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Article

Subchronic Oral Exposure to Propylparaben Induces Endocrine Disruption and Genotoxicity in Male Rats

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Abstract

Propylparaben (PP) is widely employed as a preservative in consumer products such as cosmetics, pharmaceuticals, and food items, prompting increasing safety apprehensions due to its possible endocrine-disrupting properties. A controlled experimental investigation was conducted to assess the toxicological effects of sub-chronic propylparaben exposure on the testes of adult male rats. Twenty adult male rats (n = 20) were randomly assigned to two groups (10 rats per group): a control group receiving distilled water and a treated group receiving PP orally at a dosage of 100 mg/kg body weight once daily for four consecutive weeks. This dosage was selected based on prior toxicological research indicating detrimental reproductive consequences after repeated exposure. Toxicological, hormonal, histopathological, and genotoxic assessments were performed. Data were analyzed using an unpaired Student's t-test and are presented as mean ± SEM, with statistical significance established at $p < 0.05$. Exposure to propylparaben resulted in marked toxicological disruptions and hormonal dysregulation, as reflected by a reduction in testosterone (142 ± 5.9 ng/dL, $p < 0.0001$), an increase in luteinizing hormone (10.4 ± 0.4 IU/L, $p < 0.0001$), and an elevation in follicle-stimulating hormone (9.6 ± 0.43 mIU/mL, $p < 0.0001$) compared to controls. Genotoxicity was evident through increased DNA damage, indicated by a heightened comet assay tail length (261.33 ± 4.1 µm, $p < 0.0001$). Histopathological evaluation demonstrated atrophy and structural disarray of seminiferous tubules, characterized by luminal constriction, epithelial degeneration, and localized tubular necrosis with associated inflammatory infiltration. These results indicate that repeated oral exposure to propylparaben induces measurable hormonal disturbances, genotoxicity, and structural damage to the testes, supporting concerns regarding its potential reproductive toxicity.

Keywords: Propylparaben, DNA, Hormones, Toxicity, Histology.

1. Introduction

Propylparaben (PP), or E-216, is a paraben molecule extensively utilized as a preservative in cosmetics, medicines, food items, dental products, and healthcare formulations due to its structural stability, low toxicity, and non-irritating characteristics **[1]**. Notwithstanding their prevalent application, water-based formulations are particularly susceptible to microbial proliferation, requiring robust preservation methods **[2]**. Regulatory bodies, such as the Cosmetic Ingredient Review (CIR), have generally concluded that parabens are safe when used within prescribed limits; however, mounting evidence suggests that propylparaben may act as an endocrine disruptor, raising concerns about its potential reproductive toxicity **[3]**.

Animal studies indicate that prolonged exposure to propylparaben can interfere with normal hormonal regulation, leading to alterations in testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), as well as dose-dependent reductions in sperm parameters **[4-6]**. Moreover, propylparaben has been associated with genotoxic effects, including DNA breakage, reduced mitotic activity, and chromosomal abnormalities, suggesting its potential to cause lasting cellular harm **[8-7]**. Early-life and embryonic exposures have also been linked to persistent genomic and neurodevelopmental alterations in vertebrate models, indicating long-term biological effects **[9]**.

While prior research has established the endocrine-disrupting and genotoxic properties of propylparaben, most studies have examined hormonal, histological, or DNA impacts in isolation, leaving the mechanistic connections between these endpoints inadequately explored. This highlights a clear gap in the current toxicological literature.

Therefore, this study aimed to determine whether sub-chronic oral exposure to propylparaben disrupts endocrine homeostasis, induces DNA damage, and causes histopathological alterations in the testes of male rats.

2. Materials and Methods

Chemicals:

Propylparaben:

Propylparaben (PP), with CAS No: 94-13-3, was acquired in powder form from Sigma Chemical Company, located in St. Louis, USA.

Preparation of Solution:

The test solution was generated weekly by dissolving propylparaben in distilled water at a concentration of 100 mg/kg body weight, maintaining uniformity and freshness throughout the trial **[10]**.

Experimental Animals:

Twenty healthy adult male Wistar albino rats, weighing between 160 and 200 grams, were procured from the Pharmacy College at King Abdul-Aziz University (KAU) in Jeddah, Saudi Arabia. The animals were acclimatized for one week before the experiment and kept under conventional laboratory settings (22 ± 2°C, 50–60% humidity, 12-hour light/dark cycle) with unrestricted access to food and water.

