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Review

Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species

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ABSTRACT

Due to their versatility, robustness, and low production costs, plastics are used in a wide variety of applications. Plasticizers are mixed with polymers to increase flexibility of plastics. However, plasticizers are not covalently bound to plastics, and thus leach from products into the environment. Several studies have reported that two common plasticizers, bisphenol A (BPA) and phthalates, induce adverse health effects in vertebrates; however few studies have addressed their toxicity to non-mammalian species. The aim of this review is to compare the effects of plasticizers in animals, with a focus on aquatic species. In summary, we identified three main chains of events that occur in animals exposed to BPA and phthalates. Firstly, plasticizers affect development by altering both the thyroid hormone and growth hormone axes. Secondly, these chemicals interfere with reproduction by decreasing cholesterol transport through the mitochondrial membrane, leading to reduced steroidogenesis. Lastly, exposure to plasticizers leads to the activation of peroxisome proliferator-activated receptors, the increase of fatty acid oxidation, and the reduction in the ability to cope with the augmented oxidative stress leading to reproductive organ malformations, reproductive defects, and decreased fertility.

1. Introduction

Versatility, robustness, and low cost render plastics the material of choice for many applications. Polymers that are not processable or useful in their natural form are mixed with plastic additives. This broad category of chemicals includes flame retardants, surfactants, blowing agents, and plasticizers, amongst others. Plasticizers are the most common plastic additives and provide polymers with flexibility by lowering their glass transition temperature (Daniels, 2009), and due to today's large number of plastic applications, a greater variety of plasticizers exist. However, plasticizers are often not covalently bound to the plastic matrix, and thus can slowly diffuse out of plastics leading to wide environmental contamination (Demir and Ulutan, 2013; Fromme et al., 2002; Munksgaard, 2004; Nara et al., 2009). Plasticizers are ubiquitous and numerous studies have confirmed the presence of plasticizers and their metabolites in air, soil, water, and animal and human body fluids (Horn et al., 2004). Bisphenol A (BPA) and phthalates are amongst the most important plasticizers. Several studies have shown that these compounds induce endocrine toxicity to all levels of organization in mammals. This review aims to compare and contrast the effects of plasticizers in animals, with a special focus on aquatic species. The following sections present the pathways of entry of the main plasticizers in the environment and discuss their sublethal effects on the thyroid hormone (TH) and sex steroid axes, and highlight new research directions.

2. Plasticizer uses and pathways of entry into the environment

BPA is a high production volume plastic monomer and plasticizer. About 1,150,000 metric tons were produced in the Europe Union in 2005–2006 (reviewed in Oehlmann et al., 2008). BPA is a plasticizer of choice for its cross-linking properties (Alonso-Magdalena et al., 2006); however, after polymerization, unbound monomers that remain may be released into the environment (Brotons et al., 1995). The temperature (Tan and Mustafa, 2003), pH (Xu et al., 2011), and fat content (Lopéz-Cervantes and Paseiro-Losada, 2003) of materials or tissues in contact with plasticized polyvinyl chloride (PVC) can modulate BPA leaching. BPA

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has been shown to leach from various products, including dental sealants (Olea et al., 1996), tin cans (Brotons et al., 1995), and food contact items (Biles et al., 1997; Fasano et al., 2012; reviewed in Vandenberg et al., 2007). These exposure pathways lead to the detection of BPA in human adult and fetal serum (reviewed in Vandenberg et al., 2007), urine (Calafat et al., 2005; Hauser et al., 2007), breast milk (Sun et al., 2004), and adipose tissue (Fernandez et al., 2007). Following absorption, BPA is rapidly metabolized into inactive metabolites and excreted in urine. In addition, BPA enters the environment via open disposal or recycling of products containing BPA. The amount of BPA leaching from plastic wastes can be as high as 139 mg/kg (Yamamoto and Yasuhara, 1999). Sewage and plastic leachate then lead to contamination of surface waters, groundwater, and sediment (Fromme et al., 2002). BPA degrades in aerobic environments within a few days (Fig. 1A–F; Suzuki et al., 2004a), but does not degrade in anaerobic conditions (reviewed in Rykowska and Wasiak, 2006; and in Staples et al., 1998).

Phthalates have been used in PVC since 1926 to render it flexible, pliable, and elastic, but are now used in many other plastic types and at higher concentrations (Oehlmann et al., 2009). As with BPA, phthalates are not irreversibly bound to the matrix, and therefore diffuse and evaporate out of the polymer. Food (Bradley et al., 2013), food packaging (Cao, 2010; Fasano et al., 2012), alcoholic beverages (Leitz et al., 2009), PVC flooring (Carlstedt et al., 2012), cosmetics/personal care products (Sathyanarayana et al., 2008; Shen et al., 2007), blood/intravenous solution storage bags (Kim et al., 1976; Monfort et al., 2012; Štrac et al., 2013), and medicinal products/dietary supplements (Hernández-Díaz et al., 2009; Kelley et al., 2012) are all items known for releasing phthalates. Phthalate diesters have a central ring and two esters in common (Fig. 1G). *Ortho*-phthalate diesters have the esters on consecutive carbons and are the most abundant isomers, therefore the prefix *ortho* is generally not used. *Iso*-phthalate diesters have their R groups in position 1 and 3, while *tere*-phthalate diesters exhibit chains on opposite carbons. The alkyl chain length of the esters can vary greatly, which affects the properties of the phthalates. Longer chains have higher molecular weights, and tend to have longer retention times and slower migration rates in plastics. Consequently, the industry started replacing the shorter chained di-(2-ethylhexyl) phthalate (DEHP; 6-carbon chains) by the longer chain and less mobile diisononyl phthalate (DINP; 9-carbon chains; Koch et al., 2007). After uptake, *ortho*-phthalate diesters are rapidly degraded to phthalate monoesters (Fig. 1H) and are excreted in body fluids. Monoesters harbor one ester and one carboxylic acid on the aromatic ring. As common degradation end-products, these monoester metabolites are targeted for biomonitoring and for epidemiological studies (Aylward et al., 2009; Blount et al., 2000; Huang et al., 2007; Koch et al., 2007; Mazzeo et al., 2007; Mieritz et al., 2012; reviewed in Wittassek et al., 2011). Contaminated urine and other influents represent a significant source of phthalates in the environment because wastewater treatment plants do not effectively remove plasticizers from the effluent (Barnabé et al., 2008; Clara et al., 2010; Kusk et al., 2011; Soliman et al.,

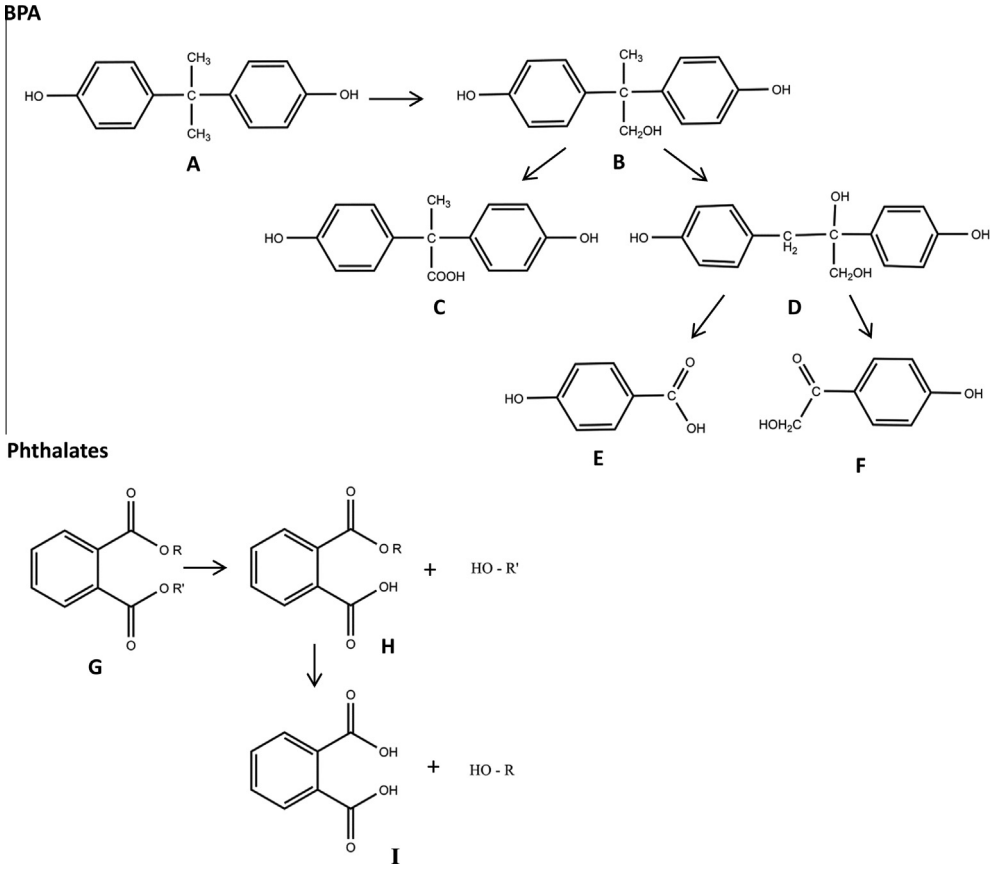

Fig. 1. Examples of biological degradation pathways of BPA and phthalates. (A) BPA consists of two aromatic rings with a hydroxyl group on either end. (B) One methyl group of BPA is oxidized to form 2,2-bis(4-hydroxyphenyl)-1-propanol (C) and further transforms to intermediate metabolites 2,2-bis(4-hydroxyphenyl)propanoic acid (D) and tetraol. (E) Tetraol is further degraded to 4-hydroxybenzoic acid (F) and 2-hydroxy-1-(4-hydroxyphenyl) ethanone. Adapted from Suzuki et al. (2004a). (G) *Ortho*-phthalate diesters have an aromatic ring and two esters on adjacent carbons. (H) Diesters are metabolised into phthalate monoesters and alcohol. (I) Further degradation yields phthalic acid and alcohol. The resulting alcohols are of added concern due to their high volatility. For most *ortho*-phthalates, the degradation is incomplete and results in a mixture of monoesters, alcohols and phthalic acid. Adapted from Horn et al. (2004) and Liang et al. (2008).

