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Perinatal Exposure to Bisphenol A Affects Body Weight and The Reproductive Function of *Wistar* Rat

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ABSTRACT

Bisphenol A (BPA) is an ubiquitous endocrine disrupting chemical reported to have many adverse health effects. It is widely used by plastic industry to produce polycarbonate plastic and epoxy resins found in enormous range of consumer products including dental sealant and composites, personal computers, compact discs, thermal papers, medical devices, baby bottles and other beverage and food containers. Our study aims to evaluate the impact of *in Utero* and lactational exposure to BPA on the reproductive function of *W*istar rat. Nine pregnant females were exposed from the sixth gestational day to the 21st post-natal day in their drinking water, to either 0.05mg/L (low dose) or 5mg/L (high dose). The obtained results showed that BPA exposure has no effect on anogenital distance. However, it induces at low dose an obesity which is more pronounced in females than males. Advancement in puberty linked to a significant decrease of body weight in males was also noticed. In addition, a significant increase of testicular and epididymides weights was observed at low dose, while, the high dose seems to cause a significant decrease in ovaries weight. Our findings suggest that perinatal BPA exposure has deleterious effects on body weight and reproductive parameters of *Wistar* rat offspring.
KEYWORDS: BPA, rat, *in utero* and lactational exposure, reproductive parameters, obesity.

1. INTRODUCTION

The reproductive function is a highly sensitive system which can be easily altered by several factors such as lifestyle (sedentarily, unbalanced diet, smoking, alcohol drinking and drugs use), genetic predisposition and some chemical compounds called endocrine disrupting chemicals (EDCs) [1]. EDCs are exogenous substances or mixture of chemicals that may interfere with any aspects of hormone action and consequently produces different adverse effects including: reproductive, developmental, neurogical and immune effects on both humans and wildlife [2, 3]. They include a wide range of substances either man-made or naturally occurring namely pharmaceuticals (diethylboestrol (DES), 17 alpha-ethynilestradiol (EE2)), dioxin and dioxin-like compounds, polychlorid rated biphenyls (PCBs), pesticides (DDT) and plasticizers such bisphenol A (BPA) [4, 5].

BPA (2,2-bis(4-hydroxyphenyl)propane) is a synthetic chemical widely utilized in the manufacture of polycarbonate plastics and epoxy resins found in everyday consumer products especially in baby bottles, water bottles, and other beverage and food containers [6,7,8,9]. Humans beings are routinely exposed to BPA due to its aptitude to migrate from containers to food particularly when they are heated at high temperature, washed with harsh detergents or simply containing an acid liquid [10,11,12].

During the last decades, much more attention was accorded to BPA health impact resulting in hundreds of studies conducted mostly on rodent model that have shown multiple adverse effects including: effects on fertility and reproductive tract, genotoxicity and carcinogenecity, oxidative toxicity, neurotoxic effects, metabolic disorders and other health effects that affect for instance the immune system [13, 14,15,16].

Regarding, the reproductive and metabolic functions, BPA exposure to low doses (lower than the lowest observed affect level (LOAEL) established at 50mg/Kg/day) has been knowledged to cause: an alteration in the time of puberty, alterations in estrous cycles, changes in morphology and histological architecture of some reproductive organs such as mammary gland and prostate that predispose animals to develop cancer, changes in the rest of reproductive organs such as: womb, ovaries, epididymides, seminal vesicles and testes, alteration in brain sexual dimorphism and socio-sexual behavior, changes in brain steroid receptors, alteration in body weight and adipogenesis, and altered glucose homeostasis [17, 18].

To verify some these findings, we conducted the present study in order to assess and compare effects of a perinatal exposure to different BPA doses in *Wistar* rat. An oral route of administration was chosen in order to reproduce as much as possible the common route of exposure to this substance in both humans and wildlife.

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2. MATERIALS AND METHODS

2.1. Chemicals and experimental animals

Bisphenol A (BPA) (CAS no. 80-05-7, purity > 99.5%) was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). It was dissolved in 1% ethanol solution($CH_3 CH_2OH$).

Nine sexually mature *Wistar* rat females (84±2 days old, 194±18g) and 3 males (approximately 1 year old, 441± 32g) were obtained from Mustapha Stombouli University (Mascara, Algeria). Animals were housed in plastic cages lined with wood bedding and maintained in temperature, humidity and light controlled conditions (24°C, 50%, 14/10h light/dark cycle). They were fed by a standard rodent chow provided by (GAO ORAVIO society, Oran, Algeria) and tap water was supplied *ad libitum* from polycarbonate bottles. All materials (food, cages and bottles) were not tested for estrogen city but we suggest that all animals were exposed to same negligible levels of BPA and phytoestrogens. Female rats were allowed to acclimatize few days before being mated to males and were then checked routinely to detect a vaginal plug which indicates the first day of gestation (GD1). Pregnant and nursing (with or without pups) dams were then housed individually.

