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Biochemical and Histopathological Effects of in Utero Di-N-Hexyl Phthalate and Di-Cyclohexyl Phthalate Exposure on the Thyroid Axes and T3, T4, TSH Hormone Levels of Male and Female Rats: at Adulthood

ORIGINAL ARTICLE

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ABSTRACT

Objective: To investigate the effects of di-n-hexyl phthalate (DHP) and dicyclohexyl phthalate (DCHP) on hypophysis/thyroid axis in utero, pregnant rats were exposed to DHP or DCHP at doses of 0, 20, 100, and 500 mg/kg bw/d, by gavage, on gestational days (GD) 6–19.

Materials and Methods: The rats were allowed to grow up until adult (PD 90) stages. Body weights were recorded weekly. After treatment period, hormone analysis was determined in serum samples. Histopathological examinations revealed histopathological changes in thyroid gland on rats that received DHP or DCHP.

Results: There were no significant differences in body weights of male adult rats among groups. However, in female rats, final body weights were statistically decreased in 100 mg/kg/d DHP group but increased in DCHP treated rats at dose of 100 and 500 mg/kg/d in dose–response experiment. There was a decrease of TSH level in DHP 100 mg/kg/d and DCHP 500 mg/kg/d groups in male and female rats. T4 levels were increased in DCHP 20 and 100 mg/kg/d groups. In male rats, an increase of TSH level was found in DCHP 20 and 100 mg/kg/d treatment groups. Similarly, an increase of T3 level was found in DCHP 100 and 500 mg/kg/d groups. Also, in thyroid tissue, increase of adipose tissue, colloidal degeneration, and follicular degeneration was observed in all treatment groups.

Conclusions: The results of this study suggest that DHP and DCHP, which was applied in pregnancy period, cause changes to T3, T4, and TSH hormone levels and thyroid histology in adult rats.

Keywords: Di-n-hexyl phthalate, dicyclohexyl phthalate, endocrine system, male and female rats, toxicity

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INTRODUCTION

Phthalates are a class of widely used industrial compounds known technically as di-alkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid and are used mainly as a specialty plasticizer for nitrocellulose, polyvinyl acetate, and polyvinyl chloride, a lubricant for aerosol valves, an antifoaming agent, a skin emollient, and plasticizer in nail polish, fingernail elongates, and hair spray (1, 2). There are many phthalates with many uses, and just as many toxicological properties. Exposure to environmental phthalates, liberated from polyvinyl chloride (PVC) materials, has recently been associated with the development of some serious problems in human health (3).

Di-n-hexyl phthalate (DHP) and dicyclohexyl phthalate (DCHP) belong to the family of phthalic acid di-esters, which are produced in large quantities, and are primarily used as softeners and plasticizers in commonly used plastics; they are found in a wide variety of media ranging from drinking water to infant formulae (4). DHP and DCHP were found in food and in indoor environments (5). DCHP was measured at a concentration of 0.07 μ/m³ in samples of indoor air from houses in Japan (6), and its monoester metabolite was found in the urine of adults of the U.S. general population (7). DCHP had the most potent estrogenic activity among phthalate esters examined, but its estrogenic potency was 1,700,000 times less than that of 17b-estradiol (8).

Human exposure to some industrial compounds may result in adverse health outcomes mediated through the neuroendocrine axis. These chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the human body that are responsible for maintaining homeostasis, reproduction, development, and/or behavior. Thyroid hormones play an important role in many physiologic systems, and alterations in thyroid hormone levels can lead to a myriad of adverse clinical conditions (9). Although much is still unknown about mechanisms and consequences involved with the relationship between environmental exposures and changes in thyroid hormone levels, phthalates and other environmental chemicals may bind to thyroid receptors and influence thyroid hormone signaling (10).

Gayathri et al. (11) reported an increase in serum T3 and T4 in adult female rats receiving a low DEHP dose (750 lg/100 g bw) (12). A dose-dependent inverse association between DBP and both triiodothyronine (T3) and tetraiodothyronine (T4) has also been reported in male rats (12). In animal study, rats with diets contaminated with DEHP were found to have thyroid alterations compared with controls (13). They found that DEHP and di-n-octyl phthalate (DNOP) at 5000 ppm caused mild histological changes in the thyroid consisting of reduced follicle size and colloid density. Study of adult men for the first time shows an association between higher urinary levels of the metabolite mono (2-ethylhexyl) phthalate (MEHP) and reduced free T4 and/or total T3 levels in blood serum (14). Also, Boas et al. (15) indicated that phthalate metabolites of di-(2-ethylhexyl) phthalate and diisononyl phthalate, which were detected in all urine samples, negatively associated with thyroid hormones and have thyroid disrupting properties in children.