2007). Considering phthalates' ability to migrate from plastics, their widespread manufacture and their high concentration in plastics, disposal of plastics is a major source of environmental contamination via landfill leachate (Zheng et al., 2007) resulting in phthalates being ubiquitous in the environment (Blair et al., 2009; Fromme et al., 2002; Michael et al., 1984; Suzuki et al., 2001; Teil et al., 2007). For example, DEHP can be found in river sediment at levels as high as 110 mg/L (Horn et al., 2004). Phthalates have half-lives ranging from a few hours to a few months in surface water, marine water, and soil (reviewed in Staples et al., 1997). Therefore, phthalates can remain in aquatic ecosystems for long period of time, which can pose a risk to aquatic living organisms.

3. PPARs as central mechanisms of action

The transcriptional function of the peroxisome proliferator-activated receptors (PPARs) is believed to be the main mechanism of action behind plasticizer-induced transcriptional changes (Gazouli et al., 2002), reproductive organ toxicity (Hurst and Waxman, 2003, reviewed in Latini et al., 2008), oxidative stress (Lee et al., 2007), and mortality (Abbott et al., 2007). PPARs are nuclear receptor proteins that bind to specific DNA sequences, and regulate DNA transcription. PPARα regulates genes that control fatty acid degradation, PPARβ regulates genes that control fatty acid metabolism, and PPARγ regulates genes that control glucose levels (Berger and Moller, 2002). Studies have shown that both BPA and phthalates can alter the expression of PPARs in mammals (Hurst and Waxman, 2003) (Fig. 2; schematic reaction #4). In an *in vitro* study conducted by Phrakonkham et al. (2008), mouse embryo fibroblasts exposed to 18 mg/L BPA for two days exhibited an increase in *Pparγ2* expression. Likewise, *Pparα* and *Pparγ* transcripts were increased in DEHP treated rats (Xu et al., 2010). Mono-(2-ethylhexyl) phthalate (MEHP) also increased the expression of *PPARα* and *PPARβ* in human liposarcoma cells (Campioli et al., 2011), and upregulated PPARγ target genes in 3T3-L1 cells (Feige et al., 2007), but reduced the expression of *PPARγ* in liposarcoma cell line (Campioli et al., 2011). Similar protein expression alteration was reported in rats following exposure to monoester phthalates (Lee et al., 2007; Xu et al., 2010). For example, MEHP, monobenzyl phthalate (MBzP), and mono-*sec*-butyl phthalate (MBuP) all activate *PPARα* and *PPARγ* in the mouse, human, and 3T3-L1 preadipocytes (Feige et al., 2007; Hurst and Waxman, 2003; Maloney and Waxman, 1999). Since monoesters can also activate PPARs, phthalate metabolites may be the active ingredients in diesters' effects in PPAR molecular disruption. This could explain why phthalates that are harboring long alkyl chains and that slowly hydrolyze, such as DEHP, are less potent in affecting PPARs than their corresponding monoesters (Gray et al., 1983; Maloney and Waxman, 1999).

Phthalate's interaction with PPARs can disrupt the expression of downstream PPAR-related genes. PPARs can form heterodimers

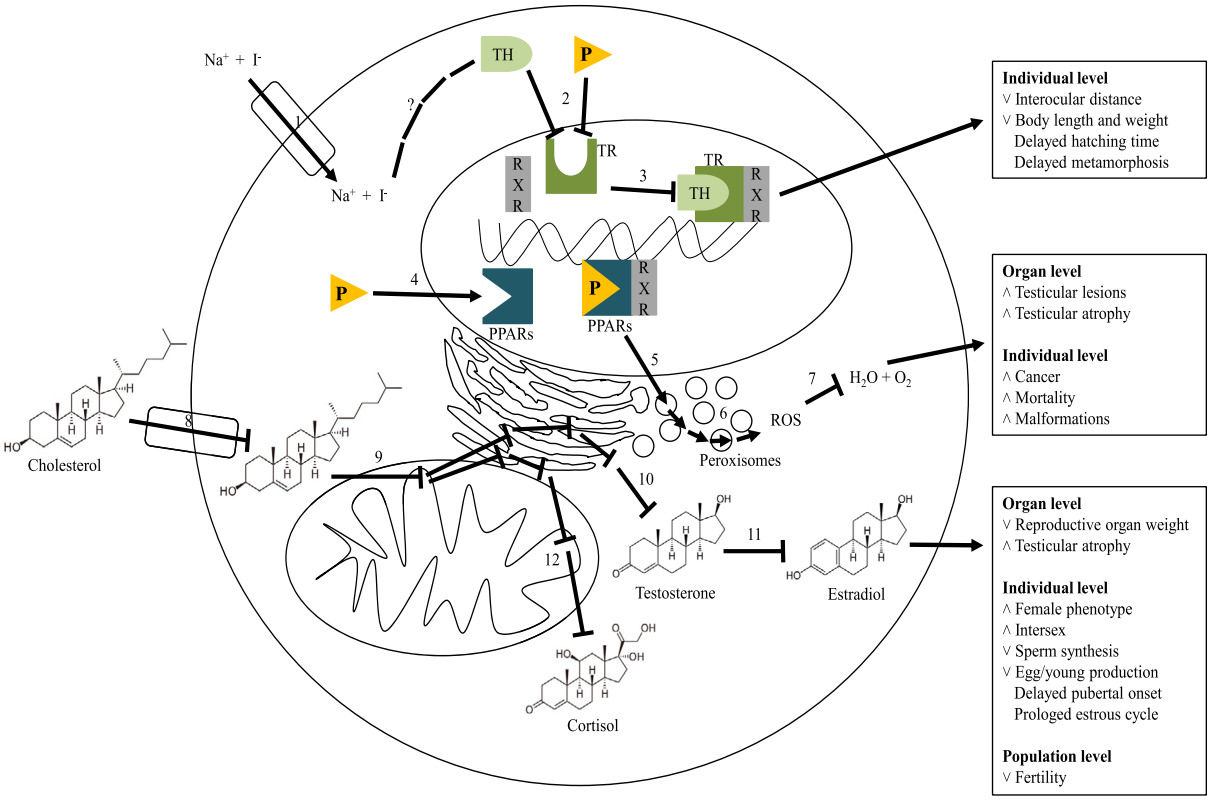

Fig. 2. Examples of the known effects and mechanisms of action of plasticizers (P), including bisphenol A (BPA) and phthalates (PAE), for each organisational level. (1) BPA/PAE increase the activity of the sodium/iodide symporter, leading to increased iodide uptake. Although iodide is necessary for thyroid hormone (TH) production, the effects of BPA/PAE on TH synthesis are unclear. (2) BPA/PAE impede with the binding of TH to thyroid hormone receptors (TR) by binding to TR themselves. (3) The gene expression of retinoid X receptor (RXR) is reduced by BPA/PAE, thus reducing the capacity to form heterodimers with TR. By doing so, the regulation of TH-related genes is disrupted, leading to effects at the individual level. (4) Peroxisome proliferator-activated receptors (PPARs) gene and protein expressions are increased. BPA/PAE can also activate PPARs. (5) After forming heterodimers and binding to response elements, peroxisome proliferation is increased. (6) Fatty acid oxidation then lead to the formation of reactive oxygen species (ROS). (7) Transcription, translation and activity of scavenger is decreased, leading to detrimental effects at the organ and individual levels. (8) PAE reduce cholesterol transport by reducing scavenger receptor class B-1, (9) steroidogenic acute regulatory protein and peripheral benzodiazepine receptor mRNA levels. (10) The gene expression of a series of enzymes necessary for steroidogenesis is repressed, leading to lower testosterone levels. (11) The gene expression, protein expression and activity of aromatase are also decreased, resulting in lessened estradiol synthesis when animals are exposed to BPA/PAE. These effects then induce changes at the organ, individual and population levels. (12) Finally, cortisol levels are reduced in BPA/PAE treated animals.

