Behavioral and Reproductive Consequences of Embryonic Exposure of *Caenorhabditis elegans* to Low doses of Bisphenol A and Its Subsitutes

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INTRODUCTION

- Bisphenol A (BPA) is a widely used endocrine-disrupting compound associated with adverse developmental, reproductive and neurobehavioral effects.
- Its structural substitutes, including bisphenol AF (BPAF), bisphenol F (BPF) and bisphenol S (BPS), are increasingly used as alternatives, although their biological safety remains insufficiently clarified.
- *Caenorhabditis elegans* provides a sensitive *in vivo* model for evaluating low-dose toxicity across developmental, reproductive and behavioural endpoints.
- In this study, we used two cuticle-related mutants, *dpy-13* and *bli-1*, which are defective in cuticle integrity.
- Because *C. elegans* cuticle acts as a protective extracellular barrier, cuticle-defective mutants may reveal genotype-dependent sensitivity that is less evident in the reference N2 strain.

AIMS

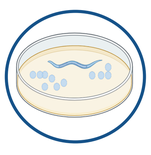
To evaluate how specific cuticle collagens affect substance penetration and organism sensitivity, linking structural matrix changes to the biological effects of bisphenols.

Specific objectives:

- To compare the effects of BPA, BPAF, BPF and BPS in *C. elegans* N2, *dpy-13* and *bli-1*.
- To assess reproductive and neurobehavioral endpoints after embryonic exposure.
- To identify mutant strains with increased sensitivity suitable for endocrine disruptor screening.

METHODS

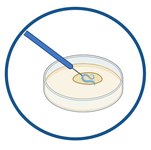
Synchronized embryos were exposed to BPA, BPAF, BPF and BPS (0.5, 1 and 5 µM) for 4 hours, followed by post-exposure cultivation and endpoint assessment.



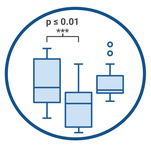
1 **Synchronized embryos**
N2 *dpy-13* *bli-1*

