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THE EFFECT OF DI-BUTYL PHTHALATES (DBP) AND DI (2-ETHYLHEXYL) PHTHALATES (DEHP) ON FEMALE RATS FERTILITY

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ABSTRACT: Phthalate esters are industrial chemicals widely used in cosmetics, plastics bottles and medical devices, and the risk of exposure to phthalates is on the increase. In recent years, many studies have been carried out on the possible health hazards of phthalates, including the effect on reproduction. In this study rats were injected twice weekly with Di-butyl Phthalates (DBP) or Di (2-Ethylhexyl) Phthalates (DEHP) (100 mg/kg) and cohabited with male rats for one month. Fertility and mortality rates as well as fecundity were impacted significantly. Fertility rate decreased from 87 % in control rsats to 67 % in DBP treated and 50 % in DEHP treated rats. Similarly mortality rate in new born litters increased from 2.8 % in control rats to 52.3 % in DBP treated and 31.3 % in DEHP treated rats. Fecundity rate which expresses the average number of litters in each delivery was reduced from 8.2 in control rats to 7.3 in DBP treated and 5.3 in DEHP treated rats. No significant changes were observed in total body weight gain, or with the relative weight of the heart, spleen, liver, or brain organs. However significant decrease in relative weight was detected in kidneys (7% $P \leq 0.05$) that have been treated with DBP (100 mg/kg). DNA oxidative stress (8-hydroxydeoxyguanosine (8-OH-dG) and hepatotoxicity (GOT and GPT) biomarkers increased considerably in female rats following continuous treatment with DBP (100 mg/kg).

INTRODUCTION: Phthalates, or phthalate esters, are a class of industrial compounds widely used as softeners of plastics, solvents in perfumes, toys, food packaging and additives to hairsprays, lubricants.

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The Environmental Working Group has focused on phthalates since 1998, when dibutyl phthalate was present in the bodies of every single person tested for industrial pollutants [1]. Previous studies have reported the presence of phthalate in soil surface, soil profiles and ground water [2].

Phthalates in the environment are subject to biodegradation, photo degradation, and anaerobic degradation and exposure can occur through direct use or indirectly through leaching and general environmental contamination.

Studies around the world have shown that there is a widespread exposure to phthalate in the general population. In the US, detectable levels for phthalate metabolite were found in over 75% of the samples [3]. Wide spread exposure to phthalates has been recently documented among pregnant women in Jerusalem [4].

Phthalates have been shown to cause a variety of effects in laboratory animals; Fertility studies have shown that active phthalates-like DEHP and DBP can decrease the fertility of rats and mice [5]. In males, Phthalates reduce concentrations of testosterone, an important androgen that contributes to the development of male sex organs. Studies in rodents exposed to doses in excess of 100 mg/kg/day DEHP clearly indicate that the testis is a primary target tissue, resulting in decreased testicular weights and tubular atrophy [6]. Within the testis, Sertoli cells appear to be the target of DEHP toxicity [7, 8]. Effects on spermatogenesis were also indicated by the appearance of damaged spermatogenic cells and abnormal sperm in rats exposed to 2,000 mg DEHP/kg/day in their diet for 15 days [9].

The co-administration of testosterone with DEHP appeared to diminish but not abolish the testicular toxicity of DEHP in rats [6]. Results from both *in vivo* and *in vitro* studies have indicated that the Sertoli cell is the main target for DEHP-induced testicular toxicity and that MEHP is the ultimately active testicular toxicant [10, 11]. However, effects on Leydig cells have also been reported previously and it was suggested that Sertoli cell and not the germ cell is the direct target of DEHP toxicity since the germinal cells affected were those inside the Sertoli cell barrier [6, 12]. Alterations in Sertoli cell cytoskeleton after exposure to phthalates have also been reported[10]. In addition to penile morphological abnormalities, hemorrhagic and undescended testis, testicular and epididymal atrophy or agenesis, and small to absent sex accessory glands [13, 14].

Recent study by Harvard School of Public Health, have shown an association between phthalate exposure and reproductive health among humans. The study recruited 168 men and found that those who had mono butyl phthalate (MBP) or mono benzyl phthalate in their urine tended to have a lower sperm count. The study showed an inverse relationship between high concentrations of these chemicals and low sperm count. Other animal studies have also shown a decrease in the weights of the testes, prostate, seminal vesicles, and caused atrophy and degeneration of the semineferous tubules upon oral exposure to DEHP which consequently altered sperm measures and reduced fertility [15].

Studies of long-term exposures in rats and mice have shown that high oral doses of DEHP caused health effects mainly in the liver and testes[16]. These effects were induced by levels of DEHP that are much higher than those received by humans from environmental exposures. Toxicity of DEHP in other tissues is less well characterized, although effects in the thyroid, ovaries, kidneys, and blood have been reported in a few animal studies [17]. It is well documented that long-term oral exposure to DEHP causes cancer of the liver in both rats and mice [17-21]. DEHP has been demonstrated to cause developmental toxicity including teratogenic effects in both rats and mice [22] and on Chicks` embryonic development [23]. This study is designed to test the effect of phthalates on female rats' fertility and whether these toxic effects are related to DNA damage and oxidative stress.

MATERIALS AND METHODS:

Chemicals and reagents: Dibutylphthalate (DBP) and Bis(2-ethylhexyl)phthalates (DEHP) were purchased from Sigma Aldrich. DNA Damage ELISA Kit from Assay Designs, Inc. 5777 Hines Drive, Ann Arbor, MI 48108 USA. Kits for biochemical assays from SEPPIM S.A.S. –Zone Industrielle - 61500 SEES France. All chemicals and drugs were of analytical grade and were purchased from Sigma Chemicals Co P.O.Box. 14508, St. Louis MO.63178 USA. Dextrostix strips were purchased from Ames, (Miles, Paris)

Experimental Design for Fertility Studies: Healthy White Albino female rats weighing 120-200 g each were used in these experiments. Animals were divided into three groups, 6 animals in different cages.

