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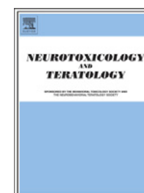

The teratogenicity and behavioral teratogenicity of di(2-ethylhexyl) phthalate (DEHP) and di-butyl Phthalate (DBP) in a chick model

Safa Abdul-Ghani [a], Joseph Yanai [b,c,*], Rula Abdul-Ghani [a], Adi Pinkas [b], Ziad Abdeen [d]

[a] Biochemistry Department Faculty of Medicine, Al-Quds University, Box 19356, East Jerusalem, Palestine
[b] The Ross Laboratory for Studies in Neural Birth Defects, Department of Medical Neurobiology, Institute for Medical Research – Israel-Canada The Hebrew University, Hadassah Medical School, Box 12272, 91120, Jerusalem, Israel
[c] Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA
[d] Al-Quds Nutrition & Health Research Institute, Faculty of Medicine, Al-Quds University, Box 20760, East Jerusalem, Palestine

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ABSTRACT

Phthalates are industrial chemicals widely used in consumer products, plastics and children toys, and the risk of exposure to phthalates, especially prenatal exposure, is a growing concern justifying the development of an animal model to better understand their effect. The present study was designed to evaluate the suitability of a chick model for phthalate DEHP teratogenicity and neurobehavioral teratogenicity, a model which is simple and devoid of potential confounding factors such as maternal toxicity, maternal-fetal unit and maternal-neonatal interactions; major findings were confirmed in the DBP study. Prehatch exposure to DEHP in doses ranging from 20 to 100 mg/kg, reduced the percent hatching from 80% in control eggs to 65%, and increased late hatchings from 12.5% in control eggs to 29.4%. In addition it induced developmental defects characterized by an opening or weakening of abdominal muscles allowing internal organs to protrude externally with or without a sac, omphalocele or gastroschisis, respectively. The effect was dose dependent ranging from 8% with DEHP (20 mg/kg) to 22% (100 mg/kg). Similar treatment with DBP 100 mg/kg has reduced percentage hatching to 57% and increased late hatching to 37.5%, with a 14% increase in gastroschisis. Biochemical evaluation revealed elevated levels of alkaline phosphatase, which reflects non-specific toxicity of DEHP at such a high dose. Behavioral evaluation using an imprinting test and locomotor activity on chicks pretreated with DEHP (100 mg/kg) has shown an abolishment of imprinting performance from the control (0.65) preference ratio. DNA damage measurements of the metabolite 8-hydroxydeoxyguanosine (8-OH-dG) in blood samples showed an increase of 39.7% after prehatch exposure to phthalates. This was statistically significant for DEHP and indicates genetic toxicity, since part of the teratogenic activity is associated with oxidative stress and DNA damage.

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1. Introduction

Phthalates, or phthalate esters, are a class of industrial compounds widely used in toys and food packages, as softeners of plastics, solvents in perfumes, and additives to hairsprays, lubricants, and insect repellents. Not surprisingly, then, the presence of phthalates has been reported in surface soil, soil profiles and ground water (Muszkat et al., 1993). This widespread presence of phthalates in consumer products and the environment has resulted in an extensive intake of these chemicals in the general population. Indeed, an NHANES extensive study in the US found detectable levels of phthalate metabolite in over 75% of the samples (Silva et al., 2004) and similarly, in another study, dibutyl phthalate was globally detected in the human sample population tested for industrial pollutants (Petersen and Breindahl, 2000).

The universal presence of these chemicals in the body is alarming because there is evidence for their deleterious effects, both in humans and in animal models. Noticeable examples are in measures related to fertility and the endocrine system which were adversely effected in many studies (Crisp et al., 1998; Gray et al., 1999; Swan et al., 2005).

Animal models provide similar results showing that active phthalates like DEHP and DBP can decrease the fertility of rats and mice through male and female-mediated effects (Lamb et al., 1987). Phthalates reduced the concentrations of testosterone and the weight of reproductive related organ (Crisp et al., 1998; David et al., 2000; Gray and Butterworth, 1980). The effect could be generalized to other unrelated organs such as the liver, thyroid, kidneys lungs and blood (David et al., 2000; Isenberg et al., 2001).