All animals used in this experiment were treated in accordance with the Guidelines for the Care and Use of Laboratory Animals of our university committee which encourage humane treatment and alleviation of animal suffering.

2.3. Treatment diets

Pregnant females were divided into three treatment groups (n=3 per group): Low Dose group (LD) and High Dose group (HD) were exposed from gestational day 6 (GD6) to post-natal day 21 (PND21) via drinking water to BPA concentrations of 0.05mg/L and 5mg/L respectively, whereas control group received only the vehicle (ethanol at 1%). Pups were supplied with BPA-free drinking water starting from the first day of weaning.

The mean exposure levels of BPA were estimated basing on measurements of daily water intake of females rats and we presume that all of this water was totally consumed by animals.

2.4. BPA effects assessment

During pregnancy dams were weighed and examined several times then, watched continuously till delivery occurs. The sex of offspring was determined on post natal day 9 (PND 9) when the entire body of pups was covered of a slight coat of hair in order to avoid any stress or cannibalism. Pups were then weighed each week from the second to the seventh post-natal week. Ano-genital distance (distance from the anterior end of the anus and the posterior end of the genital papilla [19, 20] and body length (distance from nose to anus) were measured at the third and the fourth post natal week respectively. Vaginal opening (VO) (in females) and testicular descent (in males) were checked beginning on PND28 to determine puberty onset, and body weight was also recorded at the same time. At seven weeks of age, overnight fasted pups were weighed then sacrificed using cervical dislocation technique and sexual organs were collected and cleaned with a saline solution in order to be examined and weighted.

2.5. Statistical analysis

All statistical analysis was carried out using ©Microsoft Office Excel 2013. Data were assessed by one way analysis of variance (ANOVA). Once statistical significance was established, a post hoc Student's *t*-test was performed in order to make comparison between two independent treatment groups. Results were expressed as mean ± standard deviation (SD) and considered significant at $p \leq 0,05$.

3. RESULTS

3.1. Exposure levels

The daily water intake was not affected by presence or absence of BPA in the water and was estimated about 25±1ml for all groups. The body weight of gestating females was approximately (325±53g in the LD group) and (292±18 in the HD group) at the end of gestation. Based on these measurements, we estimated the mean levels of BPA to be approximately 4µg/Kg/day in the LD group and 428µg/Kg/day in the HD group. Theses estimations did not take into consideration possible water evaporation, leakage, or spillage and we presume that all the drinking water lost from bottles was consumed.

3.2. Anogenital distance (AGD)

No BPA-related changes was observed in the AGD of both male and female *Wistar* rat pups (Fig.1) males: (Control: 15.0±1.41mm, LD: 16.1±1.19, HD: 15.2±1.28,P>0.05), Females: (Control:11.6±1.08mm, LD: 11.1±1.19, HD: 10.7±1.10,p>0.05*)*.

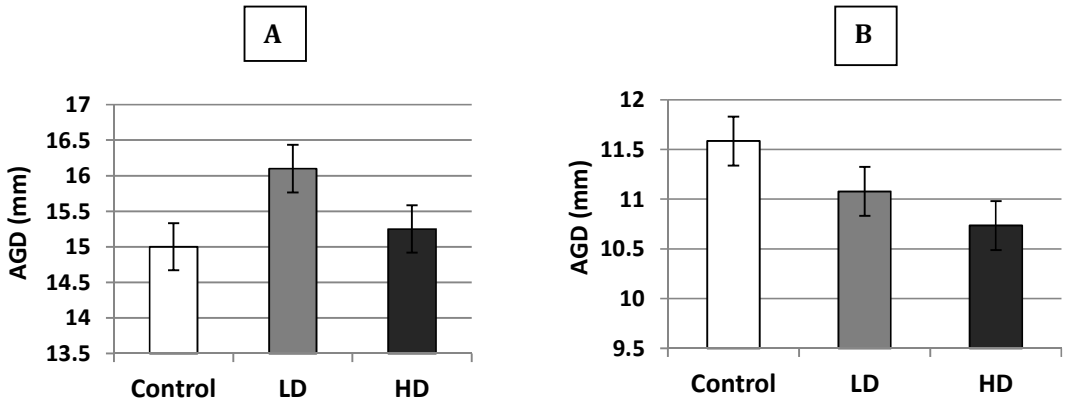

Fig. 1: BPA *in-utero* exposure did not alter the AGD of male (A) and female (B) offspring at three weeks of age (p>0,05). Control: n=9 males and 12 females. Low dose-BPA (LD): n= 10 males and 13 females. High dose (HD): n=8 males and 15 females. Data are presented as mean ± standard deviation (SD)

3.3. Puberty

In females, the average age at VO did not differ among groups (p>0.05). However, the weight at puberty was much higher in treated groups compared with the control group. The increase of body weight was more significant in the LD group (p<0.01) than the HD group (p<0.05).

