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Evidence of reproductive disruption associated with neuroendocrine changes induced by UV–B filters, phtalates and nonylphenol during sexual maturation in rats of both gender

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ABSTRACT

Endocrine disruptors (EDs) are exogenous substances or xenoestrogens natural or synthetic, capable of interacting with different systems and altering their normal hormonal regulation, being the reproductive system one of the most affected. EDs produce their effects not only by acting on nuclear steroid receptors, but also on membrane receptors, steroidal and non-steroidal synthetic enzymatic pathways and/or metabolism. The incorporation to the body depend on each EDs, which are liposoluble and easily deposited in the tissue; thus ensuring a prolonged accumulation and release, even when the exposure is not continuous. In addition to cross the placenta, EDs may act in the offspring during the reproductive system formation and maturation key stages and its regulatory mechanisms. The effects of EDs can be multiple, but most acts mediating estrogenic and/or antiandrogenic effect. Three groups of EDs are widely used: in plastics (phtalates), sunscreens (cinnamate and methylbenzylcamphor), and detergents (nonylphenol). In this paper we review the effects of the exposure to these environmental chemicals on the reproductive system and the possible mechanisms by which they occur, focusing in the hypothalamic–pituitary neuroendocrine mechanisms that regulate the reproductive system.

1. Introduction

Environmental pollution had and has a major impact on biological systems. In the last few decades huge amounts of chemicals of different origin, structure and use coming from industrialization have been released into the environment as chemical waste, causing changes to the exposed living organisms. These substances are generically considered endocrine disrupters (EDs) (Schug et al., 2011). They have been also called xenoestrogens or xenobiotics as they have the capacity to alter hormonal homeostasis through the same biological paths used by endogenous steroids (Akingbemi and Hardy, 2001; Sharpe, 2001), modifying the endocrine system, like the reproductive and the thyroid axis (Danzo, 1998; Heindel, 2006; Schmutzler et al., 2004; Zoeller, 2007) and causing adverse health effects in an organism or its progeny. One decade ago, Krimsky suggested a new hypothesis known as "Endocrine Disruption" (Krimsky, 2000), (see Table 1). At present, the US Environmental Protection Agency (EPA) defines them as: "Exogenous agents that interfere with synthesis, secretion, transport, metabolism, binding action or elimination of natural blood-borne hormones that are responsible for homeostasis, reproduction and developmental process".

There is increasing interest and research on effects of EDs on neuroendocrine systems (Dickerson and Gore, 2007; Gore, 2010). Most studies have focused on the effects of chemicals known for several years as EDs (pesticides, phytoestrogens, dioxins, etc.) on gonadal and thyroid neuroendocrine systems. This article will primarily discuss the literature on neuroendocrine alterations induced by substances used to manufacture products used daily, such as plastics (phthalates), sunscreens and cosmetics (cinnamate and methylbenzylcamphor) as well as detergents (nonylphenol), focusing in their effects on the hypothalamic–pituitary-gonadal axis and the sequelae on reproductive function.

1.1. Characteristics and mechanism of action of EDs

EDs include substances with estrogenic and/or antiestrogenic, androgenic and/or antiandrogenic actions (Carbone et al., 2010; Carou et al., 2008; Rivas et al., 2002) and mimetizers or antagonists of the thyroid hormones (Brucker-Davis, 1998; Schmutzler et al., 2004). Also EDs can interference with hormonal feedback regulation and neuroendocrine cells. Along with the direct influence of EDCs on estrogen or androgen actions, they can affect endogenous steroid production through negative and positive feedback,

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Table 1
Endocrine disruption hypothesis.

The effects occur through mechanisms of different action, all of them related to the hormonal action
There is no exposure limit without effect. It is shown a relation dose-effect not necessary lineal, which in some cases is apparently paradoxical with U or inverted U curve types. May have effect in low doses (parts per billion) while not showing manifestation at high doses. While the limits of exposure are measured in parts per million (ppm), the body concentrations of some of them exceed that limit
Most of the effects are persistent and bioaccumulative and have a latency period of decades
Their effects are specifically evident in critical development periods or in moments of particular susceptibility
Their effects may be the result of the combine action of different compounds that may trigger a synergic, antagonic and/or additive response
The expression of the effects may appear late in life in the exposed individual, and even appear over generations

effects that may differ depending on developmental stage, leading to perturbations in neuroendocrine systems function (Gore, 2010).

At the molecular level EDs can act through different mechanism (De Coster and van Larebeke, 2012): (a) activation of the classical nuclear receptors ERα and ERβ, which bind estrogen responsive elements (EREs) in the promoters of target genes, regulating its expression (Levin, 2005), (b) activation of membrane-bound estrogen receptors: mERα, mERβ, and GPR30 (non nuclear steroid hormone receptors), inducing rapid nongenomic responses and acting through second messenger-triggered signal cascades (Watson et al., 2007), (c) binding to cytosolic ER receptors, activating the kinases and leading to the activation of the Src/Ras/ERK signaling cascade (Nadal et al., 2001), (d) cross-talk between genomic and nongenomic pathways (Silva et al., 2010), (e) activation of estrogen-related receptors (ERR), which are orphan nuclear receptors (Takayanagi et al., 2006), (f) changes in DNA Methylation or Histone-Modifications, that produced epigenetic modifications (Ho et al., 2006). Also early life exposures to EDs may alter gene expression in hypothalamic nuclei via nongenomic epigenetic mechanisms (Gore, 2008), modifying the phenotype expression and explaining the fact that multi and trans-generational exposure to EDs may promote the development of a disease over future generations (Hoshino et al., 2005; Anway and Skinner, 2006), (g) acting on the enzymatic pathways involved in steroid biosynthesis and/or metabolism of hormones, such as the enzymes 3b-HSDs and 17b-HSDs, aromatase, sulphatases (Whitehead and Rice, 2006).