Expectedly, pregnant women had similar levels of phthalates to the general population as was shown in a study on the Jerusalem population (Berman et al., 2009). There are reports clearly implicating phthalates in adverse behavior among children born to phthalate exposed women. Well described are various behavioral disorders,

* Corresponding author. Tel.: +972 2 675 8439; fax: +972 2 675 8443.
E-mail address: josephy@ekmd.huji.ac.ils (J. Yanai).

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mostly attention deficit hyperactivity disorder (ADHD) (Engel et al., 2010). Animal studies supported this finding by showing alterations in the mechanistically related dopaminergic markers in mice offspring prenatally exposed to phthalates (Kim et al., 2009; Tanida et al., 2009).

The usefulness of the animal model for understanding the teratogenicity, neurobehavioral teratogenicity and the related mechanisms is apparent; however, to be further advantageous, the model developed should be simple, representing a complementary screening model. The chick provides such a model. Although rodent models are more similar to humans, there are distinct advantages to the chick model that complement mammalian studies. The rodent model has inherent methodological shortcomings mainly stemming from confounding indirect variables related to maternal effects (Sastry, 1991; Sobrian et al., 1999), including maternal care, mother–offspring interaction and disruption of maternal endocrine status, all of which affect behavioral outcomes (Barron et al., 1991; Fernandez et al., 1983; Navarro et al., 1988; Riley and Barron, 1989). The avian model avoids these confounds since the teratogen is injected into the egg causing the embryo to be directly exposed to a defined concentration. Similarly, although the rodent model exhibits a "litter effect" (Spear and File, 1996) this limitation is absent in the chick model; every individual offspring represents an independent sample. Furthermore, the chick possesses other important advantages: that embryonic development of an entire group can start at an identical point (incubation), embryonic development is mostly uniform (Kotwani et al., 1995) and the chick hatches at an advanced developmental stage that allows for immediate physical and behavioral evaluation. Lastly, chick eggs are cheap, abundant, and easy to maintain in large numbers. Developing chick embryo has been used as a method of testing the antibacterial effectiveness of wound disinfectants (Green and Birkeland, 1944) and infections in the chicken embryo have also been used to study the development of drug resistance in an embryo adapted strain of *Eimeria tenella* (Chapman, 1976).

Consequently, the present study was performed to investigate an avian model for phthalate teratogenicity and neurobehavioral teratogenicity. Fertile eggs were injected with DEHP and morphological, biochemical and DNA and behavioral alterations were ascertained at hatching age. To generalize the results, major changes were replicated with another phthalate, DBP.

2. Methods

2.1. Chemicals and reagents

Bis(2-ethylhexyl)phthalate (DEHP) CAS No. 117-81-7 EC No 204-211-0 purity 99.7% and Dibutylphthalate (DBP) CAS No. 84-74-2 EC No. 201-557-4 purity 99.8% were purchased from Sigma-Aldrich. DNA damage was assessed with ELISA Kit, Catalog No. EKS-350 which was purchased from Assay Designs Stressgen, Ann Arbon MI USA. Kits for biochemical assays were obtained from SEPPIM (Sees, France). All other chemicals and drugs were of analytical grade and were purchased from Sigma Chemicals (St. Louis, MO). Dextrostix strips were purchased from Ames (Miles, Paris).

2.2. Teratogen administration

As developed before (Izrael et al., 2004), fertile chicken eggs (*Gallus gallus domesticus*) of the Cobb broiler strain were obtained from a local breeder. To administer the DEHP, a hole was drilled in the chorioallantois end (pointed end) of the shell, the substance was thus injected into the albumen and the hole was sealed with medical silicon (type A, Dow Corning). Like other teratogens that are fat soluble, it was expected that the phthalates would disperse quickly into the whole volume of the egg. DEHP or DBP, both dissolved in corn oil, were then administered on incubation day (ID) 0. The doses for the eggs were 5, 20, 50, and 100 mg/kg for DEHP or 100 mg/kg (DBP). Control eggs received equivalent volumes (60 μl/kg of eggs) of the corn oil vehicle solution. It should be noted that these doses are high in human or even rodent terms. After the teratogen administration, the eggs were placed in a commercial incubator at 37.5 °C with 50–60% humidity. Embryonic survivals were monitored via candling and hatch rate and physical attributes at hatching were noted. The chicks were trained to follow an imprinting object and were tested for imprinting performance.