In contrast, male offsprings exposed to both BPA treatment doses seem to reach their puberty younger than controls. The difference was more significant in the LD group (p<0.01) than the HD group (p<0.05). In addition, male pups seem to reach their puberty at a lighter body weight in comparison with controls(p<0.05). All values are presented in table 1.

Table1: Age and body weight of pups at puberty

	Males			Females		
Group	**Control**	**LD**	**HD**	**Control**	**LD**	**HD**
	n=9	n=10	n=8	n=12	n=13	n=15
Age(days)	33.55±4,82	28.20±2.15**	29.50±3.55*	38.75±2.0	39.69±1.0	41.53±3.0
Bw(g)	46.89±4,70	43.4± 2.41*	41.88±5.84*	77.50±11.52	88.77±10.52**	86.8±15.76*

Values for each group are expressed as mean ± SD, n represents number of rats in each group LD: Low Dose (0,05mg/L), HD: High Dose (5mg/L). bw: body weight. Asterisks indicate a statistically significant difference between BPA treated groups an the vehicle-treated control group (** p<0.05, ** p<0.01)*

3.4. Body length

Around the time of weaning (At 4 weeks of age), BPA induced a highly significant increase of the body length in all BPA treated pup of both sex males: (Control: 114.4±7.18 mm, LD: 122.9±2.47 , HD: 123.9±0.60 , p<0.001) (Fig.2 A) and females (Control: 114.8±6.79mm, LD: 122.3±5.02 , HD: 123.3±3.46 , p<0.001) (Fig.2 B). However, the accelerated growth tends to disappear progressively with time.

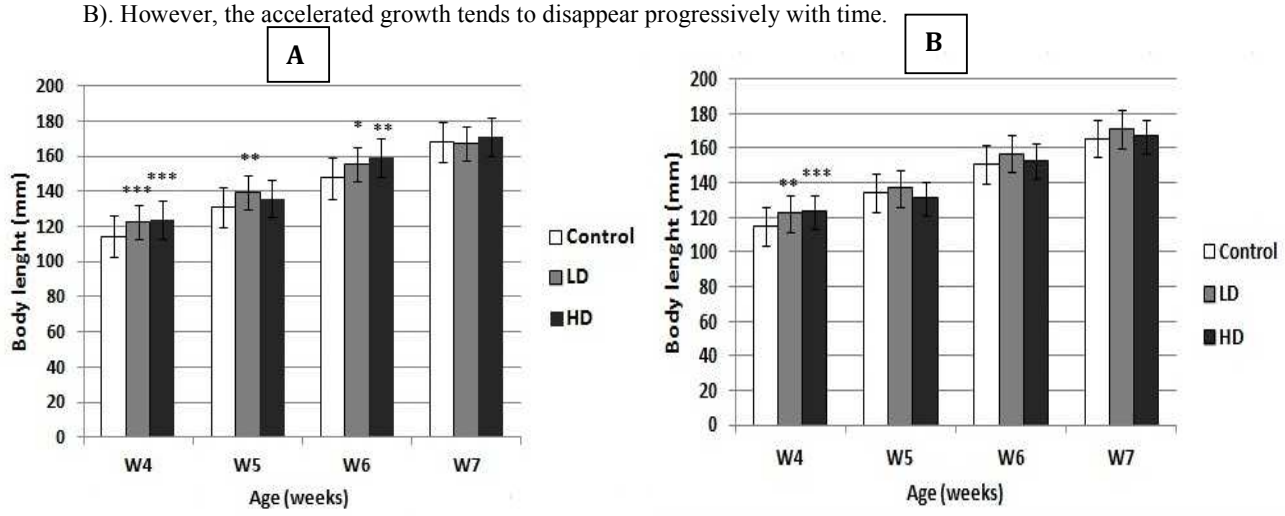

Fig. 2: Environmental doses of BPA can alter the growth of male (A) and female (B) during the first stages of life. At age of 4 weeks treated pups were longer than the control ones, this acceleration of growth tend to disappear progressively during the following weeks. Control: n=9 males and 12 females. Low dose-BPA (LD): n= 10 males and 13 females. High dose (HD): n=8 males and 15 females. W: week. Values are expressed as mean ±SD. Asterisks indicate a statistically significant difference between BPA treated groups an the vehicle-treated control group (** p<0.05, ** p<0.01) p<0,001)*

3.5. Body weight

Beginning on the fourth post natal week (PND29), male *Wistar* rat pups exposed to BPA were heavier than control animals and as usual the LD group was more affected than the HD group (Fig.3 A). In contrast, females pups born to dams treated with BPA showed a decreased body weight relative to controls ($p<0.01$) during the first weeks of life (second and third post natal (PND 15 and PND 22 respectively)). Nevertheless, body weight decrease had persisted only in the HD group and not in the LD group in which females became significantly heavier starting from 4 weeks of age (Fig.3 B).