All the experimental animals in groups A, B, C, were maintained under normal conditions of humidity, circadian cycle and temperature and with free access to food and water unless required otherwise. A standard rat pellet diet was used for all the experiments. Group B was treated with DBP 100 mg / kg, group C was treated with the same dosage of DEHP, and group A was treated with vehicle solution of Corn Oil. Experiments were performed in according to the ethical guidelines for laboratory animals.

The female rats were injected intraperitoneally (IP) twice a week with DBP or DEHP 100 mg/kg body mass and control group was injected (IP) with the same volume of Corn Oil. Each week rats were fasted for 15 hours for the measurement of body weight and blood glucose. After one week females were cohabited for one month with two male rats in each cage, and to avoid aspects of male fertility the male rats were distributed equally between the cages.

Thus the females in group A, B and C were exposed equally to the same male rats. All the male rats were not treated with the drugs. After one month of exposure the male rats were removed and we continued to measure the body weight, blood glucose, and to follow up carefully the cases of pregnancy until delivery.

During this period we recorded the number of pregnant females and the number of litters in each delivery for the measurement of mortality rate, fertility rate and fecundity rate. In addition we observed carefully the appearance of any behavioral changes including motor disorders on female rats or the new born litters.

Total Body Weight and Relative Weight of internal Organs: At the end of experiment rats were anaesthetized with ether and the following organs were removed for the measurement of absolute and relative weight of heart, kidneys, spleen, liver and brain. In all cases, body weight was measured on fasting female rats for 12 to 15 hours. Blood samples were collected from the femoral artery for separation of blood serum and measurement of blood biochemistry and DNA damage.

Biochemical measurements: At the end of the experiments female rats from group A, B and C were anaesthetised with ether, and at the stage of light anaesthesia characterized by loss of spontaneous movements and pain sensation but with positive corneal reflex, blood (1 ml) was drawn from the femoral artery into a test tube. Blood samples were centrifuged at 2000 g for 10 min; serum was isolated and stored at – 80 °C for biochemical analysis and DNA damage test.

Cholesterol Total, Cholesterol HDL and LDL, Triglycerides, Total protein and Urea levels were all determined by the colorimetric assay of (Eli-Tech diagnostics) Following the Kits instructions. Enzymatic colorimetric determination of total cholesterol was measured according to the method described previously [24, 25] .Cholesterol HDL Direct and cholesterol LDL direct were measured as described previously [26, 27]. With total protein we used the Biuret reaction as described previously [28, 29]. As for measurement of triglycerides we used the enzymatic colorimetric as described previously [27, 30]. Urea was measured as described previously [31]. Following the Kits instructions, total protein was expressed in g/dl while all the other tests as glucose, urea, triglycerides, cholesterol total, cholesterol-HDL and cholesterol-LDL were expressed in mg/dl.

Activity of several enzymes was also measured and expressed in U/L. Alanine aminotransferase (ALT/ GPT) and Aspartate aminotransferase (AST / GOT) activities were measured as described previously [32, 33] .

Measurement of DNA Damage: 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 described in details in catalog number: EKS-350, and as published previously [34-36].

DNA Damage ELISA Kit was used for detection and quantitation of 8-hydroxy-2'-deoxyguanosine in serum samples of controls and treated animals.

Statistical Analysis: All values are presented as Mean ± SEM for the number of experiments indicated in brackets and the data were analyzed using Students t-test.

RESULTS:

Effect of Phthalates on Fertility Rate, Fecundity & Mortality Rate: In control samples, female rats were injected (IP) with 200 µl corn oil twice a week for 3 months and cohabited with adult male rats for a period of one month. Female rats produced pregnancy in 87% of the cases (13 / 15). Each pregnant female rat delivered between 6 – 12 litters (an average of 8.2 liters per delivery), and average weight of each litter was 7 to 8 grams. All the new born litters developed normally. Mortality rate was 2.8 %, since 3 litters died from a total number of 106 litters. Female rats were injected (IP) with DBP 100 mg / kg twice a week and were cohabited for one month with male rats. DBP has reduced fertility rate from 87 % in control group to 67 % (6 female rats got pregnant from a total number of 9 rats as shown in **Table 1.** Fecundity rate was reduced to 7.3 liters per delivery and mortality rate was increased to 52.3 %. Litters weighed around 6 gram each. Observation studies have shown 2 cases with motor disorders, one rotating (10 rotations per min.) and one rearing (5 times per min.).

Other group of female rats was injected (IP) with DEHP 100 mg / kg for the same periods and cohabited with the same male rats for a period of one month. Treatment with DEHP decreased fertility rate to 50 % and reduced fecundity rate to 5.3 liters per delivery. Mortality rate among the new born litters was elevated from 2.8 % in control animals to 31.3 % in DEHP treated animals.

TABLE 1: EFFECT OF DIBUTYLPHTHALATES (DBP) & BIS (2-ETHYLHEXYL) PHTHALATES (DEHP) ON RATS FEMALE FERTILITY

	CONTROL	DBP	DEHP
FEMALES NUMBER:	15	9	6
FEMALES COHABITED:	15	9	6
PREGNANT FEMALES:	13 / 15	6 / 9	3 / 6
LITTER: (NEW BORN):	106	44	16
DIED LITTERS:	3	23	5
MORTALITY RATE: (% lethal cases)	3 %	52 %	31 %
FERTILITY RATE: (% Pregnant Females/Females Cohabited)	87 %	67 %	50 %
FECUNDITY RATE:	8.2 PER DELIVERY	7.3 PER DELIVERY	5.3 PER DELIVERY

Female rats weighing 120-200gm received twice a week IP injection of DBP and DEHP (100mg/kg). Control animals were injected with the same volume with corn oil. Female rats were cohabited with 2 male rats for one month. Mortality rate: is the percentage of died litters compared to new born litters. Fertility rate: is the percentage of pregnant females per the number of females cohabited. Fecundity rate: is the average number of litters per delivery.

