefficient of variation could be significantly reduced, leading to an increased confidence in interpretation of results with a reduced chance in committing a type II error.

Lastly, with the short duration (i.e., 21 days) of FSTRA, the effects on fecundity may not be as predictive as for long-term exposures to environmental conditions (Table 3, OECD 2012a). Longer studies have the advantage of providing more environmental relevance due to the pseudo-persistent nature of many pharmaceutical compounds in the receiving environment.

As the FSTRAs provide a more cost-effective means to determine the reproductive effects of PPCPs, the inherent concerns mentioned above should be taken into consideration when designing such experiments and when interpreting their results with regard to assessing PPCP environmental risk.

As mentioned previously, short-duration studies may not be as useful in assessing environmental risk of PPCPs as long term studies such as the Fish Life Cycle Toxicity Test (FLCTT) (USEPA 1996) or even multigenerational exposures. Data provided in this review (Table 3) demonstrate that fish are impacted at much lower concentrations over longer periods of exposure. The differences in LOECs due to time seem to be quite substantial in shorter-term studies (weeks) and not as large in longer-term studies (months). For example, fathead minnows exposed to methyltestosterone for 14 days had a LOEC of 200,000 ng/L, but those exposed for 21 days reported a LOEC of 5,000 ng/L, a 40-fold difference (Ankley et al. 2001, Pawlowski et al. 2004a). Whereas the difference in LOECs between zebrafish exposed to EE_2 for 91 and 118 days was only 3-fold, being 10 and 3 ng/L respectively (Fenske et al. 2005, Xu et al. 2008).

The testing duration of FLCTTs during chemical exposure occurs from one life stage to at least the same stage of the next generation (e.g., egg to egg) (USEPA 1996), which is roughly 3–6 months for common small model fish. To date, FLCTTs have not been standardized and validated, considering their cost, length, and complexity. As with the FSTRA, the fecundity endpoints associated with FLCTTs are highly variable, with relatively low statistical power (Crane et al. 2010). As a result, a multi-criteria decision analysis determined F_1 fertilization rate as a preferred endpoint for FLCTTs, as well as additional endpoints such as hatching success of F_1 eggs (Crane et al. 2010). The least preferred endpoints of FLCTTs were determined to be gonadal histopathology and measurement of biomarkers (e.g., VTG) (Crane et al. 2010). Taken into consideration, fecundity (or number of fertile eggs), fertilization rate, and hatching success endpoints should be considered together, as all may predict population-level effects of PPCPs.

In the wild, fish are likely exposed to low concentrations of PPCPs for multiple generations due to continual input into aquatic ecosystems over time. While reproduction is usually only assessed for one generation in FLCTTs, multigenerational studies may be more useful in extrapolating laboratory effects to environmental risks. However, due to their complexity, cost, and length of exposure, only a few multigenerational studies have been conducted using pharmaceuticals (Table 4). These studies demonstrate the danger of using short-term no-effect concentrations for risk assessment. While in the majority of the studies, fecundity of the parental generation was impacted at concentrations much higher than those found in the environment (except Schäfers et al. 2007), egg production of subsequent generations was affected at much lower concentrations. These effects across generations could be due to maternal transfer of bioaccumulative substances or endocrine-mediated transgenerational effects (OECD 2008). Thus, there is a need for multi-generational studies of PPCPs in fish to better understand the potential long-term reproductive effects of PPCP exposure.

Such long-term laboratory reproductive studies do not exactly simulate natural reproduction under field conditions, therefore making interpretation of the results difficult to directly apply in determining environmental risk (Crane et al. 2010). One issue is the limited genetic variation of laboratory fish as compared to their wild counterparts (Coe et al. 2009). Consequently, laboratory fish may fail to account for the full spectrum of genetic variation and response in wild populations, resulting in either over- or under-estimates of population-level effects (Brown et al. 2009). Testing chambers (i.e., aquaria or tanks) present another issue in extrapolating laboratory studies to field conditions. Wild fish are not confined to such limited space, which may result in abnormal breeding behavior. In addition, the captivity of laboratory fish results in an altered degree of stress when confined to testing chambers

Table 4. Generational effects of select pharmaceuticals on fish fecundity.

