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A Review on the Biochemical and Molecular Mechanisms of Phthalate-Induced Toxicity in Various Organs with a Focus on the Reproductive System

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ABSTRACT
Phthalates are a large group of chemicals, used in plasticizers and industrial solvents, to make them flexible and soluble, especially when these materials are applied in the production of toys, medical equipment and drugs coverings. It seems that phthalates induce multi-organ damage through a number of mechanisms such as oxidative stress via generation of Reactive Oxygen Species (ROS), DNA damage, lipid peroxidation, disrupting cell function and also altering the expression and activity of the most important antioxidant enzymes. In this study, we reviewed the recent publications that evaluated the contribution of oxidative stress in phthalate toxicity. Alteration of antioxidant enzymes such as a reduced SOD (Cu/Zn superoxide dismutase) activity as well as an increased CAT (catalase) function normally occur and can be observed particularly with higher doses of phthalates. Moreover, these compounds decrease GPX (glutathione peroxidase) and GST (glutathione S-transferase) activities. Nevertheless, controversy is found in the levels of cellular antioxidants like SOD showing a reduction in many organs like liver, kidney and reproductive system, whereas, its increase has been reported in a few studies. In summary, among various organs, reproductive system seems was affected further by oxidative stress through disruption of spermatogenesis, inducing mitochondrial dysfunction in gonocytes, impairment of cellular redox mechanism and increasing peroxiredoxin 3 and cycloxygenase 2 levels in spermatocytes. The phthalates are being replaced in some countries by other safe plasticizers.

Key words: Biological markers, molecular mechanisms, oxidative stress, phthalate, phthalic acid, review, toxicity, reproductive system

INTRODUCTION

Phthalates are one of the most important synthetic chemicals used in various materials such as children's toys, drug coatings, cosmetics and solvents to give flexibility and solubility to the materials in which they are applied (Saeidnia and Abdollahi, 2013a; Wang *et al*., 2012a). More than 8.1 million tons of phthalates are released in the world annually (Crinnion, 2010). Phthalates-exposure may happen through oral, dermal and inhalation routes in human (Bahadar *et al*., 2014). It is also demonstrated that DEHP (Bis(2-ethylhexyl) phthalate) and MEHP (Mono-(2-ethylhexyl) phthalate) are found in the environment and cause adverse health effects in humans (Yang *et al*., 2012). Phthalates cause multi-organ damage through a number of mechanisms. Some phthalates can cause hepatic toxicity through transactivation of Peroxisome Proliferator-Activated Receptor (PPARs). This activation could cause uncontrolled cell proliferation leading to hepatic tumorogenesis consequences by the alteration of hepatic enzymes activities (Yavasoglu *et al*., 2014). A recent study showed that DEHP induce necrosis and inflammation in the

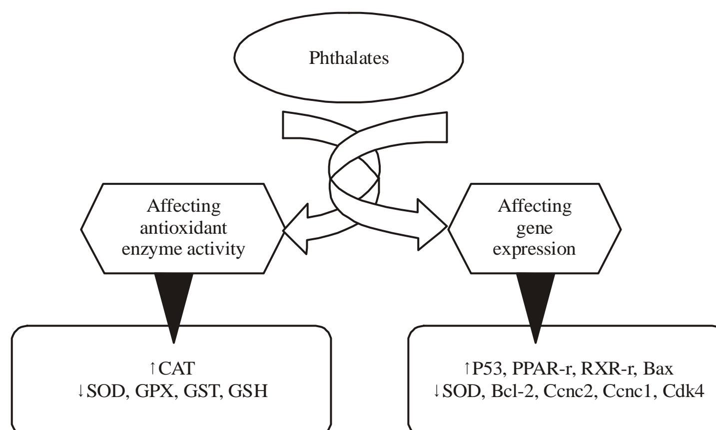

Fig. 1: Phthalates induce toxicity via affecting the antioxidant enzymes activity and gene expression

liver. Phthalate-induced oxidative stress results in decreasing the antioxidant capacity, especially in GPX (glutathione peroxidase) and GST (glutathione S-transferase). Moreover, increase in lipid peroxidation, CAT (Catalase) and SOD (Cu/Zn superoxide dismutase) activity were observed. Reactive Oxygen Species (ROS) can affect Peroxisome Proliferator (PP) leading to parenchymal cell proliferation. It is mentioned that Kupffer cells are a potential oxidant in rodent liver (Erkekoglu *et al*., 2014). Actually, phthalates induce toxicity not only via affecting the antioxidant enzymes activity but also through gene expression (Fig. 1).

In another study, Li *et al*. (2013) showed the immunotoxicity of DBP (Dibutyl Phthalate). The cytotoxicity in Peritoneal Exudate Macrophages (PEM) was observed. The PEM are used to express CD36 and CD80. Furthermore, DBP reduce phagocytosis on apoptotic cells. It also decreases cytokine production, immunogenicity and antigen presentation (Li *et al*., 2013).

In another study, neurotoxicity of phthalate was assessed. Impairment in thyroid homeostasis and activation of PPAR were reported as the most important mechanisms. Phthalates are able to change transcriptional activity of the sodium/iodine symporter leading to disruption of iodine uptake into the thyroid gland. It is shown that maternal hypothyroxinemia especially in a low level of free T4 is associated with belated cognitive and neuromotor development as well as reduced IQ. On the other hands, DEHP may overexpress PPAR-γ, resulting in apoptosis of undifferentiated neurons (Miodovnik *et al*., 2014).

The cardiotoxicity of phthalates has been also reported. A significant decrease in conduction velocity was noted 24 h after DEHP exposure. Moreover, human embryonic stem cell viability and cardiac differentiation were reduced following MEHP exposure (Posnack, 2014). In the kidney, DEHP exposure could decrease the number of nephrons in offspring at the weaning. Likewise, DEHP induce glomerulosclerosis and interstitial fibrosis in adulthood. The renin and angiotensin ll expression were decreased at birthday and enhanced at weaning (Wei *et al*., 2012).

The preliminary studies revealed that the reproductive system is more susceptible to phthalates rather than other organs (Martino Andrade and Chahoud, 2010). The mechanisms of reproductive toxicity have not been exactly elucidated so far. It is reported that these effects are correlated with anti-androgenic effect (Noriega *et al*., 2009) and activation of PPARα which can alter testosterone biosynthesis through inducing variation in the gene expression of the related enzymes. This results in down-regulation of nuclear receptors which are important in testis development (Gazouli *et al*., 2002; Ward *et al*., 1998), altering the expression and activity of antioxidant enzymes which may result in DNA damage by producing ROS (Wang *et al*., 2012b) and impairment in the function of Leydig and Sertoli cell (Martino Andrade and Chahoud, 2010).

In another study, Davis *et al*. (1994) showed that MEHP decreased the estradiol level in cultured rat granulosa cells via inhibition of aromatase activity. In the mentioned study, MEHP also decreased aromatase transcript and protein level. Literature revealed that MEHP can act through activation of PPAR-α and PPAR-γ to reduce the level of estradiol and related genes expression (Lovekamp-Swan and Davis, 2003). In the same way, activation of PPAR-γ disrupted the growth time and follicle differentiation (Lovekamp-Swan *et al*., 2003). Many of the toxic compounds can act through oxidative stress causing genotoxicity and various chronic diseases (Mostafalou and Abdollahi, 2012, 2013; Saeidnia and Abdollahi, 2013b).