Effect of Phthalates on Total and Relative Body Weight: As total body weight gain is concerned no significant changes were observed in female rats treated with DBP or DEHP compared to control animals. Similar pattern of change in total body weight gain was observed during one month matting or in pre-matting or post-matting stage as shown in **Table 2.**

TABLE 2: CHANGES IN BODY WEIGHT GAIN IN NORMAL RATS COMPARED TO RATS TREATED WITH DBP & DEHP

	CONTROL	DBP 100MG/KG	DEHP100MG/KG
INITIAL (8 days before mating)	143.5 ± 5.6 (15)	138.0 ± 7.7 (10)	120.8 ± 3.8 (6)
MATING (1ST day)	168.6 ± 5.8 (15)↑ 17.5 %	162.3 ± 8.3 (10)↑ 17.6 %	137.3 ± 4.4 (6)↑ 13.7 %
MATING (1 month)	208.8 ± 8.7 (15)↑45.5 %	186.6 ± 5.8 (10)↑35.2 %	179.7 ± 5.7 (6)↑ 48.8 %
POST MATING (after 1 month)	217.1 ± 7.5 (11)↑51.3 %	203.0 ± 6.3 (6)↑47.1 %	188.3 ± 8.7 (6)↑55.9 %

Values shown are Mean ± SEM for the number of experiments indicated in brackets. Body weight was measured one week before mating, the first day of mating, the last day of mating and one month after removal of the male rats. Significance of differences between treated and control were assessed using Student's t-test. For experimental details see legend of **Table 1** DBP, dibutylphthalates, DEHP, bis(2-ethylhexyl) phthalates. At the end of the experiments after one month matting and one month after matting, rats were anaesthetized with ether and the internal organs were removed and weight for the measurement of relative weighed of the following organs: Heart, kidneys, spleen, liver, brain.

Relative weight of the kidneys was reduced significantly in DBP treated rats from 0.685 ± 0.013 (10) gram to 0.638 ± 0.012 (9) gr. ($P \leq 0.05$). With DEHP treatment no significant changes were observed.

TABLE 3: EFFECT OF DBP & DEHP ON RELATIVE WEIGHT OF BODY ORGANS IN FEMALE RATS

	CONTROL	DBP 100MG/KG	DEHP100MG/KG
HEART	0.404 ± 0.013 (10)	0.358 ± 0.012 (9)	0.373 ± 0.018 (6)
KIDNEYS	0.685 ± 0.013 (10)	0.638 ± 0.012 (9)*↓ 7 %	0.690 ± 0.038 (6)
SPLEEN	0.281 ± 0.016 (9)	0.276 ± 0.010 (9)	0.286 ± 0.023 (6)
LIVER	3.247 ± 0.171 (10)	3.118 ± 0.173 (9)	3.490 ± 0.218 (6)
BRAIN	0.767±0.023(10)	0.769±0.026(9)	0.732±0.036(6)

Values shown are Mean ± SEM for the number of experiments indicated in brackets.

The relative weight shows the percentage weight of different organs compared to total body weight in each animal. Female rats were cohabited with male rats for one month as mentioned in table 1. At the end of experiments rats were anaesthetized with ether and the selected organs: heart, kidneys, spleen, liver and brain were removed. * $P \leq 0.05$

Biochemical Changes in Blood Serum Following Treatment with Phthalates: The level of biochemical compounds in blood serum of overnight fasted rats was measured in control animals and in animals treated with DBP or DEHP.

Table 4 shows that GPT values were increased with DBP by 42 %, from 50.33 ± 5.73 (9) to 71.25 ± 2.10 (12) U/L ($P \leq 0.02$). While GOT values were increased by DBP from 98.38 ± 6.52 (8) to 142.00 ± 11.38 (10) U / L and with DEHP they were elevated to 181.25 ± 30.80 (6) U/L ($P \leq 0.05$). Both phthalates had no significant effect on the serum levels of glucose, urea, total proteins, triglycerides, cholesterol HDL or cholesterol LDL.

TABLE 4: BIOCHEMICAL CHANGES IN RATS BLOOD SERUM TREATED WITH DIBUTYLPHTHALATES (DBP) & BIS (2-ETHYLHEXYL) PHTHALATES (DEHP)

	CONTROL	DBP (100MG/KG)	DEHP (100MG/KG)
GLUCOSE: (mg/dl)	83.71 ± 8.35 (8)	87.7 ± 4.3 (7)	68.0 ± 5.0 (6)
GOT: (U/L)	98.38 ± 6.52 (8)	142.00 ± 11.38 (10)*	181.25 ± 30.80 (6) *
GPT: (U/L)	50.33 ± 5.73 (9)	71.25 ± 2.10 (12) **	60.50 ± 12.90 (6)
UREA: (mg/dl)	61.81 ± 5.62 (13)	48.67 ± 4.30 (12)	72.00 ± 6.90 (6)
CHOLESTEROL TOTAL: (mg/dl)	7.19 ± 0.31 (13)	6.54 ± 0.19 (12)	7.74 ± 0.21 (6)
CHOLESTEROL TOTAL: (mg/dl)	115.97 ± 7.68 (12)	106.70 ± 5.69 (12)	127.60 ± 16.90 (6
CHOLESTEROL- HDL (mg/dl)	74.05 ± 5.7 (12)	72.23 ± 5.68 (12)	97.10 ± 11.20 (6)
CHOLESTEROL – LDL (mg/dl)	22.70 ± 2.73 (12)	18.55 ± 1.72 (12)	19.35 ± 1.30 (6)
TRIGLYCERIDES: (mg/dl)	62.29 ± 4.70 (8)	58.32 ± 5.68 (10)	68.90 ± 11.00 (6)

Values shown are Mean ± SEM for the number of experiments indicated in brackets.