Pharmaceutical	Species	Generation	LOEC (ng/L)	References
17β-estradiol	Sheepshead minnow	F_0	290	Cripe et al. (2009)
		F_1	82	
		F_2	82	
Estrone	Japanese medaka	F_0	>91.4	Nakamura et al. (2015)
		F_1	91.4	
17α-ethinylestradiol	Zebrafish	F_1	1.1	Schäfers et al. (2007)
		F_2	2	
	Zebrafish	F_0	50	Nash et al. (2004)
		F_1	5	
17β-trenbolone	Sheepshead minnow	F_0	870	Cripe et al. (2010)
		F_1	130	
		F_2	27	
Bicalutamide	Fathead minnow	F_0	>92100	Panter et al. (2012)
		F_1	92100	

(Crane et al. 2010). While additional stressors are controlled for in the laboratory, wild fish are exposed to many changing environmental variables (e.g., temperature changes, predation, parasitism, and competition) in addition to toxicant exposures (Brown et al. 2003). Furthermore, while many laboratory studies suggest that PPCPs can result in population-level impacts (i.e., reduced fecundity), the question of whether wild fish populations have actually been impacted by PPCPs remains unresolved (Mills and Chichester 2005).

Future considerations

PPCP prioritization

With the growing number of PPCPs being manufactured and detected in the environment, testing of each chemical for impacts on reproduction, as is required for several regulatory needs, is not practical at the present time (NRC 2007, KNAPPE 2008). Therefore, prioritizing PPCPs for further testing has been identified as a major priority amongst the scientific community (Boxall et al. 2012). Many approaches have been identified and implemented using therapeutic class, mechanism of action, and/or chemical properties as criteria for selection (Berninger and Brooks 2010, Boxall et al. 2003, Capleton et al. 2006, Kools et al. 2008, Kostich et al. 2010, Kostich and Lazorchak 2008, LaLone et al. 2013a, Roos et al. 2012). One approach that may be useful in prioritizing which pharmaceuticals require reproductive testing is the fish-plasma model (FPM) introduced by Huggett et al. (2003a). Assuming that pharmacological targets are conserved across vertebrates, the FPM compares human therapeutic plasma concentrations with either measured or predicted fish plasma concentrations to determine an effect ratio that can then be used to predict the potential impact of that pharmaceutical to fish. Chemical properties of pharmaceuticals (i.e., binding affinities to receptors) may also be useful in prioritizing pharmaceuticals, as demonstrated with progestins. As human PR-binding affinities of progestins increase, fish reproduction is impacted at lower concentrations (Figure 2). This approach could enhance the EPA's Endocrine Disruption Screening Program, as both estrogen and androgen receptor-binding assays are included as Tier 1 testing strategies (USEPA 2009a, b).

As the FPM can only be used for pharmaceuticals, other approaches such as the adverse outcome pathway (AOP) approach (Ankley et al. 2010) may also be useful in the prioritization of personal care products. The AOP approach utilizes molecular initiation events (e.g., gene expression followed by protein synthesis) to predict an adverse outcome at population levels. This approach has received great attention, with the OECD recently launching an AOP Development Program (OECD 2012c). As the number of PPCPs requiring reproductive analysis continues to increase, the use of such prioritization models will greatly enhance the identification of PPCPs that pose the greatest risks to fish reproduction.

Other research considerations

Ecotoxicological knowledge of PPCPs has greatly increased over the past decade. Most of the research has been focused on the effects of individual compounds. While knowing how individual compounds affect non-target organisms is critical to performing effective risk assessment and management measures, it is not sufficient by itself, as fish are usually exposed for multiple generations to complex mixtures composed of low levels of many potential endocrine-disrupting compounds. The synergistic effects of simple pharmaceutical mixtures have been demonstrated (Cleuvers 2003), but there are very few studies that focus on the effects of complex PPCP mixtures on reproduction. Simple concentration addition studies will greatly enhance risk assessment and allow the predictions to be more relevant to the environmental conditions. Understanding reproductive effects of complex mixtures of PPCPs is important; therefore, future studies need to consider the dynamic interactions between compounds found in the environment and their numerous modifying factors.

In addition to whole-organism results (i.e., reduced fecundity), researchers should begin to focus on the ability of fish to adapt to continuous exposure to PPCPs. For instance, much work has been conducted to understand how mummichog and Atlantic tomcod (*Microgadus tomcod*) have adapted to become more tolerant to polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dioxin-like compounds in sites contaminated with these chemicals in estuaries of the northeast US coast (Clark et al. 2013, Courtenay et al. 1999, Meyer et al. 2002, Yuan et al. 2006). It is thought that adaptations within the aryl hydrocarbon receptor (AHR) pathway have contributed to the tolerance to chemicals of this nature. Clark and Di Giulio (2012) state that the "ability of adaptation to chronic anthropogenic contamination to drive changes in response to xenobiotics with different modes of action demonstrates the ability of complex mixtures of contaminants to alter population response in a relatively broad manner." Therefore, we must consider that fish in PPCP effluent-dominated waters would likely be able to adapt to chronic exposure to these chemicals. With "omics" technologies becoming more robust and affordable, the ability of researchers to conduct such analyses will greatly enhance our knowledge of the reproductive adaptive changes of fish to chronic PPCP exposure.

Declaration of interest

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