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Emerging Pollutants: Protecting Water Quality for the Health of People and the Environment

Mutagenicity evaluation of the rubber tire oxidant by product, 6PPD quinone, using the Ames assay

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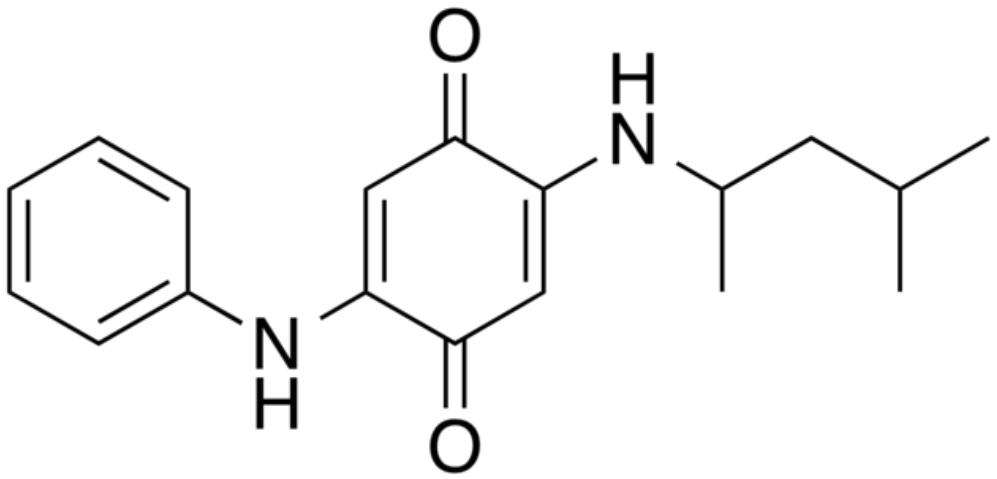
6PPD-quinone



N-(1,3-Dimethylbutyl)-N’-phenyl-p-phenylenediamine-quinone

is a transformation product of the rubber tire antioxidant, 6PPD.

It reaches the aquatic environment by stormwater and urban runoff [1].

[1] Mahoney, H, Junior, FCS, Roberts, C, Schultz, M, Ji, X, Alcaraz, AJ, Montgomery, D, Selinger, S, Challis, JK, Giesy, JP, Weber, L, Janz, D, Wiseman, S, Hecker, M, Brinkmann, M. 2022. Exposure to the tire rubber-derived contaminant 6PPD-quinone causes mitochondrial dysfunction in vitro. Environ Sci Technol Lett 9(9):765–771

6PPD-quinone studies

- There is limited information about the toxicity of 6PPD quinone but it is already considered a relevant emerging contaminant because low (ppb) concentrations are correlated with fish mortality (especially trout and salmon) in urban areas [2].

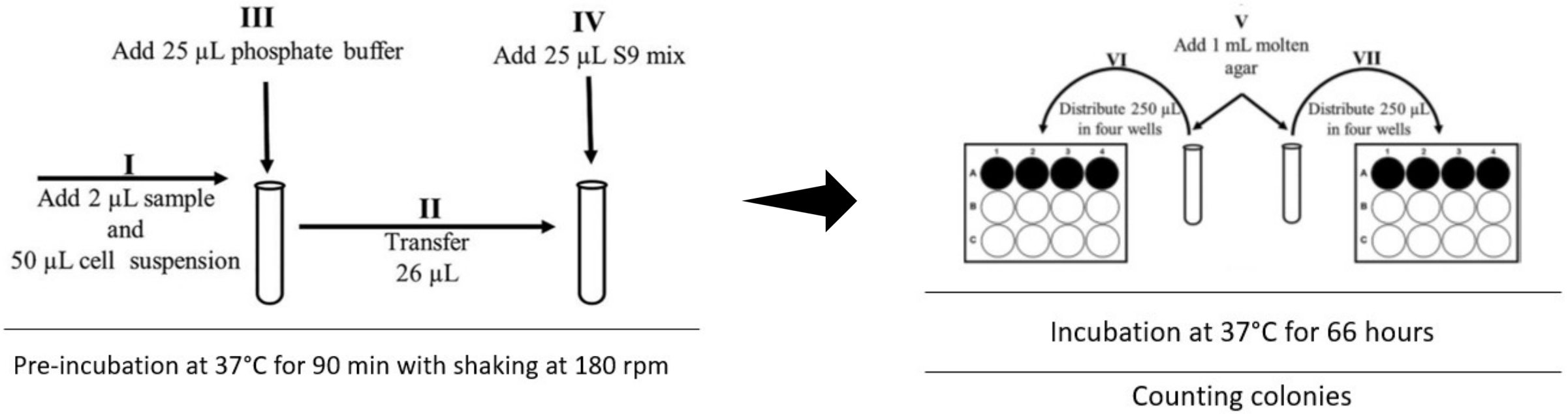
- Laboratory studies showed that 6PPD-quinone does not cause acute toxicity to *Danio rerio*, *Oryzias latipes*, *Daphnia magna,* and *Hyalella Azteca* [3].

