Review

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

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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 sub-lethal 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 (Brotens et al., 1995). The temperature (Tan and Mustafa, 2003), pH (Xu et al., 2011), and fat content (López-Cervantes and Paseiro-Losada, 2003) of materials or tissues in contact with plasticized polyvinyl chloride (PVC) can modulate BPA leaching.
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., 2015).

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 acids 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 Lung et al. (2008).
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. PPARs regulate genes that control fatty acid degradation, PPARγ regulates genes that control fatty acid metabolism, and PPARα 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 PPARγ 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 monooesters 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 TR-target 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 receptor class B-1, (8) steroidogenic acute regulatory protein and peripheral benzodiazepine receptor mRNA 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 steriodogenesis 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. Please cite this article in press as: Mathieu-Denoncourt, J., et al. Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species. Gen. Comp. Endocrinol. (2015), http://dx.doi.org/10.1016/j.ygcen.2014.11.003]
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 regulates 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 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-ethylhexyl) 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; Wirtzitter 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 (T3) and thyroxine (T4), these hormones control the rate of thyroid-stimulating hormone (TSH) release in the pituitary gland. In turn, TSH induces the synthesis of T4 in the thyroid gland. Deiodinases (dio1, 2 and 3) can convert T4 to T3 or reverse T3 (rT3), and both compounds further degrade into 3,3’-diiodothyronine (T2; Strohkeker et al., 2004). The physiological effects of T3 and T4 are mediated through the binding to nuclear thyroid hormone receptors alpha and beta (TRα, TRβ, and TRβ1; 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 T3 and/or T3 synthesis, the upregulation or downregulation of deiodinases, and/or the increase of T3 and/or T3 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 T3-response genes, including the stromelysin-3 (S3), the basic leucine zipper transcription factor (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 trα and trβ1 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 TRβ1, 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 T3 binding to human TH-binding proteins (Ishihara et al., 2003). Much of the inhibition of T3 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 T3-induced intestinal remodeling (African clawed frog: Heimeier et al., 2009), blocked the T3-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 T3 in bullfrog (Ishihara et al., 2003). Likewise, benzyll butyl phthalate (BzBP) exhibited T3-antagonist activity by impeding with the T3-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 µg/mL of both DBP and MBP altered the expression of four TH-related genes; trα

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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, 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, MEHP: monoethyl phthalate, PBR: peripheral benzodiazepine receptor, PPARα: peroxisome proliferator-activated receptor alpha, PPARγ: peroxisome proliferator-activated receptor gamma, 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 T4 transcripts were decreased, while tshx and tshβ were upregulated (Shen et al., 2011). To compensate for thyroid antagonistic activity, such as decreases in circulating concentrations of T4 and T3, 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 TR-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 T3 and T4 in the serum 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 diocetyl 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 gavage, 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 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 spermatogonia 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 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 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 T3 and T4) disappeared following cessation of DEHP exposure. In the next section, we summarize how BPA and phthalates may have other organizational 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 erz 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 (vg mRNA; Kwak et al., 2001), rainbow trout (vg 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; Kuper 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 μg/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 change 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 seminals (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; Sakae et al., 2001), in fathead minnows (0.64 mg/L BPA; Sohoni et al., 2001), and in brown trout (1.75 µg/L BPA; Lahmstein et al., 2005). Sakae 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 (Sakae 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 (Duchaud et al., 1999). BPA can also disrupt the endocrine balance by affecting sex hormone metabolism. It is believed that BPA can decrease E2 metabolization (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 metabolization (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 10.18 µM in the liver in addition to significant inhibition of E2 metabololism by 100 µM BPA (Jurgella et al., 2006). BPA also suppressed Urinary 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., 2004). Secondly, BPA can decrease T metabolization 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 (DHC7). The expression of dhc7 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 cholesterol 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 PPARa (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 3β-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...
Phthalates can bind human ERs (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, Barsel 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, Fuji 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 μg/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; Howedeshell 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 BBP (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 srds2a 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 MHP did not display affinity for the human AR at concentrations up to 3.9 μg/L and 2.8 μg/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, BBP, 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 end-point 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; Jarfert 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; Jarfert et al., 2005; Mylchreest et al., 1998, 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; Jarfert et al., 2005; Kasahara et al., 2002, 2007; 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% (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 PPARs, rather than the AR (Gazoui 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 (ins3) transcript (Wilson et al., 2004). Altogether, studies suggest that phthalates’ antiandrogenic properties 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) DIMP or diisodicyl 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 ratios. Although exposure to BPA and phthalates have impacted exposure to BPA and phthalates has been shown to bias sex ratios in mammals and non-mammalian aquatic species. Gen. Comp. Endocrinol. (2015), http://dx.doi.org/10.1016/j.ygcen.2014.11.003

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 (O2−), hydrogen peroxide (H2O2), and hydroxyl radical (·OH), 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 peroxides 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 P450 4A4 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 PPARs (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 chromatin 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 defense 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 (Lopez-Carillo et al., 2010). Although the cytochrome P450 family is very large and diverse, the major 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 (Stuve et al., 2009). For example, the expression of phase II metabolism enzymes, such as gpx, was upregulated by five phthalates in treated abalone, 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

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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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References

Abbott, B.D., Wolf, C.J., Schmid, J.E., Das, K.P., Zehr, R.D., Helfant, L., Nakayama, S., 2007. Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha. Toxicol. Sci. 98, 571–581.

