

Comparable neurotoxicity of bisphenol S and bisphenol A and its mitigation by α -lipoic acid: insights into behavior, regeneration, cardiovascular function, and oxidative stress in *Planaria* and *Daphnia*

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Research Article

Keywords: bisphenol S, bisphenol A, neurotoxicity, endocrine disruptors, *Planaria*, oxidative stress, α -lipoic acid

Posted Date: September 24th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10414411/v1>

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Abstract

Bisphenol A (BPA) is a ubiquitous plasticizer with well-documented neurotoxicity, and its regulation has driven a shift toward bisphenol S (BPS), an analogue marketed as a safer substitute yet far less studied in the neurological context. This study evaluated whether BPS is comparably neurotoxic and whether α -lipoic acid (ALA) can mitigate its effects, using *Planaria* as a model for neurotoxicity and regeneration and *Daphnia* as a model for cardiovascular toxicity. *Planaria* were exposed to 4.6–50 ppm BPA or BPS and assessed for morphology, phototactic behavior, regeneration, and oxidative stress (malondialdehyde and total glutathione); *Daphnia* heart rate was quantified by videography. BPS produced severe, concentration-dependent effects, including loss of pigment and eyes, tissue disintegration, reduced negative phototaxis, delayed regeneration, elevated lipid peroxidation, depleted antioxidant capacity, and bradycardia. Critically, no statistically significant difference was detected between BPS and BPA in photophobic behavior or lipid peroxidation, indicating that BPS is not demonstrably safer than the compound it replaced. Post-exposure ALA significantly improved regeneration, behavior, and oxidative stress and restored pigmentation and eye structures. These findings challenge the assumption that BPS is a benign alternative and identify ALA as a promising mitigation strategy, with direct relevance to consumer safety and chemical regulation.

Highlights

- BPS caused severe, concentration-dependent neurotoxicity in *Planaria* and *Daphnia*.
- BPS effects on behavior and lipid peroxidation were statistically indistinguishable from BPA.
- BPS reduced *Daphnia* heart rate from 200 to 34 bpm and impaired planarian regeneration.
- α -Lipoic acid reversed oxidative, behavioral, and regenerative damage after exposure.
- Findings challenge the safety rationale for BPS and support tighter regulation.

1. Introduction

Over 90% of Americans have detectable levels of bisphenol A (BPA) in their bodies, and the regulatory shift toward bisphenol S (BPS) has not alleviated concern about neurotoxic exposure (Toxic-Free Future 2024). BPA is an estrogenic endocrine disruptor and membrane-receptor antagonist that influences numerous cellular pathways, and since the 1990s more than a thousand studies have documented its harm to the reproductive tract, prostate, mammary glands, metabolic system, and brain structure and function, prompting bans beginning in the early 2000s (Vom Saal and Vandenberg 2021). In the brain specifically, BPA exposure drives molecular and structural change, alters gene and protein expression, elevates free-radical production, and has been linked to anxiety, autism spectrum disorder, and neurodegenerative processes including apoptosis, a particular threat given the limited capacity of the human brain for neurogenesis (Costa and Cairrão 2024; Fouyet et al. 2021).

As BPA has been restricted, BPS has become its dominant replacement, with over 80% of the global population now exposed, primarily through thermal receipts, and with BPS accounting for a rapidly rising share of thermal paper worldwide (Alharbi et al. 2022; Naderi and Kwong 2020). Despite this prevalence, BPS remains comparatively under-researched in the neurological context. The available evidence is not reassuring: BPS shows placental toxicity, metabolic and endocrine disruption, and DNA-damage-related effects comparable to or exceeding those of BPA, albeit sometimes through distinct pathways (Naderi and Kwong 2020; Thoene et al. 2020). This positions BPS as a regrettable substitution whose adoption has outpaced the evidence for its safety.

Two model organisms are well suited to interrogate these effects. Planaria have served neurotoxicology for over two centuries, offering rapid regeneration driven by pluripotent neoblasts, a centralized nervous system with mammalian-like neurotransmitters, characteristic and quantifiable gaits, and robust phototactic behavior that provides measurable behavioral endpoints (Elliott and Sánchez Alvarado 2013; Hagstrom et al. 2016; Ireland and Collins 2022). Daphnia extend the analysis to whole-organism and cardiovascular toxicity: their transparency and myogenic heart permit direct, real-time quantification of heart rate, and they are established sentinels for aquatic contaminants (Ebert 2005; Eghan et al. 2023; Kwon et al. 2021). Together they allow neurotoxicity to be probed from the cellular level through behavior to systemic cardiovascular function.

Mitigation of bisphenol toxicity remains a critical gap. α -Lipoic acid (ALA), an endogenous antioxidant, has demonstrated neuroprotective effects across Parkinson's disease, memory impairment, and oxidative damage, and the few studies examining ALA against BPA report strong reductions in oxidative stress and restoration of neurochemical balance through the Nrf2 pathway (Fahmy et al. 2024; Khan et al. 2018; Wu et al. 2021, 2023). However, no study has tested ALA against BPS-induced neurotoxicity. A complementary preventive strategy is enzymatic degradation of bisphenols prior to exposure, with horseradish peroxidase and laccases showing high efficiency against BPA (Daâssi et al. 2016; Ohore and Zhang 2019).