EDs may be transferred through placental circulation and breast secretion (Campoy Folgoso et al., 2004; Nishikawa et al., 2010; Rogan et al., 1987;Wickizer and Brilliant, 1981; Schlumpf et al., 2010).), disturbing sexual differentiation and development of exposed offspring during early prenatal or postnatal stages (Colborn et al., 1993). Most of the EDs are lipid-soluble substances and they tend to be deposited especially in adipose tissue, in a dose-dependent manner, causing a very important cumulative effect (Schlumpf et al., 2004). In addition, simultaneous exposure to several EDs produced an additive or synergic effect (Crews et al., 2003). Exposure to EDs minimum doses may cause even more drastic effects than the ones appearing in high doses exposure. This occurs because EDs exert their effects following a non classic response dose curve: in inverted U or U shape (Vandenberg et al., 2012; vom Saal et al., 2007). This would explain the fact that EDs may alter the normal function of the endocrine system even at doses that could be considered, from toxicological point of view, as "safe dose" or "safety margin".

EDs are released in the environment and can be found in natural water courses and in fish (Poiger et al., 2004). In humans, exposure occurs through air, contaminated water or food intake, dermal contact and even through medical consumables and devices such as catheters, breathing and respiratory equipment and blood bags. It is also possible that the disruptive endocrine action may come from food ingestion with some hormonal action per second. For example, it has been demonstrated that urinary concentration of the estrogenic ED genistein was about 500 times higher in the soy milk formula-fed infants than in the cow milk formula-fed infants (Cao et al., 2009).

1.2. Impact of EDs on male and female reproductive peripheral organ

Many decades ago some researchers observed the loss of reproductive capacity, malformation in the reproductive organs and abnormal sexual behavior in animals of the ecosystem (Carson, 1962).

One of the more important variable to take into account to evaluate the impact of EDs on the reproductive system is the period of exposure, being more relevant during early fetal and neonatal developmental, when the programming of the endocrine system is carried out. In males, exposure to EDs during gestation and early stages of life has been related with the presence of genital malformations as cryptorchidism, hypospadia and decreased anogenital distance (Boisen et al., 2001; Gray Jr. et al., 2000; Skakkebaek et al., 2001; Swann et al., 2005), decreased sperm quality and increased testicular cancer incidence (Landrigan et al., 2003; Olea Serrano and Zuluaga Gómez, 2001; Paulozzi, 1999). Skakkebaek et al. (2001) described the testicular dysgenesis syndrome (TDS), characterized by the presence of testicular cancer, hypospadia, cryptorchidism and low semen quality as a consequence of exposure to environmental contaminants. The reduction of the anogenital, distance parameter used as biomarker of reproductive effects, has been related with, higher levels of phthalates in urinary excretion and in the amniotic fluid in exposed gestational mothers (Huang et al., 2009; Swann et al., 2005). The decrease in sperm count in human, would be related with gonadal atrophy attributable to chronic exposure from fetal development to xenoestrogens (Sharpe et al., 1993; Toppari et al., 1996), similar to that produced by dietary 17 β-estradiol exposure (10 and 50 ppm) in adult rats (Cook et al., 1998).

In females the early contact with EDs during development has been related to an acceleration of pubertal development, polycystic ovary syndrome and cystic endometrial hyperplasia, endometriosis, uterine fibroids, premature, thelarche, early menarche, irregular menstrual cycle and a higher risk regarding breast and cervical cancer (Crain et al., 2008). It is well known the high incidence of ovarian, breast and vaginal carcinoma observed in daughters born form women treated with diethylstilbestrol (DES) during pregnancy (Blatt et al., 2003; Titus-Ernstoff et al., 2010). Direct exposure to EDs during woman reproductive stage was related to implantation failure, miscarriages and premature birth (Crain et al., 2008).

1.3. Neuroendocrine control of sexual maturation and reproduction. Possible interference by EDs

During the last two decades, an earlier onset of puberty was described (Herman-Giddens et al., 1997; Lee et al., 2001). It was not related to improvement in health and nutritional status, because they not always are accompanied by an increase of fat mass and leptin (Aksglaede et al., 2009). Thus, other factors including EDs could be involved in this early puberty (Teilmann et al., 2002). Puberty is a phenomenon that is initiated and regulated at neuroendocrine level. The control of the reproductive neuroendocrine regulation involves neurons in the basal hypothalamus that synthesize and release the decapeptide GnRH, which drives reproduction throughout the life cycle and also is the primary stimulus to the pituitary and gonadal axis. It is known that sexual hormones regulate the

reproductive axis acting on the peripheral organs, but they also change hypothalamic GnRH and pituitary gonadotropin secretion (Herbison, 1998). In males, estrogen participates in the negative feedback mechanism at hypothalamic level (Rochira et al., 2006), repressing GnRH promoter in vitro at 1 nM concentration (Roy et al., 1999). In females, estrogens are involved in the maturity of the positive feedback mechanism and in ovulation (Herbison, 1998). On the other hand, testosterone produces a tonic inhibitory feedback on neuroendocrine system in males (Mooradian et al., 1987). It is important to underline that GnRH neurons could be directly involved in responding to estrogens (Matagne et al., 2003) from early development stage, when 17 β estradiol (100 nM) stimulates the neurogenesis of precursors to GnRH neurons in the olfatory placode (Agça et al., 2008). In adulthood these estrogenic effects may become by indirect mechanism, involving other estrogen responsive cells. In immature female rats 17 β estradiol (10^{-7} M) increases the frequency of pulsatile GnRH secretion in hypothalamic explants. Similarly, early postnatal exposure of female rats to estradiol or to the insecticide DDT (dichlorodiphenyltrichloroethane), substance with estrogenic action, results in early developmental acceleration of the GnRH secretion in vitro (estradiol 10^{-9} M and DDT10^{-6} M) (Raiser et al., 2007). Neuroendocrine control of reproduction and its alteration following exposure to sex steroids during fetal or perinatal life has been described (Gorski, 1968). Prenatal exposure to testosterone propionate (100 mg twice per week from day 30 to day 90 of pregnancy) caused alteration of pubertal timing and estrous cyclicity in ewes through neuroendocrine alteration of estradiol positive feedback (Unsworth et al., 2005).

In vivo and in vitro assays have shown EDs estrogenic activity. For example, the UV filters 4-methylbenzylidene Camphor (4-MBC) and octyl methoxycinnamate (OMC) increased proliferation in breast cancer cells lines (concentration values between 1.56–3.73 μM) and in uterine weight when they were administered to rats (4-MBC 309 mg/kg/day and OMC 935 mg/kg/day in chow) (Schlumpf et al., 2001). Also, 4-MBC (35.3 μM in vitro) was able to bind to the estrogen receptor alpha (Schlumpf et al., 2004). Therefore, the probable action of an endocrine disruptor with estrogenic/antiandrogenic effect would alter normal neuroendocrine pathway causing an abnormal development and function of reproductive axis in both sexes.