2 **Exposure (4 hours)**
BPA BPAF BPF BPS
0.5 1 5 µM

3 **Post-exposure handling**
Washing + cultivation on NGM plates



4 **Endpoints assessed**
Hatching Oviposition Thrashing
Habituation



5 **Statistical analysis**
Wilcoxon test + Dunn's multiple comparisons

RESULTS

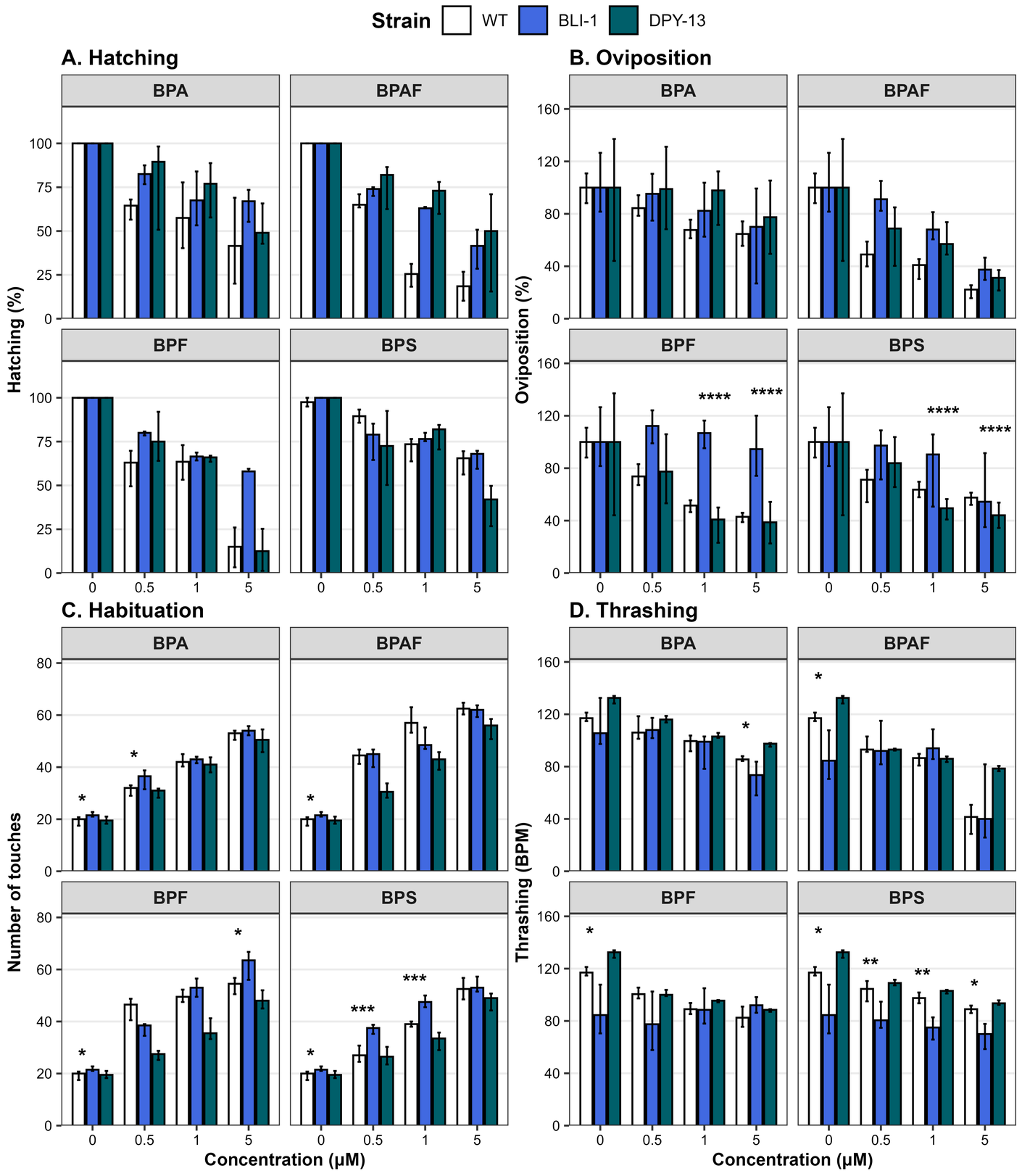


Figure 1. *Comparative effects of BPA and its analogues on hatching (A), oviposition (B), habituation (C), and thrashing (D) in C. elegans WT (N2), bli-1, and dpy-13 strains at concentrations 0.5, 1 a 5 µM. Bars represent median values and error bars indicate interquartile range (IQR). Asterisks indicate significant differences (p ≤ 0.05) between mutant strains and WT within the same chemical and concentration and are shown only when the mutant exhibited the adverse direction of change relative to WT. Oviposition is expressed as % of the corresponding within-strain baseline (0 µM).*

Key findings:

- Embryonic exposure to BPA and its analogues altered hatching, oviposition, habituation, and thrashing in a strain- and compound-dependent manner
- ***dpy-13*** showed the strongest sensitivity in **oviposition** and **hatching**
- ***bli-1*** was the most sensitive in **habituation**
- Neurobehavioral endpoints indicated that bisphenol analogues induced effects comparable to, and in some cases stronger that, BPA
- The response patterns differed across endpoints, supporting endpoint-specific genotype sensitivity

CONCLUSION

Low-dose bisphenol exposure induced strain- and endpoint-specific effects in *C. elegans*. Within the selected strains, *dpy-13* showed the highest sensitivity in reduced reproductive capacity, whereas *bli-1* displayed the most pronounced changes in habituation. Overall, BPA analogues produced effects comparable to, and in several cases stronger than, BPA, supporting the use of selected mutant strains as sensitive models for bisphenol toxicity screening.

This research was supported by the Agency for the Support of Research and Development in contract APVV-24-0093, the Cultural and Educational Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic (KEGA 013UCM-4/2025).

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