This study therefore addresses the following question: how does exposure to BPA and BPS affect oxidative stress, behavior, and regeneration in Planaria and cardiovascular function in Daphnia, and can α -lipoic acid mitigate these effects? We hypothesized that BPS would produce neurotoxic effects comparable to BPA at environmentally relevant concentrations, and that ALA would attenuate those effects following exposure.

2. Materials and Methods

2.1 Model organisms and maintenance

Brown planaria (*Dugesia dorotocephala*; syn. *Girardia dorotocephala*) and *Daphnia magna* were obtained from Carolina Biological Supply (Burlington, NC, USA). Planaria were acclimated to laboratory conditions for seven days prior to experimentation, a starvation and acclimation period consistent with established

planarian protocols (Dean and Duncan 2020; Deochand et al. 2018). During acclimation and throughout the study, planaria were maintained in Carolina proprietary spring water at pH 6.8–7.2 and approximately 22°C in darkness. Daphnia were maintained in the culture medium in which they were shipped, at room temperature and in darkness. Animals were sorted into treatment groups of 8–10 planaria and 20–25 Daphnia each. Health was monitored daily and abnormalities were documented.

2.2 Chemical preparation and exposure

Stock solutions of BPA and BPS were prepared in spring water at concentrations from 4.6 ppm (the EFSA-referenced level) to 100 ppm (an upper bound representing extreme exposure), with intermediate concentrations of 13 ppm and 50 ppm selected to reflect environmentally relevant and commonly studied levels. Masses were measured on a calibrated analytical balance and solutions were diluted and mixed to ensure accuracy. Planaria were exposed for 24–48 h before assessment; Daphnia were exposed for 24 h at 4.6 and 13 ppm BPS.

2.3 Regeneration assay

Following exposure, each planarian was sectioned transversely with a sterile razor blade approximately one-third of the body length from the anterior end. Head and tail fragments were transferred to individual dishes of spring water for independent regeneration. Regeneration was scored daily by photographic documentation over a two-week period, recording the time to visible blastema formation and to complete morphological restoration. Unexposed animals served as controls.

2.4 Behavioral testing (light maze)

Phototactic behavior was assessed 24–48 h before and after exposure using a custom light maze (dimensions in Supplementary Fig. S1). Two endpoints were recorded: the time required to reach the dark zone, expressed as a light-maze score (percentage of animals reaching the dark zone), and gait classification (gliding, scrunching, twisting/kinetotaxis). The paradigm exploits the established negative phototaxis of planaria (Paskin et al. 2014; Qian et al. 2023).

2.5 Oxidative stress assays

Oxidative stress in planaria was quantified using commercial colorimetric assay kits according to the manufacturers' protocols. Lipid peroxidation was measured as malondialdehyde (MDA) using the Malondialdehyde (MDA) Colorimetric Assay Kit (Invitrogen/Thermo Fisher Scientific, catalog EEA015), in which MDA reacts with thiobarbituric acid to form a colored adduct read spectrophotometrically at 532 nm against an MDA calibration curve (Ohkawa et al. 1979). Tissue was homogenized in phosphate-buffered saline (PBS) prior to assay. Glutathione was measured using the Glutathione Assay Kit (Cayman Chemical, item 703002), an enzymatic recycling assay employing glutathione reductase and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) to generate 5-thio-2-nitrobenzoic acid, read at 412 nm (Tietze 1969). Because this kit recycles glutathione via glutathione reductase, the values reported reflect total glutathione; samples were deproteinated prior to assay per the manufacturer's protocol. Lower glutathione indicates greater oxidative stress.

2.6 Daphnia cardiovascular and phenotypic assessment

Daphnia were imaged on a digital microscope. Heart rate was quantified from one-minute video recordings by counting beats over four 15-s intervals, which were averaged and scaled to beats per minute (Kwon et al. 2021). Phenotypic changes, including swelling and loss of pigmentation, were documented from the same recordings.

2.7 α -Lipoic acid treatment

Following BPS exposure, ALA was applied to exposed planaria groups at 10 μ M and 50 μ M. Regeneration, behavior, and oxidative stress were monitored over the subsequent week using the methods above to evaluate mitigation.

2.8 Statistical analysis

Data were analyzed using Student's t-tests for pairwise comparisons and one-way ANOVA with post-hoc Tukey HSD for multi-group comparisons. Significance was set at $\alpha = 0.05$. Values are reported as means $\pm$ standard error (SE). Analyses were performed in Microsoft Excel, Google Sheets, and GraphPad Prism.

3. Results

3.1 BPS exposure caused concentration-dependent morphological damage in planaria

BPS produced severe loss of pigment and tissue at environmentally relevant concentrations, progressing to disintegration and death at higher concentrations and longer exposures. At 4.6 ppm for 24 h, planaria showed marked depigmentation, loss of eyes, and a more translucent epidermis relative to controls (Fig. 1A–B). At 4.6 ppm for 48 h, tissue disintegration was evident (Fig. 1C), and mortality reached 40% at higher concentrations.