Abdelatif, A.G., Préat, V., Taper, H.S., Roberfroid, M., 1991. The modulation of rat liver carcinogenesis by perfluorooctanoic acid, a peroxisome proliferator. Toxicol. Appl. Pharmacol. 111, 530–537.

Akingbemi, B.T., Ge, R., Zirkin, B.R., Hardy, M.P., 2004a. Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine phenotypes. Toxicol. Appl. Pharmacol. 192, 180–192.

Akingbemi, B.T., Sottas, C.M., Koulova, A.I., Klinefelter, G.R., Hardy, M.P., 2004b. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology 145, 592–603.

Alonso-Magdalena, P., Morimoto, S., Ripoll, C., Fuentes, E., Nadal, A., 2006. The estrogenic effect of bisphenol A disrupts pancreatic β-cell function in vivo and induces insulin resistance. Environ. Health Perspect. 114, 106–112.

Andrade, A.J.M., Grande, S.W., Talsness, C.E., Grote, K., Chahoud, I., 2006. A dose-response study following utero and lactational exposure to di-(2-ethylhexyl)-phthalate (DEHP): non-monotonic dose-response and low dose effects on rat brain dopamine activities. Proc. Natl. Acad. Sci. 103, 2277–2282.

Aso, S., Saha, H., Miyata, K., Hosuyama, S., Shiraishi, K., Umano, T., Mnobe, Y., 2005. A two-generation reproductive toxicity study of butyl benzyl phthalate in rats. J. Toxicol. Sci. 30, 39–58.

Ayaward, L.L., Hays, S.M., Gagne, M., Krishnan, K., 2009. Derivation of biomonitoring equivalents for di-n-butyl phthalate (DBP), benzylbutyl phthalate (BzBP), and diethyl phthalate (DEP). Regul. Toxicol. Pharmacol. 55, 259–267.

Barber, E.D., Topping, D.C., 1995. Subchronic 90-day oral toxicology of di-(2-ethylhexyl)terephthalate in the rat. Food Chem. Toxicol. 33, 971–978.

Barlow, N.J., Phillips, S.L., Wallace, D.G., Sar, M., Gaido, K.W., Foster, P.M.D., 2003. Quantitative changes in gene expression in fetal rat testes following exposure to di-(n-butyl) phthalate. Toxicol. Sci. 73, 431–451.

Baron, V., Beausoleil, D.C., Nicol, G.A., 2008. Plasticizers and their degradation products in the process streams of a large urban physicochemical sewage treatment plant. Water Res. 42, 153–162.

Barse, A.V., Chakrabarti, T., Ghosh, T.K., Pal, A.K., Jadhao, S.B., 2007. Endocrine disruption and metabolic disturbances following exposure to di-2-ethylhexyl phthalate (DEHP). Prog. Lipid Res. 46, 388–403.

Berger, J., Møller, D.E., 2002. The mechanisms of action of PAPPs. Annu. Rev. Med. 53, 409–453.

Biles, J.F., McNeal, T.P., Begley, T.H., Hollifield, H.C., 1997. Determination of bisphenol-a in reusable polycarbonate food-contact plastics and migration to food-simulating liquids. J. Agric. Food Chem. 45, 3541–3544.

Blair, J.D., Hixon, M., Kelly, B.C., Surridge, B., Goba, F.A.P.C., 2009. Ultra-trace determination of phthalate ester metabolites in seawater, estuarine estuaries and biota from an urbanized marine inlet by LC/ESI-MS/MS. Environ. Sci. Technol. 43, 6262–6268.

Bloch, E.C., Silva, M.J., Caudill, S.P., Needham, L.L., Pirkle, J.L., Sampson, E.J., G.W., Jackson, R.J., Brock, J.W., 2000. Levels of seven urinary phthalate metabolites in a human reference population. Environ. Health Perspect. 108, 979–982.

Boes, M., Feldt-Rasmussen, U., Main, K.M., 2012. Thyroid effects of endocrine disrupting chemicals. Mol. Cell. Endocrinol. 355, 240–248.

Bonnet, R.M., Hooper, E.D., Denver, R.J., 2010. Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. Gen. Comp. Endocrinol. 166, 175–191.

Borch, J., Metzdorf, S.B., Vinggaard, A.M., Brokken, L., Dalgaard, M., 2006. Mechanisms underlying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis. Toxicology 223, 144–155.

Bozeman, C.J., Turner, K.J., Sar, M., Barlow, N.J., Gaido, K.W., Foster, P.M.D., 2005. Altered gene expression during rat Wolffian duct development following di(n-butyl) phthalate exposure. Toxicol. Sci. 86, 161–174.