At the end of experiments, blood was collected from the femoral artery of anaesthetized rats, centrifuged and blood serum was separated for the measurement of biochemical compounds. Analysis was performed using glucometer for the measurement of blood glucose and Bio- analyzer for measuring all the rest of the chemical compounds.* $P \leq 0.05$ ** $P \leq 0.02$

Female Rats DNA Damage: Treatment with DBP 100 mg/kg in female rats cause oxidative stress and DNA damage by increasing 8-OHdG concentration significantly by 29.8 % from 30.63 ± 1.33 (9) to 39.77 ± 3.30 (6), while DEHP treatment showed no significant effect. **Fig. 1** shows that DBP is more effective than DEHP in causing oxidative stress in female rats.

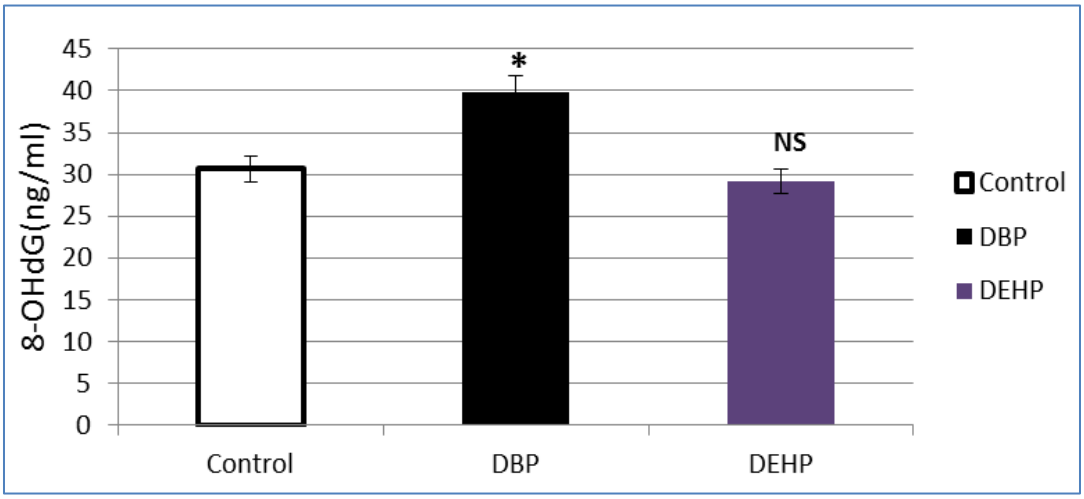
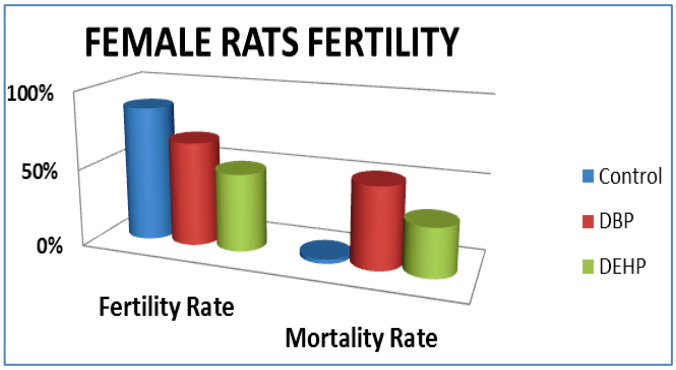


FIG. 1: MEASUREMENT OF DNA DAMAGE IN RATS

The concentration of 8-hydroxyguanosine (8-OHdG) in blood serum in (ng/ml) after intraperitoneal injection with DBP and DEHP 100 mg/ kg into female rats, Control samples were treated with the same methods of injection and with the same volume of corn oil. Values represent Mean ± SEM for the number of experiments indicated in brackets.* $P \leq 0.05$

DISCUSSION:

Effect of Phthalates on Female Fertility: The present experiments were undertaken to further investigate the action of DEHP and DBP upon female rat reproduction. The effects of DBP and DEHP on female rats fertility are summarized in **Fig 2.**



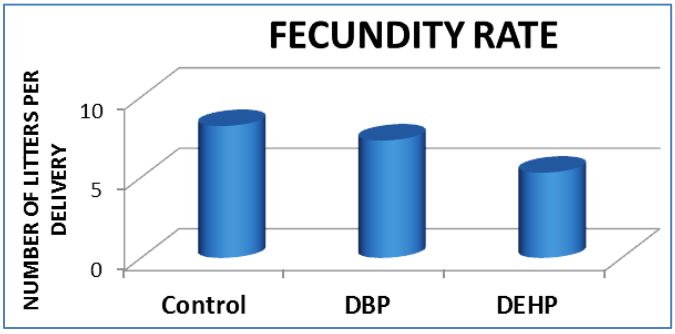


FIG. 2: SUMMARY OF THE EFFECT OF PHTHALATE ESTERS ON FERTILITY RATE, MORTALITY RATE & FECUNDITY RATE

The Fertility rate expressed as percentage of pregnant females per females cohabited was reduced by DBP from 87 % in control rats to 67 % and by DEHP to 50 %. Fecundity rate expressed as average number of new born litters per delivery was also reduced from 8.2 per delivery in control rats to 7.3 in DBP treated rats and to 5.3 per delivery in DEHP treated female rats. Mortality rate was increased significantly by DBP and DEHP from 2.8 % to 52.3 % and 31.3 % respectively.