3.2 BPS exposure altered phototactic behavior and gait

The light maze revealed reduced negative phototaxis with increasing BPS concentration, accompanied by restricted movement, reduced agility, and increased scrunching (Fig. 2A–C). Abnormal twisting and freezing under bright light, together with increased thigmotaxis, were also observed. Light-maze scores declined with concentration for both bisphenols (Fig. 2D). A t-test comparing BPA and BPS light-maze scores was not significant ($t = 1.032$, $p > 0.05$), indicating comparable behavioral effects.

3.3 BPS exposure delayed regeneration and induced oxidative stress

Regeneration slowed with increasing BPS concentration and exposure duration, with effects more pronounced at 48 h than 24 h (Fig. 3A–B). Because planarian regeneration depends on neoblast differentiation into neurons, this delay reflects a neurological component of toxicity. MDA increased with concentration for both bisphenols (Fig. 3C), indicating greater lipid peroxidation, while total glutathione decreased (Fig. 3D), indicating depleted antioxidant capacity. Each bisphenol differed significantly from control. The BPA-versus-BPS comparison for lipid peroxidation was not significant ($t = 1.706$, $p > 0.05$).

3.4 BPS reduced heart rate and altered phenotype in *Daphnia*

BPS caused pronounced swelling and clearing (loss of pigmentation) in *Daphnia* (Fig. 4A–C), paralleling the tissue effects seen in planaria. Heart rate decreased sharply with increasing BPS concentration (Fig. 4D), from 200 bpm in controls to 71 bpm at 4.6 ppm and 34 bpm at 13 ppm (ANOVA $F = 915$, $p < 0.05$; Tukey HSD significant). Some *Daphnia* eggs failed to hatch and offspring showed sustained clearing, indicating effects extending across generations.

3.5 α -Lipoic acid mitigated BPS toxicity in planaria

Post-exposure ALA treatment significantly improved outcomes across all endpoints: regeneration rate ($t = -4.02$, $p < 0.05$), light-maze score ($t = 2.45$, $p < 0.05$), and oxidative stress (MDA; $t = -6.47$, $p < 0.05$) (Fig. 5A–B). ALA restored pigmentation and eye structures in exposed planaria (Fig. 5C). Recovery was concentration-dependent, with 50 μM ALA producing greater improvement than 10 μM . Severely swollen *Daphnia* largely did not survive and could not be assessed for recovery.

4. Discussion

The central finding of this study is that bisphenol S, adopted as a safer replacement for bisphenol A, produced neurotoxic effects that could not be statistically distinguished from those of BPA on the endpoints tested. This absence of a detectable difference is itself the result of consequence. Across photophobic behavior ($t = 1.032$, $p > 0.05$) and lipid peroxidation ($t = 1.706$, $p > 0.05$), BPS matched BPA rather than improving upon it, and a post-hoc Tukey HSD likewise failed to separate the two compounds. It is important to frame this precisely: failing to reject the null hypothesis does not prove the two bisphenols are identical, and a small-sample design has limited power to detect modest differences. What the data do support is the more actionable conclusion that BPS shows no demonstrable safety advantage over the compound it was meant to replace, which is sufficient to challenge the rationale for its widespread substitution.

The morphological and behavioral results indicate that this toxicity operates at the level of the nervous system. Depigmentation, eye loss, and epidermal translucency at only 4.6 ppm point to cellular and tissue breakdown, and the accompanying behavioral shifts—slower movement, increased scrunching resembling peristalsis, kinetotactic twisting, and reduced negative phototaxis—implicate impaired

neuronal signaling and ciliary function rather than morphological damage alone. Because planaria depend on neoblast differentiation to rebuild neural tissue, the concentration- and time-dependent delay in regeneration provides a second, independent line of evidence for neurological injury: the same process that would repair the nervous system is itself impaired. The oxidative stress data supply a plausible mechanism, with elevated MDA indicating lipid peroxidation and membrane breakdown and depleted GSH indicating exhausted antioxidant defense, a combination consistent with the free-radical-mediated neurotoxicity previously described for BPA.

The *Daphnia* results extend these effects beyond the nervous system to systemic physiology. The steep, concentration-dependent fall in heart rate indicates disruption of cardiovascular function and, by extension, of the autonomic control and homeostasis on which it depends, while the failure of eggs to hatch and the persistent clearing of offspring suggest consequences that carry across generations. Taken together with the planarian data, this describes a toxicant whose reach spans the cellular, behavioral, and systemic levels in two phylogenetically distinct organisms, strengthening the inference that comparable effects could occur in humans, who lack the regenerative capacity that partially buffers these models.