Eithc Approval

All experimental procedures conformed to the regulations established by the study ethics committee at King Abdul-Aziz University (KAU), sanctioned under No: P115-2023.

Experimental Design

Rats were randomly allocated into two groups (n=10 each group):

- G1 – Control Group: Rats were administered distilled water orally on a daily basis for a duration of four weeks.
- G2 – Treated Group: Rats were administered 100 mg/kg of propylparaben orally on a daily basis for a duration of four weeks [10].

It is important to note that this pilot study did not incorporate a positive control; subsequent studies featuring numerous doses and a positive control are necessary to enhance toxicological interpretation.

Randomization was executed using simple random assignment. Histological and genotoxic assessments were performed by researchers unaware of group assignments.

Biochemical Assay

Testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) serum concentrations were quantified utilizing rat-specific ELISA kits (BioSource, USA) in accordance with the manufacturer's guidelines **[11]**.

Histological Assessment

At the end of the study, rats were euthanized with intraperitoneal injections of xylazine (80 mg/kg) and ketamine (80 mg/kg). The testes were excised, weighed, cleaned with isotonic saline, and sectioned

Table1 Effect of daily administration of PP (100 mg/kg orally) after the 4th week (G2) on serum levels of testosterone, LH, and FSH in male Wistar albino rats.

Statistical analyses were performed between control and treated (*N*=10) animals by using unpaired *t'* test. Values were expressed as

Parameters		Level of Testosterone (mean±S.E) (ng/dI)	Level of LH (mean±S.E) (IU/L)	Level of FSH (mean±S.E) (mIU/mL)
Control Treated Group	G1	328.6±4.4	4.8±0.22	4±0.2
Treated Group	G2	142±5.9*	10.4±0.4*	9.6±0.43*
%		-56%	116%	140%

mean ± S.E. *: Significant versus control (P<0.05). %: percentage of change from control.

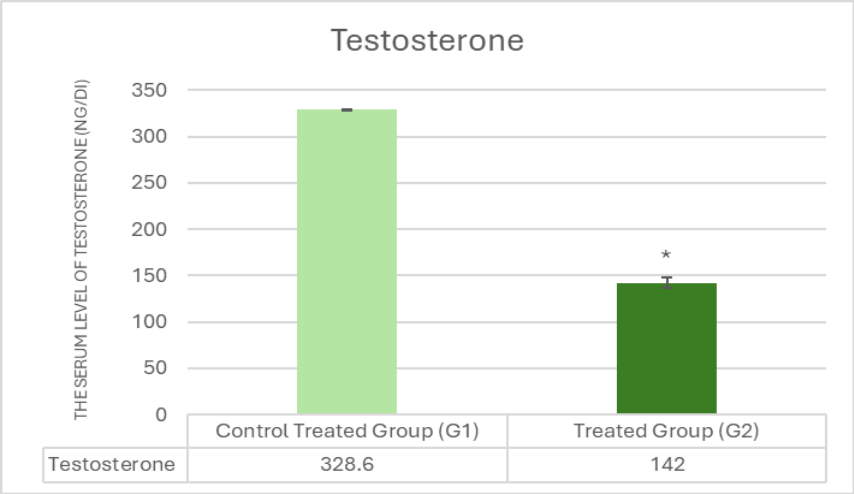

Fig.(1): Effect of daily administration of PP (100 mg/kg oral) after the 4th week (G2) on serum levels of testosterone in male Wistar albino rats. Data was expressed as mean ± standard error of the mean, significance groups made by an unpaired *t*-test. *: significance compared to control. #: significance compared to the control group.

into 0.5 cm fragments. Specimens were fixed in 10% neutral buffered formalin for histological examination **[12]**.

Histopathological changes were evaluated semi-quantitatively by scoring the severity as mild, moderate, or severe by a blinded observer. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (x400).

Genetic Procedures

DNA damage was assessed by using the Comet Assay Kit (3-well slides, ab238544), in accordance with the manufacturer's instructions. Single-cell suspensions were generated from testicular tissue and assessed for DNA strand breakage.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was conducted utilizing an unpaired Student's t-test (IBM SPSS Statistics, version 23, IBM Corp., Armonk, N.Y., USA). Statistical significance was established at $p < 0.05$.

