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Decoding hidden toxic interactions of paraquat and 2,4-dichlorophenoxyacetic acid in *C. elegans*

Rita Azevedo^{1,2,3,4,*}, Renata Silva^{3,4} and Daniel José Barbosa^{1,2}

¹ Associate Laboratory i4HB-Institute for Health and Bioeconomy, University Institute of Health Sciences IUCS-CESPU, Gandra, Portugal

² UCIBIO-Applied Molecular Biosciences Unit, Translational Toxicology Research Laboratory, University Institute of Health Sciences (IH-TOXRUN, IUCS-CESPU), Gandra, Portugal

³ Associate Laboratory i4HB-Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal

⁴ UCIBIO-Applied Molecular Biosciences Unit, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Porto, Portugal

* Correspondence: up202409826@up.pt

Abstract

Background: The widespread use of herbicides in modern agriculture, particularly paraquat (PQ) and 2,4-dichlorophenoxyacetic acid (2,4-D), raises significant concerns for human health and environmental safety [1]. Although the toxicological profiles of these compounds are well-documented individually, real-world exposures typically involve mixtures, making it essential to assess their combined effects, which may differ substantially from single-compound toxicity [1]. To address this knowledge gap, this study aimed to investigate the toxicological interactions between PQ and 2,4-D and to evaluate the potential protective role of *N*-acetylcysteine (NAC) using *Caenorhabditis elegans* as a discovery platform. **Methods:** Synchronized L1-stage animals of the DC19 strain [*bus-5(br19)*] (~200/condition) were exposed, in liquid medium, to increasing concentrations of PQ (0-10 mM) and 2,4-D (0-40 mM), either individually or in combination, for 72 h [2]. The survival rate was assessed by counting the number of live and dead worms after the exposure period. Using a sublethal concentration (0.5 mM), we further explored the influence of herbicides, either individually or in combination, on (1) animal development, lifespan and reproductive fitness [3]. **Results:** PQ and 2,4-D reduced animal survival in a concentration-dependent manner, with significant effects observed at concentrations ≥ 1 mM for PQ and ≥ 10 mM for 2,4-D ($p < 0.001$). Notably, NAC prevented the reduction in animal survival caused by both herbicides, showing a more pronounced protective effect against PQ-induced toxicity. In co-exposure experiments, the effects of PQ (5 mM) on animal survival were partially restored in the presence of 2,4-D (0.5 mM), and conversely, the effects of 2,4-D (25 mM) were also ameliorated by PQ (0.5 mM). No significant changes in animal development progression were observed for PQ or 2,4-D, either individually or in combination. Nevertheless, animal lifespan was significantly reduced across all exposed groups. **Conclusions:** These findings indicate that co-exposure to PQ and 2,4-D elicits significant toxicological interactions, emphasizing the importance of assessing pesticide mixtures under environmentally relevant exposure conditions. The protective effects of NAC on animal survival further support the involvement of oxidative stress-mediated mechanisms. Additionally, the observed reduction in animal lifespan highlights the potential long-term detrimental effects of these herbicides.

Keywords: paraquat; 2,4-dichlorophenoxyacetic acid; *C. elegans*

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References

1. Badjabaissi, S. et al. Neurotoxic Effects of Pesticide Mixtures: A Systematic Review of the Experimental Literature. *OALib* 2025, 12, 1-16. doi:10.4236/oalib.1114002.

2. Barbosa, D.J. et al. *C. elegans* as a powerful model for neurotoxicity assessment. *Neurotoxicology* **2025**, *110*, 85-110. doi:10.1016/j.neuro.2025.07.009.
3. Mendes, C. et al. Synthetic cathinones induce developmental arrest, reduce reproductive capacity, and shorten lifespan in the *C. elegans* model. *J Xenobiot* **2025**, *15*, 33, doi:10.3390/jox15010033.



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