One of the most important mechanisms for phthalates toxicity is defined regarding oxidative stress. The most common ROS are superoxide and H_2O_2 that can be converted into H_2O and O_2 by antioxidant enzymes such as SOD, GPX and CAT (Abdollahi *et al*., 2014). Phthalates are demonstrated to alter the expression and activity of these enzymes leading to disruption of the cell function (Wang *et al*., 2012a). Although there are several reports demonstrating the role of oxidative stress in this regard, the presence of a clear relationship between phthalate toxicity in some organs and oxidative stress as the main cause is still under question. Therefore, the main

purpose of the present study is to systematically review the related reports and papers published in the valid and credible journals to confirm whether there is a correlation between phthalate toxicity and oxidative stress as a main mechanism of toxicity for these compounds.

APPROACH TO A SYSTEMATIC REVIEW

Electronic searches were performed on Science Finder, PubMed, Science Direct and Google Scholar. Key words included: Phthalate, oxidative stress, antioxidant enzymes, liver toxicity and reproductive toxicity. *In vitro* and *in vivo* animal and human studies have been evaluated and included in this review. Also, oxidative stress as one of the mechanisms for phthalate toxicity has been discussed. The results of those selected reports are categorized into four sections including histopathology, hormone analysis, oxidative stress parameters and gene expression.

HISTOPATHOLOGICAL ALTERATIONS

Reproductive toxicity: Recently, the reproductive toxicity of phthalate has been evaluated by Wang *et al.* (2012b). In that study the antral follicles isolated from CD-1 mice which were received DEHP and MEHP at doses: 1, 10 and 100 μg mL^{-1} during 96 h. The results showed that the antral follicle growth decreased using all doses of DEHP and MEHP. In another study, Erkekoglu *et al.* (2010) examined the influence of DEHP and MEHP on MA-10 Leydig cell in mice. Resulted data showed that about 60-80% of the cells survived at high dose of DEHP but there is no cell survival at the dose 10 μM of MEHP.

Furthermore, Zhou *et al.* (2010) investigated the effects of DBP (dibutyl phthalate) in rats. Animals were given DBP at the doses of 100, 250 and 500 mg/kg/day orally during two weeks. Sperm count and motility significantly decreased at 250 and 500 mg kg^{-1}. Moreover, seminiferous tubules atrophy was observed in 500 mg kg^{-1}. In addition, decreased testis weight was observed in several reports (Kasahara *et al.*, 2002; Shono and Taguchi, 2014). Moreover, Botelho *et al.* (2009) investigated the DEHP toxicity on the reproductive system. The results revealed that the number of multinucleated gonocytes was increased.

Liver toxicity: Seo *et al.* (2004) assessed the effects of DEHP (Bis(2-ethylhexyl) phthalate), DBP (dibutyl phthalate) and BBP (benzyl butyl phthalate) at the doses of 50, 200 and 1000 mg kg^{-1} orally during 14 days in the rat. Hepatomegaly was observed by the DEHP administration at all doses while it happened at a dose of 1000 mg kg^{-1} for BBP and DBP. Moreover, Yavasoglu *et al.* (2014) investigated the effects of BCP (butyl cyclohexyl phthalate) toxicity in mouse liver. The animals received BCP orally at three doses of 100, 200 and 400 mg kg^{-1} for 20 days. The results showed congestions in vena centralis, an enlargement of the sinusoids, degeneration in hepatocytes, vacuole formations and presence of lipid droplets in hepatocytes in all groups (Yavasoglu *et al.*, 2014). Additionally, Zhou *et al.* (2011) evaluated the effects of DBP at three doses of 100, 250 and 500 orally during 2 weeks. A decrease in epididymal weight, an atrophy of epididymal tubules and hyperemia of interstitial vascular were also noted.

Neurotoxicity: There are a few articles on neurotoxicity of phthalates through the oxidative stress. In a study, Tseng *et al.* (2013) evaluated the influence of DEHP (2 and 20 ppm), DBP (500 and 1000 ppm) and DIBP (100 and 1000 ppm) on nematode. The results showed that intracellular ROS was increased at all doses. In the recent study, Zuo *et al.* (2014) assessed the properties of DBP on antioxidant parameters. Mice were given DBP at doses of 0.45 and 45 mg kg^{-1} orally during 32 days. A significant increase of ROS, GSH and MDA (malondialdehyde) level was observed in the brain.

SEXUAL HORMONE ALTERATIONS

Botelho *et al.* (2009) investigated the effects of DEHP on sexual hormones at a dose of 500 mg kg^{-1} from gestational day (seventh) to lactation day (second) in rats. The results showed that DEHP had no effect on testosterone in offspring. In another study, Lee *et al.* (2007) assessed DBP exposure on rat. The animals received DBP in drinking water at a single dose of 750 mg/kg/day for 30 days. Statistical analysis identified a significant decrease in testosterone level but there was no effect on thyroid hormones.

CHANGING THE OXIDATIVE STRESS PARAMETERS

In a recent study, Wang *et al.* (2012a) investigated the effect of DEHP on the activity of antioxidant enzymes. At the beginning, SOD activity significantly increased during 24 h by DEHP (10 μg mL^{-1}) but its level decreased at 72 and 96 h. GPX and CAT activities exhibited no alteration. Furthermore, Wang *et al.* (2012b) assessed MEHP properties on antioxidant enzymes activities at 0.1, 1, 10 and 100 μg mL^{-1}. It was observed that GPX activity decreased at 1, 10 and 100 μg mL^{-1} and also at 0.1 μg mL^{-1} after 96 h. CAT activity increased at two doses of 0.1 and 1 μg mL^{-1}. Moreover, SOD activity decreased at a single dose of 100 μg mL^{-1}, whereas, SOD activity increased in a single dose of 10 μg mL^{-1}. Moreover, Erkekoglu *et al.* (2010) examined the influence of DEHP and MEHP on MA-10 Leydig cell and measured the antioxidant enzymes activity. Reduction of the activity of GPX, TrxR and GST was observed with both DEHP and MEHP. Moreover, total GSH levels decreased. On the other side, Botelho *et al.* (2009) assessed the DEHP toxicity in rats. The animals were given DEHP in a dose of 500 mg kg^{-1} from gestational day (seventh day) to lactation day (second day) and the antioxidant enzymes activity was measured in offspring. Increase of CAT activity and a decrease

in GST activity were noted. In another study, Zhou *et al.* (2010) evaluated the effect of DBP on antioxidant enzymes activity. The findings showed that SOD, GSH-Px and GSH activity decreased at two doses of 250 and 500 mg kg^{-1} and MDA (malondialdehyde) level was increased at the same doses in the testes. In a previous study, Lee *et al.* (2007) examined DBP properties at a dose of 750 mg kg^{-1} orally during 30 days and measured the antioxidant enzymes activity. A significant increase was noted in MDA and 8-OHdG (8-hydroxy-2-deoxyguanosine) levels. Additionally, SOD, CAT and GPx activity increased. Bibliography revealed that Kasahara *et al.* (2002) evaluated the influence of DEHP in rat. The animals were treated with DEHP at 1 and 2 g/kg/day in drinking water during seven days. Reduction of free thiol, GSH and ascorbic acid, were reported at all doses. In addition, CAT and GPX activity increased at all doses too.