2.3. Chick model for phthalate teratogenicity and behavioral teratogenicity

As modified (Izrael et al., 2004) from earlier descriptions (McCabe et al., 1981), the imprinting apparatus contained three 20-cm diameter running wheels with the sides covered in black PVC, permitting the chicks to see only forward or backward. The imprinting objects were an illuminated red box or a blue cylinder (both 15 × 10 × 18 cm high), located 50 cm from the front open side of the running wheel, lit from within by a 40 W bulb with holes covered with red or blue filters, and rotated at 30 rpm. The chicks were hatched in total darkness and handling was done in the dark, aided by a dim green light, which has a minimal effect on imprinting (Kovach, 1971). All chicks were tagged and then transferred to an individual dark, enclosed wooden chamber warmed to 30 °C, where they were physically and visually isolated from each other. 14 to 24 h posthatch, the chicks underwent 45 min of "priming," a 30 min exposure to light (60 W bulb), followed by 15 min of darkness, conducted while recorded maternal calls were played continuously. Immediately afterward, they were placed individually on the running wheel for 60 min of training with either blue or red imprinting objects, and the wheel rotations running toward the imprinting object were recorded. After training, the chicks were returned to the enclosed chambers for 60 min, after which testing took place without any additional maternal calls.

There were four testing sessions in counterbalanced, randomized order, each lasting for 5 min; in two of the tests, the chick was allowed to run toward the imprinting rotating object and, in the other two, toward the control rotating object. For chicks trained to follow a red object, the red-light box was used as the imprinting object and the blue light box served as the control object; for the chicks trained to follow a blue object, these were reversed. Imprinting was then calculated as a preference score (Sluckin, 1972): Preference score = Rotations toward the training light / (rotations toward the training light + rotations toward the control light). The expected range of the preference score is 0–1, where 0.5 indicates no imprinting, 1 represents maximal imprinting, and 0 represents avoidance (running away from the imprinted object).

We also assessed locomotor activity of the different experimental groups (the number of rotations of the wheel made by the chick during training), since locomotion can by itself influence imprinting. The locomotor activity during imprinting testing (both training and control lights) was expressed as the total number of rotations of the wheel made by the chick.

2.4. Biochemical measurements

The chicks were killed at the end of the behavioral tests, and 1 ml blood was drawn from the common carotid artery into a test tube. The blood sample was centrifuged at 2000 *g* for 10 min and serum was isolated and stored at −80 °C for biochemical analysis and DNA damage test.

Total cholesterol, cholesterol HDL and LDL, triglycerides, total protein, urea, uric acid and creatinine were all determined by the colorimetric assay of Eli-Tech diagnostics following the Kits instructions (Fossati and Prencipe, 1982; Tietz, 1995). Total protein is expressed in g/dl while all the other compounds such as glucose, urea, uric acid, creatinine, triglycerides, total cholesterol, cholesterol-HDL and cholesterol-LDL are expressed as mg/dl.

The enzymatic activity of Alkaline phosphatase (ALP), Alanine aminotransferase (ALT/GPT) and Aspartate aminotransferase (AST/GOT) was measured as reported by the Scandinavian Society of Clinical Chemistry and the German Society for clinical chemistry (G.S.f.C. Chemistry, 1972; C.o.E. Scandinavian Society for Clinical Chemistry, 1974). In all cases, enzyme activity was expressed as U/l.

2.5. Measurement of DNA damage

Measurement was applied on a random sample of chicks and sample size was determined by power analysis where the number of animals was small. As in DBP the results can only be considered suggestive and provide the rationale for future studies. There was no correlation between enzyme activity and birth defects. Assay Designs' DNA Damage ELISA (enzyme-linked immunosorbent assay) is a fast and sensitive competitive immunoassay for the detection and quantitation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in serum as well as urine samples. 8-OHdG has become a biomarker of oxidative DNA damage and oxidative stress, the method uses an 8-OHdG monoclonal antibody to bind in a competitive manner. Details of the procedure are thoroughly described in catalog number EKS-350, and as published previously (Alam et al., 1997; Chiou et al., 2003; Lezza et al., 1999). DNA Damage ELISA Kit was used for detection and quantitation of 8-hydroxy-2′-deoxyguanosine in serum samples of controls and treated animals.

