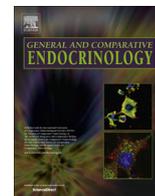




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Effects of the environmental estrogenic contaminants bisphenol A and 17 α -ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species

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ABSTRACT

Endocrine disrupting chemicals (EDCs), including the mass-produced component of plastics, bisphenol A (BPA) are widely prevalent in aquatic and terrestrial habitats. Many aquatic species, such as fish, amphibians, aquatic reptiles and mammals, are exposed daily to high concentrations of BPA and ethinyl estradiol (EE2), estrogen in birth control pills. In this review, we will predominantly focus on BPA and EE2, well-described estrogenic EDCs. First, the evidence that BPA and EE2 are detectable in almost all bodies of water will be discussed. We will consider how BPA affects sexual and neural development in these species, as these effects have been the best characterized across taxa. For instance, such chemicals have been in many cases reported to cause sex-reversal of males to females. Even if these chemicals do not overtly alter the gonadal sex, there are indications that several EDCs might demasculinize male-specific behaviors that are essential for attracting a mate. In so doing, these chemicals may reduce the likelihood that these males reproduce. If exposed males do reproduce, the concern is that they will then be passing on compromised genetic fitness to their offspring and transmitting potential transgenerational effects through their sperm epigenome. We will thus consider how diverse epigenetic changes might be a unifying mechanism of how BPA and EE2 disrupt several processes across species. Such changes might also serve as universal species diagnostic biomarkers of BPA and other EDCs exposure. Lastly, the evidence that estrogenic EDCs-induced effects in aquatic species might translate to humans will be considered.

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Abbreviations: AGD, anogenital distance; AR, androgen receptor; AOPs, adverse outcome pathways; BMPO, benzo(a)pyrene monooxygenase; BPA, bisphenol A; CYP1A1, cytochrome P450 family 1 subfamily A polypeptide 1; *Cyp19a1a*, aromatase; DES, diethylstilbestrol; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *Dnmts*, DNA methyltransferases; EDC(s), endocrine disrupting compound(s); EPA, Environmental Protection Agency; EE2, ethinyl estradiol; ER(s), estrogen receptors; ESR1, estrogen receptor 1 (alpha); ESR2, estrogen receptor 2 (beta); *Fshb*, follicle stimulating hormone beta; FW, feed weight; GnRH, gonadotropin-releasing hormone; GPER, G protein-coupled estrogen receptor 1; GSD, genetic sex determination; GSI, gonadosomatic index; IAP, intracisternal A particle; *Kiss1*, kisspeptin 1; LOD, limits of detection; *Lhb*, luteinizing hormone beta; MeCP2, methyl-CpG binding protein 2; NOAEL, no observable adverse effect level; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzodioxins; PCDFs, polychlorinated dibenzofurans; PGC, primordial germ cells; ppm, part per million; T₃, 3,5,3'-triiodo-L-thyroxine; TSP, temperature sensitive period; TSD, temperature sex determination; VTG, vitellogenin (protein product); *Vtg*, vitellogenin (transcript).

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1. Introduction

“Between animal and human medicine there is no dividing line – nor should there be” (Quote from Physician Dr. Rudolf Virchow 1856, cited by Klauder (1958)). While the idea of “one health, one medicine” was recognized two centuries ago, it has recently regained currency as it is increasingly appreciated that the genomes, gene expression, and physiologies of humans and other animals share many commonalities. Therefore, environmental-induced disruptions discovered in animals are relevant to human populations.

1.1. Endocrine disrupting chemicals

To date, one of most common classes of environmental contaminants are endocrine disrupting chemicals (EDCs). The Endocrine Society defines an EDC as any chemical that can interfere with any aspect of hormone action. EDCs typically bind to hormone receptors and can activate, or repress and/or interfere with hormone synthesis and metabolism. EDCs act via nuclear receptors, nonnuclear steroid hormone receptors (e.g., membrane, non-steroid receptors (e.g., neurotransmitter receptors such as the serotonin receptor, dopamine receptor, norepinephrine receptor), orphan receptors (e.g., aryl hydrocarbon receptor), enzymatic pathways involved in steroid biosynthesis and/or metabolism, and numerous other mechanisms that converge upon endocrine-controlled and reproductive systems (Diamanti-Kandarakis et al., 2009). In addition to binding directly to receptors, EDCs can alter expression of genes required for reproductive and immune functions through epigenetic mechanisms.

Most EDCs are manufactured chemicals (Diamanti-Kandarakis et al., 2009), and, of these, bisphenol A (BPA) is one of the most widely produced (Environment Canada, 2008; Galloway et al., 2010), with production reported to be 15 billion pounds in 2013 (GrandViewResearch, 2014; Vandenberg et al., 2013). The global BPA market is expected to reach USD 20.03 billion by 2020 (GrandViewResearch, 2014; Vandenberg et al., 2013). BPA is used in numerous products and applications, including polycarbonate plastic, the lining of metal food cans, some dental sealants and thermal receipt paper, food and water preparation and storage vessels, household products and many other uses. The pervasiveness of this chemical (Environment Canada, 2008) predicts widespread and continued exposure of animals and humans (Vandenberg et al., 2013). BPA is almost ubiquitously found in people; detectable in the urine of 93% of the US population (Calafat et al., 2008), as well as in fetal plasma, placenta (vom Saal et al., 2007), and in breast milk (Vandenberg et al., 2007).

The National Toxicology Program (2008) determined there is “some concern for effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures to bisphenol A”, although this report prepared in 2007 does not include the most current findings about BPA (Vandenberg et al., 2013). A second NIH-sponsored report published in 2007, the Chapel Hill Consensus Statement, indicated that extensive data in rodents identified the potential for adverse outcomes in humans due to exposure during critical periods of development, and that the changes would likely be irreversible (vom Saal et al., 2007). Ethical considerations, however, make any study of potential vulnerabilities in children to BPA limited to epidemiological approaches that reveal correlations but not causation (Collaer and Hines, 1995; Rochester, 2013; Trasande et al., 2012).

Another environmental estrogen that is prevalent globally is 17 α -ethinyl estradiol (EE2), the active estrogen in birth control pills (Caldwell et al., 2012; Hinteman et al., 2006; Kostich et al.,

2013; Lu et al., 2011; Pojana et al., 2004; Zhou et al., 2012). As discussed below, this chemical is also present in a range of aquatic sources and has been reported to have widespread effects in various aquatic species. Moreover, EE2 is considered the FDA-approved positive control for BPA studies that are to be used to guide policy decisions. In this review, we will consider the effects of BPA and EE2 in various taxa. This review will primarily focus on the known effects of these two chemicals in the aquatic taxa that are at the greatest risk for exposure. Effects observed in these populations will very likely translate to humans.

Past research has provided a comprehensive analysis of BPA and EE2 concentrations in a variety of water sources (Caldwell et al., 2012; Environment Canada, 2008; Flint et al., 2012; Hinteman et al., 2006; Kang et al., 2007; Kostich et al., 2013; Lu et al., 2011; Pojana et al., 2004; Zhou et al., 2012). Recent advancements in measuring estrogenic activity and assaying for select EDCs have permitted even finer-tuned assessments of aquatic contamination. BPA has been identified in both ground and surface waters, while EE2 is primarily found in surface water sources (Crain et al., 2007; Environment Canada, 2008; Flint et al., 2012; Kang et al., 2007). It is now recognized that sites deemed by the Environmental Protection Agency (EPA) as Superfund sites are contaminated with a variety of EDCs, including BPA (Agency, 1974). Fish, amphibian, aquatic reptile and mammalian species in these areas may be considered the “canaries in the mine”, as they may be at the greatest risk (Vandenberg et al., 2013). We will thus first consider the concentrations of these chemicals in the different water sources and potential bio-indicators.

Normal development of the reproductive system and programming of later adult behavioral and cognitive traits are dependent upon the correct concentration and timing of exposure of the organs to steroid hormones, in particular estrogen and testosterone (Arnold and Breedlove, 1985; Forest, 1983; Gilmore, 2002; Morris et al., 2004; Nakamura, 2010; Nugent et al., 2012; Phoenix et al., 1959; Robinson, 2006; Schulz et al., 2009). Sex steroid hormones also play a key role in the timing of the transitions between prematuration stages of development, in the scheduling of reproduction, and in determining onset of senescence. Androgens and estrogens might also affect these processes through initiation of epigenetic changes (Gabor et al., 2009, 2011; Matsuda et al., 2012; Menger et al., 2010). Moreover, these hormones influence sex determination in fish, amphibians, and reptiles (Baroiller and D’Cotta, 2001; Crews et al., 1995; Dumond et al., 2008; Elf, 2003; Jeyasuria and Place, 1998; Nakamura, 2009, 2010; Pieau et al., 1999, 2001; Ramsey and Crews, 2009; Wibbels et al., 1998; Yao and Capel, 2005). For these reasons, sexual development and later adult behaviors in various species are hypothesized to be vulnerable to developmental exposure to EDCs, including BPA and EE2. Skewed sex ratios in the above species may also serve as a barometer for the presence of these chemicals in the local environment (Guillette, 2000).

We will next consider the effects of these EDCs on sexual and brain development in fish, amphibians, and aquatic reptiles and mammals, even though specific effects in aquatic reptile species with temperature sex determination (TSD) may not fully translate to mammals and humans with genetic sex determination (GSD). In male fish, amphibians, and reptiles, BPA and other estrogenic chemicals are known to bind to ERs and induce the production of vitellogenin (VTG) (Crane et al., 2007; Goksoyr, 2006; Marin and Matozzo, 2004; Palmer and Palmer, 1995; Porte et al., 2006; Sumpter and Jobling, 1995). Therefore, this protein, along with several other genes and their protein products listed below are considered circulating biomarkers of exposure in these species, but similar diagnostic biomarkers have not been identified in mammalian species, including humans. Potential candidates for

such biomolecules of estrogenic exposure across taxa may include epigenetic or gene expression changes that occur in response to these EDCs. Consequently, we will subsequently examine the universal epigenetic and molecular alterations that may be induced by BPA and/or EE2 exposure across taxa. We will conclude this review by examining the potential relevance of the findings observed in the various aquatic species to human health.

2. Mechanisms of BPA and EE2 action

A subgroup of EDCs are exogenous chemicals that can bind to and activate estrogen receptors and are termed xenoestrogens. The gene evolutionary tree in Fig. 1 reveals strong conservation

of the two predominant estrogen receptors (and the related piscine forms) across taxa (Filby and Tyler, 2005; Nilsson et al., 2001; Warner et al., 1999). Therefore, the underlying mechanisms of BPA and EE2-induced molecular and epigenetic actions presumably have many similarities across animal species.

EE2 is a potent synthetic estrogen with >10 fold higher potency than estradiol for estrogen receptors ESR1 and ESR2 (ERs) (Thorpe et al., 2003). BPA binds both nuclear ERs with 0.1–0.01% of the affinity of estradiol (Wetherill et al., 2007). In MCF-7 human breast cancer cells, BPA competes more effectively for binding to ESR2 than ESR1, but induces ESR1- and ESR2-mediated gene expression with comparable efficacy (Matthews et al., 2001). BPA can also regulate expression of target genes by signaling through non-genomic pathways via membrane