The mitigation results are the most immediately translatable. α -Lipoic acid, applied after exposure, significantly improved regeneration, behavior, and lipid peroxidation and restored pigmentation and eye structures, consistent with its known action as an Nrf2-activating antioxidant. That a single endogenous compound reversed damage across three distinct endpoints suggests that oxidative stress is a central node in bisphenol neurotoxicity and a viable therapeutic target. The concentration dependence of recovery further supports a genuine dose-response relationship rather than incidental improvement. These findings position ALA as a candidate intervention worth testing in higher models, and they complement the preventive strategy of enzymatic bisphenol degradation discussed below.

5. Limitations

This study was conducted in a school laboratory with a modest sample size (8–10 planaria per group), and a single study cannot establish equivalence between bisphenols; the non-significant BPA-versus-BPS comparisons should be read as an absence of detectable difference under these conditions rather than as proof of equivalence. Larger samples and an extended concentration range would allow identification of the mortality plateau and improve statistical power. The mitigation results, while consistent across endpoints, are preliminary and warrant dose-optimization. Standard handling and disposal procedures were followed throughout.

6. Future Directions

Priorities include extending these findings from invertebrate models toward mammalian neuronal cultures, optimizing the timing and dose of α -lipoic acid for post-exposure remediation, and testing enzymatic degradation of BPS as a preventive measure. The latter would follow the approach outlined

for horseradish peroxidase and laccase against BPA, with attention to degradation byproducts and their toxicity: reaction mixtures of BPA/BPS with horseradish peroxidase (0.5–2.0 U/mL) or laccase (1.0–3.0 U/mL) in neutral buffer, incubated at 25°C over 0–120 min, with residual bisphenol quantified by UV-Vis spectrophotometry against calibration curves. Together, a post-exposure antioxidant strategy and a pre-exposure degradation strategy would address both existing body burden and future exposure.

7. Conclusion

Bisphenol S, marketed as a safer alternative to bisphenol A and now pervasive in receipts and plastics, produced profound neurotoxic effects in *Planaria* and *Daphnia*, including loss of tissue and eyes, altered behavior, delayed regeneration, elevated oxidative stress, and cardiac depression. Critically, its effects on behavior and lipid peroxidation could not be statistically distinguished from those of BPA, indicating that BPS offers no demonstrable safety advantage over the compound it replaced. Post-exposure α -lipoic acid significantly mitigated these effects and restored damaged structures, identifying a promising remediation strategy. These findings argue for stricter regulation of bisphenol analogues, caution against regrettable substitution, and further investigation of both antioxidant remediation and enzymatic degradation.

Declarations

Ethical Approval

This study used the freshwater invertebrate models *Dugesia dorotocephala* (planaria) and *Daphnia magna*. These invertebrates are not subject to institutional or national research ethics committee approval for animal experimentation, and no such approval was required. All organisms were handled and disposed of following standard laboratory safety procedures.

Consent to Participate

Not applicable. This study did not involve human participants.

Consent to Publish

Not applicable. This study did not involve human participants or individual person data.

Competing Interests

The author has no relevant financial or non-financial interests to disclose.

Funding

The author declares that no funds, grants, or other support were received during the preparation of this manuscript.

Author Contributions

M.G. was solely responsible for the study conception and design, material preparation, data collection and analysis, and writing of the manuscript. The author read and approved the final manuscript.

Acknowledgements

The author thanks FCS Innovation Academy for providing laboratory access and facilities that supported this research.

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information (Appendix). Additional raw data are available from the corresponding author on reasonable request.

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Appendix

A. Regeneration data

Table A1				
Planaria regeneration with 24-h BPS exposure (number regenerated out of 5; averaged across trials).				
Day	Control	BPS 4.6 ppm	BPS 13 ppm	BPS 50 ppm
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	1	0	0	0
5	2	1	0	1
6	4	3	2	1
7	5	4	3	2
8	5	4	3	2
9	5	5	4	3

Table A2
Planaria regeneration with 48-h BPS exposure (number regenerated out of 5).

Day	Control	BPS 4.6 ppm	BPS 13 ppm	BPS 50 ppm
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	1	0	0	0
5	2	1	1	0
6	4	2	2	1
7	5	3	2	1
8	5	4	3	2
9	5	5	3	2

Table A3
Planaria regeneration with 24-h BPS exposure and ALA treatment (averaged across trials).

Day	Control	BPS 50 ppm	BPS 50 + 10 μ M ALA	BPS 50 + 50 μ M ALA
1	0	0	0	0
3	0	0	0.75	1
5	2	1	2.5	3
7	5	2	4	4.5
9	5	3	4.75	5

B. Oxidative stress data

Table A4
MDA levels (nmol/mg protein).

Group	0 ppm	4.6 ppm	13 ppm	50 ppm
Control	0.25	–	–	–
BPA	–	0.65	0.85	1.10
BPS	–	0.45	0.60	0.75
BPS + 10 μ M ALA	–	–	–	0.65
BPS + 50 μ M ALA	–	–	–	0.50

Table A5
GSH levels ($\mu\text{mol}/\text{mg}$ protein).