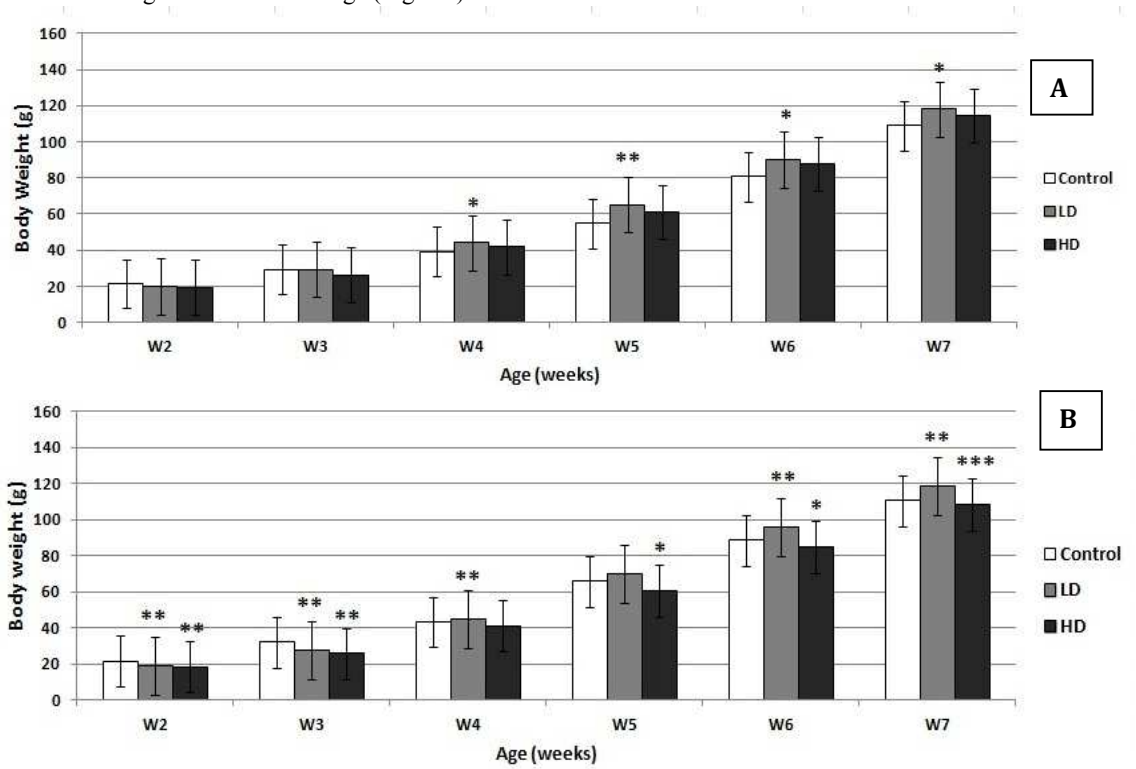

Fig. 3: Gestational and lactational exposure to low doses of BPA affects the body weight of male (A) and female (B) offsprings during early life. Body weight measurements were taken from post-natal week 2 to 7. Control: n=9 males and 12 females. Low dose-BPA (LD): n= 10 males and 13 females. High dose (HD): n=8 males and 15 females. Data are shown as mean with standard deviation (±SD). W: week. Asterisks denote a statistically significant difference of the BPA treated groups relative to the vehicle-treated control group (** p<0.05, ** p<0.01, *** p<0.001)*

3.6. Sexual organs weight

No macrosocpic abnormalities were found on sexual organs when they were examined. Regarding to reproductive organs weight, BPA caused a statistically significant increase in the absolute weight ($p< 0.05$) and the relative weight ($p<0.01$) of testes and epididymides at low dose and a significant decrease in the absolute weight ($p< 0.05$) and the relative weight ($p<0.01$) of ovaries at high dose. In contrast, uterine and seminal vesicles weights were not affected by BPA exposure (table 2).