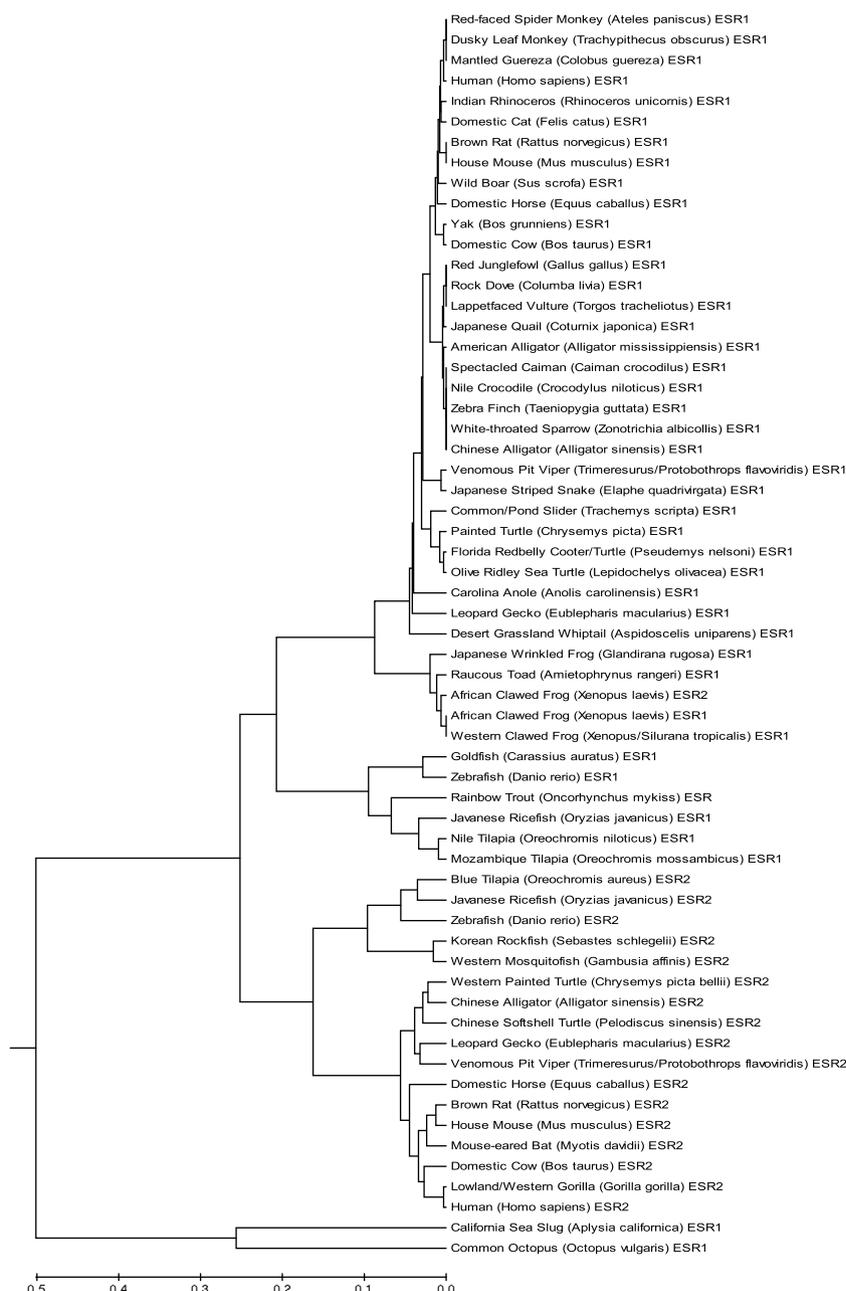


Fig. 1. Evolutionary gene tree for the entire coding sequence of ESR1 and ESR2 from various aquatic and terrestrial species. The ESR1 and ESR2 forms were downloaded from NCBI BLAST searches, aligned with ClustalW, and the evolutionary gene tree was created with the MEGA 6 program (<http://megasoftware.net/>; Matter et al., 1998). The complete coding sequences were used to create the evolutionary gene tree as there is high orthology in the DNA Binding Domain (DBD) and Ligand Binding Domain (LBD) across species.

estrogen receptors or through related signal-transduction cascades (Welshons et al., 2006). While BPA is generally less potent than estradiol for ER, it is equipotent with estradiol in activating rapid signaling systems via non-nuclear receptors (Watson et al., 2007). BPA is generally considered an ER agonist, but it can also antagonize the actions of estrogens in certain tissues, including the brain and uterus (Leranth et al., 2008a,b). BPA thus has mixed agonist/antagonist activity and is considered a selective estrogen receptor modulator (Nagel et al., 2001). In addition, BPA can bind to other steroid and non-steroid receptors, such as androgen receptor (AR) (Lee et al., 2003; Wetherill et al., 2002; Xu et al., 2005), thyroxine receptor (TR) (Zoeller et al., 2005), and PPAR γ to list a few examples (Vandenberg et al., 2013, 2009). Importantly, the affinity of BPA for nuclear ERs does not always predict the observed outcomes, which depend on the tissue, species, and life-stage.

In addition to binding to steroid receptors, BPA can also modulate their expression. The expression of *ESR1* gene is enhanced by BPA in human preadipocyte cells (Boucher et al., 2014), T cells (Cipelli et al., 2013), oocytes (Brieno-Enriquez et al., 2012), mouse prostate mesenchymal cells (Richter et al., 2007b; Taylor et al., 2012), and frog hepatocytes (Bai et al., 2011). Similar expression patterns were found *in vivo* in human leukocytes (Melzer et al., 2011), rat hypothalamus (Cao et al., 2012), mouse glandular endometrial epithelial cells (Markey et al., 2005), frog tadpole gonads (Levy et al., 2004), and fish liver and gonads (Huang et al., 2010; Yamaguchi et al., 2005). BPA was found to mimic estradiol in inducing prolactin expression via induction of *ESR1* expression in both the anterior and posterior pituitaries (Steinmetz et al., 1997). In fetal prostate mesenchyme primary cell culture studies, the expression of AR and *ESR1* was increased by BPA treatment within the range of concentrations measured in human serum and the response was comparable to the response from 17 β -estradiol at the concentration that induced permanent enlargement of prostate *in vivo* (Richter et al., 2007b). The ability of BPA to alter the expression of *Esr1* gene is thus conserved across diverse taxa.

3. BPA and EE2 in surface and ground water

Chemical contamination is nearly ubiquitous in surface and ground water. Organic wastewater pollutants have been measured in >80% of surface and ground water samples in comprehensive sampling studies in the US and Europe (Barnes et al., 2008; Kolpin et al., 2002; Loos et al., 2009, 2010). Specifically, BPA was detected in 41% and 34% and EE2 in 16% and 0% of surface water samples in the U.S. and Europe, respectively (Kolpin et al., 2002; Loos et al., 2009). BPA was detected in 30% and 40% of ground water samples in the US and Europe, and EE2 was not evaluated (Barnes et al., 2008; Loos et al., 2010).

BPA and EE2 can enter surface water through a variety of routes including industrial operations and the disposal and treatment of human waste (Petrovic et al., 2004; Pye and Patrick, 1983). They are typically present in surface water more often and at greater concentrations than in ground water due to direct contact between surface water and sources of contamination (Barnes et al., 2008; Jurado et al., 2012; Kolodziej et al., 2004; Lapworth et al., 2012; Pye and Patrick, 1983; Sychrová et al., 2012). Ground water contamination is less direct, with known sources including landfills, septic tanks, wastewater and mixing with surface water (Lapworth et al., 2012). Despite elevated concentrations in surface water, many EDCs have been reported to resist degradation in aquifers, potentially resulting in accumulation of these chemicals in ground water over time (Jurado et al., 2012; Lapworth et al., 2012; Ying et al., 2004).

3.1. BPA in surface and ground water

Median surface water concentrations between 3 and 30 ng/L tend to agree across studies (Table 1) (Esteban et al., 2014; Furuichi et al., 2004; Heisterkamp et al., 2004; Hohenblum et al., 2004; Jin et al., 2004; Kuch and Ballschmiter, 2001; Martin et al., 2014; Renz et al., 2013; Rudel et al., 1998; Sanchez-Avila et al., 2009; Selvaraj et al., 2014; Suzuki et al., 2004; Yang et al., 2014), though surface water from dense industrial areas has considerably higher concentrations (Heisterkamp et al., 2004; Kim et al., 2014; Sanchez-Avila et al., 2009). Contaminated sites across multiple studies report the presence of BPA at significantly elevated concentrations, ranging from 1 to 28 μ g/L (4.39–122.81 nM) (Barnes et al., 2008; Heisterkamp et al., 2004; Jin et al., 2004; Kolpin et al., 2002; Loos et al., 2010; Rudel et al., 1998). BPA has also been widely reported in ground water, as described above, albeit generally at lower concentrations than those reported for surface water (Table 1) (Barnes et al., 2008; Colin et al., 2014; Erickson et al., 2014; Li et al., 2013; Loos et al., 2010; Peng et al., 2014). As with surface water, while median concentrations of 0–20 ng/L tend to agree (Bono-Blay et al., 2012; Kuch and Ballschmiter, 2001), concentrations are elevated 10-fold at sites susceptible to wastewater contamination (Hohenblum et al., 2004; Rudel et al., 1998), and more than 200-fold at sites impacted by municipal septage landfills (Rudel et al., 1998). Of great concern, these concentrations are higher than the \leq 0.01 μ g/L (0.04 nM) found to cause negative effects on aquatic wildlife and laboratory animals in some studies. A review of many of these studies suggested that a 95% margin of safety for wildlife could only be expected at 0.03 μ g/L (0.13 nM) BPA, a concentration that is up to 1000 times lower than that measured in many surface and ground water sources around the world (Crain et al., 2007).

3.2. EE2 in surface and ground water

EE2 is the primary estrogen in most oral contraceptive pills. Women taking oral contraceptives excrete approximately 10 μ g EE2 per day with average use (Johnson et al., 2000; Johnson and Williams, 2004). EE2 is incompletely removed during wastewater treatment, leading to surface water contamination. Most studies report consistent values from 0.2 to 1.5 ng/L (0.88–6.58 pM) across similar types of surface water sources (Table 1) (Belfroid et al., 1999; Cargouet et al., 2004; Kuch and Ballschmiter, 2001; Li et al., 2013; Murk et al., 2002; Yang et al., 2014), with influent generally more contaminated than effluent, which is in turn more contaminated than surface water further downstream. EE2 was detected in 15% of U.S. river samples in a comprehensive sampling effort (Kolpin et al., 2002). However, many other studies have failed to detect or adequately measure EE2 due to poor limits of detection (LOD) of \geq 1 ng/L (4.39 pM) (Fine et al., 2003; Kolpin et al., 2002; Loos et al., 2009, 2010; Pawlowski et al., 2004; Williams et al., 2003). For example, EE2 was detected in 40% of samples in a German study using a 0.05 ng/L (0.22 pM) LOD (Kuch and Ballschmiter, 2001), whereas it was only detected in 1% of samples in Austria using a 0.1 ng/L (0.44 pM) LOD (Hohenblum et al., 2004). Failure of studies to report EE2 in ground water samples where EE2 is present at even lower concentrations is likely due to these poor LODs.

3.3. Other xenoestrogens in surface and ground water

In addition to BPA and EE2, water supplies are almost ubiquitously contaminated with EDCs from industrial products and processes and agricultural chemicals. There are also many household sources including human steroidal estrogens, pharmaceuticals, cleaning products, plastics, and pesticides. Steroidal estrogens are

Table 1
Select estrogenic chemical occurrence in surface and ground water.

Years	Samples (n)	Water type	Test location	Description	Bisphenol A (BPA)				Ethinylestradiol (EE2)				Estrone (E1)				Estradiol (E2)				Estriol (E3)				Researchers	
					Median conc.	% Detection	Range	Limit of detection	Median conc.	% Detection	Range	Limit of detection	Median conc.	% Detection	Range	Limit of detection	Median conc.	% Detection	Range	Limit of detection	Median conc.	% Detection	Range	Limit of detection		
2014	291	Drinking water	France	Tap water samples (paired to raw water, following treatment)	<LOD	0.7%	<LOD-50	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Colin et al.		
2014	47	Drinking water	France	Five drinking water networks with epoxy-lined pipes or water towers	<LOD	0.0%	<LOD	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Colin et al.		
2001	10	Drinking water	Germany	Drinking water reservoir and ground water sites	1.1	100.0%	0.50-2	0.02	0.35	40.0%	0.15-0.50	0.05	0.40	<0.05	40.0%	0.20-0.60	0.05	0.30	50.0%	0.20-2.1	0.1	-	-	Kuch and Ballschmiter		
1998	28	Tap water	United States	Tap water from private wells with range of wastewater impact	<LOD	7.1%	<LOD-44	3.6	<0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Rudel et al.		
2008	0	Ground water	Israel	Ground water suspected to be susceptible to contamination	-	-	-	-	-	-	-	-	-	-	-	-	0.2	50.0%	ND-~3	0.3 ng/L	-	-	-	Arnon et al.		
2008	47	Ground water	United States	Ground water sites suspected to be susceptible to contamination	<LOD	29.8%	2555 max	1000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Barnes et al.		
2012	131	Spring water	Spain	Spring water samples intended for bottling	<LOD	4.6%	<LOD-203	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Bono-Blay et al.		
2014	118	Ground water	United States	Vulnerable aquifers throughout Minnesota	<LOD	6.0%	<LOD-4411	100	<LOD	0.0%	<LOD	0.8	<LOD	0.0%	<LOD	0.8	<LOD	0.0%	<LOD	0.8	<LOD	0.0%	<LOD	2	Erickson et al.	
2003	0	Ground water	United States	Ground water susceptible to swine operation contamination	-	-	-	-	ND	ND	ND	1	ND	ND	ND	1 ng/L	ND	ND	ND	1 ng/L	ND	ND	1 ng/L	Fine et al.		
2003	11	Surface water	United States	Swine lagoon effluent samples	-	-	-	-	ND	ND	ND	40	5107	90.0%	<40-74,700	40 ng/L	88	90.0%	<40-3000	40 ng/L	261	90.0%	ND-10,900	Fine et al.		
2004	112	Ground water	Austria	Sites near urban, industrialized, or agricultural use areas	24	58.6%	ND-930	10	<LOD	0.9%	ND-0.94	0.1	<LOD	18.3%	1.6	1.6	0.07	51.8%	ND-0.79	<LOD	<LOD	1.8%	0.16	Hohenblum et al.		
2013	51	Ground water	China	Unconfined & confined aquifers recharged by reclaimed water, Beijing	4.85	80.4%	0	0.01	0.09	49.0%	0	0.01	2.01	74.5%	0	0.01	0.16	43.1%	0	0.01	0.03	29.4%	0	0.01	Li et al.	
2010	164	Ground water	23 EU Countries	Ground water monitoring stations proposed by EU labs	0*	39.6%	2299 max	1	ND	ND	ND	3	0*	0.6%	4 max	1	ND	ND	ND	3	-	-	-	-	Loos et al.	
1998	4	Ground water	United States	Ground water monitoring wells contaminated with effluent	16	100.0%	<LOD-29	3.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Rudel et al.	
1998	5	Ground water	United States	Ground water monitoring wells near municipal landfills for seepage	320	100.0%	<LOD-1410	3.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Rudel et al.	
2009	1	Ground water	Spain	Representative well known to be impacted by wastewater	0	100.0%	780	54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Sánchez-Avila et al.	
2004	43	Ground water	United States	Springs located in the Ozark Plateau Aquifer karstic basin	-	-	-	-	-	-	-	-	-	-	-	-	36.3	100.0%	12.8-79.7	4.8 ng/L	-	-	-	-	Wicks et al.	
1999	11	Surface water	The Netherlands	River samples downstream of large urban areas	-	-	-	-	<LOD	27.3%	<0.1-4.3	0.3	63.6%	<0.1-3.4	0.2-0.3	<LOD	<LOD	36.4%	<0.3-5.5	0.3-0.6	-	-	-	-	Belfroid et al.	
2005	16	Surface water	Australia	Wastewater treatment plant samplings	-	-	-	-	0	0.0%	<LOD	0.1-1	0	100.0%	29-93	0.1-1	0	100.0%	2.2-72	0.1-1	-	-	-	-	Braga et al.	
2004	7	Surface water	France	River samples up and downstream from Paris treatment plants	-	-	-	-	1.5	100.0%	1.1-2.9	0.2	1.8	100.0%	1.1-3.0	0.2	2.1	100.0%	1.4-3.2	0.2	2.1	100.0%	1.0-2.5	0.2	Cargouët et al.	
2014	291	Surface water	France	Raw water samples near industrial/commercial activities, France	<LOD	6.2%	<LOD-1430	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Colin et al.	
2014	14	Surface water	Spain	Downstream of WWTPs, Jarama & Manzanares Rivers, Spain	27	71.4%	<LOD-126	0.11	<LOD	0.0%	<LOD	0.14	<LOD	21.4%	<LOD-17	0.05	<LOD	0.0%	<LOD	0.037	<LOD	<LOD	0.0%	<LOD	0.17	Esteban et al.
2004	5	Surface water	Japan	River water samples suspected to be susceptible to contamination	33.2	100.0%	16.5-150.2	0.2	<0.2	0.0%	<0.2	0.2	44.5	100.0%	17.1-107.6	0.2	5.2	100.0%	2.6-14.7	0.2	<0.2	0.0%	<0.2	0.2	Furuichi et al.	
2004	12	Surface water	Czech Republic	River samples taken downstream of a chemical site in CR	675	100.0%	85-28,000	6	ND	ND	ND	Eluted w/matrix comp	<LOD	16.7%	<LOD-2.3	4	<LOD	0.0%	<LOD	24	ND	ND	ND	Insufficient recovery	Heisterkamp et al.	
2004	261	Surface water	Austria	Sites near urban,	<LOD	22.3%	ND-10	10	<LOD	1.5%	ND-0.1	0.35	76.1%	ND-1	ND-1	0.13	60.4%	ND-1	<LOD	1	<LOD	7.7%	ND-3	3	Hohenblum	

