Investigating the Effects of Human-Relevant DEHP Exposure on Testicular Architecture and Germ Cell Apoptosis

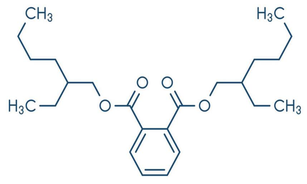
Ashley Lauver OMS-II, MS, Natalie Gartman OMS-II, Hovhannes M. Saribekyan, Estela J. Jauregui PhD
Arizona College of Osteopathic Medicine, College of Graduate Studies, Department of Physiology, Midwestern University, Glendale, AZ.

INTRODUCTION

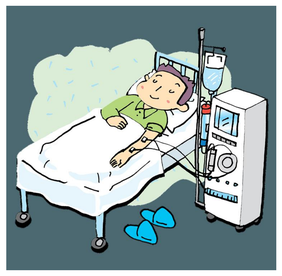
- Phthalates are chemicals commonly found in food packaging, cosmetics, and medical supplies (e.g., dialysis tubing).
- Di-2-ethylhexyl phthalate (DEHP), a common endocrine disruptor, has been linked to hormone regulation disruptions and germ cell development issues, potentially affecting fertility.[1,2]
- Endocrine-disrupting chemicals have been linked to disruption in the hypothalamic-pituitary-gonadal axis and are associated with impaired spermatogenesis.[3,4]

OBJECTIVE

Can short-term oral exposure to human-relevant doses of DEHP disrupt spermatogenesis in adult male CD-1 mice?



DEHP



METHODS

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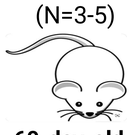
CD-1 mice
(N=3-5)



60-day-old

Pipette fed for 12 days

Vehicle Control:
-Corn oil

Euthanized following the final dose

DEHP Dosage:
- 20 ug/kg/day
- 200 µg/kg/day
-1000 mg/kg/day

1. **Testes collection**
2. **Hematoxylin and eosin staining**
3. **Caspase-3 immunostaining**
4. **Quantification of testicular phenotype**
5. **Statistical analysis using GraphPad Prism (one-way ANOVA; Dunnett's post hoc test)**

RESULTS

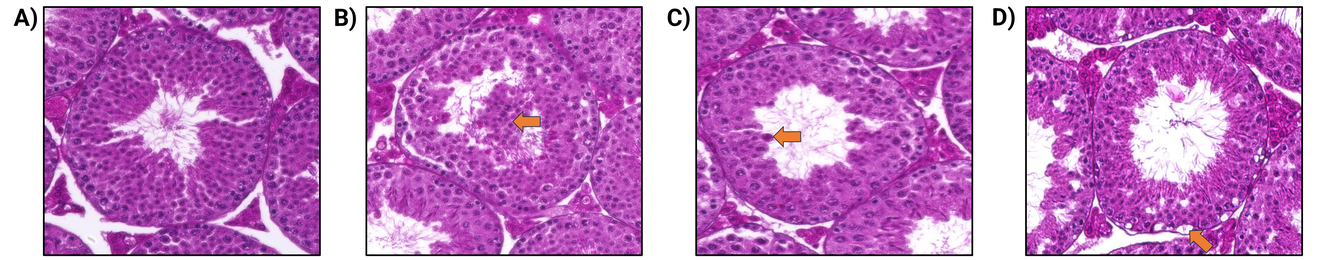


Fig. 1. Representative testicular phenotypes identified by hematoxylin and eosin (H&E) staining. (A) Normal seminiferous tubules; (B) abnormal sloughing of advanced germ cells; (C) pyknotic cells with apoptotic bodies; and (D) vacuolated seminiferous tubules.