Few studies have investigated the reproductive toxicity of DEHP in female animals. The present study coupled with other recently published studies serve to confirm some of the previously reported effects of phthalates on the female reproductive system and the effect of DEHP on decreasing fertility rate.

Fertility rate was decreased to 50% and 67% by DEHP and DBP respectively. In contrast to males, it is generally thought that female reproductive system is much less sensitive to phthalates. However, recent evidence suggests that phthalates can also induce adverse responses in females following pre and postnatal exposure [37].

Initial studies demonstrated that ovary is a target site for DEHP. Toxicity with high doses of DEHP results in prolonged estrous cycles, reduced serum estradiol levels and absence of ovulation in adult rats, which explains the reduction in pregnancy in female rats treated with DBP and DEHP[38, 39]. Fertility studies with crossover mating have also shown that active phthalates can decrease the fertility of rats and mice through male and female-mediated effects[5], which support our results. It has reported that mice ingestion of high doses of phthalates caused intrauterine growth retardation and delayed ossification with an apparently dose related manner and caused neural tube closure in developing embryo [40].

In female rats, repeated dose toxicity with DBP and DEHP induced ovarian damage expressed as vacuolation of stromal cell, increase of large atretic follicles, irregular estrous in addition to decrease of pregnancy rate [41]. Treatment with monoethylhexyl phthalates (MEHP) the active metabolite of DEHP decreased granulose cell aromatase RNA which could explain its effect in activating peroxisome proliferation [39].

In males the first finding of phthalates induced testicular injury in experimental animals[42]. The testicular effects are characterized by decreased testes weight and atrophy of seminiferous tubules. The alterations manifested in male offspring include cryptorchidism, hypospadias (ectopic opening of the urethra), atrophy or agenesis of sex accessory organs, testicular injury, reduced daily sperm production, permanent retention of nipple and decreased (feminized) anogenital distance [43]. In addition, phthalates induced testicular dysgenesis by affecting sertoli cells and leydig cells [44, 45]. DBP inhibits proliferation of somatic cells in the fetal rat testes, rather than increased apoptosis causing testicular dysgenesis [46].

However, the alteration in spermatogenesis observed after exposure to high doses of DEHP could be due to dysfunction in sertoli cells [47], or through the effect of follicle stimulating hormone action on sertoli cells [48, 49], or by targeting leydig cells which induce testosterone [12]. In general it was found that phthalates with medium side chain like DBP or branched long side chain like DEHP are more toxic and more effective than those with linear long side chains [49, 50]. Furthermore DEHP was found to reduce sperm production[50, 51], diminished the level of FSH, LH and Testosterone and decreased sperm motility [52].

Body weight and relative weight of different organs: As total body weight gain is concerned no significant changes were obtained in female rats treated with DBP or DEHP compared to control animals, these results were consistent before mating during one month mating and one month after pregnancy as shown in table 2. In studies of pregnant mice and rats orally exposed to large doses of DEHP and DBP has been demonstrated to cause developmental toxicity with significant decrease in total body weight of the fetus, including birth defects and even fetal death [22]. Previous studies in our lab, using the chick's model induced developmental defects characterized by opening of abdominal muscles, reduced percentage hatching, and reducing the total body weight of new born chicks [23].

The relative weight of the following internal organs: heart, spleen, liver, and brain were not affected following treatment with DBP or DEHP, while the relative weight of the kidney was reduced significantly by 7 % ($P \leq 0.05$) in DBP treated rats only (Table 3). In male rats a significant decrease in relative weight of sex organs was found following treatment with Phthalate esters [43]. Other studies of long term exposure in rats and mice have shown that high oral doses of DEHP reduced relative weight of testes [6, 15, 16] , ovaries and kidneys [17] and a great reduction in sex accessory glands was reported previously [13,14] .

Biochemical Levels in Blood: Our results have shown no changes in rat blood glucose level. Previous studies were performed to investigate phthalates exposure and its association with abdominal obesity and insulin resistance, suggest

that exposure to phthalates may contribute to the population burden of obesity, insulin resistance and related clinical disorders [53]. They detected an increase in serum glucose and decreased insulin when female rats were exposed to DEHP. Thyroid and adreno-cortical dysfunction was also reported previously [54]. Our results don't support that since we have not seen any change in glucose levels following treatment with DBP or DEHP. From biochemical measurements obtained in this study no significant changes were observed in serum levels of glucose, total proteins, urea, triglycerides, and cholesterol HDL or cholesterol LDL in fasted rats. In our rats model the significant increase of GOT and GPT could be explained by an idiosyncratic effect of phthalates on the liver cells, causing hepatotoxicity.

DNA damage: Our recent results with DNA damage and all other reports suggests that competitive ELISA for 8-OHdG appears to be a simple method for quantifying the extent of oxidative stress. Several evidence show that oxidative damage may be an important mechanism underlying several pathophysiological states, for example, atherosclerosis caused by oxidative modification of low-density lipoprotein [55]; diabetic complications caused by oxidative damage of lipids, protein and DNA [56, 57]; aging caused by oxidative damage of proteins and myocardial damage loss through oxidative injury. These results supported with our recent results shows that oxidative stress is increased upon exposure to DEHP and DBP in animal models and could be the mechanism underlying phthalates toxicity. Our findings of phthalate induced DNA damage in rats were consistent with those found in mice[43], and humans [58].

Previous results in our lab, using the chick model showed that phthalates are most effective in inducing teratogenic activity in developing chicks following pre-exposure to single dose of phthalate (20-100mg\kg), and for the first time it was reported to induce Gastroschisis and Omphalocele in new born chicks and in 8-20% of the cases, with DEHP, these were associated with oxidative stress and DNA damage [23]. The oxidative damage induced by DEHP or DBP is an experimental evidence for molecular mechanism of phthalates toxicity. Oxidative stress that occurs in a cell or tissue could be due to increase in oxidative activity or decrease in antioxidant capacity, because of peroxidation in lipid membrane of cells which leads to cellular injury or cell death [59]. In rats it was reported that high doses of DEHP may induce oxidative stress in testis by inducing lipid peroxidation [60, 61].