(continued on next page)

Table 1 (continued)

Years	Samples (n)	Water type	Test location	Description	Bisphenol A (BPA)			Ethinylestradiol (EE2)			Estrone (E1)			Estradiol (E2)			Estradiol (E3)			Researchers					
					Median conc.	% Detection	Range	Limit of detection	Median conc.	% Detection	Range	Limit of detection	Median conc.	% Detection	Range	Limit of detection	Median conc.	% Detection	Range		Limit of detection	Median conc.	% Detection	Range	Limit of detection
2004	41	Surface water	China	Industrialized, or agricultural use areas	34.8	100.0%	19.1–8300	0.17	-	-	-	-	-	-	-	-	-	-	-	-	et al.				
2000	20	Surface water	Italy/Netherlands	River samples suspected to be impacted by major urban area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	et al.				
2000	20	Surface water	Italy/Netherlands	Sewage treatment plant effluents	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Johnson et al.				
2014	18	Surface water	Netherlands	Hyungsan River sites near urban, industrial sources	34.3	88.9%	<LOD-636.9	0.3	-	-	-	-	-	-	-	-	-	-	-	-	Johnson et al.				
2004	11	Surface water	United States	Streams suspected to be susceptible to contamination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Kim et al.				
2004	15	Surface water	United States	Irrigation canals near suspected discharge points	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Kim et al.				
2002	70–85	Surface water	United States	Streams suspected to be susceptible to contamination (<LOD)	140	41.2%	12,000 max	90	73	15.7%	831	5	27	7.1%	112	5	9	10.0%	93	5	19	21.4%	51	5	max
2001	31	Surface water	Germany	Streams suspected to be susceptible to contamination	3.8	100.0%	4.8–47	0.04	0.40 (<0.10)	48.4%	0.10–5.1	0.1	0.40	93.5%	max	max	max	0.30 (<0.15)	0.15–3.6	0.15–3.6	100.0%	0.11	0.09 (M)	0.11	0.11
2013	64	Surface water	China	River water from along Chaobai River, Beijing	44.9	100.0%	12.0–120.8	0.1	0.52 (M)	100.0%	0	0.18	8.83 (M)	100.0%	1.1–25.4	0.1	1.46 (M)	100.0%	0	0.11	0.09 (M)	0.11	0.09 (M)	0.11	0.11
2009	122	Surface water	23 EU Countries	Streams and rivers proposed by EU labs	0	34.0%	323 max	5	ND	ND	ND	5	0 [#] (<LOD)	81	2	ND	ND	ND	ND	5	-	-	-	-	-
2014	6	Surface water	Spain	Surface water samples from Guadalquivir River, Seville	700 (M)	0	0	21.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2004	1	Surface water	Germany	River samples downstream of effluent entry	-	-	-	-	<LOD	0.0%	<1	1	0	0.0%	<1	1	0	100.0%	3.9	1	-	-	-	-	-
2013	6	Surface water	United States	Greater Pittsburgh area surface waters	0.6	83.3%	<LOD-15.4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2014	27	Surface water	India	Kaveri, Vellar, and Tamiraparani Rivers in Southern India	11.5	100.0%	2.8–136	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2004	49	Surface water	Japan	River samples both up and downstream of effluent	0	73.5%	ND-230	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2003	225	Surface water	United Kingdom	River water downstream of effluent entry	-	-	-	-	<LOD	19.1%	ND-4.6	0.5	-	ND-2.5	0.4	-	-	-	-	-	-	-	-	-	-
2014	36	Surface water	China	Reservoirs, lakes, and fish ponds in Pearl River Delta, South China	23.4	97.2%	<LOD-376.6	0.9	0.735	63.9%	<LOD-3.43	0.7	<LOD	47.2%	<LOD-1.58	1	-	-	-	-	-	-	-	-	-
1999	5	Effluents	The Netherlands	Wastewater effluents from five treatment plants	-	-	-	-	<LOD	40.0%	<0.2–7.5	0.3–1.8	4.5	80.0%	<0.1–47	0.3–1	0.9	80.0%	<0.4–12	0.5–2.4	-	-	-	-	-
2004	4	Effluents	France	Wastewater effluents from the four major Paris treatment plants	-	-	-	-	3.75	100.0%	2.7–4.5	0.2	6.35	100.0%	4.3–7.2	0.2	6.9	100.0%	4.5–8.6	0.2	6.25	100.0%	5.0–7.3	0.2	
2004	19	Effluents	Germany	Wastewater effluents from a treatment plant in Hamburg	21	100.0%	18–40	6	ND	ND	ND	Eluted w/matrix comp	<LOD	0.0%	<LOD	4	<LOD	0.0%	<LOD	24	ND	ND	ND	ND	Insufficient recovery
2001	15	Effluents	Germany	Sewage treatment plant effluents	10	93.8%	4.8–47	0.04	0.7	87.5%	0.1–8.9	0.1	1.5	93.8%	0.35–18	0.1	0.4	87.5%	0.15–5.2	0.15	-	-	-	-	-
2014	6	Effluents	Spain	Effluent wastewater from Southern Spain WWTP, Seville	4000 (M)	0	0	24.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2004	2	Effluents	Germany	Municipal wastewater treatment plant effluents	-	-	-	-	0	50.0%	<1	1	10.1	100.0%	1.2–19	1	3.3	100.0%	1–5.6	1	-	-	-	-	-
1998	3	Effluents	United States	Treated wastewater from two Cape Cod treatment facilities	38 (M)	100.0%	20–55	3.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2009	6	Effluents	Spain	Effluent samples from Mataró WWTP	620 (M)	0	0	54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2003	56	Effluents	United Kingdom	Wastewater effluents from three treatment plants	-	-	-	-	<LOD	35.7%	<0.4–3.4	1	-	91.1%	<0.4–12.2	1	-	53.6%	<0.4–4.3	1	-	-	-	-	-
2004	4	Influents	France	Wastewater influents from the four major Paris treatment plants	-	-	-	-	6.1	100.0%	4.9–7.1	0.2	13.2	100.0%	9.6–17.6	0.2	14.35	100.0%	11.1–17.4	0.2	13.6	100.0%	11.4–15.2	0.2	
1998	5	Septage	United States	Untreated septage from two	820 (M)	80.0%	110–	3.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Year	Number of samples	Location	Sample type	Number of samples	Percentage of samples	Number of samples	Reference
1998	4	United States	Influent	110 (M)	75.0%	1700	Rudel et al.
2009	14	Spain	Mixed wastewater	4130	100.0%	94–150	Sánchez-Avila et al.
2009	6	Spain	Influent	2400 (M)	0	1320–11,100	Sánchez-Avila et al.
			Influent samples from Matao WWTP	0	0	0	Sánchez-Avila et al.

All concentrations provided in table are ng/L.

(M) denotes a mean concentration for this site. This is only reported when a median value is not reported and raw values are not accessible to provide for the calculation of this value.

<LOD denotes sample concentrations that were below the method's limit of detection and cannot be accurately quantified.

ND denotes samples where the chemical could not be detected at any concentration, even below the quantitation limit specified.

Ø denotes information not provided in the referenced articles.

* Denotes median concentration calculated by including only samples with detectable concentrations above the LOD. In instances where the median of all values could be calculated, it is provided following the reported value, in parentheses.

Denotes a median concentration reported as 0 when more than half of samples were below the LOD. This should be more correctly noted as a median of <LOD, provided in parentheses following the reported value.

thus widely reported in water supplies around the world (Table 1) (Belfroid et al., 1999; Cargouet et al., 2004; Furuichi et al., 2004; Kuch and Ballschmiter, 2001; Li et al., 2013; Murk et al., 2002; Pawlowski et al., 2004; Williams et al., 2003). Given the additive nature of xenoestrogens, the presence of estradiol, estrone, and estriol should be included when considering the total estrogenic activity in water. Further, bacteria present in surface water can deconjugate inactive steroidal estrogens to biologically active ones, despite the fact that many of these are excreted in conjugated inactive forms (Ying et al., 2002).

3.4. Removal of estrogens from ground and surface water

Wastewater from human populations is a major contributor to water contamination. Wastewater treatment plants have failed to keep pace with growing populations, and are inadequate to remove many EDCs (Braga et al., 2005). Activated sludge treatment plants are capable of removing up to 90% of estrogens; however, treatment plants not utilizing this technology only routinely remove 5–10% (Braga et al., 2005). A recent report concluded that many plants do not use activated sludge treatment; the current pattern of application across the US resulted in 50% of contaminants being returned to surface water following treatment (Arvai et al., 2013). As such, many of these hormones and pharmaceuticals will eventually re-enter surface water sources due to the failure of treatment plants to adequately remove them. This can also lead to their eventual migration into shallow ground water over time through processes such as ground water recharge (Arnon et al., 2008; Fine et al., 2003; Heberer, 2002a,b) or losing reach, where the water table lies below the river bed and surface water is able to migrate through the bed into the underlying aquifer (Wicks et al., 2004).

The overall result of these different sources of EDCs is the accumulation of estrogens in downstream surface water Zoeller et al., 2005. Notably, many sites have concentrations of single chemicals that have been associated with human and environmental health effects. When additive effects are considered, the cause for concern is only magnified (NRC, 2008; Rajapakse et al., 2002; Silva et al., 2002). It is crucial to understand the full spectrum of sources through which chemicals are contributed to water sources in order to understand the potential impacts. Despite these uncertainties, it is clear that BPA is present at concentrations in surface and ground water that can have negative health consequences for fish, wildlife and humans as described below (Braga et al., 2005; Campbell et al., 2006; Colborn, 1995; Filby et al., 2007; Hinck et al., 2009; Kuch and Ballschmiter, 2001; Westerhoff et al., 2005).

4. Effects of BPA in fish

A variety of reproductive and developmental effects of BPA exposure have been observed across vertebrate taxa. The effects of BPA on fish and other aquatic species have received some attention, with notable reviews by Staples et al. (2002) and Crane et al. (2007). The effects of EE2 have been the subject of an extensive review and summary related to threshold values (Caldwell et al., 2008). Therefore, we will not attempt to evaluate the extensive literature on EE2, but rather will focus this portion of the review on current studies evaluating the effects of BPA on model fish species.