Group	0 ppm	4.6 ppm	13 ppm	50 ppm
Control	4.92	–	–	–
BPA	–	2.77	1.94	1.26
BPS	–	2.19	1.48	1.32

C. Light-maze scores

Table A6
Light-maze scores (percentage in dark zone).

Concentration	Group	Score (%)
0 ppm	Control	90
4.6 ppm	BPA	50
13 ppm	BPA	40
50 ppm	BPA	25
4.6 ppm	BPS	65
13 ppm	BPS	50
50 ppm	BPS	35
50 ppm	BPS + 10 μM ALA	50
50 ppm	BPS + 50 μM ALA	70

D. Daphnia heart rate

Table A7
Daphnia heart rate (bpm, manual counting).

Group	Heart rate (bpm)	SE	Example raw values
Control	200	–	–
4.6 ppm BPS	71	≈ 2.9	65, 71, 75
13 ppm BPS	34	≈ 2.5	31, 37, 34

Table A8
ALA remediation summary.

Group	MDA (nmol/mg)	Light-maze (%)	Regeneration (Day 9, /5)
Control	0.25	90	5
BPS 50 ppm	0.75	35	3
BPS 50 + 10 μ M ALA	0.65	50	4.75
BPS 50 + 50 μ M ALA	0.50	70	5

Figures

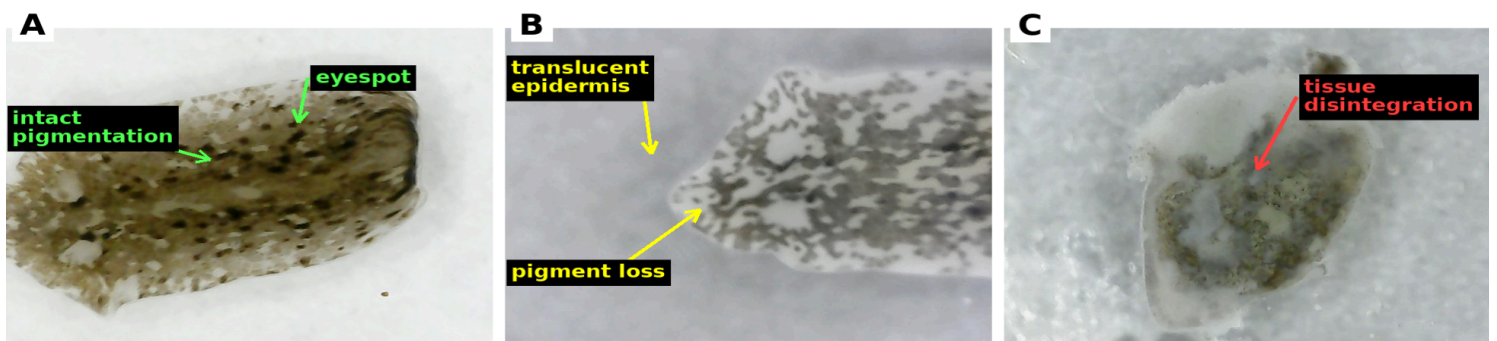
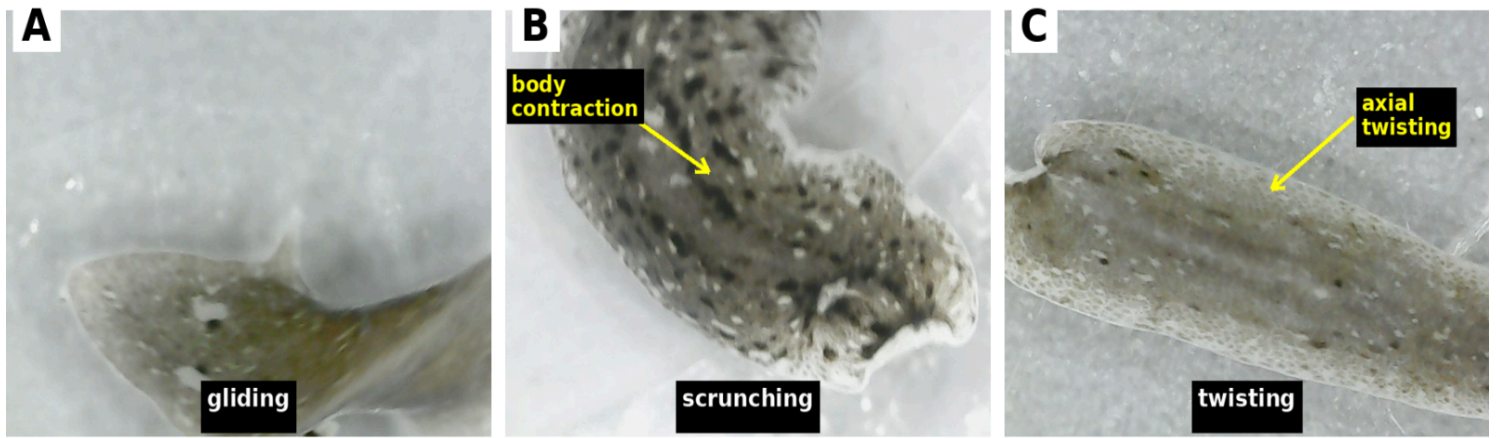


Figure 1

Concentration-dependent morphological damage in planaria. (A) Healthy control with intact pigmentation and eyespot. (B) After 24 h at 4.6 ppm BPS, showing pigment loss and translucent epidermis. (C) After 48 h at 4.6 ppm BPS, showing tissue disintegration. Arrows indicate affected features.