Neuroendocrine sexual differentiation of the reproductive system occurs during the last gestational stage and at early neonatal period (Vermeulen, 1993). Estrogens or estrogen mimetic substances act more efficiently when they are administered during gestation (Gray and Kelce, 1996). On the other hand, certain EDs might interfere in the regulation of the reproductive axis altering the gonadotropin pituitary secretion (Carou et al., 2008, 2009a, 2009b; Furuta et al., 2006). Estrogens regulate the neuroendocrine activity of the reproductive axis through receptors like the beta type estrogen expressed in GnRH hypothalamic neurons (Hrabovszky et al., 2001). Also, it is well known that at hypothalamic level, one mediator of the estrogenic action on GnRH neurons is the excitatory and inhibitory amino acids system (Donoso et al., 1990; Brann and Mahesh, 1992, 1994; Jarry et al., 1992; Gore and Roberts, 1994; Moguilevsky and Wuttke, 2001). We have demonstrated that some EDs with estrogenic, antiestrogenic and/or antiandrogenic actions on the hypothalamic-pituitary-gonadal axis (di-2-ethyl hexyl phtalate (DEHP) 30 mg/kg/day in drinking water, octyl methoxycinnamate (OMC) in vitro at 10^{-7} M and 4 methoxybenzyllidene camphor (4-MBC) 100 mg/kg/day sc.), were able to induce neuroendocrine changes modifying hypothalamic concentrations of aspartate, glutamate and gamma-aminobutyric acid (GABA) in rats exposed during gestation and lactation (Carbone et al., 2009a; Carou et al., 2008, 2009b; Szwarcfarb et al., 2008). Another indirect regulatory pathway involved in these neuroendocrine effects, could be persistent alterations of hypothalamic KiSS-1 system observed after exposure to estrogenic compounds like Bisphenol A (10 and 100 μg/rat) at critical periods of brain sex differentiation (Navarro et al., 2009).

Some evidence of the neuroendocrine reproductive effects of EDs in humans is the early hypothalamic-pituitary maturation and central precocious puberty associated with previous exposure to EDs (Krstevska-Konstantinova et al., 2001; Teilmann et al., 2007). In conclusion, EDs can interfere with steroid hormone actions altering the control of GnRH neurons, by indirect neurotransmitter system afferents (Dickerson and Gore, 2007; Gore, 2008).

2. Endocrine alterations induced UV–B filters

The use of sunscreens with UV-filters to protect the skin is increasing worldwide despite experimental animal and in vitro studies have shown that some UV-filters have adverse effects and act as EDs. The most common active ingredients used in sunscreens are 4-MBC and OMC, which at present are considered EDs (Schlumpf et al., 2001). The commercial forms of these substances are lotions or creams containing 4% of 4-MBC or OMC (Schauer et al., 2006). It has been demonstrated that the UV filters 4.MBC and OMC are absorbed through the skin. A toxicokinetic study showed that topical application of sunscreen formulation at 4% 4-MBC (w/w), resulting in a dermal dose of 22 mg/kg bw, was capable of increasing plasma concentrations of 4-MBC and its metabolites 6 h after application in humans. The same was observed in rats treated with this substance at doses of 400 and 2000 mg/kg, suggesting more intensive biotransformation of 4-MBC in rats as compared to humans after dermal application and a poor absorption of 4-MBC through human skin (Schauer et al., 2006). In other studies, 4-MBC and OMC could be detected in human plasma and urine after 1–2 h following daily whole-body application (2 mg/cm^2) of sunscreen formulation at 10% (w/w) for 4 days and for 2 weeks, being urine and plasma concentrations higher in male than in female (Janjua et al., 2008; Janjua et al., 2004) and indicating a gender difference in the metabolism, distribution and possibly also in the accumulation of UV-filters in adipose tissue.

On the other hand, UV filters OMC and 4-MBC were found in more than 96% of 2517 urine samples collected throughout 1-year from the general US population in the NHANES study (Calafat et al., 2008). These UV filters can get into the body fluids by either drinking contaminated water or contaminated fish intake (Poiger et al., 2004). Also, 4-MBC and OMC are secreted by the mammary gland being present in 85% of Swiss human milk samples, so that breastfed babies could be exposed to these substances (Schlumpf et al., 2010).

Few studies have focused on humans and have investigated the potential side effects of UV-filters, although an increasing number of experimental animal and in vitro studies indicated that some UV-filters have estrogenic action, causing endocrine and developmental adverse effects in immature female rodents (Krause et al., 2012).

2.1. 4-MBC

It was shown that 4-MBC (309 mg/kg/day in chow) produced an uterotrophic effect in rats and an increased proliferation in breast cancer cells (1.56–3.73 μM in vitro) (Schlumpf et al., 2001). Also, 4-MBC (35.3 μM in vitro) was able to bind to the estrogen receptor alpha (Schlumpf et al., 2004). These observations were confirmed by Tinwell et al., 2002, using 4-MBC 10 μM in a cell proliferation assay and doses of 500–800 mg/kg by oral gavage or 500–1000 mg/kg sc., in vivo studies. However, Maerkel et al., 2007 reported absence of the uterotrophic effect of 4-MBC when it was orally administered at doses of 7, 24 and 47 mg/kg/day. This fact

could be attributed to a decrease in the circulating levels of the 4-MBC metabolite which has a greater affinity for the alpha type estrogen receptor (Volkel et al., 2006), main mediator of the estrogenic effect on peripheral tissues (Harris et al., 2002) as well as to a lower affinity for the beta type estrogen (Volkel et al., 2006), mainly related to the neuroendocrine regulation of the reproductive axis (Klann et al., 2005; Mueller et al., 2003; Schlumpf et al., 2004). In addition, a very weak estrogenic effect of 4-MBC (57.5 and 250 mg via food), was demonstrated by Seidlová-Wuttke et al., 2006a, 2006b, in uterus and vagina of rodents. This estrogenic action of 4-MBC might interfere with the normal development of puberty and disturb the reproductive function, as it is described below.