In the Meeker's study (14), phthalate metabolite concentrations were measured in urine and thyroid hormones were measured in serum from 408 men. MEHP (mono-2-ethylhexyl phthalate) was inversely associated with free T4 and total T3 but was not associated with thyroid-stimulating hormone (TSH). Huang et al. (16) reported an inverse association between MBP and both total and free levels of T4 in 76 pregnant Taiwanese women. Unlike the study among U.S. men, they did not find an inverse association with MEHP, but there were considerable differences between the designs of the two studies (16). In addition to having a smaller study size and a vastly different study population, the Taiwanese study also did not take into account concentrations of oxidative DEHP metabolites, which served to strengthen the associations between MEHP and thyroid hormones in the U.S. study. More study is needed on the association between phthalate exposure and thyroid function, which plays an important role in many human systems including reproduction and fetal neurodevelopment.

In our previous studies, we showed that *in-utero* DHP and DCHP exposures affected the development of male reproductive tract at prepubertal, pubertal, and adult stages of life (17) and exerted genotoxic effects to testicular cells of rats at all stages of development, even at adulthood (18).

In-utero exposure is most sensitive for the elicitation of the adverse effects in the animal systems (19-21). Thus, a higher sensitivity for damage is present for *in-utero* exposure.

The aim of this study is to investigate the effects of *in-utero* di-n-hexyl phthalate and dicyclohexyl phthalate exposures on the development of male and female thyroid glands. Pregnant Wistar rats were exposed to DHP and DCHP at doses of 0, 20, 100, and 500 mg/kg/day, by gavage, on gestational days (GD) 6–19, and the effects of these phthalates on hormonal and histopathologic changes were observed at adult stages.

MATERIALS and METHODS

Test Chemicals

Di-n-hexyl phthalate (CAS No. 84-75-3) with purity of 97% and dicyclohexyl phthalate (CAS No. 84-61-7) with purity of 99% were supplied by Alfa Aesar and Aldrich Chemistry (Ankara, Turkey), respectively, and dissolved in corn oil (vehicle). Thyroxin (T4), triiodothyronine (T3), and TSH ELISA kits for rats were purchased from Calbiotech Inc. (Spring Valley, CA).

Animals

The animal experiment was approved under number 2005/46 by the Animal Experimentations Local Ethics Board of Hacettepe University (Hacettepe, Turkey). Female Wistar albino rats were purchased from the Experimental Animals Production Center, Hacettepe University in Ankara (Ankara, Turkey). The animals were allowed at least 1-week acclimation interval prior to study start. Following the acclimation period, all animals were individually wire-mesh-cages suspended over cage board. The animal room was maintained at a temperature of 22°C±2°C and relative humidity 50±5 with a 12-hour light/dark cycle (06:00–18:00 h), and given standard rat diet (Korkutelim Feed Factory, Afyon, Turkey) and water were provided *ad libitum*. The pregnant rats were distributed on a random basis into control (vehicle) and treatment groups (n=10) and housed individually.

Experimental Procedure

The animals were paired for mating in the home cage of the male. The female rats (two-month-old and 200–220 g weight) were checked twice daily to confirm mating; all the females that presented a vaginal plug were considered pregnant. The pregnant rats were distributed on a random basis into control (vehicle) and treatment groups (n=10) and housed individually. Pregnant rats were treated by gavage application at GDs 6–19. The groups in this study were classified as follows: control, vehicle control (corn oil), 20, 100, 500 mg/kg/d DHP and 20, 100 and 500 mg/kg/d. The authors decided to choose 20, 100, and 500 mg/kg/body weight (bw)/d DHP and DCHP doses, without exceeding the acute oral median lethal dose values of DHP and DCHP reported to be 29.6 and >40 g/kg bw in rats, respectively (22). The low-dose level was chosen according to no observed adverse effect level reported by Hoshino et al. (19). The high-dose level was also chosen according to lowest-observed-adverse effect level of 500 mg/kg bw/d DCHP reported by Lake et al. (23).

The solutions were prepared fresh daily according to dams' weights. The dosing volume was 0.25 ml in all groups. The rats in the vehicle control group received corn oil in equal amounts as in experimental groups. Maternal weight was recorded weekly to pregnancy period. After delivery, all pups were allowed to grow with dam for one month. Then, pups were separated from dams. Female and male pups were housed at four per cage and allowed to free access for standard rat diet and tap water *ad libitum*. Food and water intake was performed daily, and body weights for all groups were recorded weekly during the experiment to exclude that the effects of DHP and DCHP were result of a reduction in food and water intake. The rats were allowed to grow up until adult (PD 90) stage. At necropsy, the animals were weighed and sacrificed under ether anesthesia followed by decapitation, and thyroid glands were excised immediately. The tissues were dissected and weighed in order to calculate the organ/body weight ratios for each animal. The organ weight was considered as absolute organ weight, whereas organ/body weight ratio was considered as relative organ weight. All experimental procedures and animal use were confirmed as the Approval of Ethics Committee of Hacettepe University.