MEHP the active metabolite of DEHP was found to modulate cell apoptosis after toxicant-induced sertoli cells injury [62].

Environmental exposure to DEHP has demonstrated a cytotoxic and genotoxic potential, where MEHP was found to be more potent than the parent compound [63]. Higher doses of DBP led to testicular toxicity, while lower doses led to changes in the expression of proteins involved in spermatogenesis as well as changes in the number and function of sertoli and leydig cells [45]. In humans it was reported that MEHD the oxidative metabolite of DEHP show greater levels of sperm DNA damage than the parent compound [64, 65.]

DEHP applied at 1000 mg/kg dose by gavage for 10 days caused disruption and collapse of vimentin filaments and significantly induced apoptotic death of germ cells [66] and the same with DBP [67]. In addition, DEHP a sertoli and leydig cell toxicant was able to induce alterations in the expression of testicular gap and tight junction proteins when administered by gavage [68].

The mechanism of action of Phthalates could be due to induction of peroxisome proliferation, oxidative stress, or through zinc deficiency. Therefore further experiments are needed to elucidate its mechanism of action. We recommend more epidemiological studies using larger human population including (follow-up) studies of infants exposed to phthalates.

Nowadays it is well known that phthalates are the most commercially important plasticizer, and one of the serious contaminations in the whole world. DEHP and DBP are the most distributed phthalates and the greatest potential risk to human health. It has reproductive toxicity, developmental toxicity, embryonic toxicity and potential carcinogenicity.

CONCLUSION: Our recent results indicate that in addition to the effect of phthalates in reducing male fertility as reported before, it reduces female fertility and fecundity and increases mortality rate in new born litters. The reduction in female fertility is accompanied with significant increase in DNA damage, and increase in GOT and GPT activity.

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REFERENCES:

1. Petersen JH and Breindahl T: Plasticizers in total diet samples, baby food and infant formulae. Food Addit. Contam.2000; 17(2):133-141.
2. Muszkat L and Raucher L.: Liability of ground water to contam
   ination by organic xenobiotics. Water Sci. Technol.1993; 27:203
   -212.
3. Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, and Caudill SP: Urinary levels of seven phthalate metabolites in the U.S. population from the national health and nutrition examination survey (NHANES). Environ. Health Perspect. 2004; 112:331-338.
4. Berman T, Hocher-Celnikier D, Calafat AM, Needham LL Amitai Y, Wormser U, and Richter E: Phthalate exposure among pregnant women in Jerusalem, Israel results of a pilot study. Environ. Int. 2008; 1-5.
5. Lamb JC, Chapin RE, Teague J, Lawton AD, and Reel JR: Reproductive effects of four phthalic acid esters in the mouse. Toxicol. Appl. Pharmacol. 1987; 88:255-269.
6. Gray TJ and Butterworth KR: Testicular atrophy produced by phthalate esters. Arch. Toxicol. 1980; (Suppl 4) :452-455.
7. Li LH, Jester WF, and Laslett, A: A single dose of Di-(2-ethylhexl) phthalate in neonatal ratsalters gonocytes, reduces sertoli cell proliferation, and decreases cyclin D2 expression. Toxicol Appl. Pharmacol 2000; 166:222-229.
8. Saitoh Y, Usumi K, and Nagata T: Early changes in the rat testis induced by di-(2-ethylhexyl) phthalate and 2,5-hexanedione-ultrastructure and lanthanum trace study. J. Toxicol. Pathol. 1997; 10:51-57.
9. Parmar D, Srivastava SP, and Singh GB: Effect of testosterone on the testicular atrophy caused by di(2-ethylhexyl)phthalate (DEHP). Toxicol. Lett. 1987; 36:297-308.
10. Chapin RE, Gray TJ, and Phelps JL: The effects of mono-(2-ethylhexyl)-phthalate on rat Sertoli cell-enriched primary cultures. Toxicol. Appl. Pharmacol. 1988; 92:467-479.
11. Gray TJ and Beamand JA: Effect of some phthalate esters and other testicular toxins on primary cultures of testicular cells. Food Chem. Toxicol. 1984; 22(2):123-131.
12. Jones HB, Garside DA, and Liu R: The influence of phthalate esters on Leydig cell structure and function in vitro and in vivo. Exp. Mol. Pathol. 1993; 58:179-193.
13. Gray LE, Ostby J, Furr J : Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol. Sci.2000; 58:350-365.
14. Parks LG, Ostiby JS, and Lambright CR: The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. Toxicol. Sci. 2000; 58:339-349.
15. Crisp TM, Clegg ED, Cooper RL: Environmental endocrine disruption: An effects assessment and analysis. Environ. Health Perspect.1998; 106(Suppl. 1):11-56.
16. David RM, Moore MR, Finney DC, Guest D: Chronic toxicity of di(2-ethylhexyl) phthalate in rats. Toxicol. Sci.2000; 55:433-443.
17. Isenburg JS, Kamendulis LM, Ackley DC: Reversibility and persistence of di-2- ethylhexylphthalate (DEHP)- and phenobarbital-0-induced hepato-cellular changes in rodents. Toxicol Sci. 2001; 64:192-199.
18. Lake BG: Mechanisms of hepatocarcinogenicity of peroxisome-proliferating drugs and chemicals. Annual Rev. Pharmacol. Toxicol.1995; 35:483-507.
19. Lapinskas PJ and Corton JC: Phthalate ester plasticizers differentially activate the peroxisome proliferator-activated receptors. Toxicologist.1997; 36:144.
20. Ward JM, Peters JM, Perella CM: Receptor and non-receptor-mediated organ-specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor alpha-nullmice. Toxicol. Pathol.1998; 26(2):240-246.
21. Isenberg JS, Kamendulis LM, Smith JH: Effects of Di-2-Ethlhexyl phthalate (DEHP) ongap-junctional intercellular communication (GJIC), DNA synthesis, and peroxisomal beta oxidation (PBOX) in rat, mouse, and hamster liver. Toxicol. Sci. 2000; 56:73-85.
22. Sastry BV: Placental toxicology: tobacoo smoke, abused drugs, multiple chemical interactions, and placental function. Reprod. Fertil. Dev.1991; (4) 3:355-372.
23. Abdul-Ghani S, Yanai J, Abdul-Ghani R, Pinkas A, and Abdeen Z: The teratogenic activity of Di(2-ethylhexyl)Phthalates (DEHP) and Di-Butyl Phthalates (DBP) on chicks development. Neurotoxicology and Teratology 2012; 34:56–62.
24. Tietz NW: Clinical guide to laboratory tests, W.B. Saunders eds, Philadelphia (USA), Third Edition 1995.
25. Vassault A, Grafmeyer D , De Graeve J , Cohen R , Beaudonnet A , Bienvenu J : Quality specifications and allowable standards for validation of methods used in clinical biochemistry. Ann. Biol. Clin. 1999; 57(6):685-695.
26. Rifai N, Bachorik PS, Albers JJ: Lipids, Lipoproteins and Apolipoproteins. Clinical Chemistry, Theory, Analysis, Correlation. Kaplan L.A. Pesce A.J. Kazmierczak S.C. (Mosby, Inc. St, Louis (USA) Edition Four, 2001.
27. Naito HK: Coronary artery disease and disorders of lipid metabolism. Clinical Chemistry. Theory. Analysis, Correlation. Kaplan L.A., Pesce A.J. Kazmierczak S.C, (Mosby, Inc. St. Louis (USA). Edition Four, 2003.
28. Christensen SE: Proteins, Clinical Chemistry: Concepts and Application. Anderson S.C., Cockayne, S. (W.b. Saunders eds, Philadelphia (USA), 1983.
29. Scherwin, JE: Liver function, Clinical Chemistry: Theory, Analysis, Correlation, Kaplan L.A, Pesce A.J., Kazmierczak S.C. (Mosby. Inc. St. Louis (USA), Edition Four, 2003.
30. Fossati P, and Prencipe L: Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroide. Clin. Chem. 1982; 28: 2077.