Developmental toxicity of the UV filter 4-MBC was studied in rats born to parents exposed to this substance in food before mating, during pregnancy and lactation and also in the offspring exposed to 4-MBC into adulthood. It was observed that 4-MBC at the lowest observed adverse effect level (LOEAL) of 0.7 mg/kg/day, delayed male puberty and affected reproductive organ weights of adult offsprings, enhancing prostate growth (Schlumpf et al., 2008). We have demonstrated that exposure to low doses of 4-MBC (2 and 10 mg/kg during five days) can produce a decrease in LH and FSH serum and in GnRH hypothalamic release, indicating that low doses of 4-MBC inhibit the reproductive axis in adult male rats (Carou et al., 2008). Since it has been observed that 4-MBC (100 μM) is capable of generating an increase in the quantity of mRNA ER, similar to the one caused by 1 nmol/l of estradiol (Klann et al., 2005), this UV filter would facilitate the negative feedback mechanism on the reproductive axis. This fact could explain the decrease in gonadotropins observed by Carou et al. (2008) in male rats exposed to 4-MBC. In addition, the disruptive estrogenic action of 4-MBC would have its starting point at neuroendocrine regulation level and would be caused by a selective action of 4-MBC (at doses more than 1 μM) on the beta type estrogen receptors (Mueller et al., 2003; Schlumpf et al., 2004), which are expressed in the GnRH neurons (Hrabovszky et al., 2001). Taking into account that the beta estrogen receptors have an important role in the neuroendocrine regulation of the reproductive axis, 4-MBC would behave as a partial agonist of alpha and beta estrogenic receptors; but its action on the gonadal axis would be exerted mainly on the beta type receptors (Mueller et al., 2003; Schlumpf et al., 2004).

In adult rats of both sexes exposed to 4-MBC (100 mg/kg sc.,daily) from prenatal development, we have described that the 4-MBC may modify the neuroendocrine regulation of the gonadal axis, inhibiting serum GnRH hypothalamic release in males, and stimulating gonadotropin secretion in females. These changes were correlated with an important inhibitory effect on the release of the aspartate and glutamate neurotransmitters as well as a decrease in the GABA inhibitory amino acid. Therefore, these modifications in the neurotransmitter systems induced by prenatal exposure to 4-MBC would be responsible for the different effects observed in males compared to females (Carou et al., 2009b). In the same way, Maerkel et al., 2007 reported that the neuroendocrine disruptive action of 4-MBC (pre and postnatal to adulthood at doses of 7, 24 and 47 mg/kg) in male and female rats, would cause an effect consistent with a sexually dimorphic gene regulation in brain. Sexual maturation could be affected by exposure to 4-MBC during embryonic and fetal development as it was shown by Carou et al. (2009a). This UV filter administered at high doses of 100 and 500 mg/kg/day to pregnant rats was able to inhibit the testicular axis in male rats during the prepubertal stage and stimulate it during peripubertal one, producing a decrease in testicular weight, LH, GnRH and glutamate levels or an increase in serum gonadotropins and the hypothalamic aspartate concentration, respectively. These results would indicate that during embryonic and fetal development the hypothalamic sensitivity could be disturbed by 4-MBC due to its estrogenic effect, inducing changes in the expression of the beta estrogenic receptor and affecting the pattern of neuronal migration in critical periods of brain maturation (Dellovade et al., 2000). This 4-MBC (7 and 24 mg/kg bw/day) estrogenic effect on neuroendocrine brain structures can also be evidenced in female showing a reduced receptive behavior and an increased rejection behavior toward the male, accompanied by a reduction in progesterone receptor mRNA at the hypothalamus (Faass et al., 2009), that normally correlated with lordosis behavior (Ogawa et al., 1994).

Besides, the different responses of 4-MBC observed during sexual maturation, could be the result of qualitative and quantitative differences in the effect of neurotransmitters that regulate GnRH neurons. During the peripubertal stage, changes occur in the regulating mechanisms of the hypothalamic-pituitary-gonadal axis, which in turn originate changes from a prepubertal type regulation to an adult-type control mechanism. This change constitutes one of the main events involved in the pubertal development (Clarkson and Herbison, 2006; Moguilevsky and Wuttke, 2001). Excitatory amino acid glutamate and aspartate are known stimulators of the reproductive axis, during sexual maturation and in adult life; and in addition there is evidence of their involvement in the increase of the gonadotropins release at the onset of puberty (Moguilevsky et al., 1995; Losada et al., 1993. According to what was described above, prenatal exposure to 4-MBC sunscreen can alter neuroendocrine mechanisms involved in sexual maturation in an age, sex and dose dependent manner (see Table 2).

2.2. OMC

The estrogenic activity of OMC has been determined in vitro (1.56–3.73 μM) and in vivo (935 mg/kg/day to rats that received the chemicals for 4 days in powdered feed chow) studies (Schlumpf et al., 2001). In contrast, a very weak estrogenic effect in the uterus of adult rats was observed by chronic administration of OMC at lower and higher doses (57.5 or 275 mg) via pellet food (20.6 or 22.3 g) (Seidlová-Wuttke et al., 2006a). Effects of pre- and postnatal exposure to OMC (500, 750 or 1000 mg/kg/day) on the reproductive development of rat offspring were studied by Axelstad et al. (2011), finding reduced relative prostate and testis weights, and a dose-dependent decrease in testosterone levels in immature rats as well as reduced sperm counts and prostate weights in adult rats.

On the other hand, oral administration of OMC in high dose (250 mg per 20 g food) to ovariectomized adult female rats produced a mild stimulation on serum LH and in the uterine weight, suggesting that this stimulatory effect could be related to the existence of neurotransmitter-involving mechanisms exerted by OMC at hypothalamic level (Seidlová-Wuttke et al., 2006b).

In "in vitro" studies we observed changes in the release of hypothalamic GnRH, which correlated with changes in the release of excitatory and inhibitory neurotransmitters. OMC (10^{-7} M) significantly decreased GnRH release in normal male and female adult rats as well as in castrated rats with substitutive therapy. In addition, these changes correlated with a decrease in glutamate hypothalamic release and an increase in GABA inhibitory amino acid in males; while in females OMC decreased the excitatory amino acids aspartate and glutamate, but GABA was not modified. These results suggested that OMC acting in a sex-dependent manner could alter the relation between neurotransmitter-sexual hormones and GnRH in both sexes' adult rats (Carbone et al., 2010a).