Bradley, E.L., Leon, I., Mortimer, D.N., Speck, D.R., Castle, L., 2013. Determination of phthalate diesters in foods. Food Addit. Contam. A 30, 789–794.

Brotons, J.A., Olea-Serrano, M.F., Pedraza, V., Olea, N., 1995. Xenoestrogens released from lacquer coatings in foods. Environ. Health Perspect. 103, 608–612.

Calafat, A.M., Kuklenyk, Z., Reidy, J.A., Caudill, S.P., Ekong, J., Needham, L.L., 2005. Urinary concentrations of bisphenol a and 4-nonylnaphthalene in a human reference population. Environ. Health Perspect. 113, 391–395.

Campioli, E., Batarseh, A. L., J., Papadopoulos, V., 2011. The endocrine disruptor mono(2-ethylhexyl) phthalate affects the differentiation of human liposarcoma cells (SW 872). PLoS ONE 6, e28750.

Carolsfeld, F., Jönsson, B.A.G., Bornehag, C.G., 2012. PVC flooring is related to human breast cancer. Environ. Health 11, 51.

Carlsfelt, F., Jönsson, B.A.G., Bornehag, C.G., 2012. PVC flooring is related to human breast cancer. Environ. Health 11, 51.

Chahoud, I., Colborn, T., 1997. Plasticizers in aquatic non-mammalian species are critically needed. Gen. Comp. Endocrinol. 108, 345–358

Cheong, L.T., Lam, H.H.S., Teo, S.S., Tan, H.S., 2005. The influence of estriol on the endometrial estrogen receptors alpha and beta in an immortalized human endometrial cell line. V. Cytol. 36, 155–160.

Christiansen, L.B., Pedersen, K.L., Bjerregaard, P., 1998. Estrogenicity of phthalates and related compounds in fish. Mar. Environ. Res. 46, 137–140.
Christiansen, S., Scholze, M., Dalgaard, M., Vingaard, A.M., Axelstad, M., Kortenkamp, A., Hass, U. 2009. Synergic disruption of external male sex organ development by a mixture of four antiandrogens. Environ Health Perspect 117, 1820–1826.

Clara, M., Windhöfer, G., Harl, W., Braun, K., Simon, M., Gans, O., Scheffknecht, C., Chovanec, A. 2010. Occurrence of phthalates in surface runoff, untreated and treated wastewater and lake during wastewater treatment. Chemosphere 78, 1070–1084.

Crago, J., Klaper, R. 2012. A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow (Pimephales promelas) through a novel mechanism of action. Aquat. Toxicol. 110, 74–83.

Daniels, P.H., 2009. A brief overview of theories of PVC plasticization and methods used to evaluate PVC-plasticizer interaction. J. Vinyl Add. Tech. 15, 219–223.

David, R.M., Moore, M.R., Finney, D.C., Guest, D., 2001. Reversibility of the chronic effects of di(2-ethylhexyl) phthalate. Toxicol. Pathol. 29, 430–439.

Déchaud, H., Ravid, C., Claustret, F., Brac de la Perrière, A., Pugnet, M., 1999. Xenotoxnon interaction with human sex hormone-binding globulin (SHBG). Steroids 64, 328–334.

DeFoe, D.L., Holcombe, G.W., Hammesberger, D.E., Biesinger, K.E., 1990. Solubility and toxicity of eight phthalate esters to four aquatic organisms. Environ. Toxicol. Chem. 9, 623–636.

Degitz, S.J., Holcombe, G.W., Flynn, K.M., Kosian, P.A., Korte, J.J., Tietge, J.E., 2005. Progress towards development of an amphibian-based thyroid screening assay using Xenopus laevis. Organismal and thyroidal responses to the model compounds 6-propylthiouracil, methimazole, and thyroxine. Toxicol. Sci. 87, 353–364.

Demir, A.P.T., Ulutan, S., 2013. Migration of phthalate and non-phthalate plasticizers out of plasticized PVC films into air. J. Appl. Polym. Sci. 128, 1948–1954.

Duan, Z., Zhu, L., Yao, K., Zhu, X., 2008. Individual and joint toxic effects of phthalate esters, alkylphenols, bisphenol a and di(2-ethylhexyl)adipate from food packaging. Food Control 27, 1332–1339.

Foster, P.M.D., 2006. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int. J. Androl. 29, 140–147.

Foster, P.M.D., Lake, B.G., Thomas, L.V., Cook, M.W., Gangolli, S.D., 1981. Studies on the testicular effects and zinc excretion produced by various isomers of monobutyl-o-phthalate in the rat. Chem. Biol. Interact. 34, 233–238.

Fromme, H., Küchler, T., Otto, T., Pilz, K., Müller, J., Wenzel, A. 2002. Occurrence of phthalates and bisphenol A and F in the environment. Water Res. 36, 1429–1437.

Fujii, S., Yabe, K., Furukawa, M., Hirata, M., Kiguchi, M., Ikka, T., 2005. A two-generation reproductive toxicity study on diethyl phthalate (DEP) in rats. J. Toxicol. Sci. 30, 97–116.