Hormone Analysis

At the end of the study, the animals were weighed and sacrificed under ether anesthesia followed by decapitation. Blood was collected in tubes that contained heparin. Plasma was separated after centrifugation at 3000´ g for 30 min to pipette serum into silicon microcentrifuge tubes and stored at -80°C until hormone analysis. T3 (the sensitivity of the assay is 25 ng/mL), T4 (the sensitivity of the assay is 1 µg/dL), and TSH (the sensitivity of the assay is 0.2 ng/mL of serum or plasma) (Calbiotech Inc.), were measured by using commercially available ELISA kits for rats according to the manufacturer's instructions. The intra- and interassay coefficients of variations were less than 9.1%.

Histopathologic Study

For histopathological examination, thyroid (thyroid and parathyroid were removed together) tissues were dissected out and weighed in order to calculate the organ and relative organ weights for each animal. Subsequently, the tissues were fixed in Bouin's solution for 8 hours and then processed in a series of graded ethanol, embedded in paraffin, cut at 4 µm thickness, and stained with Harris hematoxylin and eosin. All slides were examined using Olympus BX51 system light microscope. The photographs were captured using Bs200prop software.

Statistical Analysis

Prior to parametric tests, Kolmogorov–Smirnov tests were used, respectively, to evaluate data for normality and homogeneity. All values presented in the text are mean±standard error (S.E.). Statistical analyses were performed using a Statistical Package for the Social Sciences (SPSS) version (SPSS Inc.; Chicago, IL, USA). Body and organ weights examined by means of univariate analysis of variance using a one-way factorial design. Body, absolute, and relative organ weights were examined by Hochberg's GT2-method or Games–Howell, which is based on unequal sample sizes to detect differences among groups. Incidences of histopathological findings were analyzed by Fisher's exact test. The value of $p<0.05$ was considered statistically significant.

RESULTS

Body and Organ Weights

Final body weight and absolute and relative (weight/body) thyroid weight ratio of male and female rats in dose–response is shown in Tables 1 and 2. There were no significant differences in body weights of male adult rats among groups. However, in female rats, final body weights were statistically decreased in 100 mg/kg/d DHP group but increased in DCHP–treated rats at dose of 100 and 500 mg/kg/d in dose–response experiment. Compared with the controls, the difference was statistically significant.

Hormone levels

T3, T4, and TSH levels of adult male and female rats in control and treatment groups are presented in Figures 1-6. In female rats, there was a decrease of TSH level in DHP 20 and 100 mg/kg/d and in male rats, a decrease was observed in 100 mg/kg/d DHP

Table 1. Final body weight (g) and selected absolute (g) and relative organ weights (mg/g) of adult male rats following *in-utero* exposure to DHP or DCHP at dose of 20, 100, or 500 mg/kg/d

		DHP (mg/kg/day)			DCHP (mg/kg/day)		
	Control	20	100	500	20	100	500
N	10	10	10	10	10	10	10
Final body weight(g)	253±12.3	256±7.4	261±5.5	265±7.1	247±10.6	260±6.3	267±6.3
Thyroid absolute organ weights (g)	0.12±0.01	0.12±0.01	0.14±0.01	0.11±0.01	0.12±0.01	0.12±0.01	0.13±0.00
Thyroid relative organ weights (mg/g)	0.49±0.02	0.45±0.01	0.52±0.01	0.43±0.03	0.50±0.02	0.47±0.01	0.49±0.020

DHP: Di-n-hexyl Phthalate; DCHP: Di-Cyclohexyl Phthalate; N: number of rats; SE: standard error
Numbers represent mean±SE

Table 2. Final body weight (g) and selected absolute (g) and relative organ weights (mg/g) of adult female rats following *in-utero* exposure to DHP or DCHP at dose of 20, 100, or 500 mg/kg/d

		DHP (mg/kg/day)			DCHP (mg/kg/day)		
	Control	20	100	500	20	100	500
N	10	10	10	10	10	10	10
Final body weight (g)	189±7.0	195±3.6[c]	166±5.1[a,b,c]	183±4.1	185±5.4	197±4.8[a]	198±4.5[a]
Thyroid absolute organ weights (g)	0.11±0.01	0.10±0.01	0.10±0.01	0.11±0.01	0.10±0.01	0.10±0.01	0.11±0.01
Thyroid relative organ weights (mg/g)	0.60±0.03	0.53±0.05	0.58±0.03	0.58±0.03	0.54±0.03	0.53±0.02	0.55±0.04

DHP: Di-n-hexyl Phthalate; DCHP: Di-Cyclohexyl Phthalate; N: number of rats; SE: standard error
Numbers represent mean±SE.
[a]Statistically different from control group ($p<0.05$)
[b]Statistically different from 20 mg/kg/day DHP group ($p<0.05$)
[c]Statistically different from 500 mg/kg/day DHP group ($p<0.05$)