The disruptive action of OMC was also observed in studies "in vitro" carried out with hypothalamic fragments of pre and peripubertal rats of both sexes. In these animals, OMC (10^{-7} M) caused a significant decrease in the release of GnRH, correlated with an increase in the GABA hypothalamic release in males, and a decrease of the excitatory amino acids aspartate and glutamate in females. These results suggested that during sexual maturation, OMC would

Table 2
Neuroendocrine changes induced by UV–B filters (4-MBC and OMC), DEHP and NPl in rats of both gender. (↑ increased; ↓ decreased; = not change).

4-MBC	Male	Adult	Prenatal, lactation, and adulthood	0.7 mg/kg/d, sc.	Delayed puberty	Schlumpf et al., 2008
	Male	Adult	Adulthood	2 and 10 mg/kg/d, sc., for 5 days	↓ LH; ↓ FSH ↓ GnRH	Carou et al., 2008
	Male	Adult	Prenatal, lactation, and adulthood	100 mg/kg/d, sc., daily	↓ LH; ↓ FSH ↓ GnRH;↓GABA ↓ Aspartate ↓ Glutamate	Carou et al., 2009b
	Male	Prepubertal	Prenatal	100 mg/kg/d, sc., daily	↓ LH no change Glutamate	Carou et al., 2009a
	Male	Prepubertal	Prenatal	500 mg/kg/d, sc., daily	↓ LH ↓ Glutamate	Carou et al., 2009a
	Male	Peripubertal	Prenatal	100 mg/kg/d, sc., daily	↑ LH no change Aspartate	Carou et al., 2009a
	Male	Peripubertal	Prenatal	500 mg/kg/d, sc., daily	↑ LH ↑ Aspartate	Carou et al., 2009a
	Female	Adult	Prenatal, lactation and adulthood	100 mg/kg/d, sc., daily	↑ LH; ↑ FSH no change GnRH ↑ Aspartate no change Glutamate ↓ GABA	Carou et al., 2009b
OMC	Male	Prepubertal	Pre and postnatal	500, 750 or 1000 mg/kg/d	↓Testosterone	Axelstad et al., 2011
	Male	Adult	Adulthood	In vitro 10^{-7} M	↓GnRH; ↑GABA ↓ Glutamate	Carbone et al., 2010a
	Male	Pre and peripubertal	Pre and peripubertal	In vitro 10^{-7} M	↓GnRH; ↑GABA no change Glutamate no change Aspartate	Szwarcfarb et al., 2008
	Female	Adult	Adulthood	In vitro 10^{-7} M	↓GnRH; =GABA ↓ Glutamate ↓ Aspartate	Carbone et al., 2010a
	Female	Adult	Adulthood	250 mg/20 g food	↓ Testosterone	Seidlová-Wuttke et al., 2006b
	Female	Pre and peripubertal	Pre and peripubertal	In vitro 10^{-7}M	↓GnRH; =GABA ↓ Glutamate no change Aspartate	Szwarcfarb et al., 2008
DEHP	Male	Prepubertal	Prenatal and neonatal	750 mg/kg/d, orally	↓ Testosterone	Parks et al., 2000
	Male	Postnatal day 3	Prenatal and neonatal	750 mg/kg/d, Orally	↓ Testosterone	Gray et al., 2000
	Male	Prepubertal	Prenatal	100 mg/kg/d, orally	↓ Testosterone ↓ LH	Akingbemi et al., 2001
	Male	Prepubertal	Postnatal days 21-48	200 mg/kg/d, orally	↑ LH	Akingbemi et al., 2001
	Male	Young adult	Postnatal days 68-89	200 mg/kg/d, orally	no change LH	Akingbemi et al., 2001
	Male	Adult	Postnatal days 21-120	200 mg/kg/d, orally	↑ LH ↓Testosterone	Akingbemi et al., 2004
	Male	Prepubertal	Prenatal and lactation	30 mg/kg/d, orally	↑ LH; ↑ FSH ↑ Aspartate no change Glutamate ↓ GABA	Carbone et al., 2012
	Male	Peripubertal	Prenatal and lactation	30 mg/kg/d, orally	no change LH; ↓ FSH ↓ Aspartate ↑ GABA	Carbone et al., 2010
	Female	Adult regulary cycling	Adulthood for 12 days	2 g/kg/d, orally,	Anovulatory cycles; ↓ LH ↓ Estradiol	Davis et al., 1994
	Female	Peri pubertal	Postnatal days 20-30	500 mg/kg/d, orally	↓ Progesterone ↓ Estradiol;↑ LH	Svechnikova et al., 2007
	Female	Prepubertal	Prenatal and lactation	30 mg/kg/day, orally	↑ Aspartate no change Glutamate ↑ GABA	Carbone et al., 2012
NP	Male	Young adult	Postnatal days 30-80	250 mg/kg/day, orally for 50 days	↓ Testosterone ↑ LH; ↑ FSH	Han et al., 2004
	Male	Adulthood	Postnatal days 30-80	250 mg/kg/day, orally for 50 days	↓ Testosterone ↑ LH	Gon and Han, 2006
	Male	Neonatal	Prenatal	25, 200, 750 ppm in diet	↓ Testosterone	Laurenzana et al., 2002
	Male	Young adult	Postnatal days 21-70	100 and 200 mg/kg/d, orally	↓ Testosterone ↑LH; ↑FSH; ↑GnRH	Ale et al., 2010
	Male	Prepubertal	Lactation	100 mg/kg/d, orally 200 mg/kg/d, orally	No change LH; ↑GnRH No change LH and GnRH	Samaniego et al., 2012
	Male	Peripubertal	Lactation	100 and 200 mg/kg/d, orally	↑ LH, ↑GnRH	Samaniego et al., 2012

Table 2 (*Continued*)

Male	Young adult	Lactation	100 mg/kg/d, orally 200 mg/kg/d, orally	No change LH; ↑GnRH ↑ LH, ↑GnRH	Samaniego et al., 2012
Male	Prepubertal	Prenatal	100 mg/kg/d, orally 200 mg/kg/d, orally	No change LH; ↑GnRH No change LH and GnRH	Samaniego et al., 2012
Male	Peripubertal	Prenatal	100 mg/kg/d, orally 200 mg/kg/d, orally	↑ LH, =GnRH No change LH and GnRH	Samaniego et al., 2012
Male	Young adult	Prenatal	100 and 200 mg/kg/d, orally	↑ LH, No change GnRH	Samaniego et al., 2012

exert an inhibitory effect on GnRH, which would be related to the action of this ED on the excitatory and inhibitory neurotransmitters in male and female rats (Szwarcfarb et al., 2008) (see Table 2).