3. Results

Biochemical Assay:

Table (1) presents a summary of serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) concentrations in control (G1) and propylparaben-treated (G2) rats after four weeks of daily oral administration of propylparaben (100 mg/kg) (n = 10 per group).

Figure (1) illustrates that serum testosterone levels in the treated group were significantly diminished (142.0± 5.9 ng/dL) relative to the control group (328.6 ± 4.4 ng/dL), indicating a mean reduction of 186.6 ng/dL, accompanied by an exceptionally large effect size (Cohen's d = −11.34) and a 95% confidence interval (CI) of −202.1 to −171.1 ng/dL, which was

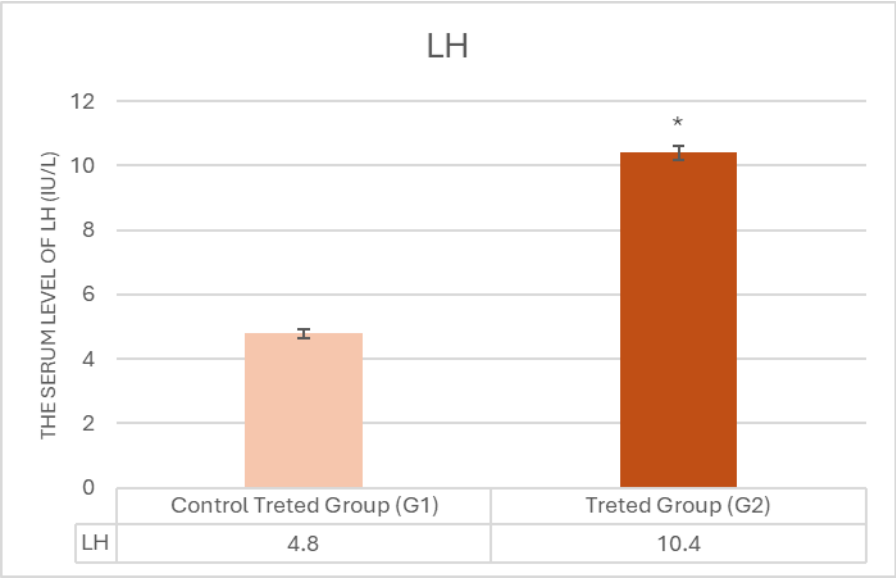

Fig.(2): Effect of daily administration of PP (100 mg/kg oral) after the 4th week (G2) on serum levels of LH in male Wistar albino rats. Data was expressed as mean ± standard error of the mean, significance groups made by an unpaired *t*-test. *: significance compared to control group. #: significance compared to the control group.

statistically significant ($P < 0.0001$).

In contrast, LH levels were markedly increased in the treated group (10.4 ± 0.4 IU/L) compared to controls (4.8 ± 0.22 IU/L), resulting in a mean elevation of 5.6 IU/L, a substantial effect size (Cohen's d = 5.49), and a 95% confidence interval of 4.64 to 6.56 IU/L ($P < 0.0001$) (Figure 2).

In the treatment group, FSH concentrations were markedly elevated (9.6 ± 0.43 mIU/mL) compared to the control group (4.0 ± 0.2 mIU/mL), indicating a mean increase of 5.6 mIU/mL, with a substantial effect size (Cohen's d = 5.28) and a 95% confidence interval of 4.60 to 6.60 mIU/mL ($P < 0.0001$) (Figure 3).

All data are shown as mean ± standard error of the mean (SEM).

Histological assessment

Control Group (G1)

Testicular sections from the control group (G1) showed typical seminiferous tubules (S.T) with an intact basal lamina (B.L), well-structured germinal epithelium, and no signs of necrosis or inflammation (Figure 4). All samples were assessed without knowledge of group assignment.

Group Treated with Propylparaben (G2)

Conversely, testis from rats treated with propylparaben (G2) exhibited significant histological changes, including:

Atrophic seminiferous tubules exhibiting restricted lumens, narrowing, and fissuring. Degeneration of the basal lamina (B.L). Focal tubular necrosis with associated inflammatory infiltration (Figure 5).

Evaluation Metrics and Quantitative Assessment

A semi-quantitative scoring method was utilized to evaluate the extent of tube damage.

Zero equals normal.