Erkekoglu *et al.* (2011) examined the oral administration of DEHP at a single dose of 1000 mg kg^{-1} in rat testis during 10 days. The results showed a decrease in both Cu.Zn-SOD activity and GSH level. Also, an increase of TBARS level was noted. In a previous study, Ambruosi *et al.* (2011) investigated the influence of DEHP on Equine COCs (cumulus-oocyte complexes) at three doses of 0.12, 12 and 1200 μM during 1 h. An increase in CC apoptosis was observed at all doses. At the lowest dose, oocyte maturation was inhibited and ROS level was decreased, whereas, at other doses, there were no changes in oocyte maturation or ROS levels.

Moreover, Zhou *et al.* (2011) evaluated the effects of DBP in rat testis. The animals received DBP orally at doses of 100, 250 and 500 mg kg^{-1} during 2 weeks. The results showed a decrease of SOD activity and an increase of MDA level in all groups. A further decrease of GSH-Px was observed at a dose of 500 mg kg^{-1}. In another study, Seo *et al.* (2004) investigated the influence of DEHP, DBP and BBP at three doses of 50, 200 and 1000 mg kg^{-1} in rat liver. The MDA level increased significantly with all compounds at doses of 200 and 1000 mg kg^{-1}. It is also increased in a dose of 50 mg kg^{-1} with DEHP. Moreover, enhancement of 8-OHdG was observed at a dose of 1000 mg kg^{-1} with DEHP. Kang *et al.* (2010) examined the effects of DEP by three consecutive daily intraperitoneal (i.p.) injections at doses of 100, 300 and 900 mg kg^{-1} in fish liver. The results showed an increase in LPO levels in all groups and decreasing the activity of CAT, AST and A LT at a dose of 900 mg kg^{-1}.

In a recent study, Yavasoglu *et al.* (2014) assessed the effects of BCP orally for 20 days at doses of 100, 200 and 400 mg kg^{-1} in mouse liver. A significant decrease was observed in all groups. In addition decrease of SOD activity and increase of TBARS level were noted at doses of 200 and 400 mg kg^{-1}.

Recently, phthalate toxicity has been evaluated by Yang *et al.* (2012). In that study, the $HepG_2$ cells were received MEHP at doses of 6.25, 12.5, 25, 50 and 100 μM during 36 h. The results showed an increase of MDA level and a decrease of GPx activity at all doses. It was also noted an increase of 8-OHdG at doses of 25, 50 and 100 μM. In another study, Erkekoglu *et al.* (2010) investigated the effects of DEHP (0.01-10 mM) and MEHP (3-30 μM) on LNCaP cells during 72 h. Furthermore, decrease of GPx activity and increase of GR activity (glutathione reductase) as well as DNA damage were all observed.

ALTERATIONS IN GENE EXPRESSION OF THE ANTIOXIDANT ENZYMES

Literature revealed that Wang *et al.* (2012a) assessed the effect of DEHP on gene expression of antioxidant enzymes. A decrease in SOD_1 expression was noted. In another study, Wang *et al.* (2012b) evaluated the effects of MEHP on the gene expression. The results revealed that SOD1 and GPX expression decreased at 100 μg mL^{-1}. Decrease of anti-apoptotic factor (Bcl-2) expression and increase of pro-apoptotic factor (Bax) expression were noted at three doses of 1, 10 and 100 μg mL^{-1}. Moreover, expression of cell cycle genes decreased at the same doses.

Erkekoglu *et al.* (2010) evaluated the DEHP and MEHP toxicity on gene expression too. In that study, p53 expression increased by MEHP (IC_{50} = 3 mM). In addition, DNA damage was observed by increased tail intensity and tail moment. Also, these authors (Erkekoglu *et al.*, 2011) investigated the effects of MEHP at a dose of 3 μM on LNCaP cell line. The results showed an increase in expression of p53 and p21. Additionally, in a previous study by Lee *et al.* (2007), the influence of DBP was evaluated on gene expression in rat. A significant increase of PPAR-r protein expression and PXRr (Retinoid X Receptors) expression were noted. All the mentioned data are exhibited in Table 1-5. Moreover, the diagrammatic representation designed for collecting data from the studies is shown in Fig. 1.

DISCUSSION

Oxidative stress and reproductive system: After conducting a vast systematic literature review regarding phthalate toxicity in reproductive organs and OS, it was pointed out that administration of the phthalate alters reproductive function by increasing of ROS (Akingbemi *et al.*, 2004). First, germ cells showed high sensitivity to phthalates (Kasahara *et al.*, 2002) and also MEHP increased Fas ligand expression in Sertoli cells which induced apoptosis in spermatocytes (Lee *et al.*, 1997, 1999). On the other side, it was reported that DEHP can induce apoptosis in pachytene spermatocytes. The differences observed were related to the GSH levels and related enzymes in the cells (Bauche *et al.*, 1994; Yoganathan *et al.*, 1989). Obviously, phthalates can produce ROS through some pathways such as activation of PPARα that is assumed to induce reproductive disorder (Rusyn *et al.*, 2006). Moreover, it is reported that phthalates can disrupt spermatogenesis and induce mitochondrial dysfunction in gonocytes (Suna *et al.*, 2007). Onorato *et al.* (2008) indicated