D

Light Maze Score based on Neurotoxin Exposure

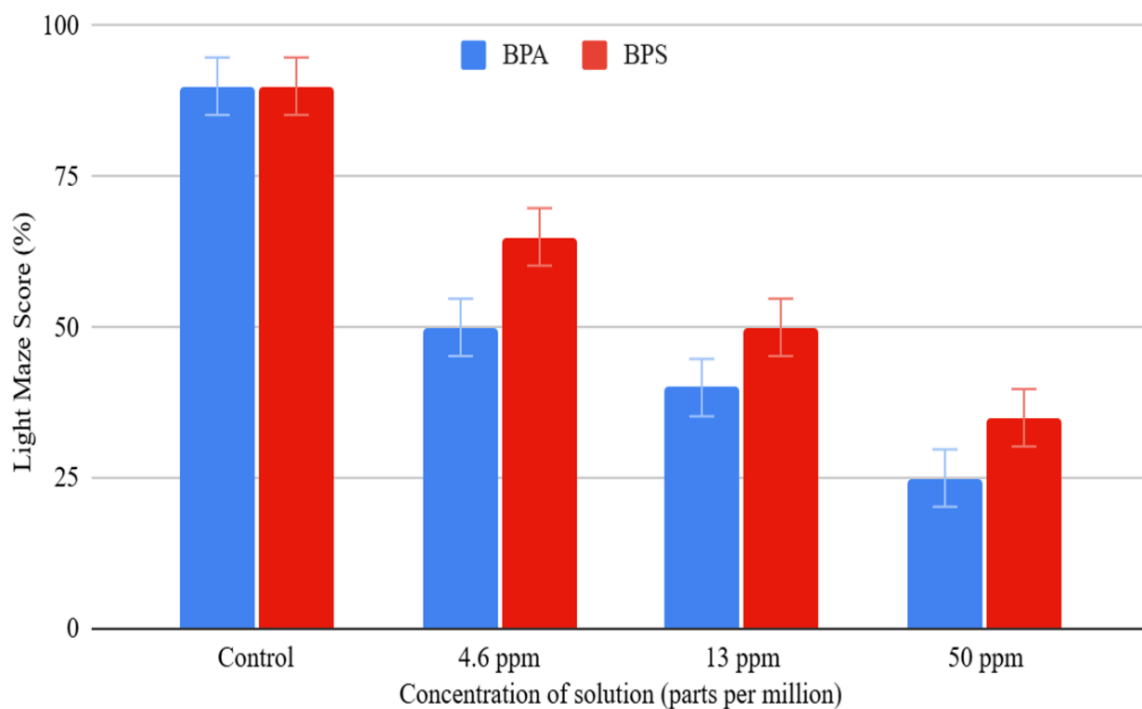


Figure 2

Behavioral alterations from BPS exposure. (A–C) Representative gaits: gliding (normal), scrunching (body contraction), and axial twisting (kinetotaxis). (D) Light-maze scores (percentage reaching the dark zone) for BPA and BPS across concentrations; error bars show $\pm$ SE. The BPA-versus-BPS comparison was not significant ($t = 1.032$, $p > 0.05$).