Table 2: Absolute and relative sexual organs' weights of both male and female offspring

	Body Weight at sacrifice (g)	Organs	Absolute weight (g)	Relative weight (%)
Control	112.38±12.51	testes	1.078±0.246	0.930±0.140
		epididymides	0.065±0.015	0.058±0.007
		seminal vesicles	0.077±0.042	0.065±0.030
	115.64±8.16	Uterus	0.292±0.105	0.290±0.100
		ovaries	0.077±0.016	0.070±0.010
LD	121.90±18.89	testes	1.386±0.251*	1.140±0.120 **
		epididymides	0.079±0.013*	0.066±0.007 **
		seminal vesicles	0.068±0.022	0.055±0.010
	126.77±9.91	Uterus	0 .285±0.084	0.280±0.080
		ovaries	0.079±0.018	0.060±0.010
HD	120.88±21.84	testes	1.271±0.272	1.020±0.100
		epididymides	0.070±0.013	0.055±0.008
		seminal vesicles	0.068±0.012	0,056±0,010
	117.00±5.79	Uterus	0.281±0.111	0.280±0.110
		ovaries	0.065±0.013*	0.050±0.010**

Control: n=9 males and 12 females. Low dose-BPA (LD): n= 10 males and 13 females. High dose (HD): n=8 males and 15 females. Values for each group are expressed as mean ± SD, n represents number of rats in each group LD: Low Dose (0,05mg/L), HD: High Dose (5mg/L). Asterisks indicate a statistically significant difference between BPA treated groups an the vehicle-treated control group (** p<0.05, ** p<0.01)*

4. DISCUSSION

A great number of recent researches have demonstrated adverse effects of BPA exposure during critical periods of life [17, 18, 21], some of these studies corroborate our results, whereas others contradict them.

Puberty is a reproductive landmark that indicates the onset of sexual maturity and early puberty was associated with a higher risk for metabolic disorders and breast cancer [22]. In the current report, we showed that perinatal exposure to low dose levels of BPA may induce deleterious effects on puberty onset of pups that concern only males but not females suggesting a possible sex-related effect of BPA. Although some studies have reported an advancement in the age of VO [23, 25]. Our results corroborate with most of studies that found no effects on sexual maturation [6, 17, 19]. Furthermore, another study conducted by Tinwell et al.on two different strains of laboratory rats (*Spargue- Dawley* (SD) and *Alderley-Park* (AP) rats) exposed to a wide range of BPA levels and DES reported a lack of effects for SD rats and a delay on VO for AP rats which indicates that BPA exposure may lead to different response according to animal species and strains[26].Concerning males and in contrast to our study, Tyl et al. have chosen preputial separation (PPS) in addition to testis descent as milestones of puberty and demonstrated in a multigenerational study that BPA exposure of *CD1-Swiss* mices and *SD* rats parents (F0 generation) to high dose of 3500 ppm (600mg/Kg/day) and 7500 ppm (500mg/Kg/day) respectively caused a significant delay in the age of the acquisition of sexual maturation in three consecutive generations of male offsprings (F1, F2 and F3 generations)[27, 28].

Anogenital distance (AGD) is a commonly evaluated endpoint in reproductive toxicity studies due its sensitivity to hormonal effects of test chemicals including BPA. AGD has been acknowledged to be a predictive landmark of decreased penile length, increased incidence of cryptorchidism and hypospadias, and seminal vesicle weight [29, 30, 31].Several studies have examined the impact of perinatal exposure to BPA on AGD of rat offspring and a number of them are consistent with our results. In our report, perinatal exposure to either low or high levels of BPA did not affect the AGD of both male and female rat pups. Although a large body of data suggests a deleterious effect of BPA exposure on the AGD of both male and female offspring [32], some authors noted a lack of effects which fit with our observation [6,20, 33].

Obesity is a multifactorial and complex endocrine disease which is becoming a serious worldwide health problem. A large body of evidence has suggested that the current increase in obesity and other metabolic disorders are correlated with EDCs that interfere with endocrine signaling which controls body growth, body

weight and metabolic processes, and BPA is a convincing example of these chemicals [34]. Our data have confirmed this evidence by revealing an increased body weight gain in both sexes of rat pups which received low doses of BPA during gestational and lactational periods. Our observation also agrees with several rodent studies that reported a body weight gain after perinatal exposure to environmentally relevant concentrations of BPA [34, 35]. In addition, the influence that may BPA exert on body weight gain was demonstrated by *in-vitro* studies, which reported effects on lipid accumulation, adipocyte differentiation, adiponectin secretion and glucose transport, the way in which BPA may exert these effects involves different potential targets that have been reported by the literature including actions on: receptors such as ER alpha and beta receptors which are expressed on adipose cells, ER related Gamma receptors and Thyroid hormone receptors; pancreatic insulin beta cells and glucagon secreting alpha cells and developing brain which may produce perturbations on circuits that regulate food intake and metabolism[35]. However, it is interesting to note that other studies have found a decrease or even no effects on body weight in response to developmental exposures to BPA [17].

Body length was also affected by BPA exposure especially at early stages of life (4 weeks of age). The same result was reported by Ryan et al. in male CD-1 mice[33]. However, the significant difference of body size increase between groupstends to disappear during the following weeks of early life and is probably due to growth acceleration caused by the influence of BPA on growth hormone.