with the retinoid X receptor (RXR) and function as metabolic ligand sensors for lipophilic hormones, fatty acids, and their metabolites (Fig. 2; schematic reactions #4-6). Amongst others, these heterodimers can regulate transcription by binding and transactivating peroxisome proliferator response elements (PPREs) located in the 5′ regulatory region of downstream peroxisome proliferator-activated genes (Hurst and Waxman, 2003). When the expression of downstream fatty acid metabolism-related genes is altered, for example, peroxisome proliferation takes place (reviewed in Schoonjans et al., 1996). Peroxisome proliferation is affected differently according to the molecular structure of the phthalates (Gray et al., 1983; Mann et al., 1985). As aforementioned, it is likely that the length of phthalate molecules drives phthalate potency to PPARs, and shorter phthalates have the greatest effect on peroxisome proliferation. Small molecules such as 2-ethylhexanol (2-EH; fatty alcohol used in phthalate production) are capable of inducing a peroxisomal response. For example, 2-EH has been shown to induce the activity of carnitine acetyltransferase (a marker of peroxisome activity) in rat hepatocytes (Gray et al., 1983) and to elevate cyanide-insensitive palmitoyl CoA oxidation (another marker of peroxisome proliferation) in *in vivo* treated rodents (Keith et al., 1992). Differences in phthalate metabolism can also affect their ability to alter PPARs and peroxisome proliferation. DEHP, DINP, and di(2-ethyhexyl) terephthalate (DEHT) did not induce peroxisome proliferation in mammals (Barber and Topping, 1995; Kurata et al., 1998; Lington et al., 1997; Topping et al., 1987), possibly because their metabolism does not lead to the formation of monoesters. For example, DEHT is known to produce no metabolites as it completely hydrolyzes, *i.e.*, yielding two moles of alcohol per mole of phthalate diester (Topping et al., 1987); whereas *ortho*-phthalates do not completely hydrolyze when they are metabolized, which results in the formation of shorter phthalate metabolites (Faber et al., 2007a; Wirnitzer et al., 2011). This difference in metabolism could contribute to explain the range of peroxisome proliferation by phthalates. In addition, branched phthalates were shown to be more potent peroxisome proliferators than their straight chain analogs (Gray et al., 1983; Mann et al., 1985). Although a lot of research effort has been focused on PPARs, it is important to continue to characterize the molecular, cellular, and organismal effects of phthalates, along with other possible mechanisms of action. Sub-lethal effects of plasticizers are presented in the following sections.

4. Development impairment via the thyroid and growth hormone axes

One of the hormonal axes disrupted by plasticizers is the TH axis. THs play a crucial role in the regulation of development, metabolism, and heart function (Hofmann et al., 2009), and in the regulation of metamorphosis in amphibian species (Shen et al., 2011). Thyrotropin-releasing hormone (TRH) is first released by the hypothalamus, and together with triiodothyronine (T_3) and thyroxine (T_4), these hormones control the rate of thyroid-stimulating hormone (TSH) release in the pituitary gland. In turn, TSH induces the synthesis of T_4 in the thyroid gland. Deiodinases (dio1, 2 and 3) can convert T_4 to T_3 or reverse T_3 (rT_3), and both compounds further degrade into 3,3′-diiodothyronine (T_2; Stroheker et al., 2004). The physiological effects of T_3 and T_4 are mediated through the binding to nuclear thyroid hormone receptors alpha and beta (TRs; TRα and TRβ; Flood et al., 2013). Contaminants have been shown to act primarily by producing a 'hypothyroidism condition', which involves either the inhibition of iodide uptake, the inhibition of T_4 and/or T_3 synthesis, the upregulation or downregulation of deiodinases, and/or the increase of T_4 and/or T_3 catabolism (Degitz et al., 2005).

4.1. Effects of BPA on the thyroid hormone axis

BPA has been shown to disrupt the expression of TH-related genes in aquatic species and mammals. BPA downregulated the expression of T_3-response genes, including the stromelysin-3 (*st3*), the basic leucine zipper transcription factor (*th/bzip*), the matrix metalloproteinase (*mmp2*), and the tissue inhibitor of metalloproteinase (*timp2*) in the African clawed frog (Heimeier et al., 2009). Likewise, Iwamuro et al. (2006) showed that BPA decreased the expression of *trs* and *rxrγ* in a frog tail culture. The authors suggested that BPA induces its effect by directly binding to TRs (Iwamuro et al., 2003, reviewed by Zoeller, 2005). However, in rodents, BPA was shown to be a weak ligand to liver *TRα* and *TRβ*, but the presence of BPA did not activate TRs in Sprague Dawley rat (Moriyama et al., 2002). Despite BPA's weak binding to *TR*, plasticizers have been shown to be potent inhibitors of T_3 binding to human TH-binding proteins (Ishihara et al., 2003). Much of the inhibition of T_3 by BPA is likely due to its ability to increase the activity of transcriptional corepressors to the *TR*, which suppresses *TR* mediated transcription and consequently causes TH antagonism (Moriyama et al., 2002). Further studies should conduct receptor binding assays in amphibians to elucidate the affinity of plasticizers to the frog TRs.

In addition, TH-related effects have been observed at the individual level. In mammals, sheep exposed to 5 mg/kg BPA per day from day 30 to day 90 prenatally had lower birth weights (Savabieasfahani et al., 2006). In amphibians, BPA hindered the T_3-induced intestinal remodeling (African clawed frog; Heimeier et al., 2009), blocked the T_3-dependent resorption of the tail, and shortened the interocular distance in tadpoles (African clawed frog; Imaoka et al., 2007; Iwamuro et al., 2003). Similarly, African clawed frogs treated with 2.3–5.7 mg/L BPA from Nieuwkoop–Faber stage 52 until stage 62 exhibited a delay of one to two developmental stages behind control animals (Iwamuro et al., 2003; Nieuwkoop and Faber, 1994) and displayed shorter body length at 4.6 mg/L BPA (Sone et al., 2004). Likewise, in fish, tail length, total length, and body weight were all reduced in Japanese medaka and swordtail fish chronically treated with BPA at concentrations as low as 2 μg/L (Kwak et al., 2001; Yokota et al., 2000). In addition, BPA delayed hatching in zebrafish (13.8 mg/L BPA; Duan et al., 2008) and delayed hatching, yolk absorption, and first feeding by about seven days in juvenile rainbow trout (30–100 μg/mL BPA; Aluru et al., 2010). The authors suggested that BPA may have increased vitellogenin (*vtg*) mRNA level, decreased in growth hormone (GH)-related gene expression and/or shifted the energy allocation from somatic growth to vitellogenesis. Furthermore, delayed hatching was observed in zebrafish exposed to 13.81 mg/L BPA at 72 hours post fertilization (Duan et al., 2008). Altogether, BPA was shown to delay development and to reduce offspring weight and size by affecting the transcription of TH-related genes in vertebrates.

4.2. Effects of phthalates on the thyroid axis

Similarly to BPA, three phthalates were shown to disrupt the expression of TH-related genes in amphibians (Fig. 3). DEHP is a potent inhibitor of the TR ligand-binding domain and was deemed to be four orders of magnitude less potent than T_3 in bullfrog (Ishihara et al., 2003). Likewise, benzyl butyl phthalate (BzBP) exhibited T_3-antagonist activity by impeding with the T_3-induced increase in *trβ* transcript in African clawed frog tadpoles exposed to BzBP (Sugiyama et al., 2005). Similarly, tadpoles of the African clawed frog exposed for 21 days to dibutyl phthalate (DBP) and its metabolite monobutyl phthalate (MBP) also led to TH disruption (Shen et al., 2011). Concentrations as low as 2 mg/L of both DBP and MBP altered the expression of four TH-related genes; *trβ*