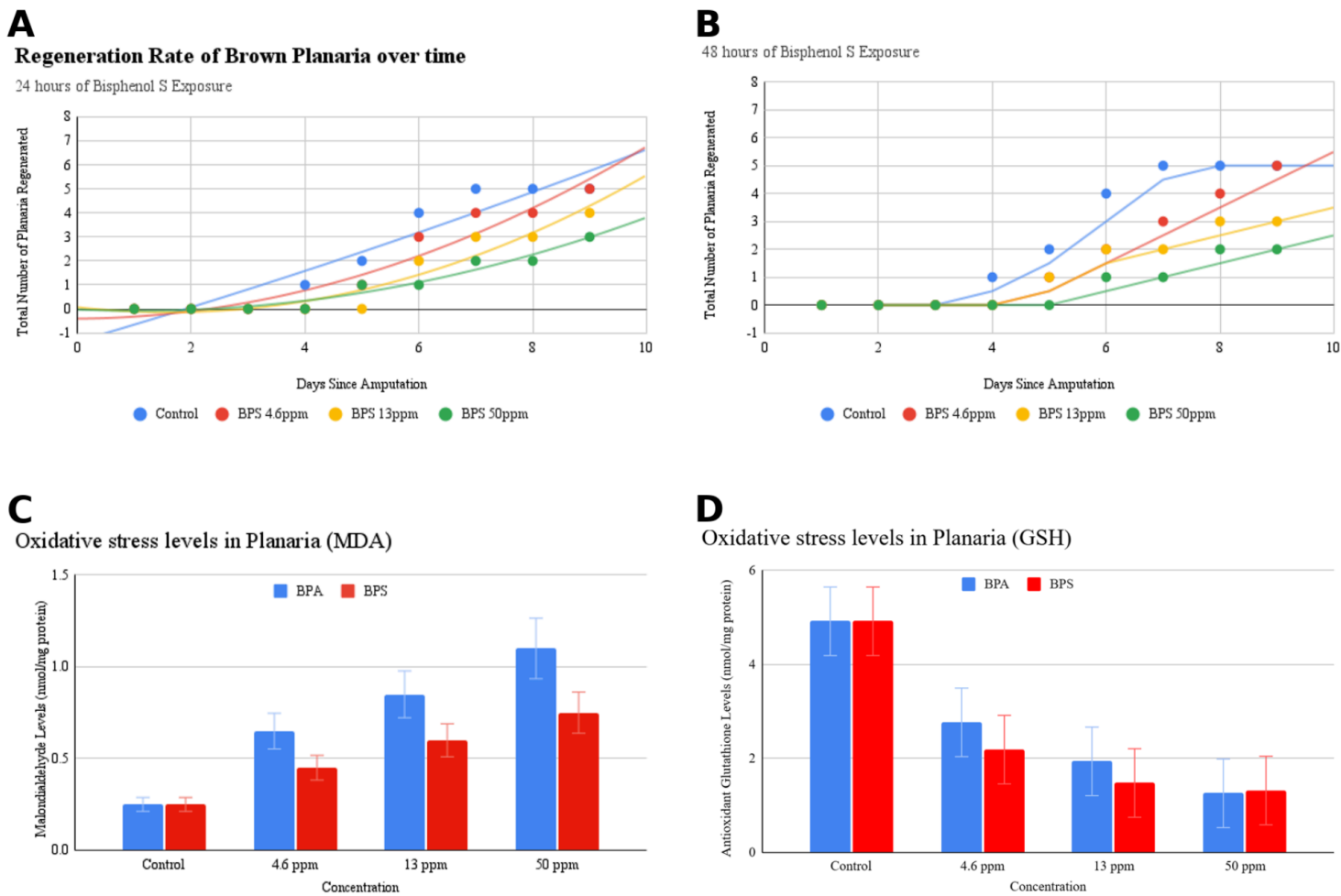


Figure 3

Delayed regeneration and oxidative stress in planaria. (A–B) Regeneration (number regenerated over days since amputation) after 24 h and 48 h BPS exposure across concentrations. (C) Malondialdehyde (MDA) and (D) total glutathione levels after BPA/BPS exposure; error bars show $\pm$ SE (MDA $t = 1.706$, GSH $t = 1.520$; each bisphenol differs from control, $p < 0.05$; BPA vs BPS not significant, $p > 0.05$).