Sexual organs weight is considered with AGD as another marker of sexual development [32]. In the present study we have seen that *in-utero* and lactational exposure to ecologically relevant doses of BPA altered the absolute and relative weight of some reproductive organs such as epididymides, testes and ovaries whereas womb and seminal vesicles were unaffected. The decline of ovaries and seminal vesicles can be related to body weight decrease observed in the high dose group although it cannot explain the lack of effects on the uterine weight. As for previous parameters, literature has reported conflicting results concerning sexual organs weight. Thus, an increase, a decrease and lack of effects on organs weight were alternatively reported by studies [28,36,37,38,39, 40].

Several lines of evidence reveals that some of these effects are associated with the ability of BPA to interact with different kinds of receptors including estrogen receptors (ER) and androgen receptors (AR) [41,42,43,44]. However, the binding activity remains extremely weak (about 1000 to 10.000 times weaker than natural hormones) and did not explain some of non-reproductive effects at low doses [45]. Recently, a new category of nuclear receptors called estrogen-related gamma (ERRγ) has been identified to be the *in-vivo* receptor of BPA that may mediate the low dose effects [46].

Conflecting results reported by the literature from rodent studies are probably due to various endogenous and exogenous factors including: study design, active components emanating from feed, periods that represent the most vulnerable window of exposure, dose and route of exposure and the power of statistical analysis used for detecting effects [17,18,32].

Overall, we can conclude that gestational and lactational exposure to environmentally relevant levels of BPA during critical developmental periods may lead to deleterious effects altering body weight and the reproductive function of *Wistar* rat. Further studies are needed to evaluate and clarify effects of BPA exposure on adipose tissue, sexual organs tissues and the homeostasis of the endocrine system.

5. REFERENCES

1.Adamao, C.,Antignac, J.P., Auger, J., Blaguer, P., Bour'chis, D., Bujan, L., Chevalier, C., Cotinot, C., Cravedi, J-P., Laudet, V., Lavera, G and Slama, R., 2010. Bisphénol A, effets sur la reproduction, rapport préliminaire d'expertise collective. Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France. (in French).

2. Gore, A.C., Crews, D., Doan, L.I., La Merrill, M., Patisaul, H. and Zota, A., 2014. Introduction to endocrine disrupting chemicals (EDCs): a guide for public interest organizations and poly-makers. Endocrine society, USA. Available at: http://www.endocrine.org/~/media/endosociety/files/advocacy-and-outreach/important-documents/introduction-to-endocrine-disrupting-chemicals.pdf[Accessed 28 Oct. 2015].

3. NIEHS., 2015. Endocrine disruptors. The National Institute of Environmental Health and Sciences, The National Institute of Health (NIH), USA: Available at:http://www.niehs.nih.gov/health/topics/agents/endocrine/. [Accessed 12 Oct. 2015].

4. Frye, C., Bo, E., Calamandrei, G., Calzà, L., Dessì-Fulgheri, F., Fernández, M., Fusani, L.,Kah, O , Kajta, M., Le Page, Y., Patisaul, H.B., Venerosi, A., Wojtowicz, A.K.. and Panzica, G.C., 2012. Endocrine distruptors: a review of some sources, effects, and mechanisms of action on behavior and neuroendocrine systems. J. Neuroendocrinol., 24(1): 144-59.

5. NIEHS., 2015. Bisphenol A. The National Institute of Environmental Health and Sciences, The National Institute of Health (NIH), USA Available at:https://www.niehs.nih.gov/health//agetopicsnts/sya-bpa/ . [Accessed 12 Oct. 2015].

6. Rubin, B.S, Murray, M.K, Damassa, D.A, King, J.C. and Soto, A.M., 2001. Perinatal Exposure to Low Doses of Bisphenol A Affects Body Weight, Patterns of Estrous Cyclicity, and Plasma LH Levels. Environ. Health. Perspect, 109(7): 675-680.

7. Newbold R.R., Jefferson W.N. and Padilla-Banks E., 2009. Prenatal exposure ton Bisphenol A at environmentally relevant doses adversely affects the murine female reproductive tract later in life. Environ. Health. Perspect., 117: 879-885.

8- Shelby, M.D., 2008. NTP-CERHR monograph on the potential human reproductive and developmental effects of Bisphenol A. The National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR). NIH publication No. 80-5994. Available at: http://ntp.niehs.nih.gov/ntp/ohat/bisphenol/bisphenol.pdf.[Accessed 18 Oct. 2015].

9. Huo X., Chen D., He Y., Zhu W., Zhou W. and Zhang J., 2015. Bisphenol –A and female infertility: a possible role of gene envrionment interactions. Int. J. Environ. Res. Public. Health.,12 : 11101-11116.