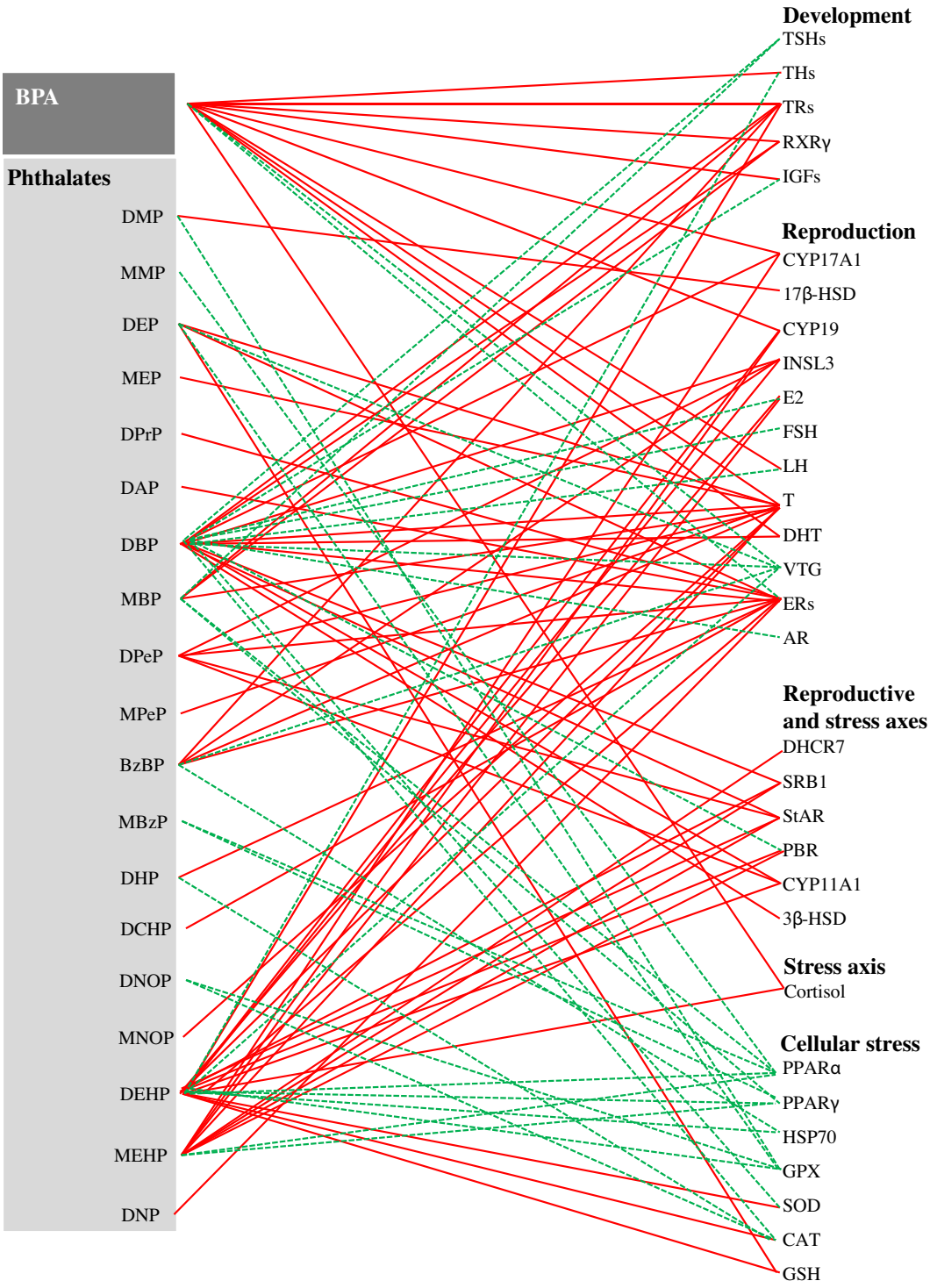

Fig. 3. BPA, phthalate monoesters and phthalate diesters affect the thyroid hormone, growth hormone, sex steroid, and stress axes. Decreases in transcription, translation, and activity are represented in black solid lines. Inhibition of endogenous hormone binding to receptors is also represented in black solid lines; whereas opposite effects are represented in gray dashed lines. 17β-HSD: 17β hydroxysteroid dehydrogenase, 3β-HSD: 3β Hydroxysteroid dehydrogenase, AR: androgen receptor, BPA: bisphenol A, BzBP: benzyl butyl phthalate, CAT: catalase, CYP11A1: cytochrome P450 side-chain cleavage, CYP17A1: cytochrome P450c17, CYP19: aromatase, DAP: diallyl phthalate, DBP: dibutyl phthalate, DCHP: dicyclohexyl phthalate, DEHP: di-(2-ethylhexyl) phthalate, DEP: diethyl phthalate, DHCR7: 7-dehydrocholesterol reductase, DHP: dihexyl phthalate, DHT: dihydrotestosterone, DMP: dimethyl phthalate, DNOP: dioctyl phthalate, DNP: dinonyl phthalate, DPeP: dipentyl phthalate, DPrP: dipropyl phthalate, E2: estradiol, ERs: estrogen receptors, FSH: follicle-stimulating hormone, GPX: glutathione peroxidase, GSH: glutathione, HSP70: heat shock protein 70, IGFs: insulin-like growth factor, INSL3: insulin-like hormone 3, LH: luteinizing hormone, MBP: monobutyl phthalate, MBzP: monobenzyl phthalate, MEHP: mono-(2-ethylhexyl) phthalate, MEP: monoethyl phthalate, MMP: monomethyl phthalate, MNOP: mono-*n*-octyl phthalate, MPeP: monopentyl phthalate, PBR: peripheral benzodiazepine receptor, PPARα: peroxisome proliferator-activated receptor alpha, PPARγ: peroxisome proliferator-activated receptor gama, RXRγ: retinoid X receptor gamma, SOD: superoxide dismutase, SRB1: scavenger receptor class B-1, StAR: steroidogenic acute regulatory protein, T: testosterone, THs: thyroid hormones, TRs: thyroid receptors, TSHs: thyroid-stimulating hormones, VTG: vitellogenin.

and *rxrγ* transcripts were decreased, while *tshα* and *tshβ* were upregulated (Shen et al., 2011). To compensate for thyroid antagonistic activity, such as decreases in circulating concentrations of T_4 and T_3, or inhibition of TR, feedback regulation may result in induction of TSH (Boas et al., 2012). Conversely, in rodents, the expression of *trα1* was upregulated in testes of rats treated with DBP (Lee et al., 2007). Shen et al. (2011) attempted to shed light on the possible mechanism of action of DBP. For this, the authors used a mammalian two-hybrid assay to test DBP and its metabolite MBP. They found that both phthalates enhanced the interactions between TRβ and its co-repressor the Silencing Mediator of Retinoic Acid and Thyroid Hormone Receptor (SMRT) in a dose-dependent manner. SMRT is a transcriptional coregulatory protein that represses transcription pathways, suggesting that DBP and MBP impair the TH system by suppressing TH-related transcripts (Shen et al., 2011).

In addition to their effects at the molecular level, phthalates can increase TH levels and the proliferation of TH-induced cells. For example, seven injections of 750 μg/100 g body weight of DEHP over 14 days yielded significant increases of T_3 and T_4 in the serum of female rats (Gayathri et al., 2004). The mechanism of action of phthalates in inducing TH levels remains unclear; however, some researchers have put forward some hypotheses. Gayathri et al. (2004) suggested that phthalates could induce hyperactivity of the thyroid gland; while Wenzel et al. (2005) proposed that phthalates could enhance the iodide intake by increasing the activity of the sodium/iodide symporter. Currently, it is unknown if plasticizers can alter the levels of TH in aquatic species.

Similarly to BPA, TH disruption by phthalates can lead to developmental abnormalities. It has been demonstrated that phthalates can affect rodent development, and that phthalate potency depends on the length of their alkyl chains (Field et al., 1993; Saillenfait et al., 2006). Short (methyl and ethyl) phthalates yield low or no developmental toxicity in rodents (Field et al., 1993), while medium weight phthalates (butyl) increase the rate of developmental malformation (Saillenfait et al., 2006). For example, pregnant rats fed 0.2–4.0 g/kg/day diethyl phthalate (DEP) or dimethyl phthalate (DMP) on gestational days 6–15 did not exhibit maternal toxicity and embryo or fetal development was not affected except for an increase in the number of ribs in the highest concentration of DEP (Field et al., 1993). However, pregnant rats fed 0.25–1.0 g/kg/day diisobutyl phthalate (DIBP) on gestational days 6–20 decreased body weight gain in the mothers and fetuses and higher incidences of fetus malformations were observed in the highest doses (0.75 and 0.1 g/kg/day; Saillenfait et al., 2006). In aquatic species, numerous studies have provided evidence that short, medium, and long chained phthalates have deleterious effects on their development. Studies have shown that exposure to 0.1 and 1.0 μg/L DEHP delayed the hatching time of Japanese medaka embryos (Chikae et al., 2004), and exposure to that same phthalate in concentrations from 0.2 to 40 μg/L affected signaling pathways involved in the maturation, growth and ovulation of zebrafish oocytes (Carnevali et al., 2010). Carnevali et al. (2010) demonstrated that DEHP increases the protein expression of bone morphogenetic protein 15 (*bmp15*), leading to a decrease in luteinizing hormone receptor (*lhr*), and membrane progesterone receptors (*mpr*) gene expression; all important genes involved in oocyte maturation and ovulation. Phthalates also alter metamorphosis in amphibians (Dumpert and Zietz, 1984; Shen et al., 2011). Dumpert and Zietz (1984) demonstrated delayed development and reduced pigmentation of African clawed frog tadpoles when exposed to 2 mg/L DEHP. In addition, Shen et al., 2011 found that exposing African clawed frog tadpoles to 2, 10, and 15 mg/L DBP and MBP for 21 days resulted in delayed metamorphosis development and significantly reduced the interocular distance and whole body length at 15 mg/L DBP and MBP. Similarly, Zhou et al. (2011a) exposed abalone embryos to DMP, DBP, DEHP, diethyl phthalate (DEP), and dioctyl phthalate (DNOP), and also noted a delay in mollusc metamorphosis. The authors demonstrated that abalone metamorphosis was suppressed in the 0.2–2 mg/L range (Zhou et al., 2011a). In summary, intermediate weight phthalates suppressed development in rodents, while all alkyl chain lengths affected amphibians, fish and mollusc development.