Table 1: Toxic effects of phthalate on the reproductive system

Phthalate compound	Model	Exposure duration	Type of administration/cell	Doses	Exposure effects	Potential mechanisms	References
DEHP	Mouse/*in vitro*	24-96 h	CD-1	1 μg mL^{-1}	↓Antral follicle growth but not significant	-	Wang *et al*. (2012a)
				10 μg mL^{-1}	↓Antral follicle growth	↑ROS/RNS, ↓Expression of SOD_1, ↑SOD_1 activity at 24 h, ↓SOD_1 activity at 72 and 96 h, -GPX and CAT	
				100 μg mL^{-1}	↓Antral follicle growth		
DEHP and MEHP	Mouse/*in vitro*	24-72 h	MA-10 Leydig cell	DEHP: 1-10 mM	~80 to 60% survival cells at a dose range of 10 μM to 0.5 mM DEHP	Both compounds at all doses: ↓GPx1, TrxR and GST activity and total GSH levels; ↑ROS, ↑P53 expression in IC_{50} dose of MEHP; ↑DNA damage by increased tail intensity and tail moment in DEHP and MEHP	Erkekoglu *et al*. (2010)
				MEHP: 1-10 μM	No cell survival at doses 10 μM and higher cell viability		
DEHP	Rat	Gestational day 7 to lactation day 2	Oral	500 mg kg^{-1}	↑Multinucleated gonocytes (MNG)	No effect on testosterone, ↑CAT activity, ↓GST activity	Botelho *et al*. (2009)
DBP	Rat	2 weeks	Oral	100 mg kg^{-1}	-	-	Zhou *et al*. (2010)
				250 mg kg^{-1}	↓Sperm count and motility,	↓SOD, GSH-Px, GSH activity and ↑MDA level in testes	
				500 mg kg^{-1}	↓Body and testicular weight, ↓Sperm count and motility, Seminiferous tubules atrophy and seminiferous epithelial cells fragmented	↓SOD, GSH-Px, GSH activity and ↑MDA level in testes	
DBP	Rat	30 days	Oral	750 mg kg^{-1}	↓Testis weight	No effect on thyroid hormones, ↑TRα-1 protein level, ↓Testosterone, ↑MDA and 8-OHdG, ↑SOD, CAT and GPx activity, ↑PPAR-r protein expression, ↑RXRr expression	Lee *et al*. (2007)
MEHP	Mouse/*in vitro*	24-96 h	CD-1	0.1 μg mL^{-1}	↓Antral follicle growth at 96 h	↑CAT activity, ↓GPX activity at 96 h	Wang *et al*. (2012b)
				1 μg mL^{-1}	↓Antral follicle growth at 72 h	↓Expression of cell cycle genes (Ccnd2, Ccne1, Cdk4), ↓Expression of antiapoptotic factor (Bcl-2), ↑Expression of proapoptotic factor (Bax), ↓GPX activity at 96 h, ↑ROS,	
				10 μg mL^{-1}	↓Antral follicle growth at 72 h	↓Expression of cell cycle genes (Ccnd2, Ccne1), ↓Bcl-2 expression, ↑Bax expression, ↓GPX activity, ↑SOD1 activity, ↑CAT activity, ↑ROS	
				100 μg mL^{-1}	↓Antral follicle growth at 72 h	↓Expression of cell cycle genes (Ccnd2, Ccne1, Cdk4), ↓Bcl-2 expression, ↑Bax expression, ↓Expression of Sod1 and GPX, ↓GPX activity, ↓SOD1 activity, ↑ROS	
DEHP	Rat	7 days	Oral	1 g kg^{-1}	↓Testes weight	↓Free thiol, GSH and ascorbic acid, ↑CAT and GPX activity, ↑Cytochrome c in cytosol	Kasahara *et al*. (2002)
				2 g kg^{-1}	↓Testes weight	↓Free thiol, GSH and ascorbic acid, ↑CAT and GPX activity, ↑Cytochrome c in cytosol	
MBP	Rat	3 days	Diet	2%	↓Testes weight, ↓Germ cell development	↑Urinary 8-OHdG concentration	Shono and Taguchi (2014)
DEHP	Rat	10 days	Oral	1000 mg kg^{-1}	↓ Weight	No change in testicular activities of GPx1, GPx4, TrxR, GST, SOD and Mn-SoD, ↓Cu,Zn-SOD activity, ↓GSH level, ↑TBARS	Erkekoglu *et al*. (2011)
DEHP	Horse/*in vitro*	1 h	Equine COCs	0.12 μM	Inhibits oocyte maturation	↑CC apoptosis, ↓ROS	Ambruosi *et al*. (2011)
				12 μM	No effect on oocyte maturation, DNA fragmentation	↑CC apoptosis, ↑ROS	
				1200 μM	No effect on oocyte maturation, DNA fragmentation	↑CC apoptosis, ↑ROS	
DBP	Rat	2 weeks	Oral	100 mg kg^{-1}	-	-	Zhou *et al*. (2011)
				250 mg kg^{-1}	For 500 mg kg^{-1}:	↓SOD, ↑MDA	
				500 mg kg^{-1}	↓Epididymal weight, Atrophy of epididymal tubules, Hyperemia of the interstitial vascular, Lumina were oligozoospermic	↓SOD, ↑MDA, ↓GSH-Px	

DBP: Dibutyl phthalate, MBP: Mono-n-butyl phthalate, DEHP: Di-(2-ethylhexyl) phthalate, MEHP: Mono-(2-ethylhexyl) phthalate, ROS: Reactive oxygen species, CAT: Catalase, GPX: Glutathione peroxidase, SOD_1: Cu/Zn superoxide dismutase, TrxR: Thioredoxin reductase, GST: Glutathione S-transferase, Trα-1: Thyroid hormone receptor α-1, PPAR-r: Peroxisome proliferator-activated receptor-r, 8-OHdG: 8-hydroxy-2-deoxyguanosine, MDA: Malondialdehyde, RXRs: Retinoid X receptors, GSH: Glutathione, COCs: Cumulus-oocyte complexes

Table 2: Toxic effects of phthalate in human

Phthalate compound form	Model	Exposure duration (h)	Type of administration/cell	Doses	Exposure effects	Potential mechanisms	References
MEHP	Human/*in vitro*	36	HepG2	6.25 μM	-	↑MDA, ↓GPX activity	Yang *et al*. (2012)
				12.5 μM	-	↑MDA, ↓GPX activity	
				25 μM	↓Cell viability	↑MDA, ↓GPX activity, ↑8-OHdG	
				50 μM	↓Cell viability	↑MDA, ↓GPX activity, ↑8-OHdG	
				100 μM	↓Cell viability	↑MDA, ↓GPX and SOD activity, ↑8-OHdG	
DEHP and MEHP	Human/*in vitro*	24-72	LNCaP cell	DEHP: 3mM	-	↑ROS,	Erkekoglu *et al*. (2011)
				MEHP: 3 μM	-	↑ROS, ↑ P_{53} and P_{21} expression	
DEHP and MEHP	Human/*in vitro*	24-72	LNCaP cell	DEHP: 0.01-10 mM	↓Cell viability	↓GPX activity, ↑DNA damage, ↑GR activity	Erkekoglu *et al*. (2010)
				MEHP: 3-30 μM	↓Cell viability	↓GPX activity, ↑DNA damage, ↑GR activity	

DEHP: Di-(2-ethylhexyl) phthalate, MEHP: Mono-(2-ethylhexyl) phthalate, ROS: Reactive oxygen species, GPX: Glutathione peroxidase, SOD_1: Cu/Zn superoxide dismutase, 8-OHdG: 8-hydroxy-2-deoxyguanosine, MDA: Malondialdehyde, GR: Glutathione reductase

Table 3: Toxic effects of phthalate on liver

Phthalate compound form	Model	Exposure duration	Type of administration/cell	Doses ($mg\ kg^{-1}$)	Exposure effects	Potential mechanisms	References
DEHP				50	Hepatomegaly	↑MDA,	
				200	Hepatomegaly, ↑liver weight	↑MDA,	
				1000	Hepatomegaly, ↑liver weight	↑MDA, ↑8-OHdG	
DBP	Rat	14 days	Oral	50	-	-	Seo *et al*. (2004)
				200	-	↑MDA	
				1000	Hepatomegaly	↑MDA	
				50	-	-	
BBP				200	-	↑MDA	
				1000	Hepatomegaly	↑MDA	
				100	-	↑LPO	
DEP	Fish (olive flounder)	3 days	I.P	300	-	↑LPO, ↓AST,	Kang *et al*. (2010)
				900	-	↑LPO, ↑GSH, ↓CAT, ↓AST, ↓ALT	
				100	Congestions in vena centralis, an enlargement of the sinusoids, Degeneration in hepatocytes, Vacuole formations and presence of lipid droplets in hepatocytes in all doses	↓CAT,	
BCP	Mouse	20 days	Orally	200		↓CAT, ↓SOD, ↑TBARS	Yavasoglu *et al*. (2014)
				400		↓CAT, ↓SOD, ↑TBARS	