10. Yamamoto, T. and Yashura, A., 1999. Quantities of bisphenol a from plastic waste samples. Chemosphere., 36 (11): 2569-76.

11. Yoshida T., Horie M. and Nakazawa H., 2001. Determination of bisphenol A in canned vegetables and fruit by high performance chromatography. Food. Addit. Contam., 18(1): 69-75.

12. EFSA, 2015. Scientific opinion on bisphenol A (2015). Fact sheet . European Food Safe Authority. TM-01-15-035-EN-N. Availabe at : http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/factsheetbpa150121.pdf. [Accessed 15 Sep. 2015]

13. Braun, J., Hauser R. and Lanphear B.P., 2011. Background Paper on Metabolic Disorders of Bisphenol A . FAO/WHO Expert Meeting on Bisphenol A (BPA) Ottawa, Canada, 2–5 November 2010.

14. Rochester J.R., 2013. Bisphenol A and human health: a review of the literature. Reprod. Toxicol.,42:132-55.

15. Konieczna A., Rutkowska A. and Rachon D., 2015. Helath risk of exposure to bisphenol A (BPA). Rocz. Panstw. Zakl. Hig., 66(1): 5-11.

16. Srivastava S., Gupta P., Chandolia A. and Alam I., 2015. Bisphenol A: a threat to human health?,J.Environ. Health., 77(6): 20-26.

17. Richter C.A., Birnbaum L.S., Farabollini F., Newbold R.R., Rubin B.S., Talsness C.E., Vandenbergh J.G., Walser-Kuntz D.R. and Vom Saal F.S., 2007. In vivo effects of bisphenol A in laboratory rodent studies**.** Reprod. Toxicol., 24(2):199-224.

18. Rubin B.S., 2011. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J. Steroid. Biochem. Mo.l Biol., 127: 27-34.

19. Ryan, B.C., Hotchkiss, A.K., Crofton, K.M. and Gray, L.E., 2009. In utero and lactationnal exposure to bisphenol A, In Contrast to Ethinyl Estradiol, Does Not Alter Sexually Dimorphic Behavior, Puberty, Fertility, and Anatomy of Female LE Rats. Toxicol. Sci., 114(1): 133-148.

20. Howdeshell, K.L., Furr, J., Lambright, C.R., Wilson, V.S., Ryan, B.C. and Gray, L.E. Jr., 2008. Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat. Toxicological Sciences,102**:**371-382.

21. Casetra D., Di Segni N., Mallozzi M., Giovanale V., Mantovani A., Marci R. and Moscarini M., 2014. Bisphenol A and the female reproductive tract: an overview of recent laboratory evidenceand epidemiological studies. Reproductive Biology and Epidemiological studies,12: 37.

22. Wolff M.S., Teitelbaum S.I, McGovern K., Pinney S.M., Windham G.C, Galvez M., Pajak A., Rybak M., Calafat A.M., Kushi L.H., Biro F.M. and The breast cancer and environment research program., 2015. Environmental phenols and pubertal development in girls. Environ. Int., 84: 174-180.

23. Howdeshell K.L., Hotchkiss A.K., Thayer K.A., Vandenbergh J.G. and vom Saal F.S.,1999. Exposure to bisphenol A advances puberty. Nature, 401(6755) : 763-4.

24. Kwon, S., Stedman, D.B., Elswick, B.A., Cattley, R.C. and Welsh, F., 2000. Pubertual development and reproductive function of Crl:CD BR Sprague Dawley exposed to bisphenol A during prenatal and postnatal development. Toxicol. Sci., 55: 399- 406.

25. Yang F., Chen L.Q., Jin M.F., Zhou W.W. and Wu H.Y. , 2014. Impact of neonatal exposure to different doses of bisphenol A on puberty in female rats. Zhongguo Dang Dai Er Ke Za Zhi, 16(7):754-8. (Article in Chinese).

26. Tinwell H., Haseman J., Lefevre P.A., Wallis N. and Ashby J., 2002. Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A.Toxicol. Sci.,68(2):339-48.

27.Tyl, R.W., Myers, C.B., Marr, M.C., Thomas, B.F., Keimowitz, A.R., Brine, D.R., Veselica, M.M., Fail, P.A., Chang , T.Y., Seely, J.C., Joiner, R.L., Butala, J.H., Dimond, S.S., Cagen, S.Z.,Shiotsuka, R.N., Stropp, G.D., Waechter, J.M., 2002.Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol. Sci., 68(1):121-46.

28. Tyl R.W., Myers C.B., Marr M.C., Sloan C.S., Castillo N.P., Veselica M.M., Seely J.C., Dimond S.S., Van Miller J.P., Shiotsuka R.N., Beyer D., Hentges S.G. and Waechter J.M. Jr., 2008. Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicol. Sci., 104(2):362-84.