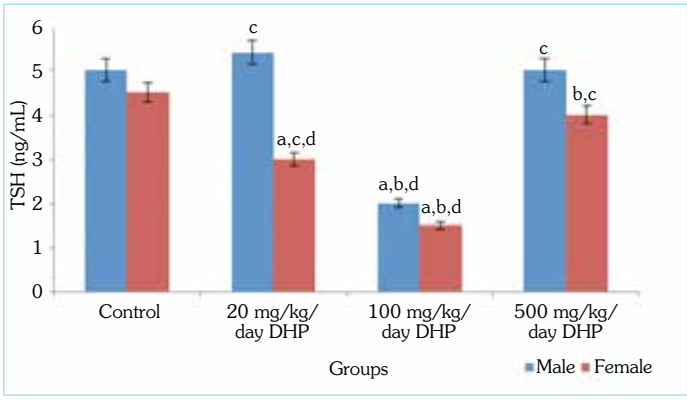

Figure 1. Serum TSH levels of male and female rats in control and DHP exposed groups. [a]p<0.05 significantly different from the control group. [b]p<0.05 significantly different from the 20 mg/kg bw/d DHP group. [c]p<0.05 significantly different from the 100 mg/kg bw/d DHP group. [d]p<0.05 significantly different from the 500 mg/kg bw/d DHP group

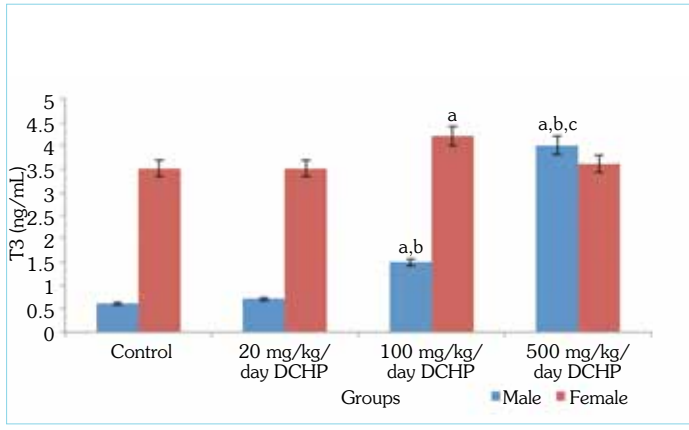

Figure 4. Serum T3 levels of male and female rats in control and DCHP-exposed groups. [a]p<0.05 significantly different from the control group. [b]p<0.05 significantly different from the 20 mg/kg bw/d DCHP group. [c]p<0.05 significantly different from the 100 mg/kg bw/d DCHP group

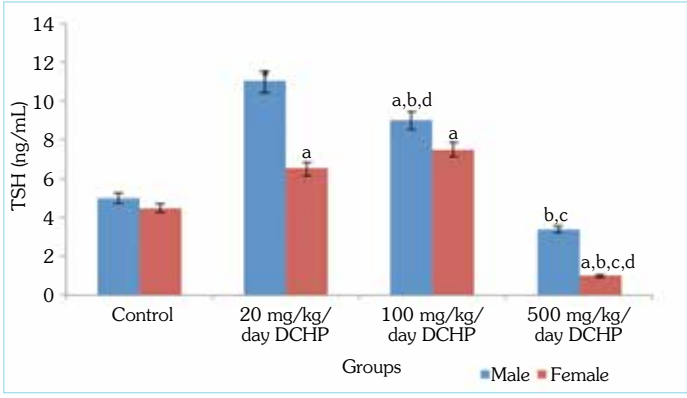

Figure 2. Serum TSH levels of male and female rats in control and DCHP-exposed groups. [a]p<0.05 significantly different from the control group. [b]p<0.05 significantly different from the 20 mg/kg bw/d DCHP group. [c]p<0.05 significantly different from the 100 mg/kg bw/d DCHP group

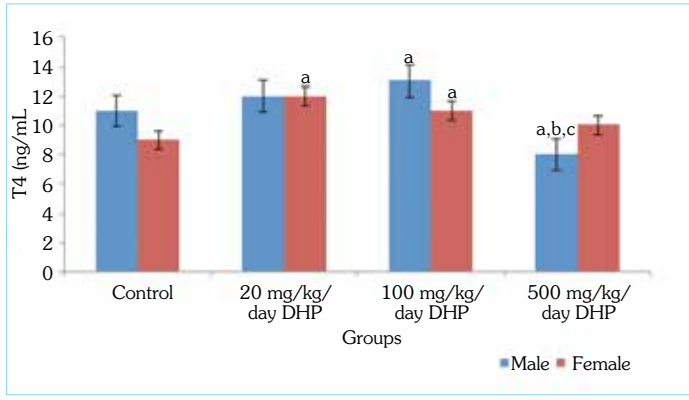

Figure 5. Serum T4 levels of male and female rats in control and DHP-exposed groups. [a]p<0.05 significantly different from the control group. [b]p<0.05 significantly different from the 20 mg/kg bw/d DHP group. [c]p<0.05 significantly different from the 100 mg/kg bw/d DHP group