The effects of BPA on fish include transcriptional activation of estrogen receptor responsive genes, increased brain aromatase activity, induction of VTG in males, disruption of gametogenesis in both males and females, altered development (neuronal, cardiac, germ cells, and sexual differentiation), and changes in sex ratios after embryonic exposure (Crane et al., 2007). These effects occur

over a wide range of exposure concentrations, and most certainly, BPA has differential tissue and species sensitivities. Therefore, routes of exposure and toxicokinetics of BPA are important considerations when attempting to evaluate hazards of this chemical in fish, or any other animal for that matter. Crane et al. (2007) conducted a hazard assessment based on effects thresholds for BPA and measured concentrations of BPA in water reported from the literature, concluding that indeed there was an overlap of effects thresholds and exposure concentrations of BPA based on the literature.

4.1. BPA-induced gene expression in adult fish

Unlike humans where the route of BPA exposure is through ingestion and personal care products, fish and aquatic wildlife are chronically exposed to these chemicals at variable concentrations. Irrespective of the route of exposure, the mode of action of this chemical is likely similar as ER-mediated transactivation mechanisms are highly conserved in vertebrates examined (Sumida et al., 2003). In an eco-toxicogenomic study, Ankley and colleagues found zebrafish to be less sensitive to effects on hepatic gene expression and steroid production than fathead minnow (Villeneuve et al., 2012). The nonmonotonic profile was consistent among species and there were nominal similarities in the functions associated with the differentially expressed genes, suggesting potential activation of common pathway perturbation motifs in the two species (Villeneuve et al., 2012). Liu et al. (2012) reported that BPA led to concentration-dependent effects on expression of steroidogenic enzyme genes, mainly *Star*, *Cyp11a1*, *3beta-hsd*, *Cyp17a1*, and *Cyp19a1a* in the gonads of rare minnow (Liu et al., 2012). In the brain, exposure to a range of BPA concentrations (0.1–10 nM or 23–2280 ng/L) suppressed the expression of aromatase (*Cyp19a1b*) in the minnow (Wang et al., 2010), zebrafish (Chung et al., 2011) and in self-fertilizing fish, *Kryptolebias marmoratus* (Rhee et al., 2011). In the pituitary of *K. marmoratus*, BPA exposure elevated the expression of *Fsh-b* and *Lhb* genes (Rhee et al., 2010). Effects of BPA in expression of genes specific for sex differentiation were examined in hermaphrodite fish (Lee et al., 2006; Rhee et al., 2010), self-fertilizing fish (Rhee et al., 2011), and viviparous fish (Kwak et al., 2001). These studies showed that BPA elevated the expression of female specific genes such as *Fig1α*, *Dax1*, and *Wt1* mRNA but repressed male specific genes, such as *Sf1*, *Dmrt1* and *Mis*.

Bisphenol A disrupts the gonadotropin-releasing hormone (GnRH) system in fish at concentrations that are environmentally relevant (15 μg/L or 66 nM) (Qin et al., 2013). Subsequently, gonadal aromatase was down-regulated in both male and female Chinese rare minnows, and GnRH receptor gene expression was up-regulated in the brains of female minnows (Qin et al., 2013), presumably due to a positive feedback mechanism as a result from depressed local estrogen production. Histological pathogenesis in these fish included increases in primary oocytes, indicative of reduced maturation and alteration of normal oogenesis. These histopathological findings in ovarian tissues of BPA-exposed fish are consistent with the follicular atresia observed in female carp exposed to much greater concentrations of BPA (Mandich et al., 2007). Effects of BPA on spermatogenesis appear to be the most sensitive reproductive endpoint in the adult fish (Crane et al., 2007). Fathead minnow exposed to 1–1280 μg/L BPA (4 nM–5.6 μM) had a concentration-dependent decrease in spermatogenesis, as indicated by increases in spermatogonia and decreases in spermatozoa after a 164-day exposure period to 16 μg/L BPA (70 nM) (Sohoni et al., 2001). Decreases in sperm motility and velocity in goldfish exposed to environmentally relevant concentrations of BPA suggest that the changes in sperm function are

associated with deficits in steroidogenesis and alterations in sperm maturation (Hatef et al., 2010).

Although consistently found to be a weak estrogen receptor agonist, BPA has also been shown to have anti-androgenic activity in fathead minnow assays and *in vitro* (Ankley et al., 2004; Ankley et al., 2010b; Ekman et al., 2012; Jolly et al., 2009). Evidence for anti-androgenic activity of BPA in fish comes from the antagonism of androgen-induced effects on breeding tubercle formation in females when BPA was tested in binary mixtures with known androgenic chemicals 17-β trenbolone, flutamide, or vinclozolin (Ankley et al., 2010b). Further evidence of anti-androgenic activity of BPA has come from *in vitro* mechanistic studies in renal cells from three-spined stickleback (*Gasterosteus aculeatus*) (Jolly et al., 2009) and inhibition of androgen receptor-mediated transcriptional activation using fathead minnow AR (Ekman et al., 2012).

Additionally, the metabolomic profile of female fathead minnows co-exposed to BPA and an androgen indicates BPA has a profile consistent with an AR antagonist (Ekman et al., 2012). Thus, BPA has the potential for working as both an estrogen-like compound and an androgen antagonist in fish, in essence enhancing the hazards posed by this ubiquitous environmental contaminant.

Bisphenol A has also been shown to potentiate the effects of the thyroid hormone T₃ (3,5,3'-triiodo-L-thyroxine) in developing fish embryos (Pelayo et al., 2012). Although not a goitrogen in developmental assays, BPA potentiated T₃-sensitive transcriptional activity in exposed zebrafish (Pelayo et al., 2012). That is, BPA caused altered transcription of three T₃-sensitive genes that are biological markers associated with thyroid hormone-dependent functions in development: skeletal development and ossification, eye development and development of the hematopoietic system (Pelayo et al., 2012).

4.2. Reproductive and neurobehavioral effects of BPA in fish

Some of the most complete assessments of the reproductive effects of BPA on fish have been evaluated in fathead minnow (Mihaich et al., 2012; Sohoni et al., 2001; Staples et al., 2011). Sohoni et al. (2001) and Mihaich et al. (2012) studied chronic exposures of fathead minnows to graded concentrations of BPA with endpoints of survival, plasma VTG, gonadosomatic index (GSI), fecundity, hatchability, and gonad histopathology. The results of these studies were fairly consistent, with no effect of BPA on egg production (fecundity), GSI, hatchability, or spawning rate up to concentrations of 640 μg/L (2.8 μM) (Mihaich et al., 2012; Sohoni et al., 2001). However, as mentioned above, changes in spermatogenesis (altered proportions of cell types, reduced spermatozoa) were reported by Sohoni et al. (2001) at 16 μg/L BPA (70 nM). While reductions in spermatozoa were noted at 160 μg/L (700 nM) BPA by Mihaich et al. (2012), this finding was not considered biologically relevant, as there was no change in hatching rate. A multigenerational study with fathead minnow performed by Staples et al. (2011) reported similar findings to relative to the effective concentrations for adverse outcomes on fecundity and GSI being 640 μg/L (2.8 μM) or greater. However, it is interesting to note that the egg production of the F₁ was significantly decreased at 1 μg/L (4 nM) and 640 μg/L (2.8 μM) BPA relative to control F₁ fecundity, and indeed egg production in all of the F₁ BPA treatment groups were approximately 50% less than F₁ control fecundity. Staples et al. (2011) dismissed these results as an outlier due to elevated control egg production in the F₁ generation; however, F₁ control egg production (26 eggs/female/day) is similar to average values of 20.5 eggs/female/day reported for fathead minnow under controlled laboratory conditions for EDCs testing protocols (Watanabe et al., 2007).

The median effect concentration of BPA in a zebrafish life-cycle reproduction assay (with endpoints of mortality, behavioral

abnormalities, growth, time until first spawning, egg production, and fertilization success) was 1400 µg/L (6140 µM) and the potency was 10⁻⁶ to 10⁻⁷ lower compared to the potency of EE2 in the same fish assay (Segner et al., 2003). The specific endpoints for determination of this ED₅₀ value were not delineated (Segner et al., 2003). The no effect concentration for reproduction in a 14 day exposure with medaka was 684 µg/L (3.0 µM) (Shioda and Wakabayashi, 2000). Taken together, these results indicate that medaka, fathead minnow, and zebrafish have similar relative species sensitivity towards the reproductive effects of BPA. In brown trout, BPA concentrations as low as 1.75 and 2.40 µg/L (7 and 9.6 nM) BPA led to reduced sperm quality, as measured by sperm density, motility, and sperm velocity (Lahnsteiner et al., 2005). This same study found dose-related delays in ovulation in females, beginning with the lowest dose tested (1.75 µg/L BPA or 7 nM), while the 5.0 µg/L (20 nM) treatment group failed to ovulate at all (Lahnsteiner et al., 2005). Egg quality in this study indicated no effect of these concentrations of BPA on standard measures of egg quality (egg mass or percent hardened) or on fertilization rates (Lahnsteiner et al., 2005).

Neurobehavioral effects of BPA on fishes have been observed upon adult exposure, as well as through developmental exposures. Male secondary sexual characteristics and female selection were compromised by BPA exposure when wild fish were brought into the lab and evaluated (Ward and Blum, 2012). Two congeneric species of freshwater fish, a native species in the Upper Coosa River Basin (Alabama, Georgia, and Tennessee, USA) and an invasive species of *Cyprinella* (genus) were acclimated then exposed to BPA for 2 weeks and behavioral assays conducted to evaluate isolation between the species. BPA caused changes in secondary sexual characteristics in male and female mate choice, and lead to a breakdown in the prezygotic isolation among these species (Ward and Blum, 2012).

4.3. Effects of BPA on early life stages of fish

Stage-specific outcomes of BPA exposure in fish development have been observed for some time. As with many chemicals, and in particular EDCs, there is a range of concentrations at which the endocrine activity of the chemical is apparent, while at greater concentrations, other modes of action (narcosis, oxidative stress, etc.) of the chemical may take precedence. This paradigm is certainly true for BPA-induced toxicity on developing fish embryos and larvae. Overt mortality, spinal curvature, pericardial edema, yolk sac edema, delayed development, and even defects in otoliths are all developmental effects of BPA that occur at greater exposure concentrations, upwards of 5000–20,000 µg/L (22–88 µM) (Alexander et al., 1988; Duan et al., 2008; Fei et al., 2010; Kishida et al., 2001; McCormick et al., 2010; Saili et al., 2012). These effects are a result of short-term exposure (hours) to elevated concentrations during critical windows of embryonic development. At lower concentrations of BPA, organizational events can be disrupted, as is observed with alteration of sex determination in medaka (Kang et al., 2002, 2007) or zebrafish (Drastichova et al., 2005) at exposures of BPA of 864 µg/L (3.79 µM) and 1000 mg/Kg-diet of fry, respectively. However, even these concentrations of BPA would only be realized in environmental settings associated with landfill leachates, which are routinely found to have BPA in the low milligrams per liter (ppm or mM) range (Oehlmann et al., 2008; Yamamoto et al., 2001).

Gene expression (*bmp4*, *cox-1*, *fgf8*, *gata4*, and *nkx2.5*) was transiently altered by embryo exposure to BPA (200 µg/L or 0.88 µM) in medaka. These are genes important in cardiac development, but the morphometric analysis of heart development indicated that these changes in gene expression did not translate into structural defects in heart development that could be readily measured

during early life stages of medaka (Huang et al., 2012). At smaller concentrations of BPA (0.1, 1, 10, and 100 µg/L; or 0.4, 4, 40, and 400 nM), other studies with medaka showed decreases in heart rate, along with concentration-related decreases in developmental time, eye density, and head growth (Lee et al., 2012). Zebrafish exposed to relatively high concentrations of BPA (100–4500 µg/L, 0.4–19.7 µM) had concentration-dependent increases in cardiac edema, craniofacial deformities, lack of swim bladder inflation, gastrointestinal developmental anomalies, and a lack of yolk resorption (Lam et al., 2011). These same authors considered gene expression during the period of development in zebrafish under exposure to BPA and found a set of endocrine-related genes were consistently dysregulated by BPA in embryo development. In particular, embryonic growth regulator 2 (*Egr2*) and specificity protein 4 (*Sp4*), transcriptional regulatory factors involved in cardiovascular and neurological development were found to be disrupted by BPA embryonic exposure and may provide good biomarkers for future studies and potentially environmental exposures (Lam et al., 2011).

Neurodevelopmental impacts of BPA exposure resulting in behavioral deficits have been observed in vertebrates, including fish. The phenotypic behavioral deficits observed in mammals from BPA exposure include hyperactivity, anxiety, learning and memory effects, and reproductive behavioral problems (Wolstenholme et al., 2011). The mechanistic basis for these behavioral deficits is not understood at this time. Fish have been less well studied; however more recently, zebrafish have been used to understand the mechanistic toxicology of BPA-induced behavioral impacts (Saili et al., 2012). Saili et al. (2012) examined locomotive behavior and learning deficits resulting from low dose, embryonic exposures to BPA and found hyperactivity in larval stages and learning deficits in adult stages of zebrafish. Concentration thresholds in these studies were 2.28 µg/L (0.01 µM) for locomotor effects in larval stages and 22.8 µg/L (0.1 µM) for learning deficits in adults, both after embryonic exposure. Thus, the same type of neurodevelopmental effects of BPA observed in mammals may well be expected to occur in fish. Further work in this area is warranted to understand species-specific alterations in brain development.