4.3. Effects of BPA and phthalates on stress axis and immune system

BPA and phthalates may also interact with TH axis, through modulating the stress and immune responses. Corticosteroids (particularly glucocorticoids) are hormones that are initially released into circulation shortly after exposure to stress, ultimately in response to stimulation by corticotropin-releasing hormones (CRH; Carrasco and Van de Kar, 2003). The complex interaction of CRH on the TH axes is regulated in both an age-dependent and tissue-specific manner (reviewed in Castañeda Cortés et al., 2014; Gray and Janssens, 1990; Suzuki and Kikuyama, 1983). Specifically, the interaction of glucocorticoids and TH axis generally occurs during periods of rapid morphological changes during development, such as during metamorphosis (Bonett et al., 2010). For example, in metamorphosing amphibians, corticosterone concentrations rise in concert with TH (Glennemeier and Denver, 2002). Phthalates decrease the expression of cholesterol transport and steroidogenesis genes, but there have been few studies on their interactions with corticosteroid-specific genes. In neonate rats exposed via the milk of dams fed BzBP through gavaging, BzBP upregulated the expression of corticotropin releasing hormone 1 (*CRHR1*) in mammary glands of neonates (Moral et al., 2007). Cortisol levels decreased in female rats exposed to DEHP for 14 days (Gayathri et al., 2004) and decreased in a concentration-dependent manner in H295R human adrenocortical carcinoma cells exposed to BPA (Zhang et al., 2011). After exposing placental JEG-3 cells to BPA at or above 25 μM, CRH mRNA expression was elevated, and as was the binding activity of the cyclic AMP response element, which is an important regulatory element of the CRH promoter (Huang et al., 2012). Following low but chronic to exposure of BPA to male and female pubescent rats, BPA-treated females had higher concentrations of basal corticosterone and lower concentrations of hypothalamic glucocorticoid receptors (Panagiotidou et al., 2014). BPA altered the basal and stress-induced activity of the hypothalamic pituitary–adrenal/interrenal (HPA) axis in a sexually dimorphic manner, as unlike females, BPA-exposed males had a higher corticosterone stress response compared with females and maintained the pre-stress concentrations of pituitary CRH-receptor 1. Conversely, in an *in vitro* experiment with rainbow trout, six phthalates failed to displace cortisol from glucocorticoid receptors from either liver or brain (Knudsen and Pottinger, 1999), which suggests that phthalates do not affect the corticosteroid axis by acting as an agonist/antagonist through glucocorticoid receptor binding, but through other pathways. Following exposure of marine medaka to 0.1 and 0.5 mg/L DEHP and MEHP from hatching to adulthood, a number of effects were found, including increased liver VTG in males, histological changes in testes and ovaries, reduced spermatozoa in testes, and increased atretic follicles in ovaries and increased plasma 17β-estradiol (E2) in both sexes (Ye et al., 2014). In addition, rainbow trout had significantly higher basal plasma cortisol concentrations when exposed to BPA as oocytes (Aluru et al., 2010). Despite these endocrinological changes, no changes in the glucocorticoid receptor mRNA were found in either sex. In summary, plasticizers impede with development by disrupting the expression of TH-related genes, by binding to TRs, by influencing THs levels, and by enhancing the interactions between TR and a co-repressor.

4.4. Effects of BPA and phthalates on the growth hormone axis

Development is also regulated by the GH axis, but studies documenting the effects of plasticizers on this endocrine axis are scarce. Aluru et al. (2010) exposed juvenile rainbow trout to 0, 30, and 100 μg/mL BPA for 3 h in ovarian fluid followed by fertilization and showed that BPA decreased the expression of genes involved in the endocrine regulation of growth and development, including the insulin-like growth factor 1 and 2 (*igf-1* and *2*), IGF-1 receptor alpha and beta (*igf-1rα* and *β*), and growth hormone receptors 1 and 2 (*ghr1* and *2*). In contrast, in rodents, dietary exposure to 500 mg/kg/day DBP for seven to nine days increased the expression of the insulin-like growth factor gene family in the Wolffian ducts of rats (Bowman et al., 2005). More specifically, DBP enhanced *IGF-1*, *IGF-2*, *IGF-1r*, and insulin-like growth factor binding protein 5 (*IGFBP-5*) mRNA levels, and DBP also increased the expression of other growth regulator genes, such as bone morphogenetic protein 4 (*BMP4*), fibroblast growth factor 10 (*FGF10*), and fibroblast growth factor receptor 2 (*FGFR2*; Bowman et al., 2005). Clear mechanisms of action for plasticizers' effects on the GH axis have yet to be elucidated.

5. Reproduction alterations mediated by plasticizers

The classic organizational–activational theory of the development of secondary sexual characteristics postulates that they are regulated by a combination of permanent (organizational) and temporary (activational) effects that are regulated by sex steroids (Phoenix et al., 1959). Organizational effects tend to have developmentally fixed alternatives, usually occur during a critical period of development, and are maintained even after the removal of the causal factor (Moore and Thompson, 1990). Activational effects tend to be traits that have developmentally plastic alternatives states, and are sexually dimorphic traits that sex hormones temporarily activate in adults (Moore and Thompson, 1990). Exposure to BPA or phthalates have been linked to both permanent organizational changes in sexually dimorphic traits (e.g., altered estrous cyclicity [Evans et al., 2004]; sex dependent behavior [Poimenova et al., 2010]) and to temporary activational changes (e.g., peroxisome proliferation [Wilkinson and Lamb, 1999]; testicular protein expression [Sobarzo et al., 2009]). Some of the effects of phthalates have been reversible, once the exposure has ended. David et al. (2001) found that changes in organ weights, erythrocyte counts, hemoglobin values, and pigmentation of Kupffer cells and renal tubules that were apparent during exposure were reversible, and disappeared after a recovery period. They argued that the reversibility of some of these effects coincided with a decrease in peroxisomal enzyme activity, and thus effects related to peroxisomal activity would reverse themselves once peroxisome proliferation returned to normal (David et al., 2001). Gayathri et al. (2004) also found that the induced effects of DEHP in rats (*i.e.*, decrease in serum cortisol and liver glycogen and increase in circulating T_3 and T_4) disappeared following cessation of DEHP exposure. In the next section, we summarize how BPA and phthalates may have other organisational and/or activational effects by acting as steroid agonists or antagonists, or by altering steroidal activity.

5.1. Effects of BPA on reproduction

BPA's deleterious effects on the sex steroid axis have been thoroughly characterized. In mammals, a dose of 2 ng of BPA per g of body weight increased the expression of both estrogen receptors in newborn mice (*ERα* and *ERβ*; Kawai et al., 2007), whereas only *ERβ* was upregulated in other rodent studies (Akingbemi et al., 2004b; Phrakonkham et al., 2008). Similarly, mice exposed to BPA during gestation displayed higher expression of ERα and ERβ in the brain (Kawai et al., 2007). In amphibians, a concentration of 0.23 mg/L BPA also upregulated *erα* mRNA in African clawed frog tadpoles exposed for two weeks (Levy et al., 2004). Other estrogen-related genes/proteins and hormones have been altered following BPA exposure, including aromatase (CYP19; an enzyme crucial in the biosynthesis of estrogens from androgens), progesterone, progesterone receptor (PR; mediates the effects of progesterone which is involved in maintaining pregnancy), and VTG (precursor protein of egg yolk essential in ovary growth and oocyte production). BPA-treated rat Leydig cells exhibited a significant decrease of *CYP19* mRNA levels at 2.28 ng/L BPA (Akingbemi et al., 2004b). Rats exposed to BPA experienced a reduction in E2 levels due to an inhibition of *CYP19* activity (Akingbemi et al., 2004b). Similarly, Grasselli et al. (2010) measured a significant decrease in E2 and progesterone production following BPA treatments in swine ovarian granulosa cells (at 1 and 10 μM; Grasselli et al., 2010). On the other hand, Olea et al. (1996) showed that a BPA-based dental sealant increased the proteic expression of the PR in MCF7 human breast cancer cells. In fish, BPA also enhanced the expression of *vtg* mRNA and VTG protein in swordtail fish (*vtg* mRNA; Kwak et al., 2001), rainbow trout (*vtg* mRNA and VTG level; Aluru et al., 2010; Christiansen et al., 1998), zebrafish (VTG level; Segner et al., 2003), and fathead minnows (VTG level; Sohoni et al., 2001). Similarly, the expression of *vtg* and VTG were increased in BPA-treated primary cultured hepatocytes of African clawed frog (Kloas et al., 1999; Nomura et al., 2006). The general consensus is that the estrogenic activity of BPA is caused by the binding of BPA to ERs in mammals (Brotons et al., 1995; Kitamura et al., 2005; Kuiper et al., 1998; Olea et al., 1996; Stroheker et al., 2004), in fish (Gibert et al., 2011), and in frogs (Lutz and Kloas, 1999; Suzuki et al., 2004b).

Previous studies have demonstrated that BPA can alter the estrous cyclicity, which interferes with female sexual maturity, and egg production; although BPA was found to be about 10- to 1000-fold less potent than endogenous estrogens (reviewed in Richter et al., 2007). For example, Suffolk ewe lambs exposed to BPA prenatally showed progressive loss of estrous cyclicity (37.4 ± 3.3 ng/mL; Savabieasfahani et al., 2006). The authors suggested that a decrease in luteinizing hormone (LH) production or release might be the cause for these observations. Interestingly, another study conducted on ewe lambs concluded that chronic BPA exposure reduced LH pulse frequency and amplitude (3.5 mg/kg BPA; Evans et al., 2004). Likewise, female rats exposed perinatally to BPA exhibited decreased plasma LH levels and permanently altered estrous cyclicity patterns in adulthood (1.2 mg/kg/day BPA; Rubin et al., 2001). Similar effects have been documented in females treated with exogenous testosterone (T) during development. In these cases, T affected the nervous system development via its local aromatization into E2. Since LH surges control the release of oocytes, alterations of estrous cyclicity might limit reproductive fertility and may decrease overall reproductive success (Rubin et al., 2001). BPA has showed to delay ovulation and egg production in non-mammalian species as well. In fish, 1.75 μg/L BPA delayed brown trout ovulation by two weeks, while 5 μg/L BPA was sufficient to completely impeded ovulation of this species (Lahnsteiner et al., 2005). Chronic treatment with BPA also inhibited fathead minnow egg production (1280 μg/L BPA; Sohoni et al., 2001). In contrast, production of eggs was enhanced in aquatic invertebrates. BPA stimulated egg production and release in treated giant ramshorn snails (7.9 ng/L BPA; Oehlmann et al., 2006), dogwhelk snails (1 μg/L BPA; Oehlmann et al., 2000), New Zealand mudsnails (5 μg/L BPA; Jobling et al., 2003), and in copepods (20 μg/L BPA; Andersen et al., 1999). Interestingly, the increase in egg production in the giant ramshorn snail was blocked by the addition of an antiestrogen (3 μg/L ICI 182,780), suggesting

once more that BPA has estrogenic properties (Oehlmann et al., 2006).