29. Bowman C.J., Barlow N.J., Turner K.J., Wallace D.G. and Foster P.M., 2003. Effects of in utero exposure to finasteride on androgen-dependent reproductive development in the male rat. Toxicol. Sci., 74(2):393-406.

30. Christiansen S., Scholze M., Axelstad M., Boberg J., Kortenkamp A. and Hass U., 2008. Combined exposure to anti-androgens causes markedly increased frequencies of hypospadias in the rat. Int. J. Androl., 31(2):241-8.

31. Welsh M., Saunders P.T., Fisken M., Scott H.M., Hutchison G.R., Smith L.B. and Sharpe R.M., 2008. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. J. Clin. Invest., 118(4):1479-90.

32. Christiansen S., Axelstad M., Boberg J., Vinggaard A.M., Pedersen G.A. and Hass U., 2014. Low-dose effects of bisphenol A on early sexual development in male and female rats. Reproduction., 147(4):477-87.

33. Ryan K.K., Haller A.M., Sorrell J.E., Woods S.C., Jandacek R.J. and Seeley R.J., 2010. Perinatal Exposure to Bisphenol-A and the Development of Metabolic Syndrome in CD-1 Mice. Endocrinology 151: 2603-12.

34. Heindel J.J., Newbold R. and Schug T.T., 2015. Endocrine disruptors and obesity. Nat. Rev. Endocrinol., 11(11):653-61.

35. Rubin B.S. and Soto A.M., 2009. Bisphenol A: Perinatal exposure and body weight,. Mol. Cell. Endocrinol., 304(1-2):55-62.

36. Cagen, S.Z., Waechter, J.M., Diamon, S.S., Breslin, W.J., Butala, J.H., Jekat, F.W., Joiner, R.L., Shiotsuka, R.N., Veenstra, G.E. and Harris, L.R., 1999. Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenolA. Toxicol.Sci., 50: 36-44.

37. Markey, C.M., Wadia, P.R., Rubin, B.S., Sonnenschein, C. and Soto, A.M., 2005. Long-term effects of fetal exposure to low doses of the xenostrogen bisphenol-A in the female mouse genital tract. Biol. Reprod.,72:1344-1351.

38. Metzdorff S.B., Dalgaard M., Christiansen S., Axelstad M., Hass U., Kiersgaard M.K., Scholze M., Kortenkamp A. and Vinggaard A.M., 2007. Dysgenesis and histological changes of genitals and perturbations of gene expression in male rats after in utero exposure to antiandrogen mixtures. Toxicol. Sci., 98(1):87-98.

39. Christiansen S., Scholze M., Dalgaard M., Vinggaard AM., Axelstad M., Kortenkamp A. and Hass U., 2009. Synergistic disruption of external male sex organ development by a mixture of four antiandrogens. Environ. Health. Perspect.,117(12):1839-46.

40. Jacobsen P.R., Axelstad M., Boberg J., Isling L.K., Christiansen S., Mandrup K.R., Berthelsen L.O., Vinggaard A.M. and Hass U., 2012. Persistent developmental toxicity in rat offspring after low dose exposure to a mixture of endocrine disrupting pesticides. Reprod. Toxicol., 34(2):237-50.

41. Krishnan AV., Stathis P., Permuth S.F., Tokes L. and Feldman D., 1993.Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology., 132(6):2279-86.

42. Olea N., Pulgar R., Pérez P., Olea-Serrano F., Rivas A., Novillo-Fertrell A., Pedraza V., Soto A.M. and Sonnenschein C., 1996. Estrogenicity of resin-based composites and sealants used in dentistry.Environ. Health. Perspect., 104(3):298-305.

43. Gaido, K.W., Leonard, L.S., Lovell, S., Gould, J.C., Babai, D., Portier, C.J. and McDonnell, D.P., 1997. Evaluation of chemicals with endocrine modulating activity in a yeast - based steroid hormone receptor gene transcription assay. Toxicol. Appl. Pharmacol., 143: 205 – 12.

44. Gould J.C., Leonard L.S., Maness S.C., Wagner B.L., Conner K., Zacharewski T., Safe S., McDonnell D.P. and Gaido K.W., 1998. Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. Mol. Cell. Endocrinol.,142(1-2):203-14.

45. Takayanagi S., Tokunaga T., Liu X., Okada H., Matsushima A. and Shimohigashi Y., 2006. Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor gamma (ERRgamma) with high constitutive activity. Toxicol. Lett., 167(2):95-105.

46. Tohmé M., Prud'homme S.M., Boulahtouf A., Samarut E., Brunet F., Bernard L., Bourguet W., Gibert Y., Balaguer P. and Laudet V., 2014. Estrogen-related receptor γ is an in vivo receptor of bisphenol A. FASEB. J., 28(7):3124-33.