4.4. Summary and future information needed in fish models

Clearly, BPA causes demonstrable effects on fish development and activational events in adults. Effects of BPA observed in fish are largely thought to occur through interactions with the estrogen receptor, but anti-androgenic mechanisms are also apparent with BPA. Major endocrine-related functions disrupted by BPA in fish are early development, sex determination and differentiation, gametogenesis, and neurobehavioral function. Many of these effects have concentration thresholds that are well above expected environmental concentrations likely to be encountered by fish. Yet some studies have demonstrated BPA-induced effects at concentrations that are closer to concentrations of BPA observed in surface waters. Exposure to environmentally relevant BPA concentrations (in the µg per liter range) disrupted spermatogenesis across fish species tested. This finding warrants further investigation. Moreover, the potential for transgenerational effects of BPA on fish has not yet been evaluated, but also warrants further investigations based on the limited findings of reductions in fecundity F₁ generation of fathead. Next, the neurobehavioral effects of BPA on development suggest that this is an area where more detailed studies are needed. Last, adverse outcome pathways (AOPs) for many of the higher level outcomes of BPA-induced toxicity in fish would be useful to understand to allow better ecological risk evaluations (Ankley et al., 2010; Kramer et al., 2011).

5. Effect of BPA and EE2 in amphibians

5.1. Sexual development and biomarkers

One of the most sensitive bioindicators of amphibian exposure to EDCs may be distorted sex ratios in favor of females. Studies have examined whether EE2 and BPA exposure can lead to feminized responses in various amphibian species. Testing with various concentrations of EE2 exposure during the larval stage in wood frogs (*Lithobates sylvaticus*) with an EC50 dose of 7.7 µg/L EE2 (26 nM) resulted in complete feminization and partial feminization was evident at 2.3 µg/L (7.8 nM) (Tompsett et al., 2013). Exposure of *Xenopus tropicalis* from hatching until metamorphosis to even lower concentrations of EE2 (6 pM or 1.8 ng/L) resulted in male to female sex reversal (Berg et al., 2009; Gyllenhammar et al., 2009). Likewise, 60 pM (18 ng/L) EE2 led to distorted female-biased ratios in *X. tropicalis* and *Rana temporaria* (Pettersson et al., 2006). Similar findings have also been replicated in the Northern leopard frog (*R. pipiens*) (Hogan et al., 2008).

BPA studies on sex ratio in amphibians have yielded mixed results. One of the first studies to examine the effects of BPA exposure on sex ratios in *X. laevis* demonstrated that tadpoles exposed to fluctuating doses of 2.28 and 22.8 µg/L BPA (10 and 100 nM) during stages 38–40 through metamorphosis demonstrated sex ratio skewing to females (Kloas et al., 1999). In a follow-up study with three doses of BPA (2.28, 22.8, and 228 µg/L; or 10, 100, and 1000 nM), only those frog populations exposed to fluctuating concentrations of the middle dose of BPA showed sexual development distortion to females (Levy et al., 2004). In contrast, when BPA concentrations ranging from 0.83 to 497 mg/L (3.6 to 2180 µM) were provided in a flow through tank design, no shifts in sex ratio were observed (Pickford et al., 2003). In this same study, a sex ratio imbalance was observed in the EE2 exposed group. The reason for these conflicting findings might relate to method of exposure (fluctuating versus continuous), number of replicates included in each study, and the statistical methods employed to analyze the categorical data. Nonetheless, these studies support the notion that even low concentrations of BPA that would be present in most environmental water sources can alter sexual development in amphibians and may be a sensitive indicator of environmental contamination. Therefore, there is strong consensus that environmentally relevant concentrations of EE2 and BPA can underpin feminization in a variety of amphibian species that could have detrimental effects on populations already in severe decline due to climate change, the chytridiomycosis pandemic and other anthropogenically driven threats (Hayes et al., 2006, 2010).

While there have not been any other studies detailing other sexual developmental abnormalities in amphibians exposed to BPA, there are reports of EE2 and other EDCs inducing such effects. Only those examining EE2 or estradiol will be further discussed. *X. tropicalis* males that were not overtly sex reversed when exposed during development to EE2 demonstrated reduced fertility and concentration of spermatozoa in the testes compared to control males (Berg et al., 2009; Gyllenhammar et al., 2009). A high percentage of females exposed during the larval stage to 600 pM (180 ng/L) EE2 lacked oviducts (Gyllenhammar et al., 2009). *Rana pipiens* exposed from the egg-stage through metamorphosis to a range of municipal wastewater effluent concentrations (0%, 10%, 50%, and 100%) that were determined to contain 1.7–2.1 mL (6.2 nM) 17β-estradiol activity (Sowers et al., 2009) displayed testicular oocytes in males treated with the 50% and 100% concentrations and delayed metamorphosis in both sexes of these groups.

Environmental assessments of EDC exposure, including BPA, have been greatly aided by the identification of gene biomarkers

in diverse species, including amphibians. As detailed above, the best characterized biomarker in estrogen-exposed vertebrates is vitellogenin (VTG) (Goksoyr, 2006; Marin and Matozzo, 2004; Porte et al., 2006; Selcer and Verbanic, 2014; Sumpter and Jobling, 1995). BPA exposure of *Bombina orientalis* and *X. laevis* males demonstrated upregulation of *Vtg* (Gye and Kim, 2005; Kloas et al., 1999). However, no surge in *Vtg* expression was observed in hepatocytes isolated from male brown frogs (*R. temporaria*) exposed to similar concentrations of BPA (22.8 µg/L or 100 nM) (Rouhani Rankouhi et al., 2005). Wood frogs exposed to concentrations of EE2 ranging from 1.08 to 80.9 µg/L (3.7 to 275 nM) exhibited increased hepatic expression of *VtgA2* (Tompsett et al., 2013). Another report with *R. temporaria* confirmed that EE2 exposure increased whole body calcium levels and egg yolk protein concentrations of vitellogenin (Brande-Lavridsen et al., 2008). EE2 concentrations of 2.96 µg/L (10 nM) up-regulated *Vtg* in exposed male *X. laevis* (Hoffmann and Kloas, 2012b). Comprehensive examination of the effects of estradiol and the mixed estrogenic/anti-estrogenic compound, tamoxifen, on a panel of candidate biomarkers revealed that estrogenic treatment of *X. laevis* induced considerable amounts of VTG protein compared to hepatic *Vtg* mRNA (Urbatzka et al., 2007). Nonetheless, *Vtg* mRNA was still a sensitive indicator of estrogenic and anti-estrogenic treatment. Additionally, transferrin (*Tf*) mRNA was suppressed by estradiol, but up-regulated in response to tamoxifen. Estradiol also decreased the expression of transthyretin (*Ttr*) and retinol-binding protein (*Rbp*) transcripts. Other biomarkers for EDCs exposure in amphibians include hepatic high density lipoprotein binding protein (*Hdp*) and 7-dehydrocholesterol reductase (*Dhcr7*) (Tompsett et al., 2013).

5.2. Neurobehavioral alterations

Later adult behaviors in other species, such as mammals, are programmed by developmental exposure to endogenous androgens and estrogens (Arnold and Breedlove, 1985; McCarthy, 2008; Morris et al., 2004; Nugent et al., 2012; O'Donnell et al., 2009; Phoenix et al., 1959; Robinson, 2006; Scott et al., 2009). Such traits in amphibians are also likely vulnerable to early contact with EDCs. While no study to date has assessed BPA effects on reproduction-associated and other behaviors in amphibians, several studies have detailed the detrimental effects of estradiol, EE2, and other xenoestrogens on neurobehavioral functions in various species (Hoffmann and Kloas, 2012a,b), although not all studies support this notion (Gyllenhammar et al., 2009). Sexual selection has resulted in many amphibian males competing for females by their advertisement call. Females may also engage in courtship vocalizations, and steroid hormones orchestrate this behavior in both sexes (Boyd, 1992; Emerson and Boyd, 1999; Gordon and Gerhardt, 2009; Hannigan and Kelley, 1986; Kelley, 1980, 1986; Moore et al., 2005; Zornik and Kelley, 2011). Developmental exposure of male *X. laevis* to varying concentrations of EE2 (0.296–296.4 µg/L; or 11000 nM) disrupts several features of this behavior, including disrupted temporal and spectral parameters of the advertisement call (Hoffmann and Kloas, 2012b). Moreover, the exposed males are less likely to become sexually aroused compared to controls, as assessed by decreased proportions of advertisement calls and increased proportion of rasping calls (signature vocalization of un-aroused males). Males exposed to a single dose of 2.96 µg/L EE2 (10 nM) for 96 h, regained their advertisement call ability within 6 weeks post-treatment. Nonetheless, females rejected these exposed males in favor of control males in female mate choice experiments (Hoffmann and Kloas, 2012b). Therefore, chronic exposure of males to EE2 could lead to long-term disruptions in reproductive success.

Co-administration of EE2 along with either partial to full estrogen receptor antagonists, tamoxifen and ICI 182, 780, respectively, abolished the effects on mate calling behavior in *X. laevis* (Hoffmann and Kloas, 2012a). These results are suggestive that the effects of this chemical are directly through targeting neural ERs (ESR1, ESR2, and/or G protein-coupled estrogen receptor 1, GPER). Vocal synapses are essential for mate calling (Kelley, 1980). Acute exposure to estradiol dampens this response; whereas chronic exposure strengthens laryngeal synapses, suggesting that proper concentrations of estradiol are essential for maintenance (Wu et al., 2001). EDC exposure may thus disturb mate calling both through direct effects on the brain and laryngeal suppression.

Another study examined the effects of estradiol (3 µg/L or 10 nM) in male and female *X. tropicalis*, which were then treated with a GnRH agonist (Schwendiman and Propper, 2012). Males exposed to estradiol showed increased approaches, touches, amplexus, and overall sum of sexual behaviors, namely increased incidence of arm waving (a potential pheromone releasing behavior). On the other hand, estradiol increased male calling behaviors compared to the unexposed group, but no effects were observed in females (Schwendiman and Propper, 2012).

In túngara frogs (*Engystomops pustulosus*), estradiol alone can stimulate the expression of female sexual responses (phonotaxis behavior) to male mate calls (Chakraborty and Burmeister, 2009). Females of this species exposed to mate choruses for 10 consecutive nights demonstrate increased estradiol concentrations, suggestive that social modulation of estradiol concentrations may maintain a female's reproductive state while males are chorusing (Lynch and Wilczynski, 2006). Sexually dimorphic expression of ESR1, ESR2, and AR has been identified in male and female túngara frogs that might account for the neural differences in hormonal sensitivities to these hormones (Chakraborty and Burmeister, 2010).

6. Effect of select EDCs in aquatic reptiles

Like the fishes and amphibians discussed above, aquatic reptiles similarly respond to environmental contaminants. For example, with exposure to contaminants, fish (Goksoyr and Forlin, 1992) and turtles (Rie et al., 2000) both upregulate hepatic detoxification enzymes. Vitellogenin production has also been observed in male frogs and turtles (Palmer and Palmer, 1995) exposed to xenobiotic estrogens. However, reptiles possess many unique traits, which make them particularly useful in studying the effects of EDCs. First, most reptiles have temperature-dependent sex determination (Bull, 1980) providing a novel and useful way to gauge exposure to environmental estrogens as these contaminants may override the temperature control, producing females at otherwise male temperatures. Second, most aquatic reptiles are oviparous, laying a large number of yolk-rich eggs on land. The eggs not only provide an easy way to measure exposure to lipophilic chemicals, but are also a link between aquatic and terrestrial habitats. Because of these features, aquatic reptiles provide a unique opportunity for research and may act as sentinels of overall ecosystem health. Of the aquatic reptiles, turtles may be a preferred group because of their relatively small size, life stages across environments (e.g., water, sediment, land), and long life spans. Additionally, an older reproductive age and strong site fidelity make aquatic turtles especially sensitive to environmental contaminant impacts and useful as sentinels of EDCs and other toxicants (Irwin and Irwin, 2006).

EDCs have been demonstrated to cause changes in a number of aquatic reptile species, especially in crocodilians and turtles. However, because this is a relatively young field as compared with fish and mammals, few studies have examined BPA and EE2 and many

of the mechanisms underlying the effects of EDCs remain largely unknown. Thus, we will draw on studies of some other EDCs to illustrate general changes in the endpoints of interest, and further studies may reveal such changes also hold for BPA and EE2.

6.1. Sex reversal and sex-related physiological effects

We can readily assess some effects of xenoestrogens by examining hatchling sex ratios. Embryonic gonadal development occurs during the temperature sensitive period (TSP) of egg incubation (Mahmoud et al., 1973; Yntema, 1968). It is the temperature of the nest during this specific time period that determines the resulting sex of the embryo. However, many studies have shown that environmental EDCs can override the effect of temperature on gonadal differentiation (Reynaud and Pieau, 1985; Wibbels et al., 1993).

In ovo exposure of alligators (*Alligator mississippiensis*) to 0.1 mg/kg EE2 during the TSP resulted in significantly more females than males at male producing temperatures (Matter et al., 1998). Similarly, caiman (*Caiman latirostris*) eggs topically exposed to 90 µg E2/egg or 9 mg BPA/egg resulted in 100% sex reversal of gonads and external genitalia (Stoker et al., 2008). Sex reversed gonads were not identical to true temperature-induced female ovaries, but were characterized by a medulla with lacunae and a cortex with follicles and oogonia. In this same study, lower doses (0.9 µg/egg E2 or 90 mg/egg BPA) did not result in sex reversal, but interestingly resulted in disorganized seminiferous tubules (Stoker et al., 2008). Thus, even low doses may impair sexual reproduction.