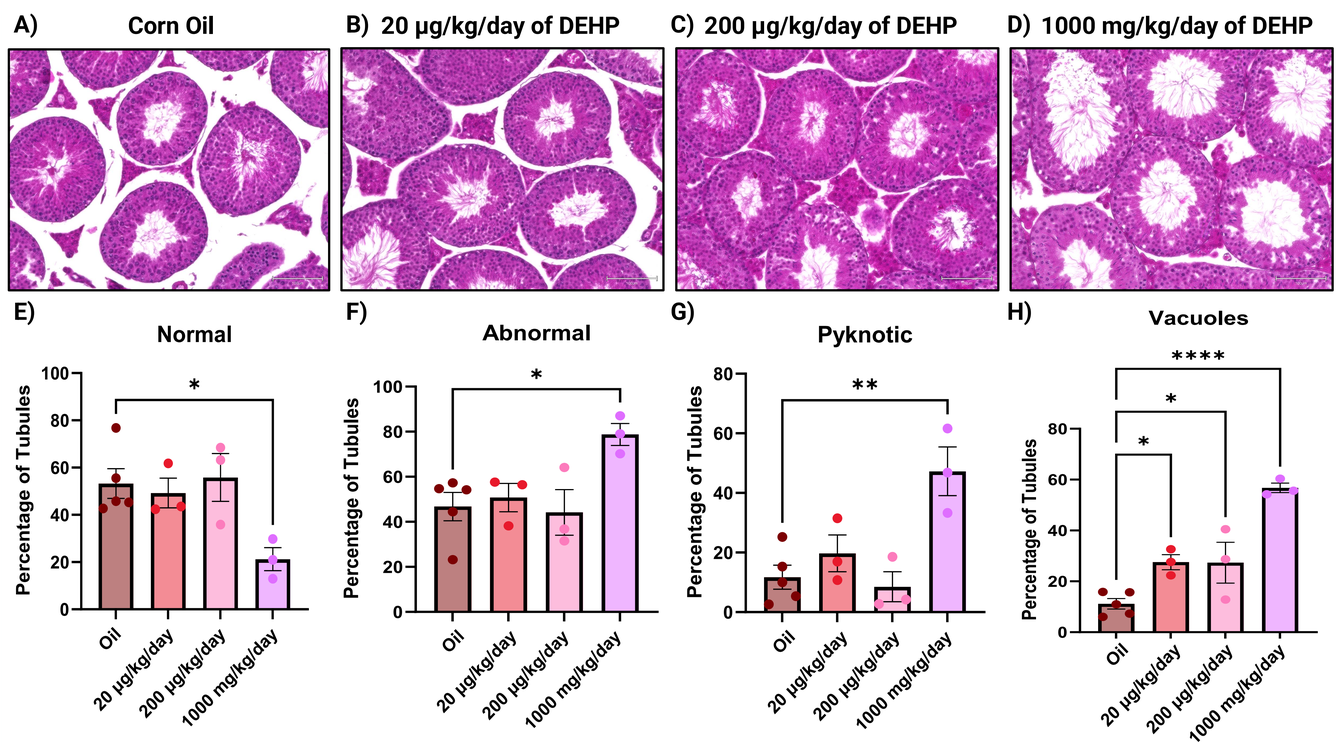


Fig. 2. Testicular morphology and phenotype quantification. Representative cross sectioned of testes from mice treated with oil (A), 20DEHP (B), 200DEHP(C), and 1000DEHP(D). Graphs depict quantification of percentage of seminiferous tubules showing (E) normal morphology, (F) abnormal morphology, (G) pyknotic cells, and (H) vacuolization in seminiferous tubules from control and DEHP-treated mice.
Data are presented as mean ± SEM. *p < 0.05

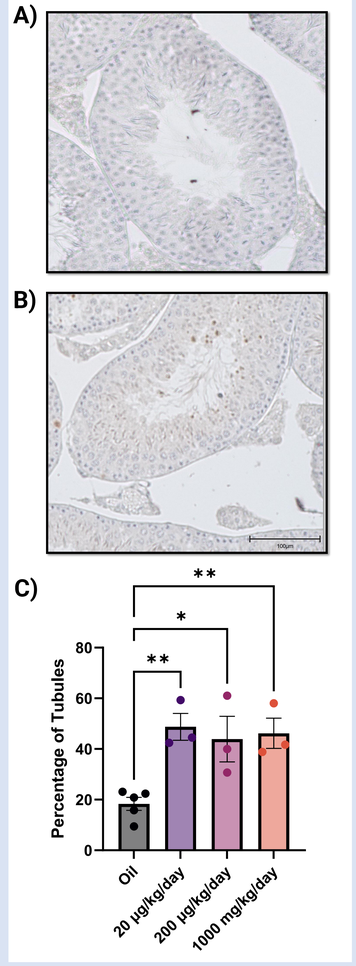


Fig. 3. Caspase-3 immunostaining and quantification. IHC of Caspase-3 in testicular cross-sections from control (A) and Caspase-3 antibody–stained sections (B). Quantification of the percentage of Caspase-3–positive seminiferous tubules across treatment groups (C). Data are presented as mean ± SEM. *p < 0.05

CONCLUSION

- CD-1 male mice exposed to 1000 mg/kg/day DEHP showed a significant increase in abnormal seminiferous tubules, including higher frequencies of pyknosis, a marker of cell death.
- A significant increase in tubules containing ≥5 vacuoles was observed in the 20 µg/kg/day, 200 µg/kg/day, and 1000 mg/kg/day DEHP groups, indicating cellular injury compared to controls.
- Caspase-3–positive cells within seminiferous tubules were significantly increased in all DEHP-exposed groups relative to controls.
- These findings suggest that short-term DEHP exposure disrupts spermatogenesis and induces testicular injury, highlighting the need for further studies to understand the molecular mechanisms and potential sex-specific reproductive effects.

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ACKNOWLEDGEMENTS

This research was supported by Midwestern University Physiology Department Startup Research Funds for EJ.