31. Newman DJ, and Price CP: None protein nitrogen metabolite in Tietz Fundamentals of Clinical Chemistry, Burtis CA & Ashwood E.R (w.b. Saunders eds Philadelphia (USA), Edition Five, 2001.
32. Schiele F: Informations complementaires concernant la method recommandee de determination de la concentration catalytique de l'Alanine aminotransferase dans le serum humain a 30 ºC. Ann. Biol. Clin.1982; 40: 87.
33. Büttner J, Borth R, Boutwell JH, Broughton PMG, Bowyer RC: Provisional recommendation on quality control in clinical chemistry. Part 2. Assessment of analytical methods for routine use. J. Clin. Chem. Clin. Biochem. 1976; 14: 265-75.
34. Chiou CC, Chang PY, Chan EC, Wu TL, Tsao KC and Wu JT: Urinary 8-hydroxydeoxyguanosineand its analogs as DNA marker of oxidative stress: development of an ELISA and measurement in both bladder and prostate cancers. Clin. Chim. Acta.2003; 334:87–94.
35. Lezza AM, Mecocci P, Cormio A, Beal MF, Cherubini A, Cantatore P, Senin U, Gadaleta MN: Mitochondrial DNA 4977 bp deletion and OH8dG levels correlate in the brain of aged subjects but not Alzheimer's disease patients. FASEB. J. 1999; 13(9):1083–1088.
36. Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, Marsden CD, Jenner P, Halliwell B: J. Neurochem. 1997; 69:1196–1203.
37. Gray LE, and Ostby J: Chronic di-n-butyl phthalate exposure in rats reduces fertility and alters ovarian function during pregnancy in female Long Evans hooded rats. Toxicol Sci. 2006; 93(1):189-195.
38. Davis BJ, Maronpot RR, Heindel JJ: Di(2-ethylhexyl) phthalate suppresses estradiol and ovalatin in cycling rats. Toxicol. Appl. Pharmacol. 1994; 128:216-223.
39. Lovekamp-Swan T and Davis BJ: Mechanisms of phthalate esters toxicity in the female reproductive system. Environ. Health Perspect. 2003; 111(2):139-145.
40. Shiotak K and Nishimura H: Teratologenicity of DEHP and DBP in mice. Eviron. Health Perspect. 1982; 45:65-70.
41. Takai R, Hayashi S, Kiyokawa J, Iwata Y, Matsuo S, Suzuki M, Mizoguchi K, S. Chiba S, and Deki T : Collaborative work on evaluation of ovarian toxicity. 10) Two-or four-week repeated dose studies and fertility study of di-(2-ethylhexyl) phthalate (DEHP) in female rats. J. Toxicol. Sci. 2009; 34 Suppl 1:SP111-119.
42. Shaffer CB, Carpenter CP, and Smyth JHF: Acute and subacute toxicity of di(2-ethylhexyl) phthalate with note upon its metabolism. J. Indust. Hyg. Toxicol. 1945; 27: 130–135.
43. Martino-Andrade AJ, and Chowhound I: Reproductive toxicity of phthalate esters. Mol. Nutr. Food Res. 2010; 54:148-157.
44. Liu K, Lehmann PK, Sar M, Young SS, and Gaido KW: Gene expression profiling following in utero exposure to phthalate esters reveals new gene targets in the ethiology of testicular dysgenesis. Biology of Reproduction. 2005; 73:180-192.
45. Bao AM, Man XM, Guo XJ, Dong HB, Wang FQ, Sun H, Wang YB, Zhou ZM , and Sha JH: Effect of di-n-butyl phthalate on male rat reproduction following pubertal exposure. Asian J. Androl. 2011; 13(5):702-709.
46. Boekelhedi K, Kleymenova E, Liu K, Swanson C, and Gaido KW: Dose-dependent effects on cell proliferation, seminiferous tubules, and male germcells in the fetal rat testis following exposure to di(n-butyl) phthalate. Microsc. Res. Tech. 2009; 72(8): 629-638.
47. Kavlock R, Bockelheide K, Chapin R, Cunningham M, Faustman E, Foster P, Golub M, Henderson R, Hinberg I, Little R, Seed J, Shea K, Tabacova S, Tyl R, Williams P, and Zacharewski T: NTP Center for the evaluation of risks to human reproduction: Phthalates expert panel report on the reproductive and developmental toxicity of butyl benzyl phthalate. Reprod. Toxicol. 2002; 16:453-487.
48. LIoyd SC and Foster PM: Effect of mono-(2-ethylhexyl) phthalate on follicle - stimulating hormone responsiveness of cultured rat sertoli cell. Toxicol. Appl. Pharmacol.1988; 96:484-489.
49. Heindel JJ, and Powell CJ: Phthalate ester effects on rat sertoli cell function in vitro: Effects of phthalate side chain and age of animal. Toxicol. Appl. Pharmacol.1992; 115:116-123.
50. Andrade AJ, Grande SW, Talsness CE and Grote K: 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 2006; 225:64-74.
51. European Food and Safety Authority (EFSA). Opinion of the scientific panel on food additives, flavorings, processing aids and materials in contact with food (afc) on a request from the commission related to bis(2-ethylhexyl) phthalate (DEHP) for use in food contact materials. The EFSA J.2004; 243:1-20.
52. Erkekoglu P, Zeybek ND, Giray B, Asan E, Hincal F: The effects of di(2-ethylhexyl) phthalate exposure and Selenium nutrition on sertoli cell vimentin structure and germ-cell apoptosis in rat testis. Arch. Environ. Contam. Toxicol. 2012; 62(3)539-47.
53. Stahlhot RW, Wijngaarden EV, Dye TD, Cook S and Swan SH: Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult U.S. males. Environ. Health Perspect. 2007; 115(6):876-882.
54. Goyathri NS, Dhanya CR, Indu AR, Kurup PA: Changes in some hormones by low doses of di(2-ethylhexyl) phthalate (DEHP), a commonly used plasticizer in PVC blood storage bags & medical tubing. Indian J. Med. Res. 2004; 119(4):139-144.
55. Ross R: Atherosclerosis-an inflammatory disease. N. Engl. J. Med1999; 340:115-126.
56. Baynes JW: Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an paradigm. Diabetes 1999; 48:1-9.
57. Dandona P, Thusu K, Cook S, Snyder B, Makowski J, and Armstrong D: Oxidative damage to DNA in diabetes mellitus. Lancet 1996; 347: 444-445.
58. Duty SM, Singh NP, Silva MJ, Barr DB, Brock JW, and Ryan L: The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral commet assay. Environ. Health Perspect. 2003; 111:1164-1169.
59. Monsefi M, Alaee S, Moradshahi A, and Rohani L: Cadmium-induced infertility in male mice. Environ. Toxicol. 2010; 25: 94-102.
60. Kasahare E, Sato EF, Miyoshi M, Konaka R, Hiramoto K, Sasaki J, Tokuda M, Nakano Y, and Inoue M: Role of oxidative stress in germ cell apoptosis induced by di(2-ethylhexyl) phthalate. Biochem. J. 2002; 365:849-856.
61. Pant N, Shukla M, Kumar PD, Shukla Y, Mathur N, Kumar GY, Saxena DK: Correlation of phthalate exposures with semen quality. Toxicol. Appl. Phrmacol. 2008; 231: 112-116.