Currently, there are no published accounts of BPA or EE2 causing sex reversal in turtles. However, *in ovo* exposure to other EDCs has produced these effects. For example, Wibbels et al. (1991, 1993) produced phenotypically female red-eared sliders (*Trachemys scripta*) at male-producing temperatures after exposing eggs to 17β-estradiol. Sex reversal was also reported after exposure to pesticides including chlordane and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) (Willingham and Crews, 1999), Aroclor 1242 (Willingham and Crews, 1999; Willingham et al., 2000), and polychlorinated biphenyls (PCBs) (Bergeron et al., 1994). The percentage of sex-reversed females increased when multiple PCBs were applied simultaneously even at low doses of 10 µg/egg (Bergeron et al., 1994) indicative of interactive effects between chemicals.

Developing turtle eggs appear to be very sensitive to exposure to estrogen and estrogenic chemicals. Very low doses of E2 (400 pg/egg or 40 ng/kg egg weight) topically applied to eggs during the onset of the TSP resulted in sex reversal in 14.4% of the hatchlings (Sheehan et al., 1999). The authors of this study developed a biologically based dose response model and concluded that there is no threshold dose for sex reversal, but that exposure to any exogenous E2 produces some level of risk (Sheehan et al., 1999). Moreover, temperature and EDCs may interact. For example, exposure to the pivotal temperature (50% male: 50% female) and simultaneous exposure to atrazine (0.5 ppm or mM atrazine) resulted in more female hatchlings than when turtles experienced either the pivotal temperature or atrazine alone (Willingham, 2005). Thus, the effects of EDCs need to be considered with respect to micro- and macro-habitat climate change.

Exposure to EDCs can have far more insidious effects than sex reversal by affecting endogenous hormones. For example, *Trachemys* eggs incubated at the pivotal temperature and exposed to aroclor 1242 (67.8 µg/L or 0.26 µM) and chlordane (89.9 µg/L or 0.22 µM) produced male hatchlings with decreased testosterone (Willingham et al., 2000). In a study examining the impacts of environmental contaminants on juvenile American alligators it was demonstrated that basal mRNA expression of inhibin and follistatin was reduced and aromatase and follistatin mRNA did not

increase, as expected, following a follicle-stimulating hormone challenge (Moore et al., 2010).

6.2. Data from select field studies

Ecotoxicological studies of turtles have correlated fitness-related parameters with contaminant concentrations in the field (Hopkins, 2006). Here we provide a few examples for illustrative purposes. Snapping turtles (*Chelydra serpentina*) sampled from the Hudson River, NY exhibited decreased egg weight and a strong correlation between PCB concentrations in maternal plasma and concentrations in eggs from within the contaminated area, but no effect on phallus size (Kelly et al., 2008). Eggs from that same site also had decreased egg lipid content and lower survival at 9 months post-hatch (Eisenreich et al., 2009). Map turtles (*Graptemys flavimaculata*) from the Pascagoula River contaminated with polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and PCBs exhibited altered estradiol patterns, higher VTG, and fewer second clutches (Shelby-Walker et al., 2009). Similarly, painted turtles (*Chrysemys picta*) from a Massachusetts Superfund site had reduced estradiol and VTG in females, but they observed no changes in reproductive hormones in males (Rie et al., 2005). Taken together, these studies would suggest PCB exposure in the field can have direct fitness consequences. The same may be true with field exposure to BPA and EE2.

6.3. Contaminant effects on behavior

Although some literature exists for the effects of contaminants on aquatic reptile behavior across a number of taxa including righting responses (Burger, 1994) and swimming performance (Neuman-Lee and Janzen, 2011), we were unable to identify any studies that documented direct effects of EDCs on specific mating behaviors. This gap in our understanding may be due to the relative difficulty in observing mating in the field (i.e. the animals are submerged during courtship and mating) and the delayed onset in reproductive maturity making laboratory studies prohibitively costly. However, there are some interesting behavioral endpoints to consider which may be affected by EDCs. One such endpoint might lie in the initial courtship stage. In many species of aquatic turtles, the male swims in front of the female and uses his sexually dimorphic longer foreclaws to stroke the female's face (Ernst and Lovich, 2009). If this behavior is driven by testosterone levels, decreased testosterone from exposure to EDCs may alter the pattern with which this behavior occurs or the duration, ultimately affecting mating success. Following courtship, the male and female turtles move to the bottom of the river or pond to copulate. Although phallus size was not seen to be smaller in one species of turtle (Kelly et al., 2008) exposed to polychlorinated biphenyls, it was shown in a study of alligators exposed to organochlorines (Gunderson et al., 2004). A smaller phallus might prohibit smaller males from being able to mount and successfully inseminate females. Finally, it is possible, that EDCs may not only cause sex reversal of the gonad, but lead to male-male pairings as has been shown with methylmercury exposure in white ibises (Frederick and Jayasena, 2011) and in atrazine-exposed frogs (*Xenopus laevis*, (Hayes et al., 2010)).

6.4. Underlying mechanisms in aquatic reptiles

The mechanisms by which EDCs exert effects on aquatic reptiles are likely similar to those discussed above for fish and amphibians. These may include direct interaction with hormone receptors as either agonists or antagonists; alteration of hormone synthesis, secretion, or bioavailability; and modifications in genes playing a significant role in reproduction.

For more than 20 years, Dr. Louis J. Guillette's group has provided robust evidence of the effects of PCBs and pesticides on the alligators of Lake Apopka and Lake Okeechobee in Florida. Much of this research is summarized elsewhere (Milnes and Guillette, 2008). In general, his lab has found decreased aromatase in females (Crain et al., 1997) and decreased testosterone (Guillette et al., 1999), increased baseline corticosterone (Gunderson et al., 2003) and smaller phallus size (Gunderson et al., 2004) in males. Contaminant exposure is not just decreasing hormone production, but also the ability of hormones to bind. Juveniles of both sexes exhibited decreased ESR2 and increased AR mRNA (Moore et al., 2010).

Efforts to understand mechanisms in turtles have only just begun. Adult female and male neonate painted turtles (*C. picta*) exposed in the lab to sediments from a Superfund site showed increased hepatic ESR1 expression (Marquez et al., 2011). In green sea turtles (*Chelonia mydas*), DDT was identified as having a possible effect on the ability of proteins to bind testosterone (Ikonomopoulou et al., 2009) thus potentially decreasing its half-life in plasma. Even if plasma concentrations remain the same, the efficacy of endogenous hormones may be altered. In the only published study regarding a mechanism for BPA exposure in turtles, researchers determined that BPA is interfering with estradiol metabolism (Clairardin et al., 2013). Recently laid and BPA-treated (40 µg/egg) *Trachemys* eggs had higher concentrations of yolk estradiol and estrone and lower concentrations of estrone sulfate than untreated control eggs. This suggests that early in development BPA is changing the metabolism of maternal estrogens and possibly making them bioavailable during later times in development including the TSP.

As illustrated above, much work is still needed with regard to not only mechanisms, but the effects of different EDCs on reproductive endpoints in reptiles. Although BPA and EE2 are prevalent in aquatic ecosystems and persistent in the sediments where turtles burrow and forage, little data have been collected as to their reproductive and physiological effects. We suggest that with the recent publication of the full genome of painted turtles (*C. picta*; (Shaffer et al., 2013)), this species is a good model organism to elucidate underlying mechanisms of EDCs in turtles and perhaps other reptiles given recent interest in reptilian genomes (Shaffer et al., 2013). With the worldwide decline in turtle populations (Rhodin et al., 2011), these data can be of vital importance to conservation of the taxa. Additionally, turtles bridge the aquatic-terrestrial interface and can be a non-piscine bioindicator of aquatic and riparian health.

7. Effect of EDCs in aquatic mammals

While it is implausible to perform controlled laboratory studies with many aquatic mammals, there are select epidemiological reports of EDC concentrations and correlative effects in these species. However, there is a paucity of data linking BPA or EE2 and health effects in aquatic mammals. For illustrative purposes, we will thus consider other studies that have examined the effects of environmental estrogens and anti-estrogens, including organochlorines and PCBs, on aquatic mammals, including whale and dolphin populations where such compounds are readily detectable (Muir et al., 1996a,b; Schantz et al., 1996), reviewed in Porte et al. (2006). A potential link between pseudohermaphroditism and PCBs has been suggested in bowhead (*Balaena mysticetus*) and beluga (*Delphinapterus leucas*) whales (Muir et al., 1996b; Tarpley et al., 1995). For cetacean species, benzo(a)pyrene monooxygenase (BMPO) and CYP1A1 may serve as reliable biomarkers for DDT, organochlorines, and possibly exposure to other EDCs (Fossi et al., 1992, 2003). Dichlorodiphenyltrichloroethane (DDT) and PCBs have also been implicated in a sharp population decline in

seals in the Baltic Sea. These seals had a high incidence of reproductive tract abnormalities, including uterine leiomyomas, colonic ulcers, and adrenocortical hyperplasia suggesting a possible role for EDCs (Bergman, 1999).

Organophosphate EDCs have also been linked to a decrease in penis weight in river otters (*Lontra canadensis*) (<http://www.chemtrust.org.uk/documents/Otter%20Health%20&%20Pollutants%20V8%20DesignedV4%20FINAL.pdf>; <http://megasoftware.net/>). A more recent study demonstrated that 72% of 235 wild Eurasian otters (*Lutra lutra*) from Sweden examined possessed remnant Müllerian duct cysts on the spermatic duct (vasa deferentia) (Roos and Agren, 2013). While not definitively assayed, exposure to EDCs was postulated to be one likely cause for incomplete regression of the female reproductive tract in these males. Overall, effects of EDCs on aquatic mammals have been reported, but this is an area in need of more extensive research.

8. Potential for EDCs to induce epigenetic/gene disruption in a wide range of species

Microarray gene expression analyses show the significant shift both BPA and EE2 make in expression of thousands of genes in the gonads of a number of laboratory vertebrate models (Bredhult et al., 2009; Duan et al., 2010; Heimeier et al., 2009; Imanishi et al., 2003; Kishi et al., 2008; Naciff et al., 2002). In the hypothalamus of the female rat, neonatal BPA treatment diminished *Kiss1* expression (Cao et al., 2012). Maternal exposure to a complex cocktail of endocrine disruptors, including BPA, suppressed fetal hypothalamic *Kiss1* mRNA expression in the sheep and induced reproductive health consequences later in life (Tena-Sempere, 2010). *KISS1* is an essential upstream component of the neuroendocrine system regulating reproduction in vertebrates. Expression of aromatase (*Cyp19a1a*), the enzyme that catalyzes the conversion of estrogen from androgens, is significantly reduced by BPA exposure in the JEG-3 trophoblast cells (Huang and Leung, 2009). In the mouse brain, BPA (50 mg/kg/day) treatment increased the expression of aromatase (Chung et al., 2011) and *GnRH* *in vivo* (Xi et al., 2011), but decreased the expression of *GnRH I* in *GnRH* expressing cells *in vitro* when treated with the concentration of 200 μ M (Warita et al., 2013). In the embryonic stem cells, BPA treatment induced meiotic marker gene (*Stra8*, *Sycp3*) expression along with up-regulation of ovarian markers (*Foxl2* and *Wnt4*) and suppression of testicular markers (*Sox9* and *Fgf9*) (Aoki and Takada, 2012). These observations suggest that BPA affects testicular and ovarian development as well as germ cell differentiation in developing mammals as highlighted above in fish gonadal development (Section 3).

BPA has been shown to affect implantation and organogenesis in a number of species. *Hox10a* gene expression was elevated in the reproductive tract of embryos by BPA injection in CD1 mice, whereas the post-implantation loss caused by neonatal exposure of male rats to BPA involved suppressed expression of DNA methyltransferase genes (*Dnmts*) and related transcription factors in resorbed embryos (Doshi et al., 2012). In the frog, BPA downregulated genes in the thyroid hormone network required for metamorphosis (Heimeier and Shi, 2010).

Environmental estrogenic chemicals increase the expression of female specific genes in females and suppress male specific genes in males (Villeneuve et al., 2012). Additionally, they induce the expression of female specific genes in the male tissue and thus are considered the biomarkers of exposure. Examples of such anomalies are the induced expression of *Cyp19a1* gene in the rat Leydig cells (Kim et al., 2010) and testis of medaka (Scholz and Gutzeit, 2000), and of *Vtg* in a number of vertebrate species. In egg laying vertebrates, both BPA and EE2 increase the expression of *Vtg I* gene in the liver of birds (Lorenzen et al., 2003), turtles (Custodia-Lora et al., 2004; Tada et al., 2008), frog (Bai et al.,

2011), and fish (Ferreira et al., 2013; Schmid et al., 2002). These biomarker expression studies involve invasive techniques. Hayashi et al. (Hayashi et al., 2007) found the environmentally-relevant concentration of BPA (0.23 and 2.3 μ g/L; or 1 and 10 nM) to induce *ESR1* expression on the anal fin of female medaka, suggesting medaka anal fins may be a sensitive bioindicator for screening of environmental estrogenic chemicals. Biomarker gene expression in the fin, mucus, blood, urine, or scales could provide fast and reliable prediction of endocrine disruption and would not involve invasive surgical procedures.

