



## Supplementary materials.

# Products of bisphenol A degradation induce cytotoxicity in human erythrocytes (*in vitro*)

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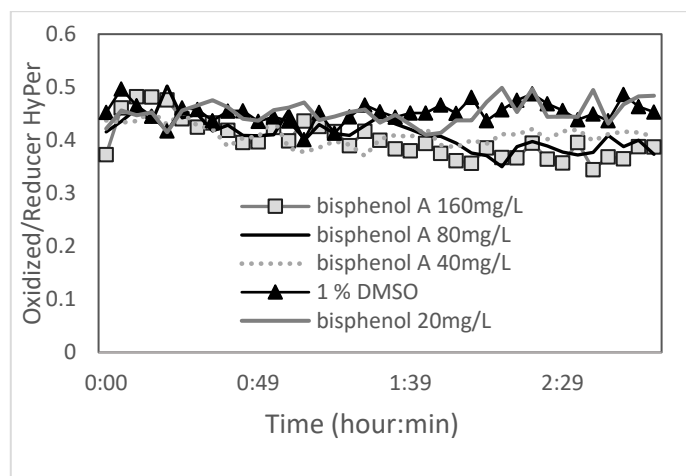
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### 1. Toxicity studies on N2 worms

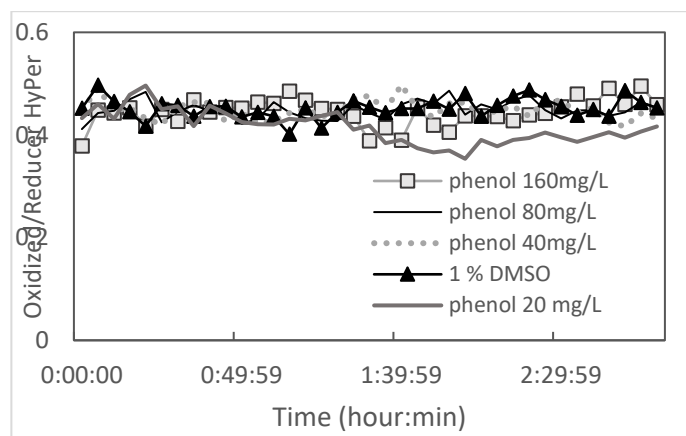
N2 worms is commonly used to study toxicity of heavy metals, various environmental pollutants, including pesticides and neurotoxins, for example bisphenol A. No data about phenol, hydroquinone or 4-isopropylphenol were reported. Previous studies reported effects of bisphenol A on the development of *C.elegans* rather at higher concentrations of bisphenols (228mg/L), causing abnormalities in mitochondria, short organismal lifespan, age-related changes in neurons, decrease ROS and increase ATP levels. [1] Here we did not observe any mortality of the nematodes in the range of concentrations 5-160mg/L.

#### Oxidative stress

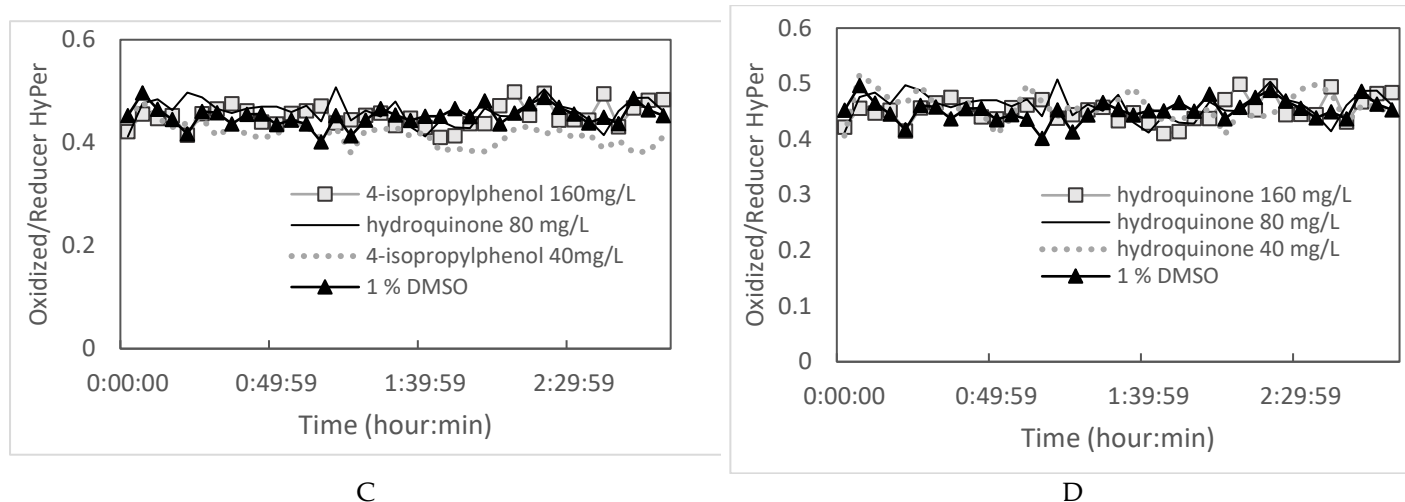
To get information about oxidative stress induced by bisphenol A, phenol, hydroquinone or 4-isopropylphenol on *C.elegans*, we used transgenic worms that express the biosensors HyPer. Previously, it was shown that oxidized-to-reduced ratios of HyPer can be used as proxy for H<sub>2</sub>O<sub>2</sub> level *in vivo*. [2] None of the compounds in the studied range of concentrations caused significant changes in oxidized-to-reduced ratios of HyPer (Fig.1)