2.6. Statistical analysis

Data are presented as Mean ± SE, with differences between treatments established by ANOVA groups. Significance for all tests was assumed at $P<0.05$. $\chi2$ was applied for nonparametric variables.

3. Results

3.1. Effect of phthalates on hatching rates and gross malformations and functional defects

As is shown in Table 1 there was a slight decrease in hatching (from 80% to 60–70%) among the phthalate exposed chicks which, however, did not reach statistical significance (χ^2). The proportion of chicks that were late to hatch (1 day) did not differ between groups. A slight decrease in% hatching in the control group is below the level of commercial hatcheries and may reflect the effect of the injection per se. Further decrease is to be expected after administration of the insult. Gross malfunctions and severe motor dysfunctions appeared in the phthalate treated animals except for the low 5 mg/kg dose (DEHP). These included gastroschisis, a disorder resulting in an opening in or weakening of abdominal muscles allowing internal organs to protrude externally without a sac, omphalocele, a disorder resulting in an opening in or weakening of abdominal muscles, allowing internal organs to protrude externally in a translucent sac. Pictures showing gastroschisis and omphalocele as checked in chicks that failed to hatch on ID 22 are presented in Fig. 1. Defects in chicks that failed to hatch are presented because they are similar to those observed in chicks that hatched. They were found only in the doses and the phthalates shown in the figure and not in the rest of the phthalates and dose groups.

Table 1
Percent hatching, incubation length and congenital malformation in chicks with prehatch exposure to DEHP or DBP.

Prehatch exposure	Control	DEHP mg/kg				DBP mg/kg
Dose (mg/kg)	0	5	20	50	100	100
% Hatching	80	64	62	68	68	57
(number of eggs incubated)	(40)	(11)	(13)	(19)	(9)	(14)
% Late hatching (of those that hatched)	16	14	13	54	17	38
% Defects of those* that hatched	0	0	13[G]	15[G,O]	33[O]	13[G]

* $P<0.05$ for the difference in defects between the control and phthalate exposed group pooled (χ^2).
[G] Gastroschisis: An opening in or weakening in abdominal muscles, allowing internal organs to protrude externally without a sac.
[O] Omphalocele: An opening in or weakening in abdominal muscles, allowing internal organs to protrude externally in a translucent sac.

3.2. Biochemical measurements in chicks serum

A variety of biochemical measures was assessed in the blood serum of the DEHP-exposed chicks (Table 2). Among them, a statistically significance increase from the control was shown for Alkaline phosphatase activity (296%, $P<0.05$), and significant reductions were shown for GPT (76%, $P<0.05$), urea (41%, $P<0.05$), and creatinine (69%, $P<0.05$). The decrease in GPT and urea could be postulated as a necrotic side effect of phthalate on the liver cells. However, since creatinine is a by-product of muscle catabolism the loss of muscle bulk as a teratogenic side effect of phthalates compounds could account for its decreased levels.

3.3. DNA damage induced by phthalates (100 mg/kg)

DNA damage was estimated by measuring the concentration of 8-OHdG in blood serum of newly hatched chicks. As shown in Fig. 2, prehatch exposure to DEHP increased 8-OHdG by 39.7% ($P<0.05$), while the 25% increase induced by DBP was not significant.

3.4. Behavioral test

3.4.1. Imprinting test

The test was conducted on chicks exposed to 100 mg/kg DEHP. Imprinting preference score of the control group was 0.65 (Fig. 3), well above the “no preference” score of 0.5 and prehatch exposure to DEPH abolished imprinting (preference score 0.51, $P<0.05$ for the difference between the control and DEPH treated group F.).

3.4.2. Locomotor activity

The general activity (number of rotations during training (1 h) and imprinting test (20 min)) was monitored to exclude potential confounding effects on activity in the imprinting evaluations (Table 3). The apparent inconsistent changes during training and testing were not statistically significant.