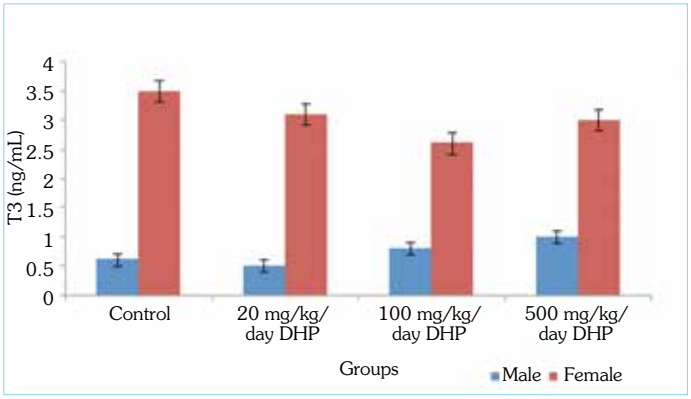

Figure 3. Serum T3 levels of male and female rats in control and DHP-exposed groups. [a]p<0.05 significantly different from the control group. [b]p<0.05 significantly different from the 20 mg/kg bw/d DHP group. [c]p<0.05 significantly different from the 100 mg/kg bw/d DHP group. d p>0.05 significantly different from the 500 mg/kg bw/d DHP group

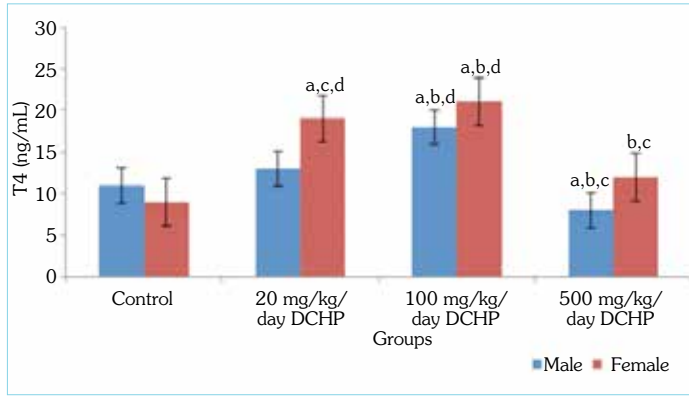

Figure 6. Serum T4 levels of male and female rats in control and DCHP-exposed groups. [a]p<0.05 significantly different from the control group. [b]p<0.05 significantly different from the 20 mg/kg bw/d DCHP group. [c]p<0.05 significantly different from the 100 mg/kg bw/d DCHP group. dp<0.05 significantly different from the 500 mg/kg bw/d DCHP group

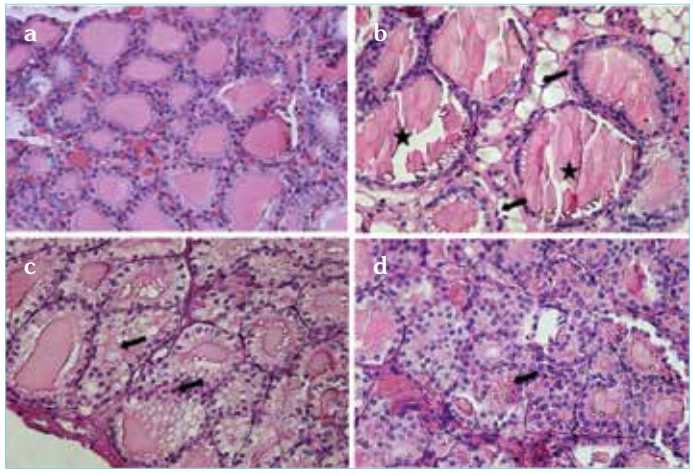

Figure 7. a-d. Photomicrograph of thyroid gland in control group (H&E stain, ×200) (a), photomicrograph of thyroid gland in 500 mg/kg/d DCHP treatment group. Increase of adipose tissue (→) and colloidal degeneration are shown (★) (H&E stain, ×200) (b), colloidal degeneration (→) in thyroid gland in 100 mg/kg/d (c), follicular degeneration in 20 mg/kg/d DCHP treatment group (→) are shown (H&E stain, ×200) (d)

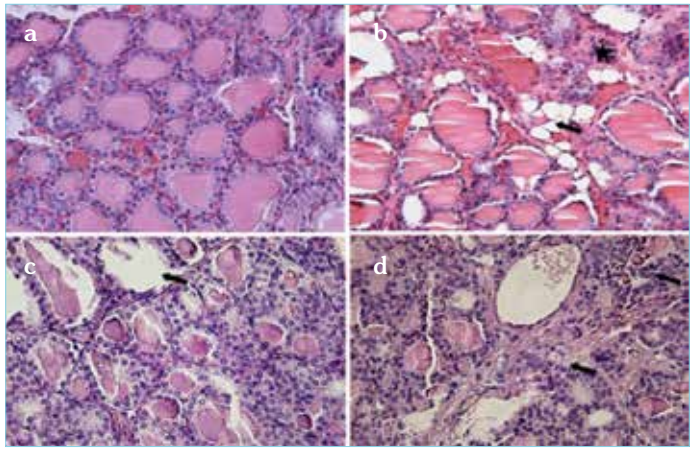

Figure 8. a-d. Photomicrograph of thyroid gland in control group (H&E stain, ×200) (a), photomicrograph of thyroid gland in 500 mg/kg/d DHP treatment group. Increase of adipose tissue (→) and follicular degeneration are shown (★) (H&E stain, ×200) (b), follicular degeneration (→) of thyroid gland in 100 mg/kg/d (c), DHP 20 mg/kg/d treatment groups are shown (H&E stain, ×200) (d)