8.1. Epigenetic changes induced by BPA/EE2 exposure-potential unifying EDC-induced disruption across taxa

Epigenetics is the study of heritable changes in gene expression occurring without changes in DNA sequence (Jaenisch and Bird, 2003). Given that developmental BPA/EE2 exposures cause adult onset diseases, these effects are thought to be mediated, at least in part, through epigenetic mechanisms (Cortessis et al., 2012; Singh and Li, 2012). Epigenetic mechanisms include DNA methylation, histone modifications, and expression of non-coding RNAs (including microRNAs). DNA methylation has been considered a key player in mediating environmental signals. Genetically engineered agouti mice (A^{vy}) have been considered a reliable model for studying environmental estrogen-induced adverse health outcomes and related DNA methylation (Dolinoy et al., 2007) and histone modifications (Dolinoy et al., 2010). In this mouse model, coat color development toward yellow is linked to adverse health outcomes, e.g. obesity. Studies by Dolinoy et al. (2007, 2006) found that maternal exposure to BPA (50 mg/kg feed) favored the birth of greater numbers of yellow, presumably more unhealthy mice with a hypomethylated intra-cisternal A particle (IAP), whereas the phytoestrogen genistein [250 mg/kg feed weight (fw)] caused a shift in coat color balance toward brown and more healthy offspring. However, Rosenfeld et al. (2013) repeated these same experiments by treating mice with BPA alone and in combination with genistein within the “no observable adverse effect level (NOAEL)” and found that none of the diets provided any significant differences in relative numbers of brown, yellow, or intermediate coat color $A^{vy/a}$ offspring (Rosenfeld et al., 2013). These conflicting findings warrant further validation of this animal model particularly for BPA and EE2 related epigenetic research.

The ability of estrogens to modulate epigenetic control of gene regulation has been well demonstrated. Studies have found that, in normal healthy cells, estrogen receptor signaling induces transient formation of multiple DNA loops in the chromosome 16p11.2 region by bringing 14 distant loci to focal *ESR1*-docking sites for coordinate repression (Hsu et al., 2010). Replication dependent histone H2A isotype (H2ac) mediates regulation of estrogen receptor target genes by recruiting *ESR1* and facilitating the formation of a chromatin loop between the promoter, enhancer and 3'-untranslated region of the respective genes (Su et al., 2013). Estrogen regulation of *Esr1* expression involves interactions with methyl-CpG-binding protein 2 (MeCP2) and histone deacetylase (Fuks et al., 2003; Sharma et al., 2005; Westberry et al., 2010). These findings suggest the ability of estrogens to regulate transcriptional activity of its targets through epigenetic mechanisms.

Epigenetic alterations caused by BPA exposure are limited to “treatment and effect” studies and no mechanistic studies have been published so far, except for those in the Endocrine Disruptor Knowledge Base (Ding et al., 2010). The ability of BPA to alter gene transcription via estrogen receptors and DNA methylation predict the mode of epigenetic action to be similar. BPA generally causes a hypomethylation of the promoter that leads to an increased binding of estrogen receptor to their targets and to untimely activation of silenced genes during development that

may cause early onset of adult diseases (Bromer et al., 2010; Gore et al., 2011). In human breast epithelial cells, BPA exposure was found to suppress apoptosis of *Bcl2l11* via induction of hypermethylation of the promoter (Fernandez et al., 2012). Bisphenol A exposure during early stages of imprinting in mice caused a decrease in DNA methylation of imprinted genes, mainly *Igf2r*, *Peg3* and *H19* in the fetal mouse germ cells (Zhang et al., 2012), whereas exposure during late stages of oocyte development and early stages of embryonic development significantly disrupted imprinted gene expression in embryonic day 9.5 and 12.5 embryos and placentas and these alterations were in parallel with alterations of DNA methylation of the imprinting control region (Susiarjo et al., 2013). The affected genes included *Snrpn*, *Ube3a*, *Igf2*, *Kcnq1ot1*, *Cdkn1c*, and *Ascl2*. Mutations and aberrant regulation of these genes are associated with imprinting disorders in humans.

Information on epigenetic studies in lower vertebrates is limited. A two-week exposure of adult zebrafish to EE2 (100 ng/L or 0.33 nM) increased *Vtg1* expression and decreased the DNA methylation levels on 5' region of the gene in the liver in both females and males (Stromqvist et al., 2010), whereas estradiol-17 β treatment did not make any change to *Cyp19a1a* promoter in fish with temperature dependent sex determination (Navarro-Martin et al., 2011). In the gonads of alligators (*A. mississippiensis*), male-producing temperature (33.5 °C) caused hypermethylation of *Cyp19a1a* promoter, and female producing temperature (30 °C) hypermethylation of *Sox9* promoter (Parrott et al., 2014b). However, no difference in global DNA methylation levels was found in whole blood and ovaries of juvenile alligators collected from three different contaminated sites (Parrott et al., 2014a). Contractor et al. (2004) measured ESR1 methylation in various tissues of EE2 (500 ng/L or 1.69 nM) treated medaka and found aberrant ESR1 promoter CpG island methylation which did not correlate with tissue specific expression of ESR1 gene.

A vast amount of literature is available showing DNA methylation pattern in a whole embryo or whole organ or a dissected portion of a tissue. DNA methylation profile is highly variable, with a remarkable variation even in a homogeneous population of the cell (Kantlehner et al., 2011). Examining DNA methylation of a promoter of the cell-specific gene in a tissue does not necessarily reflect the cell-specific pattern of DNA methylation. The reason is that a tissue is composed of a heterogeneous population of cells and there are several cell types that do not express the gene of interest. For transcriptional repression of the same gene in other non-expressing cells, DNA methylation and other epigenetic silencers are engaged. In such a case, data represents DNA methylation on the promoter of the gene of interest in both target cell and the non-target cell where gene transcription is repressed. Such analyses usually take a huge amount of background noise of DNA methylation into account. Therefore, future epigenetic analyses should be conducted in homogeneous cell populations if at all possible.

8.2. Epigenetic transgenerational inheritance of phenotype

There is tremendous interest in the potential for environmentally induced alterations to pass to subsequent generations. There are many reports that EDCs can induce transgenerational diseases in laboratory models (Anway et al., 2005; Crews et al., 2012, 2007; Doyle et al., 2013; Guerrero-Bosagna et al., 2012, 2010; Jirtle and Skinner, 2007; Manikkam et al., 2012a,b, 2013; Nilsson et al., 2012, 2008; Skinner et al., 2008, 2013, 2012; Tracey et al., 2013; Wolstenholme et al., 2012) and epidemiological information further supports the fact that this phenomenon is already in place in humans (Painter et al., 2008). By definition, epigenetic transgenerational inheritance implies that altered epigenetic

changes already in parents' germ cells are transmitted to its offspring, whose cells were not directly exposed. In mammals, only epigenetic marks transmitted to the F3 generation are truly transgenerational, whereas in egg laying species, epigenetic marks transmitted to the F2 generation are considered transgenerational. Most studies performed to test the ability of EDCs to induce transgenerational diseases used laboratory rodents; therefore mechanisms presented are based on findings in mice and rats.

Primordial germ cells (PGCs) are precursors to eggs and sperm. They undergo epigenetic reprogramming at the time of male sex determination (Sasaki and Matsui, 2008). A global erasure of DNA methylation marks gives rise to a stem cell state for PGCs and *de novo* methylation starts allowing a controlled gene expression pattern in germ cells in a sex specific manner (Hajkova et al., 2002). Any perturbations of global epigenetic reprogramming events in PGCs have reproductive consequences later in life and adverse health outcomes in descendants (Skinner, 2007, 2011; Skinner et al., 2013). Epigenetic alterations can cause a global shift in the epigenome and associated transcriptome, and these shifts can be permanently programmed and transmitted to subsequent generations via both sperm (Skinner, 2007, 2011; Skinner et al., 2013) and eggs (Morgan et al., 1999; Skinner et al., 2013). Environmental EDCs can influence epigenetic programming of germ cells with adverse outcomes in later generations. Examples of such transgenerational effects are: early onset of puberty in females, reduced sperm number, polycystic oocytes, prostate disease, kidney disease, and behavioral abnormalities (Anway et al., 2005; Crews et al., 2012, 2007; Doyle et al., 2013; Guerrero-Bosagna et al., 2012, 2010; Guerrero-Bosagna and Skinner, 2014; Jirtle and Skinner, 2007; Manikkam et al., 2012a, b, 2013; Nilsson et al., 2012, 2008; Nilsson and Skinner, 2014; Skinner et al., 2008, 2013, 2012; Tracey et al., 2013; Wolstenholme et al., 2012). It is notable that many studies reviewed here used pharmacological doses of estrogenic chemicals to induce transgenerational phenotype. It will be important to determine if environmentally relevant concentrations of estrogenic chemicals are able to induce similar transgenerational phenotypes and epigenetic changes.

8.3. Non-coding RNAs

The role of non-coding RNA (ncRNA), including microRNA (miRNA), in epigenetic control of gene transcription and epigenetic transgenerational disease onset is poorly understood. A recent study by Rechavi et al. (Rechavi et al., 2014) highlighted the involvement of miRNAs in transgenerational transmission of acquired traits in *Caenorhabditis elegans* induced by a week-long food deprivation. All miRNAs that were transgenerationally transmitted belonged to the subfamily that controls the expression of genes related to nutrition. Given that the environmental estrogens are able to induce transgenerational abnormalities in vertebrates via epigenetic mechanisms, it will be interesting to examine the role of miRNAs in mediating transgenerational transmission of altered traits and their exposure specificity in organisms across taxa. In the sheep, prenatal BPA treatment altered fetal ovarian microRNA expression of relevance to gonadal differentiation, folliculogenesis, and insulin homeostasis (Veiga-Lopez et al., 2013). BPA exposure elevated overexpression of miR-146a in placental cell lines, which was associated with slower cell proliferation and higher sensitivity bleomycin-induced DNA damage (Avisar-Whiting et al., 2010). Newer techniques to measure miRNA expression have recently evolved (Meng et al., 2013). By using a microarray approach, Ma et al. (2012) revealed several maternal miRNAs in the rainbow trout egg. Among these unique miRNAs, *Let7* and *miRNA-21* were dominantly expressed, while other known miRNAs which were abundantly expressed included *miR-24*, *miR-202*, *miR-148*, *miR-30*, *miR-10*, *miR-146*, *miR-25*, and *miR-143*

(Ma et al., 2012). A repertoire of egg and sperm miRNAs will ultimately provide grounds for further investigation of parent of origin in transgenerational inheritance of phenotype.

9. Human health effects

9.1. Human health trends

There has been a dramatic increase in many human endocrine, reproductive and metabolic diseases over the last 40 years. Endocrine and reproductive tumors, including breast, endometrial, ovarian, prostate, thyroid and testicular cancers, and metabolic diseases, including obesity, hypertension, diabetes and heart disease have increased 2 to 3-fold since the 1970s. While all of these diseases are multifactorial, mounting evidence suggests that exposure to EDCs during development and in adulthood is a risk factor.

9.2. Developmental exposure to EDCs and reproductive health and cancer in adulthood

Much research supports the idea that the most sensitive window of exposure to EDCs is during development—both prenatal and during childhood. One of the best examples of developmental exposure to a xenoestrogen remains that of children whose mothers took the pharmaceutical estrogen diethylstilbestrol (DES) during pregnancy during the 1940s until 1971 in a mistaken attempt to prevent miscarriage. (Barnes et al., 1980; Herbst et al., 1979, 1971; Robboy et al., 1982). The children, termed “DES Daughters and Sons”, have a wide range of long-term negative consequences (Barnes et al., 1980; Herbst et al., 1979, 1971; Robboy et al., 1982). Daughters have an increased risk of breast cancer (Palmer et al., 2006), fibroids, endometriosis, vaginal cancer, early menarche (Hatch et al., 2011), early menopause (Hatch et al., 2006), vaginal and cervical squamous cell neoplasia (Hatch et al., 2001), reproductive tract abnormalities and negative pregnancy related outcomes, including preeclampsia (Troisi et al., 2007), ectopic pregnancy, miscarriage, premature birth, neonatal death and small size for gestational age babies (Hatch et al., 2011). Although the third generation is still relatively young, an assessment of DES granddaughters suggests increased risk of birth defects (Titus-Ernstoff et al., 2010), infertility (Titus-Ernstoff et al., 2006), and ovarian cancer (Titus-Ernstoff et al., 2008) that will require further study.

In men, the Testicular Dysgenesis Syndrome is a prevailing hypothesis that developmental exposure to EDCs is associated with declining semen quality and increasing rates of hypospadias and testicular cancer (Skakkebaek et al., 2001). Although less studied, DES sons have an increase in urogenital anomalies, such as hypospadias, and a possible increased risk of testicular cancer (Swan et al., 2000). While long-term studies of perinatal exposure to BPA and adult reproductive health are lacking, prenatal BPA exposure has been correlated with reduced anogenital distance (AGD) in babies (Li et al., 2011). Importantly, AGD is positively associated with semen quality in adulthood, which suggests that developmental exposure to BPA in humans may result in decreased semen quality in adulthood (Mendiola et al., 2011).