3. Endocrine alterations induced by phtalates

Di-(2-ethylhexyl) phthalate (DEHP) is the most widely used phthalate to convey flexibility and transparency to numerous plastic products made of polyvinyl chloride (PVC) (Latini et al., 2003). Humans are daily exposed to this chemical through ingestion, inhalation and by dermal contact (Calafat et al., 2004; Doull et al., 1999; Faouzi et al., 1999; Koch et al., 2006; Latini et al., 2003; Moore et al., 2001). DEHP is not covalently bound to the polymer and therefore, it can leach from plastic products into foods, beverages or directly into body fluids (Lovekamp-Swan and Davis, 2003; Moore et al., 2001). Moreover, also occupational and medical exposure (e.g., tubing, blood bags and dialysis equipment) increase body burden levels, reaching much higher concentrations in this population. Pre and postnatal oral exposure to DEHP may be associated in animals with male reproductive development and function alteration. DEHP in rats is rapidly hydrolyzed in the gut to mono-(2-ethylhexyl) phthalate (MEHP), which pass into breast milk and cross the placental barrier (Latini et al., 2003; Stroheker et al., 2005).

It was shown that the exposure to DEHP during early development can cause birth defects in male reproductive tract. For example, DEHP administered by gavage during gestation and lactation at doses of 405 mg/kg/day (high dose) produced nipple retention, reduced anogenital distance and caused histological changes in the testis, indicating that this chemical could act as an anti-androgen at high dose exposure (Andrade et al., 2006). Also, testicular effects in males rats exposed to DEHP (300 and 750 mg/kg/day by gavage) were observed at different stages of sexual maturation (Borch et al., 2005). In the same way, Parks et al. (2000) reported that DEHP maternal exposure to DEHP (750 mg/kg/day by gavage) induced testicular malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat offspring. Pre and postnatal exposure to DEHP (100 mg/kg/day/by oral gavage) or prepubertal and adult rat exposure (100 and 200 mg/kg/day) was able to induce alterations in Leydig cell development, that may occur through modulation of testosterone- biosynthetic enzyme activity and serum LH levels (Akingbemi et al., 2001). Male offspring exposed to DEHP (0, 20, 100 and 500 mg/kg per day by gavages) in uterus and during lactation, showed reduced absolute and relative weights of androgen-dependent tissue organs (ventral prostate and seminal vesicle) and altered spermatogenic processes, demonstrating the ability of DEHP to disrupt the androgen-regulated development of the male reproductive tract at the highest dose level tested and indicating permanent effects of in uterus and lactational DEHP exposure (Dalsenter et al., 2006). Perinatal exposure to DEHP (750 mg/kg/day from gestational day (GD) 14 to postnatal day (PND) 3) induced a significant incidence of reproductive malformations in male pups such as reduced anogenital distance and testis weights (Gray et al., 2000). These alterations could be directly associated with epidemiological evidence indicating that boys born from women exposed to phthalates during pregnancy have an increased incidence of inborn genital malformations and spermatogenic dysfunction (Hu et al., 2009; Ge et al., 2007). Also, the presence of phthalate monoesters in human breast milk was positively correlated with cryptorchidism and LH increase, and negatively associated with free testosterone in infants three months of age. This fact could indicate that human Leydig cell development and function may also be vulnerable to perinatal exposure to some phthalates (Main et al., 2006).

It has been proposed that DEHP acts in an anti-androgenic manner which appears to result from an androgen receptor independent mechanism of anti-androgenicity. Although DEHP does not bind the androgen receptor but suppresses androgen-stimulated sexual differentiation, they are considered prototype antiandrogen. DEHP could act by interfering the esteroidogenesis (Akingbemi et al., 2001). Chronic postnatal exposure to DEHP (100 mg/kg/day by gavage from weaning to adulthood) was capable of inducing an increase in serum LH and estradiol biosynthesis in Leydig cells, a decrease in testosterone biosynthesis and Leydig cell hyperplasia, suggesting that the decrease in T biosynthesis together with increased estradiol production could be due to LH induction of aromatase activity in Leydig cells (Akingbemi et al., 2004). In uterus exposure to DEHP (750 mg/kg/day) was able to produce a reduction in testosterone production, but neither DEHP nor its metabolite MEHP displayed affinity for the human androgen receptor at concentrations up to 10 μM in vitro, indicating that DEHP could disrupt male rat sexual differentiation by reducing testosterone in the fetal male rat during a critical stage of reproductive tract differentiation (Parks et al., 2000).

Others mechanisms, as reduction in the expression of steroid genesis related factors and in nuclear receptors that regulate cholesterol transport, could explain the suppressive effect of DEHP on testosterone levels. In male fetuses of pregnant rats exposed to DEHP (100 ad 300 mg/kg/day by gavages), Borch et al. (2006) have found a reduction in testicular testosterone production that it was correlated with reduced mRNA expression of the steroidogenesis related factors, the nuclear receptors SF-1 and PPAR gamma, which regulate certain steps in steroid synthesis, and the cryptorchidism-associated Insl-3. Howdeshell et al. (2007) have demonstrated cumulative effects on reproductive tract of male fetuses exposed to phthalates (500 m/kg/day) and alterations in fetal steroid hormones and genes. Wilson et al. (2004) have reported that exposure to phthalates (750 mg/kg/day during gestational day14–18) caused decrease in mRNA expression of key steroidogenic enzymes and also the peptide hormone insulin-like peptide 3 (insl-3) from the fetal Leydig cells.