group (Figure 1). In DCHP 500 mg/kg/d, groups TSH levels were decreased in male and female rats. On the other hand, in male and female rats, serum TSH values statistically significant increase were observed in 20 and 100 mg/kg/d DCHP treatment dose groups when compared to control values (Figure 2). There was no statistically significant difference in serum T3 levels of adult rats in control and all DHP treatment groups (Figure 3), but an increase of serum T3 levels was found in DCHP 100 of female rats and DCHP 100 and 500 mg/kg/d of male rats (Figure 4). Changes with statistically significant difference included elevated serum T4 levels of female rats in 20 and 100 mg/kg/d DCHP treatment groups increased, but high-dose DCHP treatment groups was found to be not significant compared to control. Also, in male rats, serum T4 levels were increased in 100 mg/kg/d DCHP treatment group but decreased in high DHP and DCHP treatment groups compared with control and other treatment dose groups (Figures 5 and 6).

Histopathology

The incidence of exposure-related histopathologic lesions of male and female rats in the control and treatment groups are given in Table 3 and light microscopic evaluation of the thyroid gland in control and 20, 100, or 500 mg/kg/d DHP and DCHP treatment groups are shown in Figures 7 and 8. The thyroid of control rats showed normal structure with cubical follicular epithelial cells and medium-sized colloid mass. In the adult male and female rats' thyroid gland, the elevated incidences of follicular and colloidal degeneration, increase in connective tissue, and adipose tissue between follicular tubules were observed (Figures 7 and 8) in all DHP and DCHP treatment groups. There was evidence of in male and female rats that had been exposed. Also, same follicles had been damaged and joined together in thyroid gland of treatment groups.

DISCUSSION

The present study was investigated the effects of DHP and DCHP on the thyroid gland of male and female rats *in utero*. Pregnant rats were exposed to DHP and DCHP at doses of 0, 20, 100, and 500 mg/kg/d, by gavage, on gestational days (GD) 6–19, and the effects of these phthalates on morphologic, hormonal, and histopathologic changes were observed at adult (PD 90) stages.

Thyroid hormones are involved in numerous physiological processes as regulators of metabolism. Maintenance of normal thyroid function is essential for psychological and physiological wellbeing. However, thyroid hormones are of special importance in fetal development. During the first part of pregnancy, the fetus relies entirely on transplacental transfer of maternal thyroid hormones and thus on a normal maternal thyroid function, but maternal thyroid homeostasis is also contributing substantially to fetal development during the remaining part of pregnancy (15, 24). Thus far, only few studies in humans on thyroid disrupting effects of phthalates have been carried out, whereas their adverse effects on rat's endocrine health are better investigated. In studies, urinary concentrations of phthalate metabolites are measured as a proxy for phthalate exposure. In 76 pregnant women, a significant negative association between the metabolite of DBP and free and total T4 was found (16). Likewise, negative associations between DEHP exposure and free T4 and total T3 have been reported in adult men (14). Boas et al. (15) performed a large cohort study of children, documenting that children are exposed to similar amounts of phthalates as adults. Their exposure was negatively associated with serum levels of T3 and height attainment (15). Animal studies on thyroid-disrupting effects of phthalates are also scarce. In rats, di-n-butyl phthalate (DBP) decreased T3 and T4 in a dose-dependent manner (12), and several studies have shown morphological changes in the thyroid after exposure to phthalates (13, 25). In study of Howard et al. (26) the thyroids of animals treated with either DEHP, DnHP, or a mixture of the compounds showed evidence for hyperactivity, as indicated by a reduction in follicular size and increase in the proportion of follicular cells with a columnar appearance.

Experimental studies suggest different mechanisms of action of phthalate effects on the thyroid homeostasis. Some phthalates (DBP, BBP) to inhibit T3 uptake in cells (27). Furthermore, phthalates competitively bind to transthyretin (TTR) (28) and inhibit the expression of the TR-beta gene (29). Intermediate results from an ongoing prospective birth cohort (n=106) in Puerto Rico studying urinary phthalate metabolites in relation to maternal serum thyroid and sex hormone levels revealed significant inverse associations between urinary phthalate metabolites and fT3 and fT4 (30). Also, Meeker and Ferguson (31) explored the cross-sectional relationship between urinary concentrations of metabolites of di(2-ethylhexyl) phthalate, dibutyl phthalate, and BPA with a panel of serum thyroid measures among a representative sample of U.S. adults and adolescents, and they observed that significant inverse relationships between urinary DEHP metabolites and total thyroxine (T4), free T4, total triiodothyronine (T3), and thyroglobulin and positive relationships with thyroid-stimulating hormone (TSH).