Fukuoka, M., Tanimoto, T., Zhou, Y., Kawasaki, N., Tanaka, A. 1989. Mechanism of testicular atrophy induced by di-n-butyl phthalate in rats. Part 1. J. Appl. Toxicol. 9, 277–283.

Gazouli, N.S., Dhamla, C.R., Indu, A.R., Kurup, P.A., 2004. Changes in some hormones by low doses of di (2-ethyl hexyl) phthalate (DEHP), a commonly used plasticizer in PVC blood storage bags & medical tubing. Indian J. Med. Res. 119, 139–144.

Gazouli, M., Yao, X.Z., Bourjard, N., Corton, J.C., Culky, M., Papadopoulos, V. 2002. Effect of peroxisome proliferators on leydig cell peripheral-type benzodiazepine receptor gene expression, hormone-stimulated cholesterol transport, and steroidogenesis: role of the peroxisome proliferator-activator receptor α. Endocrinology 143, 2571–2583.

Gilbert, Y., Sassi-Messai, A., Fini, J.B., Bernard, L., Zalio, D., Cravedi, J.P., Balaguer, P., Andersson-Lendahl, M., Demeneix, B., Lauvet, V., 2011. Bisphenol a induces otothil malformations during vertebrate embryogenesis. BMC Dev. Biol. 11, 1–17.

Giribabu, N., Sainath, S.B., Reddy, P.S., 2014. Prenatal di-n-butyl phthalate alters reproductive functions at adulthood in male rats. Environ. Toxicol. 29, 529–538.

Glenneneer, K.A., Denver, R.J. 2002. Developmental changes in interrenal responsiveness in anuran amphibians. Integr. Comp. Biol. 42, 565–573.

Gonzalez, F.J., 2005. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. Mutat. Res. 569, 101–110.

Grasselli, F., Barta, L., Baiso, L., Russo, R., Grolli, S., Basini, G., 2010. Bisphenol A disrupts granulosa cell function. Domest. Anim. Endocrinol. 39, 34–39.

Gray, K.M., Janssens, P.A., 1990. Gonadal hormones inhibit the induction of metamorphosis by hormones in Xenopus laevis tadpoles in vivo, but not in vitro. Gen. Comp. Endocrinol. 77, 202–211.

Gray, T.J.B., Bagam, J.A., Foster, J.R., Gangolli, S.D., 1983. Peroxosomal effects of phthalate esters in primary cultures of rat hepatocytes. Toxicology 28, 167–179.

Gray, L.E.J., Barlow, N.J., Howeswell, K.L., Ostby, J.S., Furr, J.R., Gray, C.L., 2009. Transgenerational effects of di-(2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: added value of assessing multiple offspring per litter. Toxicol. Sci. 110, 411–425.

Gupta, R.K., Singh, J.M., Leslie, T.C., Meachum, S., Flaws, J.A., Yao, H.H.C., 2010a. Di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate inhibit growth and increase estradiol levels of antral follicles in vitro. Toxicol. Appl. Pharmacol. 242, 224–230.

Gupta, S.C., Sharma, A., Misra, M., Choudhuri, D.K., 2010b. Heat shock proteins in toxicology: how close and how far? Life Sci. 86, 377–384.

Hannas, B.R., Furr, J., Lambright, C.S., Wilson, V.S., Foster, P.M.D., Gray, L.E.J., 2011. Dibutyl phthalate dosing during sexual differentiation disrupts fetal testis function and postnatal development of the male Sprague-Dawley rat with greater relative potency than other phthalates. Toxicol. Sci. 120, 184–193.

Harnois, S., Sinno, H., Nishimoto, M., 1998. Comparative study of specific cytochrome P450 isoforms by bisphenol A in rat liver. Arch. Toxicol. 72, 387–394.

Hanss, R.R., Furr, J., Lambright, C.S., Wilson, V.S., Foster, P.M.D., Gray, L.E.J., 2011. Dibutyl phthalate dosing during sexual differentiation disrupts fetal testis function and postnatal development of the male Sprague-Dawley rat with greater relative potency than other phthalates. Toxicol. Sci. 120, 184–193.

Hausen, R., Meeker, J.D., Singh, N.P., Silva, M.J., Ryan, L., Duty, S., Calafat, A.M., 2007. DNA damage in human sperm is related to urinary levels of phthalate monoesters, and increases from age 17 to age 45. Environ. Health Perspect 115, 167–179.

Hofmann, P.J., Schomburg, L., Köhle, J., 2009. Interference of endocrine disruptors with the thyroid hormone receptor – dependent transactivation. Toxicol. Sci. 110, 125–137.

Horn, O., Nalli, S., Cooper, D., Nicell, J. 2004. Plasticizer metabolites in the environment. Water Res. 38, 3693–3698.

Hoshino, N., Iwai, M., Okazaki, Y. 2005. A two-generation reproductive toxicity study of diclofuryl phthalate in rats. J. Toxicol. Sci. 30, 79–96.