DBP: Dibutyl phthalate, BBP: n-butylbenzyl phthalate, BCP: Butyl cyclohexyl phthalate, DEHP: Di-(2-ethylhexyl) phthalate, CAT: Catalase, SOD: Superoxide dismutase, 8-OHdG: 8-hydroxy-2-deoxyguanosine, MDA: Malondialdehyde, GSH: Glutathione, LPO: Lipid peroxide, TBARS: Thiobarbituric acid reactive substances

Table 4: Tixuc effects of phthalate on kidney

Phthalate compound form	Model	Exposure duration	Type of administration/cell	Doses ($mg\ kg^{-1}$)	Exposure effects	Potential mechanisms	References
DEP	Fish (olive flounder)	3 days	I.P	100	-	↑LPO	Kang *et al*. (2010)
				300	-	↑LPO	
				900	-	↑LPO, ↓GST, ↓GR activity	

DEP: Diethyl phthalate, LPO: Lipid peroxide, GST: Glutathione S-transferase and GR: Glutathione reductase

Table 5: Neurotoxicity of phthalate

Phthalate compound form	Model	Exposure duration	Type of administration/cell	Doses	Exposure effects	Potential mechanisms	References
DEHP, DBP, DIBP	Nematode *C. elegance*	24 h	Direct exposure in plate	DEHP (2 and 20 ppm) DBP (500 and 1000 ppm) DIBP (100 and 1000 ppm)	At all doses of these compounds: ↓Body bends and head thrashes	↑Intracellular ROS in all groups	Tseng *et al*. (2013)
DBP	Mice	32 days	Oral	OVA + 45 $mg\ kg^{-1}$ DBP	-	↑ROS in brain, ↑GSH and MDA level	Zuo *et al*. (2014)
				OVA + 0.45 $mg\ kg^{-1}$		↑ROS in brain, ↑GSH and MDA level	

DEHP: Di (2-ethylhexyl) phthalate, DIBPL: Diisobutyl phthalate, DBP: Dibutyl phthalate, OVA: Ovalbumin-immunized, MDA: Malondialdehyde, GSH: Glutathione

that the impairment of cellular redox mechanism increased peroxiredoxin 3 and cyclooxygenase 2 levels in spermatocytes. Thus, increase of intracellular ROS is undoubtedly one of the main mechanisms of phthalate-induced reproductive toxicity (Erkekoglu *et al*., 2010).

Hormonal evaluations in the reproductive system demonstrated that DEHP exposure in prenatal period increased MNGs in the rats possessed testosterone insufficiently (Botelho *et al*., 2009; Andrade *et al*., 2006; Mahood *et al*., 2007). In addition, administration of DEHP or MEHP was able to decrease the testosterone level in mice. Although DEHP reduced testosterone, the injection of testosterone had no effect on the prevention of testicular atrophy. Thus, it is possible that testosterone has no effect on the pathogenesis of testicular atrophy (Kasahara *et al*., 2002). On the other hand, superoxide radical and H_2O_2 that are able to increase the ROS levels might play more important roles, where MEHP (but not DEHP) increased the release of cytochrome c (a key factor for inducing apoptosis) in testis mitochondria in low concentration (Lee *et al*., 1999). DEHP and MEHP can also affect Sertoli cells and induce vacuolization (Kasahara *et al*., 2002).

A literature review for other kinds of phthalates showed that even DBP increased ROS by DNA hunting (increase of 8-OHdG) and lipids as well (increase of MDA) in testis which prevented cell growth and decreased testosterone level (Lee *et al*., 2007). Moreover, it was shown that prepubertal exposure to DBP can cause a significant abnormality in male reproductive track (Mylchreest *et al*., 2002). In addition, DBP is reported to induce atrophy in testis and hyperplasia in Leydig cells in neonatal rats (Kim *et al*., 2004). Some pathways for DBP toxicity have been suggested. These are including the decrease of testosterone level (Parks *et al*., 2000), anti-androgenic activity (Mylchreest *et al*., 1998) and zinc depletion (Fukuoka *et al*., 1995).

More investigations on DBP demonstrated that this compound was able to block Sertoli cell maturation and consequently AR expression level decreased in testis (Lee *et al*., 2007). Also, it is observed that DBP decreased the number of sperms by affecting Sertoli cells (Kleymenova *et al*., 2005). Likewise, DBP (750 mg kg^{-1}) enhanced TRα-1 protein level that decreased testosterone synthesis and thus testicular size reduced. In order to explain these alterations, it should be noted that there are peroxisomes in the testis (Corton and Lapinskas, 2005) and peroxisome proliferators can cause the protection of antioxidant enzymes against ROS (Mehrotra *et al*., 1999). CAT is one of the most important enzymes located in peroxisome in testis, nevertheless previous studies revealed that DEHP enhanced GPx and CAT activity while decreased GSH level in testis (Kasahara *et al*., 2002).

Although DEHP is known to cause alterations in the activity and expression of the antioxidant enzymes (Erkekoglu *et al*., 2010; Botelho *et al*., 2009), it is reported that DEHP inhibited antral follicle growth and reduced SOD_1 activity and expression via an *in vitro* study (Wang *et al*., 2012a). SOD is an antioxidant enzyme converting superoxide into H_2O_2 while GPX and CAT convert this product into H_2O and O_2. Further, there are two major enzymes in rodent ovarian cells, including copper/zinc superoxide dismutase (SOD_1) and manganese superoxide dismutase (SOD_2). SOD_1 and SOD_2 are located in the cytoplasm and mitochondria, respectively (Matzuk *et al*., 1998; Tilly *et al*., 1995).

Beside the above mentioned studies, it was previously reported that DEHP (10 and 100 μg mL^{-1}) decreased the estradiol levels and inhibited the antral follicle growth *in vitro* (Gupta *et al*., 2010). In addition, only DEHP-10 (but not DEHP-100) enhanced ROS/RNS levels. Low and high doses of DEHP might act through different pathways. Lower dose of DEHP may work via oxidative stress while high dose of DEHP may affect the follicle growth through decreasing the estradiol levels. Moreover, DEHP-100 decreased gene expression of the so-called cell cycle regulators Ccnd2 (cyclin-D2), Cdk4 (cyclin-depended-kinase 4) and aromatase (Wang *et al*., 2012a). MEHP is also found to decrease the expression of the cell cycle regulators (Ccnd2, Cdk4 and Ccne1) and anti-apoptotic factor Bcl-2 (B-cell lymphoma 2) while increased the expression of pro-apoptotic factor Bax (Bcl-2-associated X protein) (Wang *et al*., 2012b).

Probably, the inhibition of GPX activity is one of the main mechanisms of MEHP reproductive toxicity. Accumulation of H_2O_2 due to inhibition of GPX can activate other antioxidant enzymes. DEHP mainly affects the expression and activity of SOD_1 leading to the accumulation of superoxide, whereas MEHP decreased the expression and activity of GPX resulting in accumulation of H_2O_2 that caused more toxicity in antral follicles.

Oxidative stress and liver/kidney toxicity: Literature reviews demonstrated that PPs can increase H_2O_2 by induction of enzymes activities. In the same way, H_2O_2 is able to increase the 8-OHdG levels and carcinogenesis. Also, reduction of the hepatic activity of CYP1A1, 1A2 and 3A4 and selective induction of PPs on CYP4A1 which involved in lipid metabolism, were both demonstrated (Seo *et al*., 2004). Some studies are summarized in Table 3 indicating the correlation between liver toxicity of phthalates and OS parameters. In fact and in conclusion, by decreasing CAT and SOD, increasing of TBARS and MDA occurred.