1 signifies mildness

2 signifies mild

3 signifies severe

All slides underwent assessment by a blinded histopathologist.

Control group (G1): 0 ± 0

Treated group (G2): 2.8 ± 0.2, signifying significant tubular disruption.

The unpaired t-test statistical analysis verified that the intergroup difference was extremely significant ($P < 0.0001$).

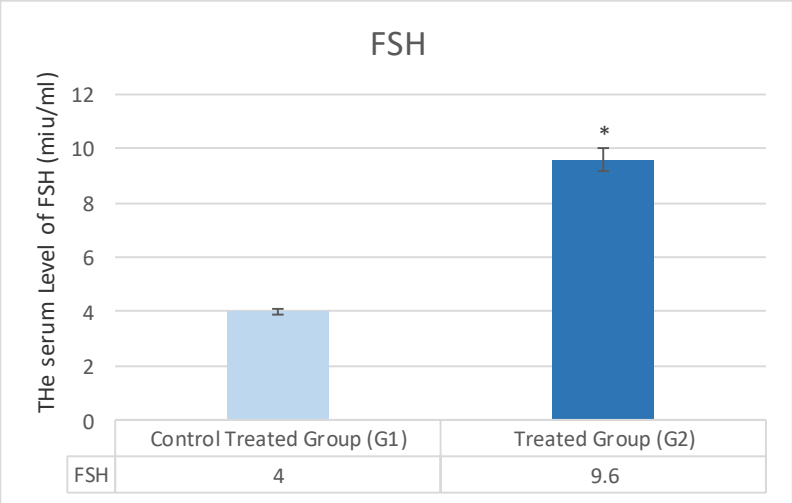

Fig.(3): Effect of daily administration of PP (100 mg/kg oral) after the 4th week (G2) on serum levels of FSH in male Wistar albino rats. Data was expressed as mean ± standard error of the mean, significance groups made by an unpaired *t*-test. *: significance compared to control group. #: significance compared to the control group.

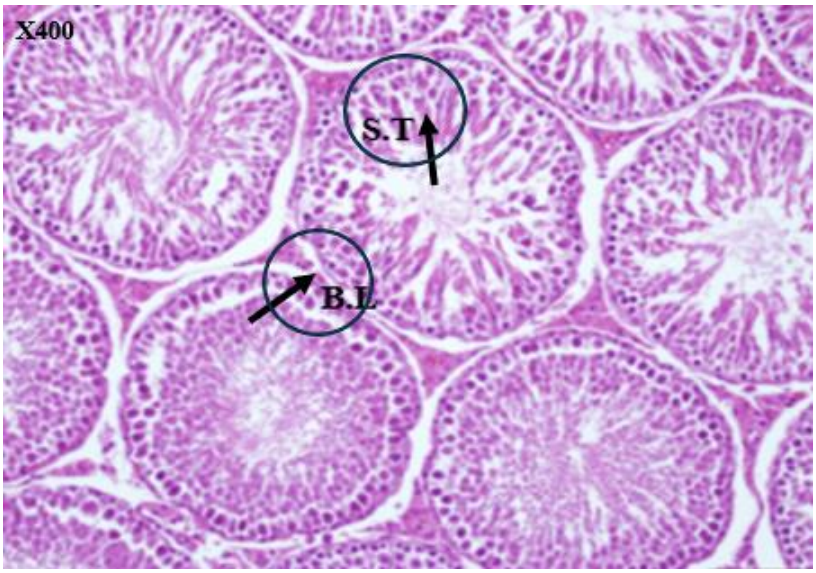

Fig. 4. Light microphotograph of treated control group (G1) testis section of male rat showing the normal histological structure of seminiferous tubules (S.T), lamina (L), and basal lamina thickness. (B.L). (H&E, ×400)

Genetic Results:

Table 2 and Figure 6 depict the impact of sub-chronic propylparaben exposure on DNA integrity in rat testicular cells, assessed via the Comet assay.

- **Control group (G1):** Head length = 228.15 ± 5.6 µm, Tail length = 12.23 ± 5.9 µm
- **Treated group (G2):** Head length = 98.33 ± 4.4 µm, Tail length = 261.33 ± 4.1 µm

These results indicate **substantial DNA damage** in G2 compared with controls.