Follicular and colloidal degeneration and ligaments and fatty tissue increase between follicles was detected in thyroid tissue. It has been reported that phthalates adversely affect thyroid's hormonal mechanism. Exposure to high levels of phthalates changed (TSH, T3, and T4) hormone levels. Increased levels of these hormones affect iodine intake. Also, it may affect thyroidal function by changing colloid content. The two-generation key study reports an increased absolute and relative thyroid weight and signs of increased thyroid activity (hypertrophy of follicular cells) in the high-dose group (19). This was not seen in our study and the study by Yamasaki et al. (20); however, the period for dosing in the latter was only GD6 PND 20.

In this study, it can be concluded that histologic differences are caused by changed thyroid hormones that had indirect exposure to phthalates. The increases in plasma TSH levels and thyroid hyperplasia were consistent with a consequent stimulation of pituitary cells. The elevated TSH levels resulting from a decreased negative feedback contributed to the observed morphological alterations of the thyroid. Our study found that serum TSH levels were significantly and negatively associated with daily intake of DHP and DCHP.

It is not clear how much phthalates pass on and affect offspring during pregnancy. However, with results at hand, it could be maintained that enough phthalate make its way through placenta barrier. Also, a chemical such as this, that is abundantly used, that has clear adverse effects for endocrine systems could very well reversely affect human embryo. Increased usage of phthalates in this century brings forth increased issues in sperm count, fertility, and endocrine and hormone systems when compared to previous century.

There are few data to understand the effects of DHP and DCHP on endocrine system. Our previous and present studies indicate that DHP and DCHP have anti-androgenic and weak estrogenic effects at fetal period on reproductive systems in rats (17, 32).

CONCLUSION

Findings of this study demonstrate that DHP and DCHP, which mimics the estrogen caused adverse effects on male and female endocrine system at the fetal period and later phases of life. Histopathological effects on thyroid gland, hormone levels, and morphological changes of male and female rats are quite important for endocrine system. Although in the present study, the exact amount of DHP and DCHP that passes from dam to pups is not clear; our findings suggest that the amount of DHP and DCHP that has passed through placental barrier is enough to adversely affect male pups.

In summary, our findings of this study indicate that exposure of DHP and DCHP at doses of 20, 100, or 500 mg/kg/d by gavage during fetal life may cause adverse effects on endocrine system of adult male and female Wistar albino rats. Further experimental research is needed to clarify the mechanism and action of DHP and DCHP in the body used in plastics.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the Animal Experimentations Local Ethics Board of Hacettepe University.

Informed Consent: Informed consent is not necessary for this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Conceived and designed the experiments or case: NB. Performed the experiments or case: EG. Analyzed the data: NB., EG. Wrote the paper: NB., EG. All authors have read and approved the final manuscript.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Bhattacharya N1, Dufour JM, Vo MN, Okita J, Okita R, Kim KH. Differential effects of phthalates on the testis and the liver. Biol Reprod 2005; 72(3): 745-54. [CrossRef]
2. Zhang Y, Jiang X, Chen B. Reproductive and developmental toxicity in F1 Sprague-Dawley male rats exposed to di-n-butyl phthalate in utero and during lactation and determination of its NOAEL. Reprod Toxicol 2004; 18(5): 669-67. [CrossRef]
3. Annex XV report, Proposal for identification of a substance of very high concern on the basis of the criteria set out in reach article 57, Submitted by: Sweden, In cooperation with: Denmark. 2015; 77p.
4. Kavlock R, Boekelheide K, Chapin R, Cunningham M, Faustman E, Foster P, et al. NTP center for the evaluation of risks to human reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di-n-hexyl phthalate. Reprod Toxicol 2002; 16(5): 709-19. [CrossRef]
5. Rakkestad KE, Dye CJ, Yttri KE, Holme JA, Hongslo JK, Schwarze PE, et al. Phthalate levels in Norwegian indoor air related to particle size fraction. J Environ Monit 2007; 9(12): 1419-25. [CrossRef]
6. Otake T, Yoshinaga J, Yanagisawa Y. Exposure to phthalate esters from indoor environment. J Expo Anal Environ Epidemiol 2004; 14(7): 524-8. [CrossRef]
7. Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, et al. Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect 2000: 108(10); 979-82. [CrossRef]
8. Okubo T, Suzuki T, Yokoyama Y, Kano K, Kano I. Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay in vitro. Biol Pharm Bull 2003; 26(8): 1219-24. [CrossRef]