In adult female rats, DEHP (2 g/kg by gavages during 12 days) produced hypoestrogenic anovulatory cycles and polycystic ovaries (Davis et al., 1994). The reproductive toxicity of DEHP was attributed to suppression of ovarian granulosa cell estradiol production by its metabolite MEHP (mono-(2-ethylhexyl) phthalate), which was also capable of suppressing aromatase transcript levels in cultured rat granulosa cells at 200 μM concentration (Lovekamp and Davis, 2001). Pre and perinatal exposure to DEHP (405 mg/kg/day) has been associated with an increase in the number of ovarian atretic tertiary follicles in adult female offspring

rats (Grande et al., 2007). Also, in vitro assays demonstrated that DEHP (1–100 μg/ml) and the metabolite MEHP (0.1–10 μg/ml) may directly inhibit antral follicle growth in adult mice, via a mechanism that includes reduction in levels of estradiol production and decreased expression of cell cycle regulators (Gupta et al., 2010). It has been reported that on the pituitary–gonadal axis of prepubertal female rats DEHP (500 mg/kg/day by oral gavages for 10 days) exerted dual effects stimulating the hormonal function of the pituitary and, at the same time, inhibiting steroidogenesis by the ovarian granulose cells (Svechnikova et al., 2007).

Few studies of reproductive neuroendocrine effects of phthalates are available in the literature. We have studied the effect of pre and perinatal exposure to DEHP on the neuroendocrine parameters that regulate reproduction in prepubertal male and female rats. DEHP at low doses of 3 and 30 mg/kg/day was administered orally in the drinking water to dam rats since pregnancy onset until the moment of pups sacrifice at 15 days of age. No changes in gonadotropin levels and amino acid neurotransmitters were detected at the low dose in both sexes. However, 30 mg/kg/day of DEHP produced a significant decrease in the hypothalamic inhibitory neurotransmitter GABA and an increase in the stimulatory neurotransmitter aspartate in prepubertal male offspring rats, accompanied by gonadotropin serum levels increase. Therefore, we detected different effects by sex on hypothalamic–pituitary axis of prepubertal offspring rats produced by in uterus and lactational exposure to DEHP. The impact of this chemical at hypothalamic level could have very important consequences on the onset of puberty and on the reproductive function in adult life (Carbone et al., 2012).

In other work (Carbone et al., 2010), we investigated the effect of the pre- and perinatal exposure to DEHP on the hypothalamic-pituitary-gonadal axis in peripubertal male rats. We observed that DEHP (30 mg/kg bw/day) was capable to reduce testis weight and serum FSH, in correlation with a significant increase in the inhibitory GABAergic tone and a reduction in the stimulatory effect of aspartate on gonadotropin levels.

Our results showed that exposition to DEHP has different effect depending of stage of sexual maturation, time exposition and sex. This fact could be related with quantitative and qualitative changes in the regulation of the reproductive axis during sexual maturation, previously described (Moguilevsky and Wutke, 2001; Moguilevsky et al., 1991, 1995; Losada et al., 1993; Szwarfarb et al., 1994).

On these bases, the phthalate DEHP could modify the neuroendocrine regulation of the hypothalamic–pituitary axis in immature rats offspring from dams exposed to DEHP at dose of 30 mg/kg bw/day during gestational and lactational periods. Moreover this effect would be dependent on sex and period of sexual maturation to be evaluated. These findings would indicate a possible action to DEHP at central level, in addition to direct effect on gonads evidenced by the presence of testicular and ovarian histological alterations that were already mentioned (see Table 2).

4. Endocrine alterations induced by nonylphenol

Nonylphenol (NP) is an alkylphenol, industrial intermediary in the production of non ionic detergents, latex paint, adhesives and plastics as the polystyrene, polymer stabilizers to package food, herbicides and pesticides, and also antioxidant agents and lubricant additives (Shaw and McCully, 2002; Inoue et al., 2001; Soto et al., 1991). Some decades ago, the presence of NP in the environment was first reported (Ginger et al., 1984). Sixty percent (60%) of these alkylphenols end finally in environmental waters (Daughton and Ternes, 1999; Heberer et al., 2002; Petrovic et al., 2003; Snyder et al., 2003; Naylor et al., 1992), and also in treated potable water (Servos et al., 2007; Stackelberg et al., 2007; Yu et al., 2007). In humans it can be observed that the most direct contact with NP is through food (Inoue et al., 2001). NP is considered an ED that has chemical and physical properties very similar to natural estrogens and has the possibility to link the estrogenic receptors. NP (50 mg/kg) administered to ovariectomized adult female rats treated with estradiol (15 ng at 12, 13 and 14 days after ovariectomy) was capable to increase the mitotic activity in rat endometrium (Soto et al., 1991). Also in "in vitro" assays this chemical (10^{-6} M) induced cellular proliferation and expression of the progesterone receptor in human breast tumor cells sensitive to estrogens (Soto et al., 1991). In male fish, the exposure to NP (10 μg/l) caused vitellogenin expression, a protein associated with the reproduction in female fish (Schawaiger et al., 2002; Sumpter and Jobling, 1995) as well as inhibition of spermatogenesis (at in vitro concentration of 10 μM) and the appearance of intersexes (Schawaiger et al., 2002). In rodent models exposed to NP (250 mg/kg) it has been described a decrease in the testicular size and sperm production, as well as an increase of the intertubular space and low seminal quality (Jager et al., 1999; Lee et al., 1999). Recently we have demonstrated that exposure to NP (50 and 100 mg/kg by gavages) in early stages of sexual maturation, led to a histological disorganization of the epithelium seminiferus in rat testis and changes in the neuroendocrine regulation of the reproductive system (data unpublished). Coincidentally, immature rats exposed neonatally to NP (20.8 mg/kg) showed a reduction in testis, epididymis and seminal vesicles size, as well as an increase of cryptorchidism up to a 60% (Lee, 1998). Similar results were found by Nagao et al. (2001), using NP at dose of 50 mg/kg. The testicular and epididymis atrophy also occur in a dose-dependent manner in adult male rats exposed to NP (50, 100 or 200 mg/kg by gavages) during gestational and lactational periods (Fan et al., 2001). These changes are similar those observed in adult male rats treated neonatally with 0.1, 1 or 10 μg sc. of diethylstilbestrol, an ED with estrogenic action (Atanassova et al., 1999). In addition, Han et al. (2004) have shown a significant decrease in epididymis weight, sperm density and testosterone level, as well as histopathological changes in the seminiferus tubulus of rats treated with NP (250 mg/kg/day by gavage for 50 days).