- **Mean difference (tail length):** 249.1 µm
- **Effect size (Cohen's d):** 45.0 → extremely large effect
- **95% CI (tail length):** 238.5–259.7 µm
- **P-value:** <0.0001 (unpaired t-test)

The significant decrease in head length, along with the notable rise in tail length, signifies substantial genomic impairment in testicular cells after sub-chronic exposure to propylparaben. Although other conventional comet metrics, including tail moment, Olive tail moment, and percentage of DNA in the tail, were not assessed in this work, the data unequivocally indicate substantial genotoxicity.

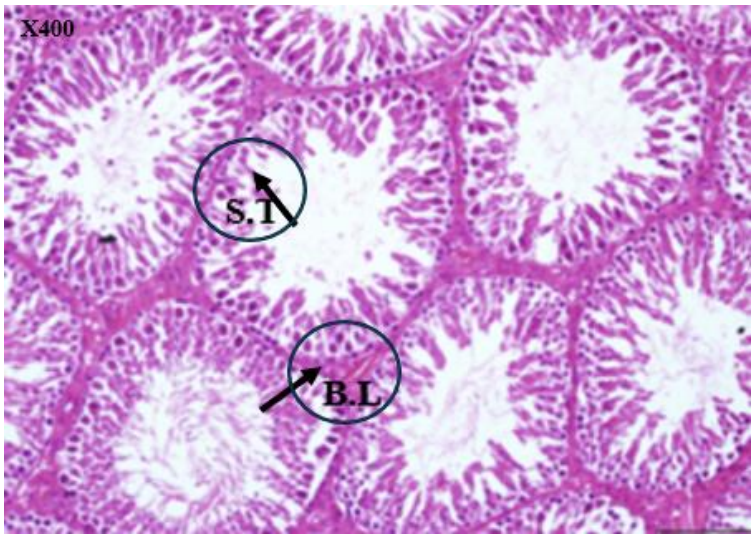

Fig 5. Light microphotograph of testicular sections from propylparaben-treated rats (G2) showing atrophic seminiferous tubules (S.T) with constricted lumens, tubular splitting, and atrophy of the basal lamina (B.L). Focal tubular necrosis with associated inflammatory infiltration is also evident. (H&E, ×400)

Table (2): Effect of daily administration of PP (100mg/kg/day) for four weeks on treated group (G2) compared to control treated group (G1) on head and tail length of DNA in male rats.

Group	DNA head length Mean± SE	DNA tail length Mean± SE
Control Treated Group (G1)	228.15±5.6	12.23±5.9
Treated Group (G2)	98.33±4.4*	261.33±4.1*
	-57%	2036%

Data was expressed as mean ± standard error of the mean, significance groups made by an unpaired *t*-test. *: significance compared to control group. #: significance compared to the control group.

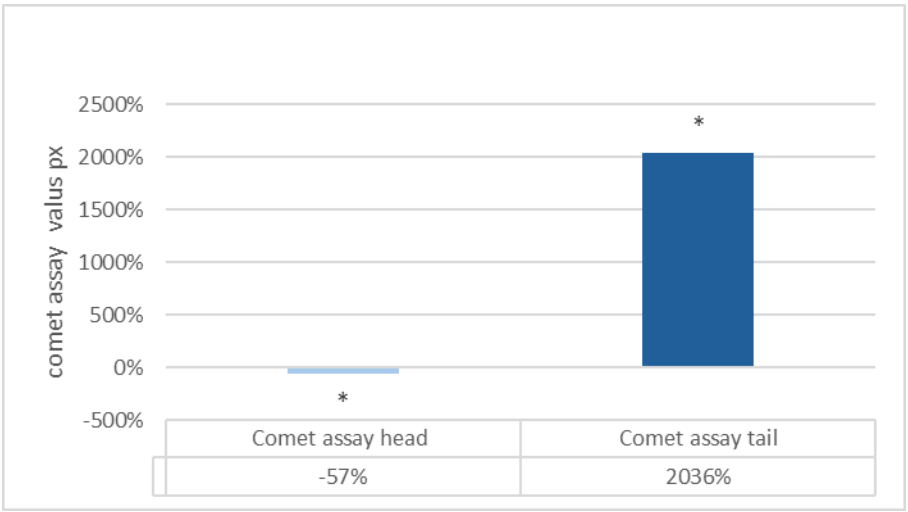

Fig.(6): Effect of daily administration of PP (100 mg/kg. oral) and subsequent treatment for 4 weeks on head and tail length of DNA of male Wistar albino rats. Data was expressed as mean ± standard error of the mean, significance groups made by *t*-test. *: significance compared to control group. #: significance compared to the control group.