In addition to its effects on the female reproductive axis, BPA exhibits antiandrogenic properties. BPA decreased luciferase induction by dihydrotestosterone (DHT) in MCF-7 cells (Stroheker et al., 2004) and inhibited the production of androstenedione and T in H295R cells (Zhang et al., 2011). A reduction in T synthesis was also observed in dietary exposed rats to BPA (Akingbemi et al., 2004b). The authors suggested that this hormonal decrease was due to a diminution of cytochrome P450c17 (CYP17A1) expression (an important steroidogenic enzyme; Akingbemi et al., 2004b). Similar trends were observed in a mice cell line where BPA inhibited the activity of DHT in fibroblast cells (NIH3T3; Kitamura et al., 2005). More interestingly, Salian et al. (2009) has shown that perinatal exposure of male rats to BPA reduced the proteic levels of steroid receptor coactivator-1 (*SRC*-1) and nuclear corepressor (*NCoR*). These testicular steroid receptor coregulators are involved in the activation or repression of gene expression, thus they affect the regulation of the male reproductive axis. Along with alteration with testicular transcription, BPA can also interfere with testis growth and spermatogenesis. In invertebrates such as the dogwhelk snails, BPA exposure also altered spermatogenesis by reducing the volume of sperm stored in the vesicular seminalis (1 μg/L BPA; Oehlmann et al., 2000). In rodents and fish, BPA significantly reduced testis weight and sperm cell quality, e.g., in rats (20 μg/kg BPA; Sakaue et al., 2001), in fathead minnows (0.64 mg/L BPA; Sohoni et al., 2001), and in brown trout (1.75 μg/L BPA; Lahnsteiner et al., 2005). Sakaue et al. (2001) suggested that BPA alters spermatogenesis by acting as an estrogen antagonist. The authors observed a decrease in daily sperm production in treated rats, suggesting that BPA might prevent E2 from inhibiting germ cell apoptosis (Sakaue et al., 2001).

The fragile balance between the levels of androgens and estrogens was reported to be disrupted by BPA exposure. BPA has been identified as a potential human sex hormone-binding globulin ligand, in which BPA could displace endogenous sex steroids from binding sites and disrupt the balance between estrogens and androgens (Déchaud et al., 1999). BPA can also disrupt the endocrine balance by affecting sex hormone metabolism. It is believed that BPA can decrease E2 metabolism (Jurgella et al., 2006; Zhang et al., 2011). For example, in human adenocarcinoma cells exposed to BPA, cellular steroidogenesis was affected mainly through the inhibition of E2 metabolism (Zhang et al., 2011). Also, lake trout kidney and liver exposed for 1 h to 0.1–100 μM BPA exhibited IC50 values between 40-108 μM in the kidney and 11–18 μM in the liver in addition to significant inhibition of E2 metabolism by 100 μM BPA (Jurgella et al., 2006). BPA also suppressed Uridine 5′-diphospho-(UDP)-glucuronosyltransferase (UGT) activities, UGT2B1 protein and *UGT2B1* mRNA in adult male rats, and therefore affected the glucuronidation of sex hormones (Shibata et al., 2002). Secondly, BPA can decrease T metabolism as BPA-treated rats exhibited significantly decrease in the activity of male-specific cytochrome P450 isoforms, such as testosterone 2α-hydroxylase (T2AH) and testosterone 6β-hydroxylase (T6BH) (Hanioka et al., 1998). Together these studies suggest that BPA exposure may lead to increased sex steroid levels by inhibiting their catabolism. In summary, BPA is mostly estrogenic and antiandrogenic, and acts by binding to ER, repressing ER coregulators, and by impeding with androgen production, which results in sperm production inhibition, gonad size reduction, deregulation of estrous cyclicity, and a decrease in egg production.

5.2. Effects of phthalates on reproduction

Unlike BPA, studies have shown that phthalates can disrupt the expression of several genes involved in cholesterol transport and steroidogenesis (reviewed in Euling et al., 2013). Before cholesterol can be uptaken in the cell for steroidogenesis, double bonds are removed by the enzyme 7-dehydrocholesterol reductase (DHCR7). The expression of *dhcr7* is found to be reduced in rats exposed to a single high dose of MEHP (10 mg/kg; Lahousse et al., 2006). Similarly, the expression of the scavenger receptor class B-1 (*SRB1*), which is responsible for transporting high-density lipoprotein cholesteryl esters into the cell, was downregulated by DBP and DEHP in rats (Barlow et al., 2003; Borch et al., 2006; Lehmann et al., 2004; Thompson et al., 2004). Then, once the cholesterol has crossed the cellular membrane, the steroidogenic acute regulatory protein (StAR) and the peripheral benzodiazepine receptor (PBR) transport it to the inner mitochondrial membrane. In amphibians, a recent study have demonstrated that dicyclohexyl phthalate (DCHP) significantly increased *star* mRNA levels following an acute exposure of larvae of the Western clawed frog to 1.5 and 4.1 mg/L DCHP (Mathieu-Denoncourt, 2014), while in mammals, *STAR* expression was reduced in rat testes by several phthalates, including DEHP (Borch et al., 2006), MEHP (Lahousse et al., 2006), DBP (Barlow et al., 2003; Lehmann et al., 2004; Thompson et al., 2004), and dipentyl phthalate (DPeP; Hannas et al., 2011). In contrast, the expression of *PBR* can be either up or downregulated by phthalates in rodents (Borch et al., 2006; Gazouli et al., 2002; Lehmann et al., 2004). This regulation of *pbr* by phthalates is believed to be mediated by PPARα (Gazouli et al., 2002).

Thereafter, the first step of steroidogenesis involves the transformation of cholesterol into pregnenolone by the enzyme cytochrome P450 side-chain cleavage (CYP11A1). DBP, DEHP, and DPeP were all shown to reduce the expression of *CYP11a1* in rats (Barlow et al., 2003; Borch et al., 2006; Hannas et al., 2011; Lehmann et al., 2004; Shultz et al., 2001; Thompson et al., 2004). Progestogens are then transformed into androgens by CYP17A1, which mediates both 17α-hydroxylase and 17, 20 lyase activities. The gene expression of *CYP17a1* was lowered in rats exposed to DBP and MEHP (Barlow et al., 2003; Lahousse et al., 2006; Lehmann et al., 2004; Shultz et al., 2001; Thompson et al., 2004). The product of the 17, 20 lyase transformation is dehydroepiandrosterone, which is transformed into androstenedione by the enzyme 3*β* hydroxysteroid dehydrogenase (3β-HSD) and DBP is known to decrease the expression of *3β-hsd* in rats (Barlow et al., 2003; Lehmann et al., 2004). Furthermore, the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD), which has the ability to transform androstenedione into T, and estrone (E1) into E2, was found to be downregulated in abalone embryos exposed to DMP or DBP (Zhou et al., 2011a,b). Finally, T can be transformed into E2 by CYP19. Dramatic decreases in *cyp19* transcript levels were observed in a human adrenocortical carcinoma cell line and in rodents treated with DEHP and MEHP (Gupta et al., 2010a; Lee et al., 2009; Lovekamp and Davis, 2001; Noda et al., 2007; Xu et al., 2010). In addition, Wistar rat male pups exposed to DEHP during gestation and lactation showed decreased *CYP19* activity at low doses and increased activity at high doses (Andrade et al., 2006). *CYP19* activity was also reduced in a human cell line treated with MEHP, which is believed to be due to a rapid increase in nerve growth factor IB (*NUR77*) mRNA and protein levels, a member of the nuclear receptor 4A subfamily (Noda et al., 2007). Since CYP19 is an important enzyme in the biosynthesis of estrogens, phthalate exposure could result in disturbances of the normal balance between androgens and estrogens. Indeed, increases in E2, follicle stimulating hormone, and LH were observed in rats exposed to DBP and DEHP (Akingbemi et al., 2004a; O'Connor et al., 2002). Contrarily, DEHP and MEHP decreased E2 levels in rodents, likely due to the decrease in CYP19 protein expression (Gupta et al., 2010a; Lovekamp and Davis, 2001; Xu et al., 2010). Similar to BPA, numerous studies have shown the estrogenic activity of phthalates in human cell lines, mammals, amphibians and

fish. Phthalates can bind human ERα (Ohashi et al., 2005), rat ERs (Zacharewski et al., 1998), rainbow trout ERs (Knudsen and Pottinger, 1999), and African clawed frog ERs (Lutz and Kloas, 1999; Suzuki et al., 2004b). Upon binding to the ERs, phthalates can alter the production of VTG in aquatic species. In fish, phthalates increase the transcription of VTG including DEHP in zebrafish (500 mg/kg, Uren-Webster et al., 2010; 2 μg/L, Carnevali et al., 2010), butyl benzyl phthalate (BBP) in rainbow trout (500 mg/kg; Christiansen et al., 1998), DBP in medaka (776 μg/kg/day, Patyna and Cooper, 2000), and DEP in common carp (0.1–5 mg/L, Barse et al., 2007). In contrast, DBP, BBP, benzyl benzoate (BB), and butyl phthalyl butyl glycolate (BPBG) did not increase VTG activity in primary-cultured hepatocytes of the African clawed frog (Nomura et al., 2006).