9. Nussey S, Whitehead S. Endocrinology: An Integrated Approach. Oxford, UK: Bios Scientific Publishers Ltd. 2001. [CrossRef]
10. Zoeller RT. Environmental chemicals as thyroid hormone analogues: new studies indicate that thyroid hormone receptors are targets of industrial chemicals? Mol Cell Endocrinol 2005; 242(1-2): 10-5. [CrossRef]
11. Gayathri NS, Dhanya CR, Indu AR, Kurup PA. Changes in some hormones by low doses of di (2-etyl hexyl) phthalate (DEHP), a commonly used plasticizer in PVC blood storage bags & medical tubing. Indian J Med Res 2004; 119(4): 139-44.
12. O'Connor JC, Frame SR, Ladics GS. Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. Toxicol Sci 2002; 69(1): 92-108. [CrossRef]
13. Poon R, Lecavalier P, Mueller R, Valli VE, Procter BG, Chu I. Sub chronic oral toxicity of di-n-octyl phthalate and di (2-ethylhexyl) phthalate in the rat. Food Chem Toxicol 1997; 35(2): 225-39. [CrossRef]
14. Meeker JD, Calafat AM, Hauser R. Di (2-ethylhexyl) Phthalate metabolites may alter thyroid hormone levels in men. Environ Health Perspect 2007; 115(7): 1029-34.[CrossRef]
15. Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebæk NE, Hegedüs L, Hilsted L, et al. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor i, and growth. Environ Health Perspect 2010; 118(10): 1458-64. [CrossRef]
16. Huang PC, Kuo PL, Guo YL, Liao PC, Lee CC. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. Hum Reprod 2007; 22(10): 2715-22. [CrossRef]
17. Aydoğan Ahbab M, Barlas N. Developmental effects of prenatal di-n-hexyl phthalate and dicyclohexyl phthalate exposure on reproductive tract of male rats: postnatal outcomes. Food Chem Toxicol 2013; 51: 123-36. [CrossRef]
18. Ahbab MA, Ündeğer Ü, Barlas N, Başaran N. In utero exposure to dicyclohexyl and di-n-hexyl phthalate possess genotoxic effects on testicular cells of male rats after birth in the comet and TUNEL assays. Hum Exp Toxicol 2014; 33(3): 230-9. [CrossRef]
19. Hoshino N, Iwai M, Okazaki Y. A Two-generation reproductive study of dicyclohexyl phthalate in rats. J Toxicol Sci 2005; 30: 79-96. [CrossRef]
20. Yamasaki K, Okuda H, Takeuchi T, Minobe Y. Effects of in utero through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. Toxicol Lett 2009; 189(1): 14-20. [CrossRef]
21. Saillenfait AM, Gallissot F, Sabate JP. Differential developmental toxicities of di-n-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. J Appl Toxicol 2009; 29(6): 510-21. [CrossRef]
22. Shibko SI, Blumenthal H. Toxicology of phthalic acid esters used in food-packaging material. Environ Health Perspect 1973; 3: 131-7. [CrossRef]
23. Lake BG, Foster JR, Collins MA, Stubberfield CR, Gangolli SD, Srivastava SP. Studies on the effects of orally administered dicyclohexyl phthalate in the rat. Acta Pharmacol Toxicol (Copenh) 1982; 51(3): 217-26. [CrossRef]
24. Crofton KM, Craft ES, Hedge JM, Gennings C, Simmons JE, Carchman RA, et al. Thyroid-hormone disrupting chemicals: evidence for dose-dependent additivity or synergism. Environ Health Perspect 2005; 113(11): 1549-54. [CrossRef]
25. Howarth JA, Price SC, Dobrota M, Kentish PA, Hinton RH. Effects on male rats of di-(2-ethylhexyl) phthalate and di-nhexylphthalate administered alone or in combination. Toxicol Lett 2001; 121(1): 35-43. [CrossRef]
26. Breous E, Wenzel A, Loos U. The promoter of the human sodium/iodide symporter responds to certain phthalate plasticizers. Mol Cell Endocrinol 2005; 244(1-2): 75-8. [CrossRef]
27. Shimada N, Yamauchi K. Characteristics of 3,5,3'-triiodothyronine (T3)-uptake system of tadpole red blood cells: effect of endocrine-disrupting chemicals on cellular T3 response. J Endocrinol 2004; 183(3): 627-37. [CrossRef]
28. Ishihara A, Sawatsubashi S, Yamauchi K. Endocrine disrupting chemicals: interference of thyroid hormone binding to transthyretins and to thyroid hormone receptors. Mol Cell Endocrinol 2003; 199(1-2): 105-17. [CrossRef]
29. Sugiyama S, Shimada N, Miyoshi H, Yamauchi K. Detection of thyroid system-disrupting chemicals using in vitro and in vivo screening assays in Xenopus laevis. Toxicol Sci 2005; 88(2): 367-74. [CrossRef]
30. Johns LE, Ferguson KK, Soldin OP, Cantonwine DE, Rivera-González LO, Del Toro LV, et al. Urinary phthalate metabolites in relation to maternal serum thyroid and sex hormone levels during pregnancy: a longitudinal analysis. Reprod Biol Endocrinol 2015; 13: 4. [CrossRef]
31. Meeker JD, Ferguson KK. Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. adults and adolescents from the National health and Nutrition Examination Survey (NHANES) 2007-2008. Environ Health Perspect 2011; 119(10): 1396-402. [CrossRef]
32. Aydoğan Ahbab M, Barlas N. Influence of in utero di-n-hexyl phthalate and dicyclohexyl phthalate on fetal testicular development in rats. Toxicol Lett 2015; 233(2): 125-37. [CrossRef]