An appraisal on Table 3 and 4 shows that LPO level generally increased in liver and kidney. DEP exposure increased detoxifying enzymes and anti-oxidative enzymes such as GSH, GPx and GR to deal with ROS. In contrast, there was no change in GST and CAT activity (Kang *et al*., 2010). In addition, it is revealed that MEHP decreased the viability of HepG2 cells while increased apoptosis (Yang *et al*., 2012). A significant increase of 8-OHdG at 24 h and a decrease of 8-OHdG at 36 h suggested that oxidative DNA damage might be repaired after 24 h. MEHP also induces mitochondrial dysfunction and reduces cellular ATP content. Moreover, up-regulation of cytochrome c in cytosol and increase of caspase-9 and caspase-3 were noted as well. DEHP and MEHP are reported to increase the DNA damage and apoptosis while decrease GPx1 activity in LNCaP cells. In that study, MEHP (not DEHP) caused augmentation in TrxR activity. TrxR and

GPx1 are two important defense systems (Gromer *et al*., 2004) that play roles in many biological processes such as apoptosis and DNA repair (Arner and Holmgren, 2000). An increase of H_2O_2 may lead to a reduction in TrxR activity in small-cell lung carcinoma that has previously been reported (Gandin *et al*., 2009).

Oxidative stress and neurotoxicity: Previous studies have demonstrated that OS involves in neurological and psychiatric disorders, especially in depression (Ng *et al*., 2008; Sahin and Gumuşlu, 2004). Likewise, phthalates could induce these disorders as a result of imbalance in antioxidants and ROS levels (Zuo *et al*., 2014). Moreover, the accumulation of ROS has been related to a variety of neurodegenerative diseases (Bilici *et al*., 2001). On the other hand, OS cause impairment in learning behavior and reduce motor activity (Kumsta *et al*., 2001; Murakami and Murakami, 2005). Furthermore, oxidative stress might be considered as a main factor in neurotoxicity of phthalates, a factor by which disruption of neuronal systems may lead to neurobehavioral abnormalities (Tseng *et al*., 2013).

An appraisal of the present reports (Table 1-4) on phthalate compounds and oxidative stress shows us there are some controversies in augmentation of oxidative stress parameters after exposure of different organs to the various phthalate derivatives. For instance, although DEHP, DBP and DIBP could increase the levels of ROS, GSH and MDA in brain (Table 5), the level of ROS decreased following exposure to the lowest dose of DEHP 0.12 μM in the reproductive system (Table 1). But the most controversy can be seen in the levels of cellular antioxidants like SOD which generally shows decrease in many organs like liver, kidney and reproductive while in some studies its augmentation has been reported (Table 1). For example, Lee *et al*. (2007) reported SOD enhancement after exposure to 750 mg kg^{-1} of DBP in rat testis. Studies on human cell lines (*in vitro*) exhibited an increase of MDA, a decrease of GPx activity and enhancement of 8-OHdG, as well as increase in DNA damage and GR activity following exposure to different phthalate derivatives. Nevertheless, extrapolation of the *in vitro* investigations to animal and human studies may bring some different results due to the lack of intracellular connections and normal physiological states (Shetab-Boushehri and Abdollahi, 2012), a review on the present data shows that, except a few reports almost all the animal studies revealed the critical role of oxidative stress in phthalate toxicity. An explanation for such differences can be found in possibility of compound accumulation in certain organs, tissues or cell types resulting in prolonged or increased exposure of the target tissue and then an *in vitro* model system may lead to an underestimation of biological activity of the compound under study.

CONCLUSION

Phthalates are among the most important synthetic chemicals playing roles in inducing toxicity in various organs such as liver, kidney and the reproductive system in both human and animals. Their mechanisms of toxicity, especially in reproductive system have not been exactly elucidated so far, however, altering the expression and activity of antioxidant enzymes that result in DNA damage via augmentation of ROS is one of the noteworthy mechanisms involved.

Various kinds of phthalates are able to interfere with antioxidant enzymes in different organs. For instance, DEHP enhances GPX and CAT activity, whereas decreases GSH level in the testis or DBP that acts via increasing ROS by DNA and lipid hunting and increasing MDA in the testis. Moreover, MEHP (but not DEHP) can cause an increase in TrxR activity. TrxR like GPX1 is an important defense system that displays in many biological processes such as apoptosis and DNA repair. An increase of H_2O_2 may lead to the increase of TrxR activity in a small-cell lung carcinoma cell line which has been previously reported. Since, various phthalate derivatives are able to cause irreversible tissue and organ damages especially in reproductive system via a number of mechanisms such as oxidative stress, recently, these compounds have been replaced in the USA, Canada and European Union by other plasticizers. Considering the U.S. Environmental Protection Agency (EPA), application of the following eight phthalates should be managed carefully: Dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), Butyl Benzyl Phthalate (BBP), di-n-pentyl phthalate (DnPP), di(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DnOP), diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP), of which, BBP, DEHP and DBP cause the most toxicity to terrestrial organisms, fish and aquatic invertebrates.

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ABBREVIATIONS

BBP: Benzyl butyl phthalate
CAT: Catalase
DBP: Dibutyl phthalate
DEHP: Bis(2-ethylhexyl) phthalate
DIBP: Diisobutyl phthalate
DIDP: Diisodecyl phthalate
DINP: Diisononyl phthalate
DnOP: Di-n-octyl phthalate
DnPP: Di-n-pentyl phthalate
EPA: U.S. environmental protection agency
GPXx: Glutathione peroxidase
GST: Glutathione S-transferase
MDA: Malondialdehyde
MEHP: Mono-(2-ethylhexyl) phthalate
PP: Peroxisome proliferator
PPARs: Peroxisome proliferator-activated receptor
ROS: Reactive oxygen species
SOD: Cu/Zn superoxide dismutase

REFERENCES

Abdollahi, M., M.Y. Moridani, O.I. Aruoma and S. Mostafalou, 2014. Oxidative stress in aging. Oxid. Med. Cell. Longev, Vol. 2014. 10.1155/2014/876834

Akingbemi, B.T., R. Ge, G.R. Klinefelter, B.R. Zirkin and M.P. Hardy, 2004. Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proc. Nat. Acad. Sci. USA., 101: 775-780.

Ambruosi, B., M.F. Uranio, A.M. Sardanelli, P. Pocar and N.A. Martino *et al*., 2011. *In vitro* acute exposure to DEHP affects oocyte meiotic maturation, energy and oxidative stress parameters in a large animal model. PloS One, Vol. 6. 10.1371/journal.pone.0027452

Andrade, A.J., S.W. Grande, C.E. Talsness, K. Grote, A. Golombiewski, A. Sterner-Kock and I. Chahoud, 2006. A dose-response study following *in utero* and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology, 225: 64-74.

Arner, E.S. and A. Holmgren, 2000. Physiological functions of thioredoxin and thioredoxin reductase. Eur. J. Biochem., 267: 6102-6109.

Bahadar, H., F. Maqbool and M. Abdollahi, 2014. Consumption of phthalates coated pharmaceutical tablets: An unnoticed threat. Int. J. Pharmacol., 10: 78-81.