Hotchkiss, A.K., Rider, C.V., Blystone, C.R., Wilson, V.S., Hartig, P.C., Ankley, G.T., Foster, P.M., Gray, C.L., Gray, L.E. 2008. Fifteen years after «wingspread» – an environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. Toxicol. Sci. 105, 235–259.

Hosseiwad, K.L., Wilson, V.S., Furr, J., Lambright, C.M., Rider, C.V., Blystone, C.R., Hotchkiss, A.K., Gray, L.E.J., 2008. A mixture of five phthalate esters inhibits fetal testicular P450 aromatic oxidase activity.
testicular testosterone production in the Sprague–Dawley rat in a cumulative, dose-additive manner. Toxicol. Sci. 105, 153–165.

Hu, F., Smith, E.E., Carr, J.A., 2008. Effects of larval exposure to estradiol on spermatogenesis and in vitro gonadal steroid secretion in African clawed frogs, *Xenopus laevis*. Gen. Comp. Endocrinol. 155, 190–200.

Huang, P.C., Kuo, P.L., Guo, Y.L., Liao, P.C., Lee, C.C., 2007. Associations between urinary phthalate metabolites and thyroid hormones in pregnant women. Hum. Reprod. 22, 2715–2722.

Huang, H., Tan, W., Wang, C.C., Leung, L.K., 2012. Bisphenol A induces corticotropin-releasing hormone expression in the placental cells JEG-3. Reprod. Toxicol. 34, 317–322.

Hurst, C.H., Waxman, D.J., 2003. Activation of PPARα and PPARγ by environmental phthalate monooesters. Toxicol. Sci. 74, 297–308.

Imaoka, S., Mori, T., Kinoshita, T., 2007. Bisphenol a causes malformation of the head region of *Xenopus laevis* and decreases the expression of the ESR-1 gene mediated by Notch signaling. Biol. Pharm. Bull. 30, 371–374.

Isenberg, J.S., Kamendulis, L.M., Smith, J.H., Ackley, D.C., Smith, J.H., Lington, A.W., Pugh, G.J., 2007. Effects of di(2-ethylhexyl) phthalate (DEHP) on gap-junctional intercellular communication (CJCL), DNA synthesis, and peroxisomal beta oxidation (PBOX) in rat, mouse, and hamster liver. Toxicol. Sci. 56, 73–85.

Isenberg, J.S., Kamendulis, L.M., Ackley, D.C., Smith, J.H., Pugh, G.J., Lington, A.W., McKeever, R.H., Klauing, J.E., 2001. Reversibility and persistence of di(2-ethylhexyl) phthalate (DEHP)- and phenobarbital-induced hepatic cellular changes in rodents. Toxicol. Sci. 64, 192–199.

Ishihara, A., Sawatubashi, S., Yamashita, K., 2003. Endocrine disrupting chemicals: interference of these hormones binding to transferrin and to thyroid hormone receptors. Mol. Cell. Endocrinol. 199, 105–117.

Iwamuro, S., Sakakibara, M., Terao, M., Ozawa, A., Chigeura, T., Kato, M., Kikuyama, S., 2003. Teratogenic and anti-metamorphic effects of bisphenol a on embryonic developmental of *Xenopus laevis*. Gen. Comp. Endocrinol. 133, 189–196.

Iwamuro, S., Yamada, M., Kato, M., Kikuyama, S., 2006. Effects of bisphenol a on thyroid hormone-dependent up-regulation of thyroid hormone receptor α and β and down-regulation of retinoid X receptor α in *Xenopus* tadpole life cycle. Sci. Total. Environ. 363, 217–221.

Jarfelt, K., Dalgaard, M., Hass, U., Borch, J., Jacobsson, H., Ladeffoged, O., 2005. Antiandrogenic effects in male rats perinatally exposed to a mixture of di(2-ethylhexyl) phthalate and di(2-ethylhexyl) adipate. Reprod. Toxicol. 19, 505–512.

Jobling, S., Casey, D., Rodgers-Gray, T., Oehlmann, J., Schulte-Oehlmann, U., Pawlowski, A., Baumbek, T., Turner, A.P., Tyler, C.R., 2003. Comparative reproductive endocrine disruption of fish to environmental estrogens and an estrogenic effluent. Aquat. Toxicol. 65, 205–220.

Johnson, E.F., Palmer, C.N., Griffin, K.J., Hsu, M.H., 1996. Role of the peroxisome proliferator-activated receptor in cyctochrome P450 4A gene regulation. FASEB J. 10, 1241–1248.

Jurgella, G.F., Marwah, A., Malison, J.A., Peterson, R., Barry, T.P., 2006. Effects of xenobiotics and steroids on renal and hepatic estrogen metabolism in lake trout. Gen. Comp. Endocrinol. 148, 273–281.

Kaihara, E., Sato, K.F., Kouchi, T., Nakanou, Y., Inoue, M., 2002. Role of oxidative stress in germ cell apoptosis induced by di(2-ethylhexyl)phthalate. Biochem. J. 365, 849–856.