4. Discussion

Propylparaben (PP) is extensively utilized as a preservative in cosmetics, pharmaceuticals, and food items; nevertheless, increasing evidence suggests it may function as an endocrine disruptor with possible reproductive and genotoxic consequences. The hypothalamic-pituitary-gonadal (HPG) axis is pivotal in male reproductive function, as pulsatile GnRH secretion prompts the release of pituitary LH and FSH, which subsequently govern testosterone synthesis by Leydig cells and spermatogenesis in Sertoli cells [**13-14]**.

This study demonstrated that sub-chronic oral administration to PP at 100 mg/kg/day for four weeks significantly decreased testosterone levels while increasing LH and FSH levels, indicating disturbance of the hypothalamic-pituitary-gonadal axis. These hormonal imbalances were associated with histological changes, including atrophic seminiferous tubules[**15-16]** , atrophy of the basal lamina, tubular necrosis, and inflammation, as well as significant DNA damage in testicular cells, as assessed by the comet assay. The results align with other research indicating PP-induced testicular damage and endocrine disruption in rodents, along with genotoxic effects in mammalian cells**[17-20]**.

Although these findings corroborate the harmful potential of PP in rodents, some caveats must be recognized. The study utilized a single dose without evaluating dose–response, and did not incorporate a positive control group. The sample size (n=10 per group) was comparatively limited, and several comet test parameters (tail moment, Olive tail moment, % DNA in tail) were not assessed. Moreover, direct extrapolation to humans is constrained, as the administered dose (100 mg/kg) above standard human exposure thresholds from cosmetics, and interspecies variations in absorption, metabolism, and elimination may affect systemic toxicity.

Notwithstanding these limitations, the data underscore that even sub-chronic PP exposure can disturb endocrine homeostasis, cause anatomical testicular injury, and impair genomic integrity in male rats. Subsequent research ought to investigate various dosages, incorporate positive and vehicle controls, evaluate comprehensive comet assay characteristics, and analyze long-term reproductive effects, preferably utilizing pharmacokinetic modeling to more accurately correlate animal dosages with human exposure.

5. Conclusions

This study elucidates the mechanisms behind PP-induced testicular toxicity, revealing a correlation among endocrine disturbance, histopathological damage, and genotoxicity in a sub-chronic exposure context. These observations highlight the necessity for additional research to elucidate potential human health hazards linked to propylparaben.

Author Contributions: All authors contributed to the study conception and design. Hypothesis and Experiment design N. A. Material preparation, data collection were performed by S.M. The analysis S.M. and N.A. The first draft of the manuscript was written by S.M. and N.A. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of AI use: No conflicts of intelligence tools were used in the preparation of this study.

Supplementary Materials: Not applicable.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Institutional Review Board Statement: All experimental procedures conformed to the regulations established by the study ethics committee at King Abdul-Aziz University (KAU), sanctioned under No: P115-2023.

Informed Consent Statement: Not applicable.

References:

1. Sun L, Yu T, Guo J, Zhang Z, Hu Y, Xiao X, Sun Y, Xiao H, Li J, Zhu D, Sai L, Li J. The estrogenicity of methylparaben and ethylparaben at doses close to the acceptable daily intake in immature Sprague-Dawley rats. Sci Rep. 2016;6:25173. doi:10.1038/srep25173
2. Gilani A, Hussain F, Aslam T. Environmental and health risks of paraben preservatives in low-quality cosmetics. Rev J Neurol Med Sci. 2025;3(7):391–404. doi:10.12345/rjnms.2025.391
3. Hadir M, Alzoman NZ, Almeshal MA, Alotaibi HA, Alotaibi NN, Al-Showiman H. Quantitative screening of parabens in ready-to-eat foodstuffs available in the Saudi market

using high performance liquid chromatography with photodiode array detection. Arab J Chem. 2020;13(1):2897–2911. doi:10.1016/j.arabjc.2019.10.014