62. Lin YC, Yao PL, and Richburg JH: FasL gene-deficient mice display a limited disruption in spermatogenesis and inhibition of mono-(2-ethylhexyl) phthalate-induced germ cell apoptosis. Toxicol. Sci. 2010; 114(2):335-345.
63. Erkekoglu P, Rachidi W, Yuzugullu OG, Giray B, Favier A, Ozturk M, and Hincal F: 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. Appl. Pharmacol. 2010; 248(1):52-62.
64. Hauser R: Urinary phthalate metabolites and semen quality: A review of a potential biomarker of susceptibility. Int. J. Androl. 2008; 31:112-117.
65. Han SW, Lee H, Han SY, Lim DS, Jung KK, Kwack SJ, Kim KB, and Lee BM: An exposure assessment of di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) in human semen. J. Toxicology and Environmental Health, Part A. 2009; 72:1463-1469.
66. Erkekoglu P, Zeybek ND, Giray B, Asan E, Arnaud J, and Hincal F: Reproductive toxicity of di(2-ethylhexyl) phthalate in Selenium- supplemented and selenium-deficient rats. Drug Chem. Toxicol. 2011; 34(4):379-389.
67. Alam MS, Ohsako S, Tay TW, Tsunekawa N, Kanai Y, Kurohmaru M: Di (n-butyl) phthalate induces vimentin filamentsdisruption in rat sertoli cells: a possible relation with spermatogenic cell apoptosis. Anat. Histol. Embryol. 2010; 39(3)186-193.
68. Sobarzo CM, Lustig L, Ponzio R, Suescun MO, and Denduchis B: Effect of di(2-ethylhexyl) phthalate on gap and tight junction protein expression in the testis of prepubertal rats. Microsc. Res. Tech. 2009; 72(11):868-877.

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