Kawak, K., Murakami, S., Senba, E., Yamanaka, T., Fujiwara, Y., Arimura, C., Nozaki, T., Takahashi, Y., 2007. Changes in estrogen receptor α and β and androgen receptor in the brain of mice exposed prenatally to bisphenol A. Regul. Toxicol. Pharmacol. 46, 177–180.

Keith, Y., Cornu, M.C., Canning, P.M., Foster, J., Lhuguenot, J.C., Elcombe, C.R., 1992. Effects of (2-ethylhexyl) phthalate on thyroid hormone metabolism in rats. Reprod. Toxicol. 7, 195–202.

Kikuyama, S., 2003. Teratogenic and anti-metamorphic effects of bisphenol a on embryonic developmental of *Xenopus laevis*. Gen. Comp. Endocrinol. 133, 189–196.

Keith, Y., Cornu, M., Canning, P.M., Foster, J., Lhuguenot, J.C., Elcombe, C.R., 1992. Effects of (2-ethylhexyl) phthalate on thyroid hormone metabolism in rats. Reprod. Toxicol. 7, 195–202.

Leitz, J., Kubballa, T., Rehm, J., Lachenmeier, D.W., 2009. Chemical analysis and risk assessment of dietary intake of phthalate monoesters. Arch. Toxicol. 66, 321–326.

Leitz, J., Kubballa, T., Rehm, J., Lachenmeier, D.W., 2009. Chemical analysis and risk assessment of dietary intake of phthalate monoesters. Arch. Toxicol. 66, 321–326.

Linn, D.A., Zhang, T., Fang, H.J.P., He, J., 2008. Phthalates biodegradation in the environment. Appl. Microbiol. Biotechnol. 80, 183–198.

Linton, A.W., Bird, M.G., Plutnic, R.T., Stubbfeld, W.A., Scala, R., 1999. Chronic toxicology and carcinogenic evaluation of diisononyl phthalate in rats. Fundam. Appl. Toxicol. 44, 211–222.

Lizcano, T., Takii, M., Kubo, C., 2007. Changes in estrogen receptors α and β and decreases the expression of the ESR-1 and ESR-2 genes in male rats exposed to di (n-octyl) phthalate. Toxicol. Appl. Pharmacol. 211, 60–68.

López-Carillo, L., Hernández-Ramírez, R.U., Calafat, A.M., Torres-Sánchez, L.,Galván-Portillo, M., Needham, L.L., Ruiz-Ramos, R., Cebríai, M.E., 2010. Exposure to phthalates and breast cancer risk in northern Mexico. Environ. Health Perspect. 118, 539–544.

López-Cervantes, J., Paseiro-Losada, P., 2003. Determination of bisphenol A in, and its migration from, PVC stretch film used for food packaging. Food Addit. Contam. 20, 596–600.

Loveland, T.N., Davis, B.J., 2001. Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. Toxicol. Appl. Pharmacol. 172, 217–224.

Lutz, I., Klose, W., 1999. Amphibians as a model to study endocrine disruptors: I. Environmental pollution and estrogen receptor binding. Sci. Total. Environ. 225, 49–57.

Lutz, I., Klose, W., Springer, T.A., Holden, L.R., Wolf, J.C., Krueger, H.O., Hosmer, A.J., 2003. Amphibians as a model to study endocrine disruptors: I. Environmental pollution and estrogen receptor binding. Sci. Total. Environ. 225, 59–68.

Knudsen, F.R., Pottinger, T.G., 1999. Interaction of endocrine disrupting chemicals, single compounds and mixtures, with estradiol-, antiandrogen-, and corticosteroid-binding sites in rainbow trout (Onchorhynchus mykiss). Aquat. Toxicol. 44, 159–170.

Koch, S.M., Müller, J., Angerer, J., 2007. Determination of secondary, oxidised di-iso-nonylphthalate (DINP) metabolites in human urine representative for the exposure to commercial DINP plasticizers. J. Chromatogr. B 847, 114–125.

Kiquer, G.G., Lemmen, J.G., Carlsson, B.O., Corton, J.C., Safe, S.H., Van der Saag, P.T., Ver Dieur, B., Gustafsson, J.A., 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139, 4252–4263.

Kurata, Y., Kidachi, F., Yokoyoma, M., Toyota, N., Tsuchitani, M., Kato, M., 1998. Subchronic toxicity of di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. Toxicol. Sci. 42, 49–56.
di(2-ethylhexyl)phthalate and mono-(2-ethylhexyl)phthalate in seminal plasma. Biomed. Chromatogr. 21, 1166–1171.

McCarthy, J.F., Whitmore, D.K., 1981. Chronic toxicity of di-n-butyl and di-octyl phthalate to Daphnia magna and the fathead minnow. Environ. Toxicol. Chem. 4, 167–179.