4. Discussion

Since phthalates are common in products, have leached into the environment, are present in human and animal bodies and are affecting human physiology and behavior (Patisaul and Adewale, 2009; Swan et al., 2005), especially after prenatal exposure, it is important to establish a simple complementary screening model for their neurobehavioral teratogenicity. The current investigation was designed for that purpose. Our present finding suggests that phthalate teratogenicity is expressed in all levels examined: congenital malformations, defects in major biochemical markers, DNA damages and an abolishment of the behavioral function studied. These results were demonstrated with the di(2-ethylhexyl) phthalate (DEHP) and generalized using one dose of an additional phthalate, di-butyl Phthalate (DBP). Increased alkaline phosphatase could be explained by toxic effects of phthalate on the liver, and could be related to the yolk-sac of the chick embryo. This is consistent with previous studies where alkaline phosphatase activity was increased on gestation days 12–14 after the exposure of pregnant rats with folic acid antagonist, 9-methyl pteroylglutamic acid (Johnson and Spinuzzi, 1968; Padykula, 1958). Significant reductions were shown for GPT, urea and creatinine with uncertain toxicological significance. Since the biochemical changes don't explain the teratogenic

A) Normal Chicks (Control)
B) 20 mg/kg DEHP
D) 100 mg/kg DEHP
Omphalocele
Normal
Gastoschisis
C) 50 mg/kg DEHP
E) 100 mg/kg DBP
Gastroschisis
Omphalocele
Gastroschisis

Fig. 1. Gastroschisis and omphalocele (see text) after prehatch exposure to phthalates in chicks that failed to hatch (ID 22).

defects directly, it was not relevant to measure biochemical changes with low doses of DEHP. For the present study, we focused on the dose where the highest percent of defects occur, 100 mg/kg and mostly on DEHP. A more thorough study including dose–response curves and a complete comparison of the two phthalates will be the subject of future investigation.

Table 2
Biochemical measures in the blood of control chicks and chicks with prehatch exposure to DEHP.

Value (units)	Control	DEHP (100 mg/kg)
Glucose: (mg/dl)	232.6 ± 11.4 (6)	207.0 ± 10.2 (10)
Alkaline phosphatase: (U/l)	90.0 ± 25.5 (4)	357.0 ± 73.0 (8)*
GPT: (U/l)	32.0 ± 8.2 (6)	7.82 ± 2.1 (11)*
GOT: (U/l)	148.8 ± 11.7 (5)	164.3 ± 15.4 (12)
Urea: (mg/dl)	64.2 ± 4.1 (4)	37.7 ± 2.8 (12)**
Creatinine (mg/dl)	1.7 ± 0.4 (4)	0.5 ± 0.1 (10)*
Uric acid: (mg/dl)	7.4 ± 1.9 (6)	5.2 ± 0.6 (12)
Total protein: (g/dl)	3.1 ± 0.6 (6)	2.3 ± 0.1 (11)
Cholesterol—total: (mg/dl)	396.0 ± 36.1 (6)	378.9 ± 16.9 (11)
Cholesterol—HDL (mg/dl)	155.6 ± 11.8 (5)	149.6 ± 7.8 (12)
Cholesterol—LDL (mg/dl)	261.0 ± 35.0 (6)	207.0 ± 10.2 (10)
Triglycerides: (mg/dl)	103.5 ± 16.0 (6)	68.2 ± 3.5 (12)

Values shown are Mean ± SE for the number of samples indicated in parentheses. At the end of each experiment, blood was collected from the common carotid artery of chicks, centrifuged and the serum separated for the analysis. Analysis was performed using Bio-analyzer for measuring all the chemical compounds.
* $P<0.05$ (ANOVA).
** $P<0.02$ (ANOVA).

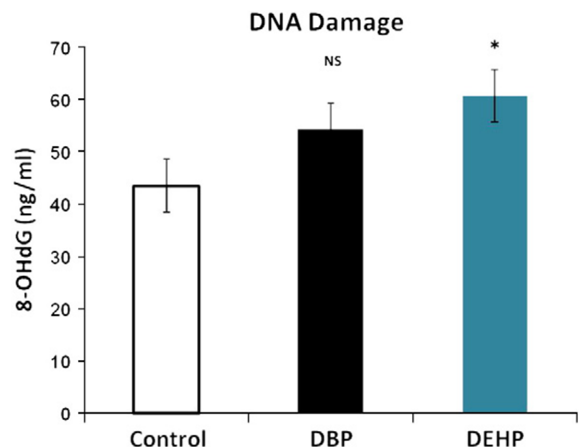

Fig. 2. DNA damage in control chicks and chicks with prehatch exposure to DEHP or DBP. Values are Mean ± SE expressed as ng 8-OHdG/ml blood. Sample sizes: Control, 6 chicks; DBP, 3; DEHP, 6. * $P<0.05$, NS = non-significant (ANOVA).