Bauche, F., B. Fouchard and B. Jegou, 1994. Antioxidant system in rat testicular cells. FEBS Lett., 349: 392-396.

Bilici, M., H. Efe, M.A. Koroglu, H.A. Uydu, M. Bekaroglu and O. Deger, 2001. Antioxidative enzyme activities and lipid peroxidation in major depression: Alterations by antidepressant treatments. J. Affect. Disord., 64: 43-51.

Botelho, G.G., A.C. Bufalo, A.C. Boareto, J.C. Muller and R.N. Morais *et al*., 2009. Vitamin C and resveratrol supplementation to rat dams treated with di (2-ethylhexyl) phthalate: Impact on reproductive and oxidative stress end points in male offspring. Arch. Environ. Contamination Toxicol., 57: 785-793.

Corton, J.C. and P.J. Lapinskas, 2005. Peroxisome proliferator-activated receptors: Mediators of phthalate ester-induced effects in the male reproductive tract? Toxicol. Sci., 83: 4-17.

Crinnion, W.J., 2010. Toxic effects of the easily avoidable phthalates and parabens. Altern. Med. Rev., 15: 190-196.

Davis, B.J., R. Weaver, L.J. Gaines and J.J. Heindel, 1994. Mono-(2-ethylhexyl) phthalate suppresses estradiol production independent of FSH-cAMP stimulation in rat granulosa cells. Toxicol. Applied Pharmacol., 128: 224-228.

Erkekoglu, P., W. Rachidi, O.G. Yuzugullu, B. Giray, A. Favier, M. Ozturk and F. Hincal, 2010. Evaluation of cytotoxicity and oxidative DNA damaging effects of di(2-ethylhexyl)-phthalate (DEHP) and mono(2-ethylhexyl)-phthalate (MEHP) on MA-10 Leydig cells and protection by selenium. Toxicol. Applied Pharmacol., 248: 52-62.

Erkekoglu, P., W. Rachidi, O.G. Yuzugullu, B. Giray, M. Ozturk, A. Favier and F. Hincal, 2011. Induction of ROS, p53, p21 in DEHP- and MEHP-exposed LNCaP cells-protection by selenium compounds. Food. Chem. Toxicol., 49: 1565-1571.

Erkekoglu, P., N.D. Zeybek, B.K. Giray, W. Rachidi and M. Kizilgun *et al*., 2014. The effects of di (2-ethylhexyl)phthalate on rat liver in relation to selenium status. Int. J. Exp. Pathol., 95: 64-77.

Fukuoka, M., T. Kobayashi and T. Hayakawa, 1995. Mechanism of testicular atrophy induced by di-n-butyl phthalate in rats. Part 5. Testicular iron depletion and levels of ferritin, haemoglobin and transferrin in the bone marrow, liver and spleen. J. Applied Toxicol., 15: 379-386.

Gandin, V., C. Nystrom, A.K. Rundlof, K. Jonsson-Videsater and F. Schonlau *et al*., 2009. Effects of the antioxidant Pycnogenol® on cellular redox systems in U1285 human lung carcinoma cells. FEBS J., 276: 532-540.

Gazouli, M., Z.X. Yao, N. Boujrad, J.C. Corton, M. Culty and V. Papadopoulos, 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.

Gromer, S., S. Urig and K. Becker, 2004. The thioredoxin system-from science to clinic. Med. Res. Rev., 24: 40-89.

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

Kang, J.C., J.H. Jee, J.G. Koo, Y.H. Keum, S.G. Jo and K.H. Park, 2010. Anti-oxidative status and hepatic enzymes following acute administration of diethyl phthalate in olive flounder *Paralichthys olivaceus*, a marine culture fish. Ecotoxicol. Environ. Safety, 73: 1449-1455.

Kasahara, E., E.F. Sato, M. Miyoshi, R. Konaka and K. Hiramoto *et al*., 2002. Role of oxidative stress in germ cell apoptosis induced by di(2-ethylhexyl) phthalate. Biochem. J., 365: 849-856.

Kim, H.S., T.S. Kim, J.H. Shin, H.J. Moon and I.H. Kang *et al*., 2004. Neonatal exposure to di (n-butyl) phthalate (DBP) alters male reproductive-tract development. J. Toxicol. Environ. Health Part A, 67: 2045-2060.

Kleymenova, E., C. Swanson, K. Boekelheide and K.W. Gaido, 2005. Exposure in utero to di (n-butyl) phthalate alters the vimentin cytoskeleton of fetal rat Sertoli cells and disrupts Sertoli cell-gonocyte contact. Biol. Reprod., 73: 482-490.

Kumsta, C., M. Thamsen and U. Jakob, 2011. Effects of oxidative stress on behavior, physiology and the redox thiol proteome of *Caenorhabditis elegans*. Antioxidants Redox Signal., 14: 1023-1037.

Lee, J., J.H. Richburg, S.C. Younkin and K. Boekelheide, 1997. The Fas system is a key regulator of germ cell apoptosis in the testis. Endocrinology, 138: 2081-2088.

Lee, J., J.H. Richburg, E.B. Shipp, M.L. Meistrich and K. Boekelheide, 1999. The Fas system, a regulator of testicular germ cell apoptosis, is differentially up-regulated in Sertoli cell *Versus* germ cell injury of the testis. Endocrinology, 140: 852-858.

Lee, E., M.Y. Ahn, H.J. Kim, I.Y. Kim and S.Y. Han *et al*., 2007. Effect of di(n-butyl) phthalate on testicular oxidative damage and antioxidant enzymes in hyperthyroid rats. Environ. Toxicol., 22: 245-255.

Li, L., H.S. Li, N.N. Song and H.M. Chen, 2013. The immunotoxicity of dibutyl phthalate on the macrophages in mice. Immunopharmacol. Immunotoxicol., 35: 272-281.

Lovekamp-Swan, T. and B.J. Davis, 2003. Mechanisms of phthalate ester toxicity in the female reproductive system. Environ. Health Perspect., 111: 139-145.

Lovekamp-Swan, T., A.M. Jetten and B.J. Davis, 2003. Dual activation of PPARα and PPARγ by mono-(2-ethylhexyl) phthalate in rat ovarian granulosa cells. Mol. Cell. Endocrinol., 201: 133-141.

Mahood, I.K., H.M. Scott, R. Brown, N. Hallmark, M. Walker and R.M. Sharpe, 2007. *In utero* exposure to di (n-butyl) phthalate and testicular dysgenesis: Comparison of fetal and adult end points and their dose sensitivity. Environ. Health Perspect., 115: 55-61.

Martino Andrade, A.J. and I. Chahoud, 2010. Reproductive toxicity of phthalate esters. Mol. Nutr. Food Res., 54: 148-157.

Matzuk, M.M., L. Dionne, Q. Guo, T.R. Kumar and R.M. Lebovitz, 1998. Ovarian function in superoxide dismutase 1 and 2 knockout mice. Endocrinology, 139: 4008-4011.

Mehrotra, K., R. Morgenstern, M.B. Ahlberg and A. Georgellis, 1999. Hypophysectomy and/or peroxisome proliferators strongly influence the levels of phase II xenobiotic metabolizing enzymes in rat testis. Chemico-Biol. Interact., 122: 73-87.