Metcalfe, C.D., Metcalfe, T.L., Kiparissis, Y., Koenig, B.G., Khan, C., Hughes, R.J., Kiparissis, Y., Koenig, B.G., Khan, C., Hughes, R.J., 2005. Endocrine disrupting effects of veterinary pharmaceuticals: a review of the potential risks to wild aquatic ecosystems. Environ. Pollut. 138, 1–12.

Miyazawa, Y., Miyata, K., 1990. Developmental screening of endocrine-disrupting chemicals using in vitro assays. Environ. Pollut. 66, 1–9.

Moore, M.C., Thompson, C.W., 1990. Field endocrinology of reptiles: hormonal control of alternative male reproductive tactics. In: Epple, S., Coggins, C.G., Stetson, H. (Eds.), Progress in Comparative Endocrinology. Wiley-Liss, New York, NY, USA, pp. 685–690.

Morales, R., Wang, R., Russo, L.H., Maillo, D.A., Lamontagne, C.A., Russo, L.H., 2007. The plastic bottle butyl phthalate induces genomic changes in the rat young mammalian gall bladder after perinatal/pupal exposure. BMC Genomics 8, 453.

Morisawa, T., Tagami, T., Kamuzu, T., Usui, T., Saji, M., Kanamoto, N., Hayata, Y., Shimatsu, A., Kuzuya, H., Nakao, K., 2002. Thyroid hormone action is disrupted by diethylhexyl phthalate. J. Clin. Endocrinol. Metab. 87, 513–518.

Mugański, E.C., 2004. Leaching of plasticizers from urine: indications of autologous blood transfusion in sports. Transfer 52, 647–657.

Moen, M., Thompson, C.W., 1990. Field endocrinology of reptiles: hormonal control of alternative male reproductive tactics. In: Epple, S., Coggins, C.G., Stetson, H. (Eds.), Progress in Comparative Endocrinology. Wiley-Liss, New York, NY, USA, pp. 685–690.

Morita, R., Wang, R., Russo, I.H., Russo, J., 2007. The chronic toxicity of di-n-butyl phthalate in the laboratory. Part I: bisphenol A and octylphenol as xeno-estrogens. Environ. Toxicol. Chem. 26, 1005–1009.

Mitra, S., Skulski, S., Kubo, T., 1999. Hormonal reversal and the genetic control of sex differentiation in Xenopus. Zoolog. Sci. 16, 335–340.

Montfort, N., Ventura, R., Platon, P., Hurschts, T., Brixius, K., Schönheit, P., Thevis, M., Geyer, H., Segura, J., 2012. Plasticizers excreted in urine: indications of autologous blood transfusion in sports. Transfer 52, 227–235.

Miyata, S., Ito, K., Kubo, T., 1999. Di(2-ethylhexyl) phthalate. In: Schulte-Oehlmann, U., Oehlmann, J., Schulte-Oehlmann, U., Tillmann, M., Markert, B., 2000. Effects of environmental endocrine disruptors on gonadal sex differentiation of genetic male and female grass carp (Ctenopharyngodon idella). J. Expo. Sci. Environ. Epidemiol. 10, 595–601.

Miyata, S., Okita, K., Kubo, T., 1999. Hormonal reversal and the genetic control of sex differentiation in Xenopus. Zoolog. Sci. 16, 335–340.

Miyata, S., Okita, K., Kubo, T., 1999. Hormonal reversal and the genetic control of sex differentiation in Xenopus. Zoolog. Sci. 16, 335–340.

Miyata, S., Okita, K., Kubo, T., 1999. Hormonal reversal and the genetic control of sex differentiation in Xenopus. Zoolog. Sci. 16, 335–340.
Schoonjans, K., Staelens, B., Auwers, J. 1996. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. Biochem. Biophys. Acta 1302, 1–9.

Segner, H., Caroll, K., Fenske, M., Janssen, C.R., Maack, G., Pascoe, D., Schafer, C., Vandenbergh, G.F., Watts, M., Wenzel, A. 2003. Identification of endocrine-disrupting effects in aquatic vertebrates and invertebrates: report from the non-mammalian aquatic species workshop. Environ. Toxicol. Chem. 22, 302–314.

Shen, H.Y., Jiang, H.L., Mao, H.L., Pan, G., Zhou, L., Cao, Y.F. 2007. The peroxisome proliferator alpha agonist, clofibrate, disrupts thyroid hormone metabolism in H295R cells. Toxicol. Sci. 98, 299–308.

Sone, K., Hinago, M., Kitayama, A., Morokuma, J., Ueno, N., Watanabe, H., Iguchi, T. 2011a. Potency of phthalate ester induced cervicothoracic fusion defects in Mullerian ducts. Toxicol. Sci. 121, 320–327.

Sone, K., Hinago, M., Kitayama, A., Morokuma, J., Ueno, N., Watanabe, H., Iguchi, T. 2011b. Potency of phthalate ester induced cervicothoracic fusion defects in Mullerian ducts. Toxicol. Sci. 121, 320–327.

Struve, M.F., Gaido, K.W., Hensley, J.B., Lehmann, K.P., Ross, S.M., Sochaski, M.A., Suffet, I.H. 2007. Human pharmaceuticals, antioxidants, and plasticizers in wastewater treatment plant and water reclamation plant effluents. Water Environ. Res. 79, 159–166.