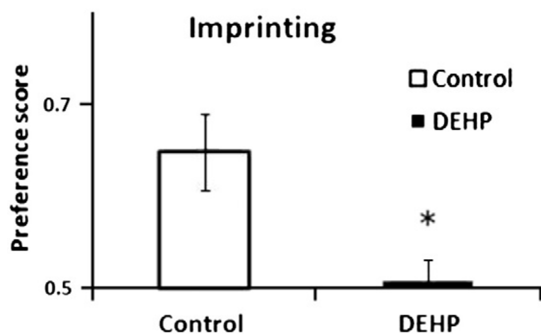

Fig. 3. Imprinting preference scores of control chicks and chicks with prehatch exposure to 100 mg/kg DEHP. *$P<0.05$ for the difference between control and DEHP-exposed groups (ANOVA).

4.1. Gross malformations: gastroschisis and omphalocele

The congenital malformations, gastroschisis and omphalocele, that occurred all point to teratogenesis related to insult occurring in early embryonic development, and only DBP was associated with motor dysfunction. Other defects, particularly the behavioral defects, are related to the effect of the teratogen during later stages of embryonic development and hatching (Hutchings, 1978). Human studies on the abdominal malformations of the present research have shown that gastroschisis better represents an outcome of teratogenic insult and omphalocele is better correlated with advanced maternal age and genetic factors (Van Dorp et al., 2010). However, it is also recognized that different species may respond differently to the same substance (Ekwall et al., 1998; Hurtt et al., 2003). Indeed our results point to species differences in the response to phthalates between the chick and rodents. This is an important factor which also provides potential benefit in understanding the mechanism of phthalates, providing the rationale for further studies. Similarly our results do prove that similar defects will occur in humans, an issue which was often raised with regards to similar studies in other experimental animals (Robinson et al., 2001; Schardein, 2000). This issue is being further complicated by the difference in doses between animal studies and human exposure', which again points to the necessity of future studies on this issue (ACSH, 1997).

4.2. Chemical levels in blood

Furthermore, in chicks that did not have gross malformations, blood samples revealed significant changes in chemical levels. Alkaline phosphatase, which is formed by the mucosal cells that line the bile system of the liver, serves as an important indicator of liver or bone diseases. Bile ducts or gallbladder functional failures or blockage prevents alkaline phosphatase from being discharged through the bile, thus resulting in the release of alkaline phosphatase into the blood stream, and as shown in our study, leading to a decrease in creatinine and urea. Additionally, osteoblasts release alkaline phosphatase during bone growth (Fernandez and Kidney, 2007), suggesting that the increased levels of alkaline phosphatase in our avian model of phthalate teratogenicity may similarly signify bone failures. The present proposition of possible bone tissue failure is further supported by related previous studies where crania bifida, anophthalmia, resulting from an absence of bone tissue forming the orbit of the eye, and blindness was found after developmental exposure to other types of phthalate esters like dibutoxyethyl phthalates, di-2-methoxyethyl phthalates and octylisodecyl phthalates (Bower et al., 1970). On the other hand, the increase in alkaline phosphatase in the present model may not be a valid indication of liver failure due to the decrease in GPT activity. There is no direct evidence to link these biochemical events to induction of congenital malformation; it may reflect non-specific toxicity of phthalates.

Table 3
Activity in the imprinting apparatus in control chicks and in chicks with prehatch exposure to DEHP (100 mg/kg).

Treatment	Activity during training	Activity during testing
Control	158.6 ± 45.1 (7)	50.0 ± 8.6 (8)
DEHP	203.0 ± 55.4 (10)	42.2 ± 11.2 (11)

Values are the mean number (±SE) of wheel rotations during training and the total number of wheel rotations (toward the training and the novel light) during testing.
() = sample size.
There were no significant differences between groups in this test.