4. Oishi S. Effects of propyl paraben on the male reproductive system. Food Chem Toxicol. 2002;40(12):1807–1813. doi:10.1016/S0278-6915(02)00176-6
5. Cherian P, Zhu J, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JG Jr, Shank RC, Slaga TJ, Snyder PW, Heldreth B. Amended safety assessment of parabens as used in cosmetics. Int J Toxicol. 2020;39(1_suppl):5S–97S. doi:10.1177/1091581820907655
6. Shin GS, Park Y, Kim JY, Kim CH, An MJ, Lee HM, Kim JW. Propylparaben-induced endoplasmic reticulum stress triggers G2/M phase cell cycle arrest and initiates caspase-3-dependent apoptosis in human lung cells. Genes Genomics. 2025;47(2):223–233. doi:10.1007/s13258-024-01763-1
7. Ahn J, Kim S, Lee J. Genotoxic effects of parabens evaluated by comet assay and micronucleus test in human cells. Environ Res. 2020;182:109077. doi:10.1016/j.envres.2019.109077
8. Agarwal D, Bhatt U, Soni V. Ecotoxicological impacts of parabens on flora and fauna. npj Emerg Contam. 2025;5:12–20. doi:10.1038/s41545-025-0012-0
9. Pan ZN, Li LZ, Zhao HS, Yin SY, Chu M, Liu XY, Bao HC. Propylparaben exposure impairs G2/M and metaphase anaphase transition during mouse oocyte maturation. Ecotoxicol Environ Saf. 2025;283:116798. doi:10.1016/j.ecoenv.2024.116798
10. Salem AM, Said MM, Badawi MM, Rabo MMA. Subchronic toxicity of propyl paraben in adult male rats. Egypt J Biochem Mol Biol. 2013;31:1–20. doi:10.21608/ejbmb.2013.5461
11. Ilgin S, Kilic G, Baysal M, Kilic V, Korkut B, Ucarcan S, Atli O. Citalopram induces reproductive toxicity in male rats. Birth Defects Res. 2017;109(7):475–485. doi:10.1002/bdr2.1053
12. Mustafa FEZA, Elhanbaly R. Histological, histochemical, immunohistochemical and ultrastructural characterization of the testes of the dove. Zygote. 2021;29(1):33–41. doi:10.1017/S0967199420000220
13. Yang Y, Workman S, Wilson MJ. The molecular pathways underlying early gonadal development. J Mol Endocrinol. 2019;62(1):R47–R64. doi:10.1530/JME-18-0164
14. Petric Z, Ružić J, Žuntar I. The controversies of parabens – an overview nowadays. Acta Pharm. 2021;71(1):17–32. doi:10.2478/acph-2021-0002
15. Junjie A, Qiu W, Huo X, Wang Y, Wang W, Zhang Q, Liu Z, Zhang J. Paraben exposure and couple fecundity: a preconception cohort study. Hum Reprod. 2023;38(4):726–738. doi:10.1093/humrep/deac276
16. Majhi PD, Sharma A, Roberts AL, Daniele E, Majewski AR, Chuong LM, Black AL, Vandenberg LN, Schneider SS, Dunphy KA, Jerry DJ. Effects of benzophenone-3 and propylparaben on estrogen receptor-dependent R-loops and DNA damage in breast epithelial cells and mice. Environ Health Perspect. 2020;128(1):17002. doi:10.1289/EHP5167
17. Todorovac E, Durmisevic I, Cajo S, Haverić A, Mesic A. Evaluation of DNA and cellular damage caused by methyl-, ethyl- and butylparaben in vitro. Toxicol Environ Chem. 2020;103(1):85–103. doi:10.1080/02772248.2020.1760192
18. Guo Y, Yang Y, Zhou Z, et al. Propylparaben induces reproductive toxicity in human extravillous trophoblast cells via apoptosis and cell cycle pathways. Environ Health. 2024;2(5):301–310. doi:10.1186/s12940-024-01012-3
19. Guerra MT, Sanabria M, Cagliarani SV, Leite GA, Borges CD, De Grava Kempinas W. Long-term effects of in utero and lactational exposure to butyl paraben in female rats. Environ Toxicol. 2017;32(3):776–788. doi:10.1002/tox.22345
20. Hwang Y, Kim Y, Choi D, Lee JH. Effects of long-term treatment with low concentration butylparaben on prostate organoids. Environ Pollut. 2025;366:125502. doi:10.1016/j.envpol.2025.125502