Sun, Y., Irie, M., Kishikawa, N., Wada, M., Kuroda, N., Nakashima, K. 2004. Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. Biomed. Chromatogr. 18, 501–507.

Teil, M.J., Blanchard, M., Dargnat, C., Larcher-Tiphagne, K., Chevreul, M. 2007. Occurrence of phthalate diesters in rivers of the Paris district (France). Hydro. Process. 25, 251–255.

Thibault, S., Porte, C. 2004. Effects of endocrine disruptors on sex steroid synthesis and metabolism pathways in fish. J. Steroid Biochem. Mol. Biol. 92, 485–494.

Til, R.W., Myers, C.B., Marr, M.C., Fail, P.A., Seeley, J.C., Brine, D.R., Barter, R.A., Butala, J.H. 2004. Reproductive toxicity evaluation of dietary butyl benzyl phthalate (BBP) in rats. Reprod. Toxicol. 18, 241–264.

Urein-Webster, T.M., Lewis, C., Filby, A.L., Paul, G.C., Santos, E.M. 2010. Mechanisms of toxicity of di(2-ethylhexyl) phthalate on the reproductive health of male zebrafish. Aquat. Toxicol. 99, 360–369.

Vandenbarg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V. 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24, 139–172.

Ward, J.M., Peter, J.M., Perella, C.M., Gonzalez, F.J. 1998. Receptor and nonreceptor-mediated organ-specific toxicity of di(2-ethylhexyl) phthalate (DEHP) in porcine proliferator-activated receptor a-null mice. Toxicol. Pathol. 26, 240–246.

Warren, J.R., Lalwani, N., Reddy, J.K. 1982. Phthalate esters as peroxisome proliferators. Environ. Health Perspect. 45, 35–40.

Woo, E., Wang, Y., Zhao, Y., Berber, C., Giesy, J.P., Wilcoxon, J.C., 2012. Brominated flame retardants in the Great Lakes region. Environ. Sci. Technol. 46, 7616–7619.

Xia, L., Sun, X., Zhang, H., He, P.J., Shao, L.M. 2011. Leaching behaviour of bisphenol A from polystyrene bottles in water. Food. Chem. Toxicol. 49, 2140–2146.

Xu, S.Y., Zhang, H., He, P.J., Shao, L.M., 2011. Leaching behaviour of bisphenol A from polystyrene bottles in water. Food. Chem. Toxicol. 49, 2140–2146.

Yamasaki, K., Okuda, H., Takeuchi, T., Minobe, Y. 2009. Effects of in utero through postnatal exposure to bisphenol A and di(2-ethylhexyl)-phthalate on gonadal development of male mice. Environ. Sci. Pollut. Res. 16, 2515–2527.

Zeng, W., Yang, L., Zha, J., Zhang, X., Li, W., Li, Z., Wang, Z. 2010. Alterations in mRNA expression of steroid receptors and heat shock proteins in the liver of rare minnow (Gobiocephalus rarus) exposed to atrazine and p,p′-DDE. Aquat. Toxicol. 98, 381–387.

Ye, T., Kang, M., Huang, Q.S., Fang, C., Chen, Y.J., Hong, D.J. 2014. Exposure to DEHP and MEHP from bathing to adulthood causes reproductive dysfunction and endocrine disruption in male medaka (Oryzias melasigma). Aquat. Toxicol. 146, 115–125.

Yokota, H., Tsuruya, Y., Umeda, M., Oshima, Y., Tadokoro, H., Nakazato, A., Honjo, T., Kobayashi, K. 2000. Effects of bisphenol A on the early life stage in Japanese medaka (Oryzias latipes). Environ. Toxicol. Chem. 19, 1525–1530.

Zacharewski, T.R., Meek, M.D., Clemens, J.H., Wu, Z.F., Fielden, M.R., Matthews, J.B. 1998. Examination of the in vitro and in vivo estrogenic activities of eight phthalates- the human biomonitoring approach. Mol. Nutr. Food Res. 53, 7–31.

Zhou, J., Cai, Z.H., Xing, K.Z. 2011a. Potential mechanisms of phthalate ester related adverse effects on spermatogenesis in rats. Arch. Toxicol. 85, 121–125.

Zhou, J., Cai, Z.H., Xing, K.Z. 2011b. Potential mechanisms of phthalate ester related adverse effects on spermatogenesis in rats. Arch. Toxicol. 85, 121–125.

Zhou, J., Zhu, X.S., Cai, Z.H. 2011b. Influences of DMP on the fertilization process and subsequent embryogenesis of abalone (Haliotis discolor discolor) by gametes. Aquat. Toxicol. 98, 2595–2599.

Zoeller, R.T. 2005. Environmental chemicals as thyroid hormone analogues: new studies indicate that PPARα and PPARγ receptors are targets of industrial chemicals? Mol. Cell. Endocrinol. 242, 10–15.