A



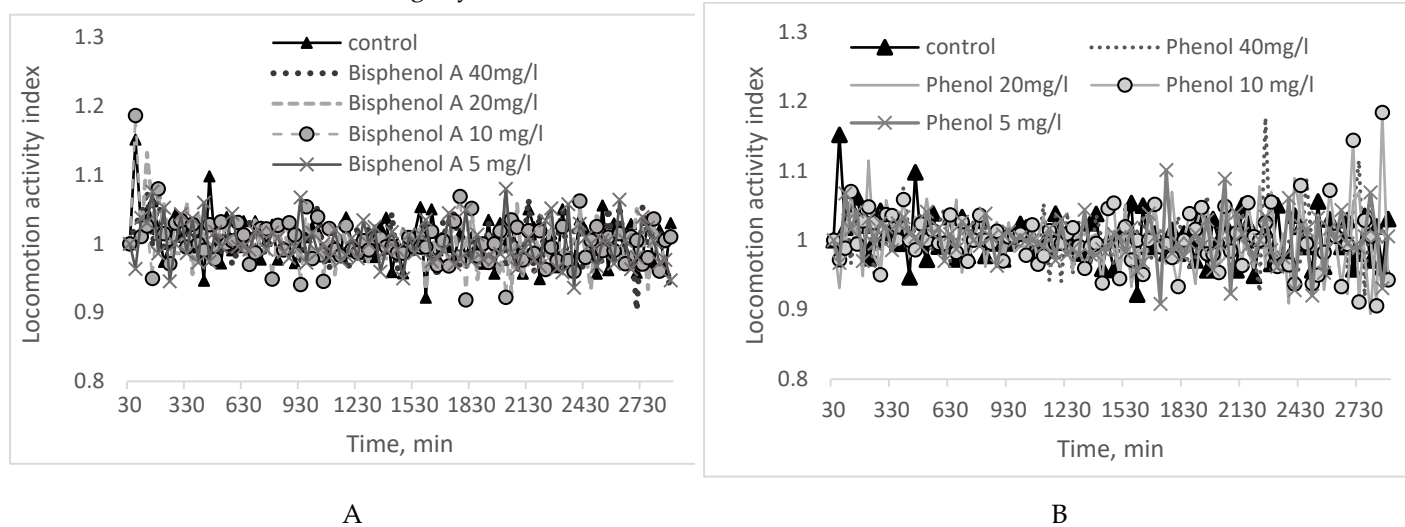
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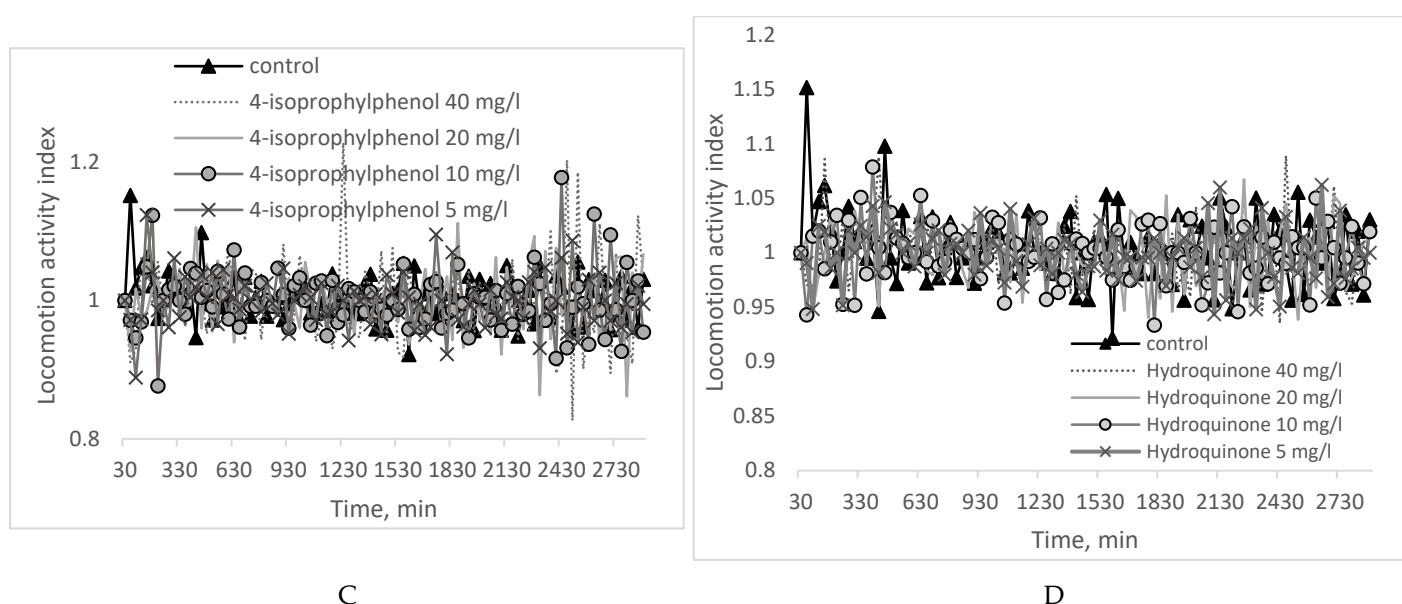