The objective of this study was to verify its mutagenicity using the Salmonella/microsome assay

[2] Tian, Z, Zhao, H, Peter, KT, Gonzalez, M, Wetzel, J, Wu, C, Hu, X, Prat, J, Mudrock, E, Hettinger, R. 2021. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. Science 371(6525):185– 189.
[3] Hiki, K, Asahina, K, Kato, K, Yamagshi, T, Omagari, R, Iwasaki, Y, Watanabe, H, Yamamoto, H. 2021. Acute toxicity of a tire rubber-derived chemical, 6PPD quinone, to freshwater fish and crustacean species. Environ Sci Technol Lett 8(9):779–784

Microplate agar, MPA

Miniaturized version of the Ames test [4]



[4] Zwarg, JRRM, Morales, DA, Maselli, BS, Brack, W, Umbuzeiro, GA. 2018. Miniaturization of the microsuspension Salmonella/microsome assay in agar microplates. Environ Mol Mutagen 59:488– 501.

Microplate agar mutagenicity test (MPA

- 6PPD-quinone was purchased from Toronto Research Chemicals (Canada) with the purity of ≥97%
- 6PPD-quinone was dissolved in DMSO at the limit of solubility (5000 ng/µL)
- Tested in concentration-response with a maximum concentration 100 ng/µL
- Five strains were selected YG1041, TA100, TA98, TA102, and TA1535 tested with and without metabolic activation (rat liver S9, 5%)
- An additional test with TA100 was performed 10% of S9

Results

- No statistical increase in the number revertants per well was observed for YG1041, TA98 and TA1535 in the presence and absence of S9 and for TA100 in the absence of S9 in any concentration tested, except for TA100 in the presence of S9.

- No signs of toxicity was observed in the background of the agar plates.

Results

	Mean of number of revertants/well and standard deviation (SD) for **TA100**		
	A	**B**	**C**
	+S9 5%	+S9 5%	+S9 10%
Concentration (µg/µL)	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	16.50 ± 2.65	19.75 ± 2,87	14.50 ± 1.73
0.003125	16.75 ± 3.20	21.25 ± 0.96	15.00 ± 3.27
0.00625	25.25 ± 2.22*	26.50 ± 1.91*	15.75 ± 3.59
0.0125	19.75 ± 5.91	17.50 ± 3.87	20.75 ± 2.06*
0.025	20.25 ± 4.11	23.75 ± 0.96	27.75 ± 2.22**
0.05	23.00 ± 5.48	31.00 ± 0.82**	15.00 ± 1.83
0.1	18.50 ± 1.73	26.25 ± 4.03	17.50 ± 1.29
Positive Control	>150	>150	>150

*significant at 5% | **significant at 1%

Discussion & Conclusion

- We conclude that 6PPD quinone showed a weakly mutagenic response with TA100 (causing base pair substitution) only in the presence of metabolic activation (S9 mix)
- An increase in the number of revertants per well was observed starting from 6.25 ng/uL, in two independent experiments performed with 5% S9.
- The increase of S9 percentage to 10% seems to turn the compound less active because mutagenicity started from 12.5 ng/uL.
- More tests will be conducted using less concentrated starting solutions to verify if the lack of mutagenicity in the higher concentrations are due the compound aggregation in the incubation mixture.

Mutagenicity *in vivo* tests are being conducted (micronucleus) using aquatic invertebrates to confirm the *in vitro* findings

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