Unsurprisingly, female reproduction is also affected by phthalate exposure. Xu et al. (2010) has demonstrated that DEHP prolonged the estrous cycle duration in rats. Likewise, a reproductive study conducted by Hoshino et al. (2005) showed that DCHP also prolonged the estrous cycle in F0 female rats. In addition, puberty and vaginal opening were both delayed in rats treated with BBP (750 mg/kg/day, Tyl et al., 2004) and DEP (15 g/L; Fujii et al., 2005). In aquatic species, phthalates also affected female reproduction, although mainly in altering with egg production and biasing sex ratio. Exposure to phthalates decreased the number of eggs or young produced by daphnids (3-30 μg/L DEHP, Mayer and Sanders, 1973; 1.8 ng/L DBP, McCarthy and Whitmore, 1985; 0.64–1.91 mg/L DBP, DeFoe et al., 1990) and medaka (776 μg/kg/day DBP, Patyna and Cooper, 2000). Moreover, female zebrafish exposed to 40 μg/L DEHP for 3 weeks produced 1% of the eggs laid by the control animals (Carnevali et al., 2010). The authors suggested that this reduction was due to a decrease in the expression of the prostaglandin-endoperoxide synthase 2 (*ptgs2*) expression, which codes for the enzyme essential for the ovulation process, cyclooxygenase (COX; Carnevali et al., 2010).

As seen with BPA, in addition to their estrogenic activity, phthalates are also known to have antiandrogenic properties. In mammalian studies, exposure to several phthalates have been associated with lower T levels in dogs (Pathirana et al., 2011), rabbits (Higuchi et al., 2003), and rodents (Borch et al., 2006; reviewed in Foster, 2006; Hannas et al., 2011; Howdeshell et al., 2008; Lee et al., 2007, 2009; Lehmann et al., 2004; Noriega et al., 2009; Pereira et al., 2006; Shultz et al., 2001; Thompson et al., 2004; Wilson et al., 2004). For example, rat pups exposed to MBP during gestation exhibited a mean testicular T content of about 6% of that of the control group (Shono et al., 2000). Other androgens such as androstenedione and DHT were also decreased in rats and rabbits exposed to DBP (O'Connor et al., 2002; Shultz et al., 2001). In aquatic species, the activity of the steroid 5α-reductase type 2 (SRD5A2), one of the enzymes converting T into the more potent DHT, was inhibited by DBP and DEHP in the common carp (Thibaut and Porte, 2004); however, MMP, DMP and DCHP did not alter *srd5a2* mRNA levels in larvae of the Western clawed frog (Mathieu-Denoncourt, 2014). Unlike other antiandrogens, phthalates are not likely acting by binding to the AR. *In vitro* studies showed that DEHP and MEHP did not display affinity for the human AR at concentrations up to 3.9 mg/L and 2.8 mg/L, respectively (Parks et al., 2000). However, the antiandrogenic toxicity of phthalates appear to be additive with other antiandrogens, and can produce cumulative developmental effects (Christiansen et al., 2009; Rider et al., 2008), even if the mechanism of action differ amongst the antiandrogens.

Phthalate plasticizers can disrupt male reproduction in many species. Several studies reviewed the effects of phthalates in mammalian reproduction (Ema, 2002; Hotchkiss et al., 2008; Makris et al., 2013; Talsness et al., 2009). In rats and rabbits, BzBP, DBP, and DEHP reduced sperm synthesis, sperm concentration, sperm motility, ejaculate volume, and number of motile sperms (Aso et al., 2005; Giribabu et al., 2014; Gray et al., 2009; Higuchi et al., 2003; Lee et al., 2009; Tyl et al., 2004). In invertebrates, sperm cells exposed to 100 μg/L DMP also exhibited a low fertilization rate of 38.5% when compared to approximately 80% in control abalone (Zhou et al., 2011b). To assess antiandrogenic effects, the anogenital distance (*i.e.*, the distance from the anus to the anterior base of the penis) is widely used as another morphological endpoint in mammals. Anogenital distance is positively correlated with fertility, sperm density, and mobile sperm count in men, suggesting that it is associated with fatherhood and may predict male reproductive potential (Eisenberg et al., 2011). Previous studies have showed that the anogenital distance was decreased in rodents after being exposed to BzBP, DCHP, DBP, DEHP, DPeP, and DPP (dipropyl phthalate; Aso et al., 2005; Ema et al., 2003; Gray et al., 2009; Hannas et al., 2011; Hoshino et al., 2005; Jarfelt et al., 2005; Mylchreest et al., 1998, 1999, 2000; Parks et al., 2000; Saillenfait et al., 2011; Tyl et al., 2004; Yamasaki et al., 2009). The reduction in anogenital distance was believed to be due to a decreased T production during the critical stage of the reproductive tract differentiation (Parks et al., 2000). In addition, phthalates inhibited transabdominal testicular descent in rats, a T-dependent event (Ema et al., 2003; Mylchreest et al., 1998; Saillenfait et al., 2006, 2011; Shono et al., 2000). Other frequent results of phthalate exposure are permanent retention of nipples (Gray et al., 2009; Hannas et al., 2011; Hoshino et al., 2005; Jarfelt et al., 2005; Mylchreest et al., 1999, 2000; Tyl et al., 2004; Yamasaki et al., 2009), delayed preputial separation (Aso et al., 2005; Mylchreest et al., 1999; Yamasaki et al., 2009), and delayed pubertal onset in male rodents (Noriega et al., 2009 ; Tyl et al., 2004). Testicular lesions have also been observed in treated rats (Foster et al., 1981; Gray et al., 2009; Tyl et al., 2004) and in African clawed frogs (Lee and Veeramachaneni, 2005). In addition to these adverse effects, phthalates and their monoester metabolites reduced the size of the testes and the weight of androgen-dependent organs, such as the prostate and seminiferous tubules in rats (Aso et al., 2005; Foster et al., 1981; Gray et al., 2009; Hoshino et al., 2005; Jarfelt et al., 2005; Kasahara et al., 2002; Lake et al., 1982; Mylchreest et al., 1998; Noriega et al., 2009; Parks et al., 2000; Srivastava et al., 1990; Yamasaki et al., 2009). This effect is also believed to be due to the decreased T production (Parks et al., 2000). Furthermore, subchronic exposures of 0.1–10.0 mg/L DBP to African clawed frog tadpoles from stage Nieuwkoop-Faber 52 to Nieuwkoop-Faber 66 affected spermatogenesis through malformations of the male reproductive system (Lee et al., 2005). Three possible mechanisms of action have been put forward. The first mechanism of action involves the induction of oxidative stress, which injures mitochondrial function leading to the release of cytochrome *c*, and inducing apoptosis of spermatocytes (Kasahara et al., 2002). The second proposed mechanism of action involves a decrease in sorbitol dehydrogenase activity, fructose level and phospholipids levels (Fukuoka et al., 1989). The third possible mechanism of action suggests that phthalates act on T and DHT levels, as mentioned in previous paragraphs (Mylchreest et al., 1998). Testicular atrophy and lesions were not observed in rats treated with-tert monoesters, providing evidence for the effect of branching in testicular toxicity (Foster et al., 1981). Similarly, ring substitution is an important factor determining reproductive damage in an animal exposed to phthalates. For example, adverse effects were not evident when rats were exposed through diet to 0–1.0% di(2-ethylhexyl) terephthalate as it did not induce histopathologic changes (Faber et al., 2007b) and it did not induce peroxisome proliferation (Barber and Topping, 1995; Topping et al., 1987). The antiandrogenic properties observed in *in vivo* studies are thought to be related with PPARα, rather than the AR (Gazouli et al., 2002). However, Ward et al. (1998) demonstrated

that in PPARα-null mice, lesions did develop in kidney and testis when fed 12 g/L DEHP, suggesting the testicular toxicity of DEHP can act independently of PPARα. Other authors suggested that the testicular toxicity may be mediated by another PPAR form (Hurst and Waxman, 2003) or by decreased insulin-like hormone 3 (*insl3*) transcript (Wilson et al., 2004). Altogether, studies suggest that phthalates' antiandrogenic proprieties are mainly triggered by the reduced T production, which could be due to PPAR-dependent transcriptional changes.