Miodovnik, A., A. Edwards, D.C. Bellinger and R. Hauser, 2014. Developmental neurotoxicity of *ortho*-phthalate diesters: Review of human and experimental evidence. Neurotoxicology, 41: 112-122.

Mostafalou, S. and M. Abdollahi, 2012. Current concerns on genotoxicity of pesticides. Int. J. Pharmacol., 8: 473-474.

Mostafalou, S. and M. Abdollahi, 2013. Pesticides and human chronic diseases: Evidences, mechanisms and perspectives. Toxicol. Applied Pharmacol., 268: 157-177.

Murakami, S. and H. Murakami, 2005. The effects of aging and oxidative stress on learning behavior in *C. elegans*. Neurobiol. Aging, 26: 899-905.

Mylchreest, E., R.C. Cattley and P.M. Foster, 1998. Male reproductive tract malformations in rats following gestational and lactational exposure to di (n-butyl) phthalate: An antiandrogenic mechanism? Toxicol. Sci., 43: 47-60.

Mylchreest, E., M. Sar, D.G. Wallace and P.M.D. Foster, 2002. Fetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di(n-butyl) phthalate. Reprod. Toxicol., 16: 19-28.

Ng, F., M. Berk, O. Dean and A.I. Bush, 2008. Oxidative stress in psychiatric disorders: Evidence base and therapeutic implications. Int. J. Neuropsychopharmacol., 11: 851-876.

Noriega, N.C., K.L. Howdeshell, J. Furr, C.R. Lambright, V.S. Wilson and L.E. Gray, 2009. Pubertal administration of DEHP delays puberty, suppresses testosterone production and inhibits reproductive tract development in male Sprague-Dawley and Long-Evans Rats. Toxicol. Sci., 111: 163-178.

Onorato, T.M., P.W. Brown and P.L. Morris, 2008. Mono (2 ethylhexyl) phthalate increases spermatocyte mitochondrial peroxiredoxin 3 and cyclooxygenase 2. J. Androl., 29: 293-303.

Parks, L.G., J.S. Ostby, C.R. Lambright, B.D. Abbott, G.R. Klinefelter, N.J. Barlow and L.E. Gray, 2000. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol. Sci., 58: 339-349.

Posnack, N.G., 2014. The adverse cardiac effects of Di (2-ethylhexyl) phthalate and bisphenol A. Cardiovasc. Toxicol., 14: 339-357.

Rusyn, I., J.M. Peters and M.L. Cunningham, 2006. Modes of action and species-specific effects of di-(2-ethylhexyl) phthalate in the liver. CRC Crit. Rev. Toxicol., 36: 459-479.

Saeidnia, S. and M. Abdollahi, 2013a. Are medicinal plants polluted with phthalates? DARU J. Pharm. Sci., Vol. 21. 10.1186/2008-2231-21-43

Saeidnia, S. and M. Abdollahi, 2013b. Toxicological and pharmacological concerns on oxidative stress and related diseases. Toxicol. Applied Pharmacol., 273: 442-455.

Sahin, E. and S. Gumuşlu, 2004. Alterations in brain antioxidant status, protein oxidation and lipid peroxidation in response to different stress models. Behav. Brain Res., 155: 241-248.

Seo, K.W., K.B. Kim, Y.J. Kim, J.Y. Choi, K.T. Lee and K.S. Choi, 2004. Comparison of oxidative stress and changes of xenobiotic metabolizing enzymes induced by phthalates in rats. Food Chem. Toxicol., 42: 107-114.

Shetab-Boushehri, S.V. and M. Abdollahi, 2012. Current concerns on the validity of *in vitro* models that use transformed neoplastic cells in pharmacology and toxicology. Int. J. Pharmacol., 8: 594-595.

Shono, T. and T. Taguchi, 2014. Short-time exposure to mono-n-butyl phthalate (MBP)-induced oxidative stress associated with DNA damage and the atrophy of the testis in pubertal rats. Environ. Sci. Poll. Res., 21: 3187-3190.

Suna, S., F. Yamaguchi, S. Kimura, M. Tokuda and F. Jitsunari, 2007. Preventive effect of D-psicose, one of rare ketohexoses, on di-(2-ethylhexyl) phthalate (DEHP)-induced testicular injury in rat. Toxicol. Lett., 173: 107-117.

Tilly, J.L. and K.I. Tilly, 1995. Inhibitors of oxidative stress mimic the ability of follicle stimulating hormone to suppress apoptosis in culture rat ovarian follicles. Endocrinology, 136: 242-252.

Tseng, I.L., Y.F. Yang, C.W. Yu, W.H. Li and V.H.C. Liao, 2013. Phthalates induce neurotoxicity affecting locomotor and thermotactic behaviors and AFD neurons through oxidative stress incaenorhabditis elegans. PloS one, Vol. 8. 10.1371/journal.pone.0082657

Wang, W., Z.R. Craig, M.S. Basavarajappa, R.K. Gupta and J.A. Flaws, 2012a. Di (2-ethylhexyl) phthalate inhibits growth of mouse ovarian antral follicles through an oxidative stress pathway. Toxicol. Applied Pharmacol., 258: 288-295.

Wang, W., Z.R. Craig, M.S. Basavarajappa, K.S. Hafner and J.A. Flaws, 2012b. Mono-(2-ethylhexyl) phthalate induces oxidative stress and inhibits growth of mouse ovarian antral follicles. Biol. Reprod., 87: 152-152.

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

Wei, Z., L. Song, J. Wei, T. Chen and J. Chen *et al.*, 2012. Maternal exposure to di-(2-ethylhexyl)phthalate alters kidney development through the renin-angiotensin system in offspring. Toxicol. Lett., 212: 212-221.

Yang, G., X. Zhou, J. Wang, W. Zhang and H. Zheng *et al.*, 2012. MEHP-induced oxidative DNA damage and apoptosis in HepG2 cells correlates with p53-mediated mitochondria-dependent signaling pathway. Food Chem. Toxicol., 50: 2424-2431.

Yavasoglu, N.U., C. Koksal, M. Dagdeviren, H. Aktug and A. Yavasoglu, 2014. Induction of oxidative stress and histological changes in liver by subacute doses of butyl cyclohexyl phthalate. Environ. Toxicol., 29: 345-353.

Yoganathan, T., W. Eskild and V. Hansson, 1989. Investigation of detoxification capacity of rat testicular germ cells and sertoli cells. Free Radical Biol. Med., 7: 355-359.

Zhou, D., H. Wang, J. Zhang, X. Gao, W. Zhao and Y. Zheng, 2010. Di-n-Butyl Phthalate (DBP) exposure induces oxidative damage in testes of adult rats. Syst. Biol. Reprod. Med., 56: 413-419.

Zhou, D., H. Wang and J. Zhang, 2011. Di-n-Butyl Phthalate (DBP) exposure induces oxidative stress in epididymis of adult rats. Toxicol. Ind. Health, 27: 65-71.

Zuo, H.X., J.Q. Li, B. Han, C.J. Ke and X.D. Liu *et al.*, 2014. Di-(n-butyl)-phthalate-induced oxidative stress and depression-like behavior in mice with or without ovalbumin immunization. Biomed. Environ. Sci., 27: 268-280.