4.3. DNA damage

The present study also demonstrated DNA damage after prehatch exposure to phthalates, which is significant in light of the wide spectrum of physiological changes that it indicates. Recent studies employing comet assay to measure the DNA damage induced by DEHP demonstrated that DEHP induced DNA damage in the nervous system of mice and oxidative damage in the internal organs of mice in an apparently dose-dependent manner (Martino-Andrade and Chahoud, 2010). Other studies demonstrated DNA damage induced by DEHP, as indicated by long term induction of 8-OH-dG, in the liver of male rats (Cattley and Glover, 1993; Takagi et al., 1992). In humans, urinary monoethyl phthalate at environmental levels is associated with increased DNA damage in sperms (Duty et al., 2003).

We used 8-OHdG as a quantitative indicator for DNA damage which points to oxidative stress. This is in line with previous studies all demonstrating the efficacy of the competitive ELISA for 8-OHdG as a simple method for quantifying the extent of oxidative stress (Takane et al., 2005). There is much evidence to show that oxidative damage may be an important mechanism underlying several pathophysiological states including: atherosclerosis caused by oxidative modification of low-density lipoprotein (Ross, 1999); diabetic complications caused by oxidative damage of lipids, protein (Baynes and Thorpe, 1999) and DNA (Dandona et al., 1996); aging caused by oxidative damage of proteins and myocardial damage\loss through oxidative injury. These results support the possibility that an increase in oxidative stress is at least one mechanism by which DEHP and DBP exert their teratogenic effect in the chick model.

4.4. Imprinting deficits

On the neurobehavioral teratological level, the phthalate-exposed chicks had deficits in imprinting, suggesting a defect in a specific brain region and innervation. Imprinting in the chick is mechanistically related to the intermediate hyperstriatum ventrale (IMHV) (McCabe et al., 1981) and to several innervations (Gruss and Braun, 1997; Horn, 1998; Tsukada et al., 1999). In our previous studies, after prehatch exposure to various teratogens (Yanai et al., 2010), the chicks had deficits in imprinting behavior with concomitant reduction in the basal level of PKCγ and PKCβ and an abolishment of the cholinergic-induced activation/translocation of PKCγ and in cholinergic and serotonergic biomarkers (Izrael et al., 2004). While the involvement of several neurotransmitter innervations in the phthalate's effect on imprinting is mechanistically important, studies have reported a correlation between phthalates exposure and developmental attention disorders in humans (Engel et al., 2010; Kim et al., 2009) which are known to largely be related to the dopaminergic innervation. These studies, which were corroborated both in a mouse study where early exposure to DEHP (Tanida et al., 2009) affected DA innervation and in a chick study where auditory imprinting was shown to be related to DA (Gruss and Braun, 1997), imply the usefulness of further studies on DA innervations as a possible mechanism by which phthalates exert their teratogenic effect on imprinting behavior.

4.5. Comparison of the chick model to previous studied model of higher species

One-to-one comparison with previous studies in rodents cannot be made because the studies investigate different parameters, often dictated by the difference between the species. Generally, however, our findings were similar to that of the rodent model in showing gross malformations, even though they were of a different nature (Gray et al., 2000; Gray et al., 1999; Gray and Butterworth, 1980; Lamb et al., 1987; Parks et al., 2000; Parmar et al., 1987); omphalocele and gastroschisis defects were unique to the chick. A better comparison can be made with certain cellular parameters where our findings of phthalate-induced DNA damage were consistent with those found in mice (Martino-Andrade and Chahoud, 2010; Takagi et al., 1992) and humans (Duty et al., 2003). Consistently, phthalate-induced behavioral deficits, although of a different nature, have been found both in our chick model (imprinting) and in humans (ADHD) (Engel et al., 2010).

5. Conclusions

In conclusion, phthalates, administered to the chick embryo, induced gross malformations, alterations in biomarkers indicating damage of vital organs, DNA damage indicating dysfunctions, and deficits in imprinting behavior which represent neurobehavioral teratogenicity with specific etiology. The chick model can be relatively easily applied to the understanding of the mechanism by which phthalates exert their deleterious effect on development, an important step in the establishment of a chick model for the reversal of phthalate induced deficits, toward eventual clinical application (Yanai et al., 2010).

Conflict of interest statement

The authors state that they have no conflicts of interest.

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