Decreased testicular and epididymal masses in rats exposed to NP (250 mg/kg/day) in utero until 10 weeks of age were reported by Jager et al. (1999), suggesting an adverse effect of this ED on the fertility potential of male rats after gestational, lactational and direct exposure.

In humans the infertility male factor is commonly observed in the idiopathic oligoasthenotetarospermia (IOAT) syndrome, being possible that estrogenic substances as NP are related with its physiopathology. Multiplication of Sertoli cells, controlled by FSH, occurs during fetal, neonatal and prepubertal life. The inhibition of FSH secretion reduces the multiplication of these cells. It is important to have in mind that in neonates FSH secretion is quite sensitive to the inhibition of exogenous estrogens (Sharpe and Skakkebaek, 1993). Therefore, the NP estrogenic activity could explain this effect. On the other hand, in female offspring's of female rats exposed to NP (12.31 mg/kg/day sc., during pregnancy), an increase of ovary weight has been observed (Kimura et al., 2006) without significant changes in uterine weight. However, when this particular ED at dose of 100 mg/kg/day sc. was directly administered to immature rats, uterine weight, diameter of uterine duct and vaginal luminal epithelial height were significantly increased (Kang et al., 2000).

The effect of NP on the regulating mechanisms of the reproductive system has not been studied so thoroughly as the morphometric parameters. However, it has been described an abrupt decrease in the testosterone level with NP (250 mg/kg/day by gavages) administered during adulthood (Gong and Han, 2006) and also in animals treated with dietary doses of NP (0, 25, 200 and 750 ppm) from fetal development until adulthood (Laurenzana

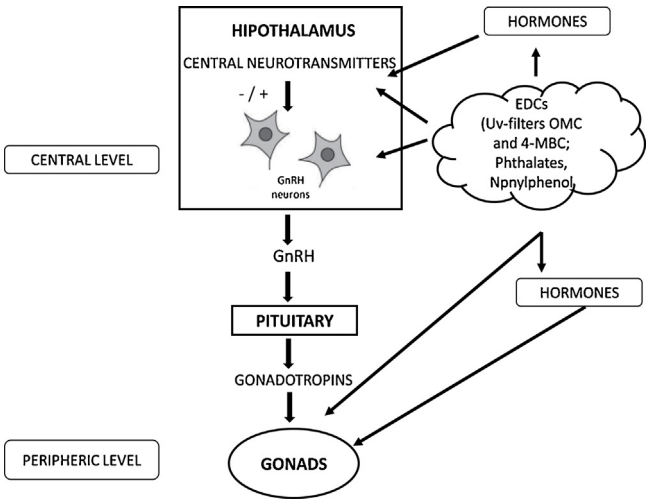

Fig. 1. Schematic description of how hypothalamic neuroendocrine systems are targets of environmental endocrine disruptors. In the brain, hypothalamic neuroendocrine cells are regulated by neurotransmitter systems and by both direct and indirect hormone actions. The hypothalamic gonadotropin – releasing factor (GnRH) is secreted and released from GnRH neurons to the pituitary gland, regulating the secretion of gonadotropins which exert their effect on gonads. Environmental endocrine-disrupting chemicals (EDCs) may mimic or block some of these hormonal effects, disrupting at central or peripheral levels the neuroendocrine processes involved in the reproductive function.

et al., 2002). Recently we have reported that chronic exposure to NP (100 and 200 mg/kg/d by gavages) since weaning to adulthood, in addition to cause an important decrease of plasmatic testosterone, induces a significant increase in the hypothalamic release of GnRH and pituitary gonadotropins (Ale et al., 2010). This exposure to NP would lead to a decrease of the inhibitory control on the neuroendocrine regulation. Similarly, 30 and 70 days old male rats exposed to the same doses of NP during lactation, showed an increase in GnRH and gonadotropins secretion (Samaniego et al., 2012).

In ovariectomized rats treated with NP (10 mg/kg sc.) a significant decrease in plasmatic LH level was observed (Furuta et al., 2006). However, chronic administration of NP (100 and 200 mg/kg/day, from weaning to adulthood) stimulated GnRH and LH release "ex vivo" in non ovariectomized rats in diestrum phase. Also this exposure was associated with an early vaginal opening (Ale et al., 2012).

Administration of NP (50 mg/kg, orally) in the last stage of gestation produced a concentration of NP in the fetal serum within a range from 30 to 40% of the observed in maternal plasma. In these animals, NP was present in the central nervous system (Doerge et al., 2002), suggesting that NP goes through the placenta, and accumulates in the brain. This could cause alterations in the hypothalamic regulation of the excitatory/inhibitory amino acids–GnRH–gonadotropins of the reproductive system as we have already described by others EDs (see Table 2).

5. Conclusion

In conclusion, the exposure to different substances with endocrine disruptive action could produce severe alterations, mainly in the reproductive axis. Recent studies demonstrate that the three reproductive levels are also responsive to environmental EDs. Recently findings in experimental animals have shown new evidences of reproductive disruption associated with neuroendocrine changes induced by UV–B filters, phtalates and nonylphenol during sexual maturation in rats of both gender. The adverse effects of these compounds could be exercised in a sex, age and exposure period dependent manner, on the white organs of the reproductive system and/or at central level, modifying the regulatory neuroendocrine mechanisms (see Fig. 1). One of the neuroendocrine alterations could be the changes in the amino acid neurotransmitters system described by us, as well as the modifications in others neurotransmitters which have not been studied yet. If the exposure to these substances occurs during critical periods of development, such as intrauterine, early neonatal and pubertal stages, when the neuroendocrine mechanisms are very sensitive to changes in the action of estrogenic and androgenic hormones, the impact caused can be even greater and permanent. Given this background, future study should explore other neurotransmitter systems and neuropeptides that could be involved in the reproductive system changes induced by EDs.

Also, considering that neuroendocrine systems do not work in isolation, a possible cross talk with the effects of these EDs on other neuroendocrine axes, which could interfere with the normal functioning of the reproductive axis, should be revised in the future..

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