Another possible mechanism of action for the adverse effects of phthalates in the reproductive axis is via the increase of T metabolism. Crago and Klaper (2012) have observed a reduction in T levels following treatment to 12 μg/L DEHP in fathead minnows, and have suggested that this decrease was associated with an increase in catabolism enzymes. Indeed, the authors showed that the expression of the phase I-metabolising enzyme cytochrome P450 3A4 (*cyp3a4*) and phase II-metabolising enzyme sulfotransferase dehydrogenase 2A1 (*sult2a1*) was increased following exposure. Similarly, Patyna et al. (2006) demonstrated that exposure to 20 μg/g (1 μg/g fish/day) DINP or diisodecyl phthalate (DIDP) increased T hydroxylase activity in Japanese medaka. In mammals, male rats exposed to 15 g/L DEP in their diet had increased cytochrome P450 3A2 (CYP3A2) content (Fujii et al., 2005). The role of this enzyme is to hydroxylate T at the 6β-position. Overall, phthalate esters have been shown to be estrogenic and antiandrogenic. The former is mediated mostly by CYP19, while the latter is accomplished through a reduction in cholesterol transport and a potential increase in T metabolism.

5.3. Effects of BPA and phthalates on sex ratios

Exposure to plasticizers has shown to have varying effects on sex ratios. Although exposure to BPA and phthalates have impacted reproductive development in male rats (see above), sex ratios of offspring of exposed mothers are no different from sex ratios of offspring from mothers in control treatments (375 mg/kg monobenzyl phthalate, Ema et al., 2003; 750 mg/kg DBP, Mylchreest et al., 1998; 2.4 μg/kg BPA, Salian et al., 2009). In contrast, dietary exposure of 1.0–10 mg BPA combined with DEHP/kg/day to mice during pregnancy resulted in a significant decrease in the male to female sex ratio of the pups (Wei et al., 2012). In non-mammalian animals, exposure to BPA and phthalates has been shown to bias sex ratios and induce hermaphrodites. For example, medaka exposed to 1.8 mg/L BPA (Yokota et al., 2000) and to 0.01 μg/L DEHP exhibited a biased sex ratio towards females (Chikae et al., 2004). In amphibians, treatment with 0.23 mg/L BPA induced approximately 62–70% females in African clawed frog (Kloas et al., 1999; Levy et al., 2004), while chronic exposure to 1.4 mg/L BPA slightly increase female phenotypes (5 males:15 females) in Western clawed frog (Mathieu-Denoncourt, 2014). In contrast, lower concentration of BPA did not affect frog sex ratio (49.7 μg/L BPA; Pickford et al., 2003); while higher levels of BPA (e.g., 0.23 mg/L BPA) produced intersexed testes in male African clawed frogs (Levy et al., 2004). Ohtani et al. (2000) showed that treating genetically male Japanese wrinkled frogs with 2.8 mg/L DBP during gonadal differentiation led to ovarian formations in 17% of the gonads. Similarly in medaka, concentrations as low as 0.01–1.82 mg/L BPA (Metcalfe et al., 2001; Yokota et al., 2000) and up to 776 μg/kg/day DBP also induced testis-ova phenotype in male fish (Patyna and Cooper, 2000). Altogether, these studies show that plasticizers behave like estrogens, agreeing with the literature where E2 and ethinyl estradiol are well known for affecting sex ratios in amphibians (Hu et al., 2008; Levy et al., 2004; Lutz et al., 2008; Miyata et al., 1999; Pettersson et al., 2006; Pettersson and Berg, 2007). The mechanism of action for BPA involves its binding to ER and the upregulation of *ER* mRNA levels (Levy et al., 2004). However, the mechanism of action for phthalates remains unclear. One possibility could be that phthalates alter the CYP19 activity (Andrade et al., 2006), which would disrupt the balance between androgens and estrogens.

6. Plasticizer-induced cellular stress

Oxidative stress is an imbalance between the endogenous formation of reactive oxygen species (ROS), which are formed naturally by aerobic metabolism, and the organism's capacity to detoxify or eliminate the ROS or to repair damage caused by ROS. Cellular ROS, such as superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (·HO), are generated by mitochondrial oxidative phosphorylation. Alternatively, ROS can be formed by the activity of cytochrome P450 enzymes that are induced by the presence of xenobiotics. Through the process of hydroxylating the xenobiotics, P450s can inadvertently produce peroxides that react with other endogenous substrates. Oxidative stress can also be caused by xenobiotics activating peroxisomes, which metabolize long chain fatty acids through β oxidation, which in turn produces peroxide by direct transfer of electrons to oxygen. Xanthine oxidase, NADPH, and oxidases and ATP production during oxidative phosphorylation can also produce superoxide. P450s, such as P4504A which differs from other P450 enzymes by their capability to hydroxylate medium and long chain fatty acids (Johnson et al., 1996), are themselves induced by increased activity of PPARα (Gonzalez, 2005). ROS are highly reactive towards biological macromolecules, including nucleic acids, proteins, and lipids. ROS can damage DNA by degrading it, breaking strands, crosslinking DNA, inducing scissions, leading to chromatid breaks, exchanging chromatids and inducing unscheduled DNA synthesis. The excessive production of hydrogen peroxide or the diminished rate of hydrogen peroxide degradation by catalase (CAT) can induce mutations and may lead to cancers (Abdellatif et al., 1991; Feinstein et al., 1978; Warren et al., 1982). The cellular defence mechanism against oxidative stress include the production of antioxidants such as CAT, superoxide dismutase (SOD), glutathione peroxidase (GPX) and thiols, amongst others, which neutralize ROS.

Phthalates disrupt the oxidative stress balance in a cell by activating peroxisomes and by inducing P450 activity, and thus produce reactive oxygen species (ROS). By increasing peroxisome proliferation, phthalates increase peroxisomal β oxidation (Isenberg et al., 2000, 2001), increase ROS production (Erkekoglu et al., 2012; Kasahara et al., 2002; Pereira et al., 2006), induce DNA damage in sperm and testes (Duty et al., 2003; Hauser et al., 2007; Lee et al., 2007), and are associated with breast cancer (Lopéz-Carillo et al., 2010). Although the cytochrome P450 family is very large and diverse, the main P450 that is associated with the metabolism of xenobiotics and that is induced by phthalates is P450 3A, (Takeshita et al., 2011; Zhou et al., 2011a); however, P450 4A and 2B have also been induced in mice by DEHP (Ren et al., 2010). Following the induction of P450 enzymes, phase II metabolites are produced, which are water soluble metabolites formed following P450 mediated metabolism. Phase II metabolites, such as glucuronide conjugates, have been found in tissue following exposure to DBP (Struve et al., 2009). For example, the expression of phase II metabolism enzymes, such as *gpx*, was upregulated by five phthalates in treated abalones, in addition to the induction of P450 3A (Zhou et al., 2011a).

Heat shock proteins are important components of a generalized stress response, and are chaperone proteins that stabilize denatured proteins, and protect organisms from oxidative stress by preventing the irreversible loss of vital proteins (Gupta et al., 2010b; Yang et al., 2010). Phthalates have been shown to increase the concentrations of heat shock proteins, which are induced to protect the organism from damage by the phthalates. DEHP and BzBP

induced a two and four-fold increase in the expression of heat shock protein 70 (*hsp70*), respectively in harlequin flies (Planelló et al., 2011). BzBP also led to an increase in the expression of the heat shock factor 2 (*hsf2*) in the rat mammary gland in neonate rats exposed to BzBP through the milk of exposed dams (Moral et al., 2007). These transcriptional changes correlate with disrupted proteic expression and activity. The activity of SOD, which are antioxidant enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide, was found to be increased in rats exposed to DBP and decreased in kidneys of rats exposed to DEHP (Erkekoglu et al., 2012; Lee et al., 2007). Similarly, the activity of CAT, an antioxidant that catalyzes the decomposition of hydrogen peroxide to water and oxygen, increased in response to exposure to DBP, DHP and DNOP, and decreased after exposure to DEHP in rats (Lee et al., 2007; Mann et al., 1985). DEP and DEHP both depleted glutathione content in rat tissues (Erkekoglu et al., 2012; Kasahara et al., 2002; Pereira et al., 2006), presumably through depletion by the formation of glutathione conjugates of the phthalates. GPX activity was either increased by DBP or decreased by DEHP in rats (Erkekoglu et al., 2012; Lee et al., 2007), while thiol and ascorbic acid content was reduced in kidney and testes of rats exposed to DEHP (Erkekoglu et al., 2012; Kasahara et al., 2002). Ultimately, the cells' battle against ROS was lost in rats treated with MEHP; the monoester induced the release of cytochrome *c* and induced apoptosis in the testes (Kasahara et al., 2002). Although the effects of phthalates on the mechanisms of defence against ROS have been investigated, BPA-induced cellular stress is still not well characterized.

4. Conclusions

In conclusion, three main chains of events are happening in BPA and phthalate treated animals (Figs. 2 and 3). Firstly, plasticizers can (1) disrupt the TH and GH axes, affecting development, albeit the mechanisms of action are not completely characterized yet; (2) decrease cholesterol transport to the mitochondria, reducing cholesterol intake and steroidogenesis, thereby increasing the rate of reproductive defects and decreased fertility; and (3) activate PPARs, increasing fatty acid oxidation and reducing the animal's ability to cope with the high level of ROS, increasing the occurrence of malformations. Considering that plasticizers are continuously released into water bodies and taking into account their relatively short half-lives, additional studies on the adverse health effects of plasticizers in aquatic non-mammalian species are critically needed.

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