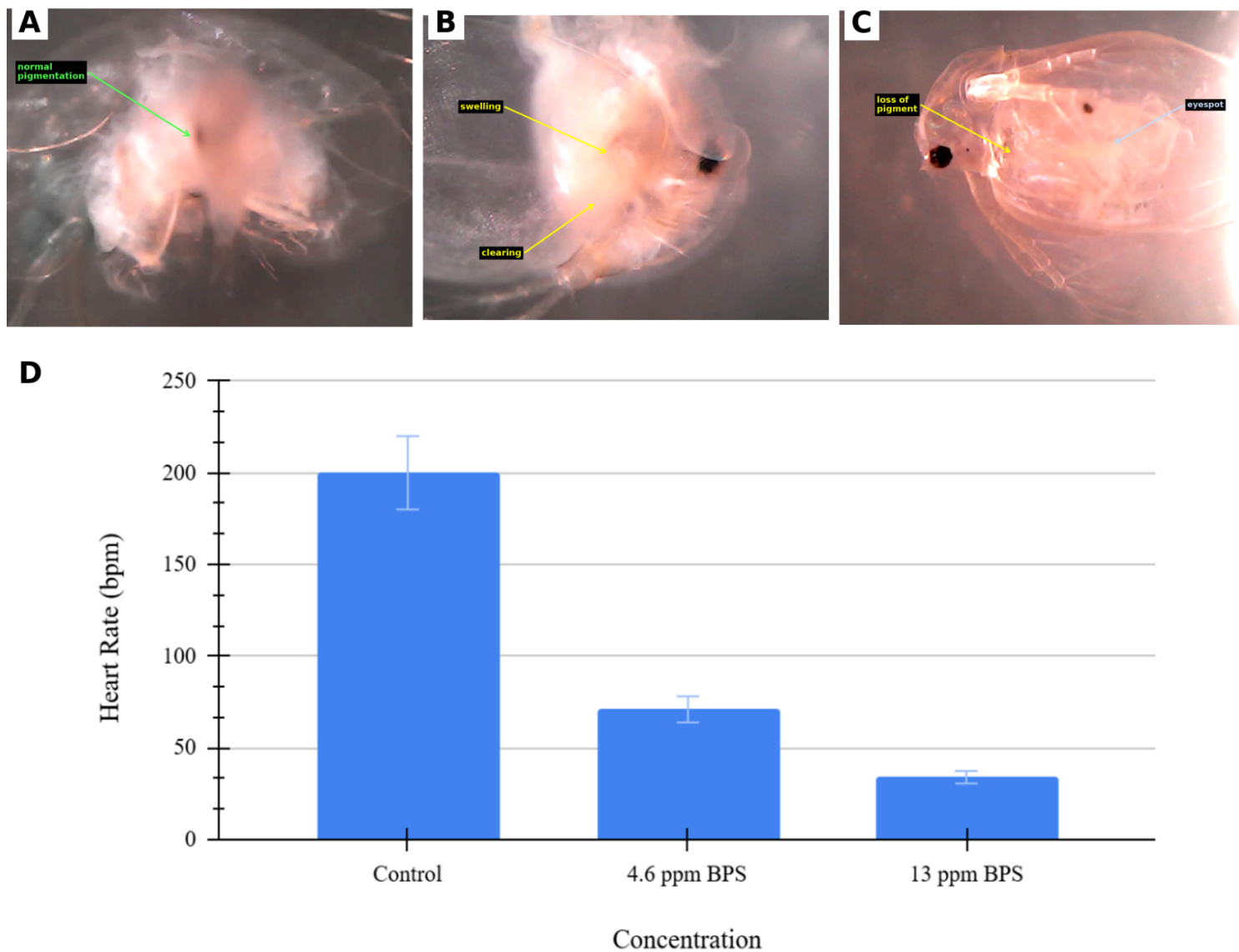
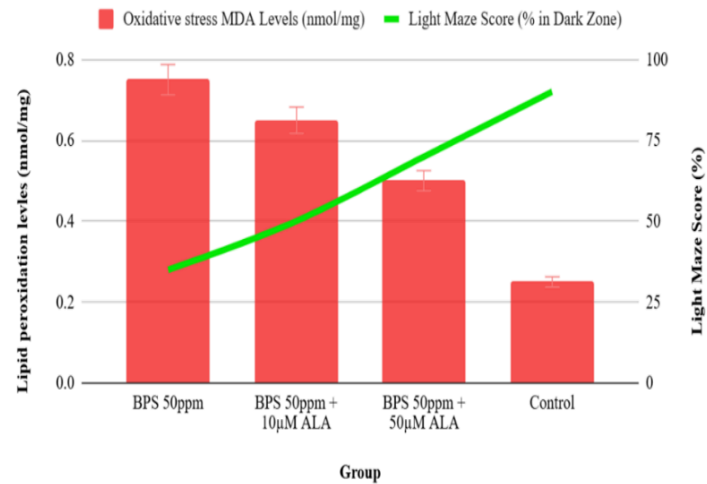


Figure 4

Cardiovascular and phenotypic effects in *Daphnia*. (A) Healthy control with normal pigmentation. (B–C) After 24 h at 4.6 ppm BPS, showing swelling, clearing, and loss of pigment; the eyespot is indicated for orientation. (D) Heart rate across BPS concentrations; error bars show $\pm$ SE (ANOVA $F = 915$, $p < 0.05$).

A

Improvement in Planarian health through Light Maze scores and reduced oxidative stress with ALA treatment



B

Development of Regeneration Rate of Brown Planaria over time with ALA treatment

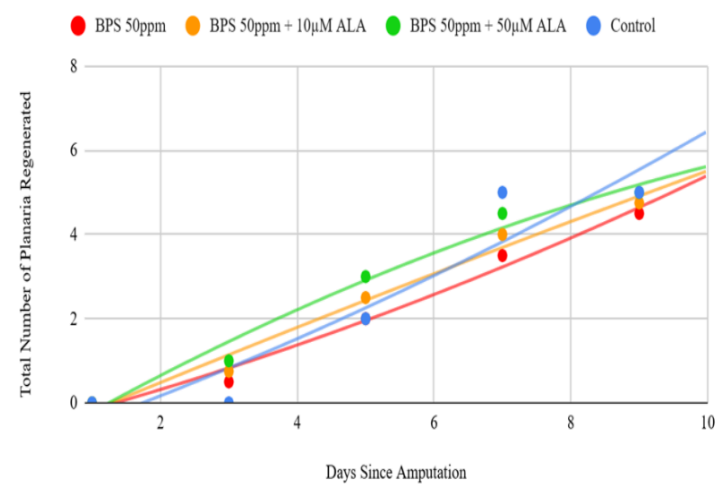


Figure 5

Mitigation of BPS toxicity by α-lipoic acid (ALA) in planaria. (A) MDA levels (bars) and light-maze scores (line) across BPS and BPS + ALA groups. (B) Regeneration over time for BPS 50 ppm with 10 μM and 50 μM ALA versus control. (C) Restored pigmentation following ALA treatment.

Supplementary Files

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- [GraphicalAbstract.docx](#)
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