Several studies have shown that oral contraceptive use during pregnancy is associated with increased risks for birth defects in children (Chen et al., 2009; Janerich et al., 1980; Leite et al., 2002; Rothman and Louik, 1978; Smithells, 1981), though a large meta-analysis yielded conflicting results (Bracken, 1990). Developmental exposure to EE2 has been associated with many adverse reproductive outcomes in fish (Caldwell et al., 2008), and increased prostate size and decreased sperm production (Thayer et al., 2001) and altered mammary gland development (Shiorta

et al., 2012; Wadia et al., 2013) and folliculogenesis in mice (Nagel unpublished). Rodent studies suggest that perinatal exposure to BPA and EE2 can alter AGD, reduce fertility and pubertal age, increase reproductive abnormalities, and cause other adverse reproductive health outcomes (Honma et al., 2002; Richter et al., 2007a; Ryan et al., 2010).

In addition to exposure to xenoestrogens, the human fetus is also sensitive to endogenous fetal steroidal estrogens. Increased endogenous estrogens have been associated with disease in adulthood including breast cancer and endometriosis (Cerhan et al., 2000; Ekblom et al., 1997; Missmer et al., 2004). Taken together, it is clear that the developing human fetus is sensitive to both endogenous and exogenous estrogen and exposure can result in many long-term negative consequences. Importantly most of the adverse human health outcomes associated with developmental DES exposure have been recapitulated in rodent laboratory studies. Rodent studies have reported a plethora of adverse health outcomes from perinatal exposure to BPA, including but not limited to increases in mammary and prostate cancers, reproductive developmental issues, obesity, diabetes, reduced age of pubertal development, and neurological developmental issues (Acevedo et al., 2013; Diamanti-Kandarakis et al., 2009; vom Saal et al., 2007). This demonstrates the applicability of these models to human hazard assessment for other xenoestrogens and also suggests broad species conservation of these developmental pathways (Richter et al., 2007a; vom Saal et al., 2005).

9.3. Developmental exposure to EDCs and altered neurodevelopment and behavior

Altered behavior in children has been associated with perinatal BPA exposure, and laboratory studies suggest neurodevelopment and subsequent behavior are some of the most sensitive endpoints of EDC exposure. Higher mean maternal urinary BPA concentrations have been correlated with increased externalizing behaviors such as hyperactivity and aggression in girls; whereas, BPA concentrations during the first trimester were correlated with increased externalizing scores in girls and boys (Braun et al., 2009). Prenatal BPA exposure has also been correlated with greater anxiety and depression, and reduced emotional control and inhibition in three-year-old girls (Braun et al., 2011). Rodent studies support a cause and effect relationship between perinatal BPA exposure and altered brain physiology, structure, sexual differentiation, and behaviors such as aggression, hyperactivity, and parenting behavior (Richter et al., 2007a); and between perinatal exposure to EE2 and altered sexual behavior in rats (Ryan et al., 2010), and masculinized, sexually dimorphic, non-reproductive behaviors in mice (Ryan and Vandenberg, 2006).

9.4. EDC exposure and adult reproductive health

In men, BPA exposure has been correlated with altered hormone levels (Meeker et al., 2010a), decreased sperm counts, decreased motility and mobility (Li et al., 2011; Meeker et al., 2010b), and increased rates of self-reported infertility and reduced sexual desire (Li et al., 2010). In women, adult BPA exposure has been correlated with increased risks for recurrent miscarriages (Benachour and Aris, 2009; Sugiura-Ogasawara et al., 2005), endometriosis (Cobellis et al., 2009), and polycystic ovarian syndrome (Takeuchi et al., 2004).

9.5. EDC exposure and metabolic syndrome in adults

Rates of obesity, type II diabetes, heart disease, and hypertension in humans have risen over the last several decades, spurring research into the possible role of estrogenic chemicals in the

development of Metabolic Syndrome (obesity, cardiovascular disease, and diabetes) (vom Saal et al., 2012). Urinary BPA concentrations of children and adolescents have been correlated with higher rates of obesity (Trasande et al., 2012), and adult urinary BPA concentrations have been correlated with increased obesity and diabetes mellitus, independent of other traditional risk factors (Carwile and Michels, 2011; Lang et al., 2008; Shankar and Teppala, 2011). Urinary BPA levels have also been positively correlated with cardiovascular disease; angina, coronary heart disease, and heart attacks (Lang et al., 2008). A causative association is suggested by *in vitro* studies where 1 μM (228 $\mu\text{g/L}$) BPA blocked the human heart sodium channel, hNav1.5 (O'Reilly et al., 2012). Rodent studies have found that BPA treatment results in decreased insulin sensitivity and impaired glucose tolerance (Angle et al., 2013), increases in body weight, and cardiovascular abnormalities (Belcher et al., 2012; Richter et al., 2007a; vom Saal et al., 2012).

9.6. EDC exposure and cancer in adults

Lifetime exposure to estrogens is associated with increased risks of breast and prostate cancer (Clemons and Goss, 2001; Prins, 2008). BPA has been found to stimulate human breast cancer cell proliferation *in vitro* (Krishnan et al., 1993). Further, BPA concentrations were positively correlated with several risk factors for breast cancer in a study of Korean women (Yang et al., 2009) and establishment and maintenance of aggressive breast cancer tumors in an *in vitro* model following 100 nM (22.8 $\mu\text{g/L}$) BPA treatment (Dairkee et al., 2008).

Concentrations of BPA as low as 1 nM (0.228 $\mu\text{g/L}$) increase proliferation of prostate carcinomas with mutant ARs and may lead to relapse of androgen-independent prostate cancer in patients (Wetherill et al., 2005). In addition, 1–30 nM (0.228–6.84 $\mu\text{g/L}$) BPA resulted in greater proliferation rates and tumor growth when prostate cancer cells were grafted into mice (Wetherill et al., 2006). Recently, BPA has been reported to increase renewal of human prostate stem-progenitor cells and gene expression, suggestive that even exposure to low doses of this chemical can increase later prostate cancer risk (Prins et al., 2014). Rodent studies support that BPA may act as a complete mammary gland carcinogen, and exposure may also contribute to the development of prostate and testicular cancers (Acevedo et al., 2013; Keri et al., 2007; Richter et al., 2007a; Tharp et al., 2012). For EE2, epidemiological studies have reported increased risks of cervical cancer (Appleby et al., 2007), nonmalignant liver cancer (Rooks et al., 1979), and breast cancer (Kahlenborn et al., 2006) for women using oral contraceptives. Rodent studies show support for exposure to EE2 leading to the development of prostate and mammary cancers (de Assis et al., 2012; Thayer et al., 2001).

10. Conclusions and future perspectives

For over a half-century, xenoestrogens, such as BPA and EE2, have been mass-produced on a global scale (Vandenberg et al., 2009). In addition, the inability of wastewater treatment plants to remove these and excreted endogenous estrogens, has resulted in complex mixtures in aquatic sources. The continued production and persistence of these chemicals in the environment has resulted in xenoestrogens being detected now in almost all aquatic sources tested to date and in many cases at bioactive concentrations (Hoffmann and Kloas, 2012a).

It is therefore not surprising that some of the earliest reports that these chemicals had the ability to disrupt sexual development

originated from an aquatic species, i.e. alligators (Gunderson et al., 2004). Studies with alligators have confirmed a variety of effects from EDC exposure that include decreased aromatase in females (Crain et al., 2007) and decreased testosterone (Guillette et al., 1999), increased corticosterone (Gunderson et al., 2003) and smaller phallus size (Gunderson et al., 2004) in males. Mounting evidence suggests that these chemicals can induce similar sexual development disruptions in a wide range of aquatic species, including fish, amphibians, and reptiles. Consequently, distorted sex ratios are a potential bioindicator for regional environmental contamination (Guillette, 2000). Another potential risk that xenoestrogens have on the individual and population level is disruption of reproductive-associated behaviors. Heightened anxiety and shoaling behaviors have been reported in adult male zebrafish exposed to EE2 (Reyhani et al., 2011). Bisphenol A and other estrogenic EDCs have been reported to disrupt reproductive mating behaviors of other fish species (Soffker and Tyler, 2012; Ward and Blum, 2012). Estrogenized male frogs exhibit reduced and altered properties in the advertisement calls, indicative of an aroused state (Hoffmann and Kloas, 2012b). In species of reptiles, altered adult behaviors, including righting responses, (Morgan et al., 1999) swimming, and foraging performance (Morris et al., 2004) have been demonstrated with EDC exposure. Further work should be directed to determine if reproductive-related behaviors in reptiles are also under BPA/EE2 influence. For example, the behavior of male aquatic turtles to use their sexually dimorphic longer foreclaws to stroke the female's face may be driven by testosterone levels and thus vulnerable to these chemicals. In piscine and reptilian taxa, a number of impacts on physiology (e.g., hepatic enzymes, vitellogenin production) (Contractor et al., 2004; Palmer et al., 2006; Rie et al., 2005), reproduction (e.g., phallus size in alligators) (Greathouse et al., 2012), and fitness costs (e.g., lower post-hatch survival) (Doyle et al., 2013) have been shown to be associated with EDC exposure.

In contrast to humans and rodent models where currently no specific biomarkers of exposure to BPA or other estrogenic EDCs exist, *Vtg* is a useful biomarker of such exposure in fish, amphibians, and reptiles (Goksoyr, 2006; Marin and Matozzo, 2004; Porte et al., 2006; Sumpter and Jobling, 1995). Other potential candidate biomarkers of exposure include in fish: *ncl1*, *apoeb*, *mdm1*, *mycl1b*, *sp4*, *UISNRNPBP* homolog in fish (Lam et al., 2011); amphibians: *Ttr*, *Rbp*, hepatic *Hbp*, and *Dhcr7* (Tompsett et al., 2013; Urbatzka et al., 2007); and reptiles: *Sox9*, *sf1*, *wt1*, *mis*, *foxL2*, and *Rspo1*, which are associated with gonadal development (Shoemaker and Crews, 2009). While identification of these gene expression changes are useful in ecotoxicology, biomarkers of estrogen, including BPA and EE2, exposure across taxa, including humans, are essential to identify those populations at risk for various diseases due to exposure to these chemicals. Characterization of universal EDCs-induced epigenetic alterations, such as hyper- or hypo-DNA methylation changes in select candidate genes, may allow for development of diagnostic tests for humans and wildlife species. It is now apparent from a variety of rodent and fish studies that BPA and EE2 exposure leads to DNA methylation and gene expression changes that might account for reproductive and developmental abnormalities (Anderson et al., 2012; Bromer et al., 2010; Chao et al., 2012; Dolinoy et al., 2007; Doshi et al., 2012; Fernandez et al., 2012; Greathouse et al., 2012; Hanna et al., 2012; Ho et al., 2006; Jang et al., 2012; Prins et al., 2008; Tang et al., 2012; Weng et al., 2010; Yaoi et al., 2008; Zhang et al., 2012). Circulating miRNAs (including other ncRNAs) may be essential in epigenetic inheritance in mammals (Sharma, 2014). The fact that these ncRNAs can be identified in the serum also renders them as potential candidate biomarkers. Various miRNAs are altered in response to BPA or xenoestrogen exposure (Avisar-Whiting

et al., 2010; Meunier et al., 2012; Veiga-Lopez et al., 2013). Comparative studies that simultaneously examine BPA and other EDCs-induced epigenetic changes occurring in diverse aquatic and terrestrial species might help elucidate prospective diagnostic biomarkers that can be followed-up on in blood samples of human populations suspected of being at risk to EDCs exposure. These epigenetic changes might also govern transgenerational inheritance (Avisar-Whiting et al., 2010; Bannon et al., 2009; Bleck et al., 2013; Cui et al., 2012; Fukushima et al., 2007; Izzotti et al., 2011; Kure et al., 2013; Marczylo et al., 2012; Meunier et al., 2012; Rosenfeld, 2014; Sonkoly and Pivarcsi, 2011; Tilghman et al., 2012; Wilker et al., 2011; Zhang and Pan, 2009). Future studies are thus needed to screen the global sperm and oocyte epigenome in a wide-range of taxa to determine if commonalities exist in how BPA and EE2 might lead to transgenerational propagation.

Lastly, these aquatic animal models might serve as sentinels for human populations, who are also increasingly being exposed to these same chemicals. An understanding of the cross-species behavioral alterations and reproductive abnormalities might guide human epidemiological studies, where such phenotypic changes might serve as barometers of exposure. By dissecting out the potential underpinning mechanisms in comparative models, it may also yield potential preventative and remediation strategies in humans. There has been a surge of evidence linking BPA exposure and various human health outcomes, including increased incidence of several cancers, as well as cardiovascular, neurobehavioral, reproductive, and metabolic disorders. Future policy decisions on potential reductions and even elimination of exposure to BPA and other estrogenic chemicals in human and other animal populations is unlikely without definitive mechanisms in naturally exposed populations. In summary, the “One health, one medicine” approach might illuminate how BPA and EE2 lead to harmful effects across taxa and thereby provide key essential mechanistic data that can be exploited for diagnostic and therapeutic purposes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ygcen.2014.09.014>.

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