**Figure S1** In vivo HyPer response to (a) BPA; (b) phenol; (c) 4-isopropylphenol; (d) hydroquinone treatment. Treatment with the selected compounds induced a transient dose-dependent increase in the ratio of oxidized to reduced HyPer in transgenic worms. The fluorescent transgenes were excited at 405 (reduced HyPer) and 490 nm (oxidized HyPer), and emission at 535 nm was quantified in a spectrofluorometer.

#### Locomotive activity

The movements of nematodes were disturbed, indicating neurotoxicity. Among studied compounds (Fig. S1) 4-isopropylphenol causes changes in *C. elegans* locomotion pattern. Increased oscillating values suggests that the compound irritates N2 worms and is slightly neurotoxic





**Figure S2.** Normalized locomotive activity for N2 worms exposed to (a) BPA; (b) phenol; (c) 4-isopropylphenol; (d) hydroquinone

## 2. Materials and Methods

*C.elegans* N2 (Bristol) worms were cultured according to standard procedures [3]. For neurotoxicity assessment worms were washed 3 times with K-medium (2.36 g KCl, 3 g NaCl, in 1 L dH<sub>2</sub>O). Approximately 100 mix stage worms were placed in 100 µL of K-medium per 96 well plate. The overall locomotion activity was assessed in Worm MicroTracker (InVivo Biosystems, OR, US). The data were integrated every 30 min during 48 hours. The concentration range from 5 to 40 mg/L was used.

Construction of HyPer transgenic strain and spectrofluorometry measurements were performed as described elsewhere. [2]

## References

1. Hyun M, Rathor L, Kim H-J, et al.: Comparative toxicities of BPA, BPS, BPF, and TMBPF in the nematode *Caenorhabditis elegans* and mammalian fibroblast cells. *Toxicology*. 2021, 461:152924.
2. Back P, De Vos WH, Depuydt GG, et al.: Exploring real-time in vivo redox biology of developing and aging *Caenorhabditis elegans*. *Free Radical Biology and Medicine*. 2012, 52:850-859.
3. Brenner S. The genetics of *Caenorhabditis elegans*. *Genetics*. 1974 May;77(1):71-94.