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Aquatic Neurotoxins:

Multi-Parametric Analysis of Saxitoxins Effects on

Daphnia magna

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Background

Aquatic toxins are potent natural toxins synthesized by some cyanobacteria and marine algae species during harmful cyanobacterial and algal blooms (HABs). HABs constitute a human and environmental health concern worldwide.

Among these waterborne toxins, there is the neurotoxin saxitoxin (STX) and its derivatives. Despite being extensively studied for more than eighty years, the full biological effects of STX remain elusive, especially its effects in the aquatic biota.

Aim

This work aims at comprehensively evaluating the effects of short exposure (24h) of the model cladoceran Daphnia magna to an environmentally relevant concentration of STX (30 µg L-1), that also corresponds to the safety guideline established by the World Health Organization for this toxin in recreational freshwaters [1], simulating a possible pulse exposure that may occur in a freshwater ecosystem.

Methods Exposure ➤ Daphnia magna juveniles (4 days old) > 24-hour exposure STX Control > 5 replicates per condition **Biomarkers** > Total cholinesterases activity Colorimetric assay > Heart rate > DNA methylation analysis Visual counting using EpigenTek™ MethylFlash Global slow motion videos DNA Methylation ELISA Easy kit > Antioxidant enzymes Lipid peroxidation (TBARS) Colorimetric assay Colorimetric assay

References

[1] World Health Organization (2020) Cyanobacterial toxins: saxitoxins. World Health Organization. Available at: https://iris.who.int/bitstream/handle/10665/338069/WHO-HEP-ECH-WSH-2020.8-eng.pdf

[2] Sanchez, W. et al. (2013) 'A novel "Integrated Biomarker Response" calculation based on reference deviation concept', Environmental Science and Pollution Research, 20(5), pp. 2721-2725. Available at: https://doi.org/10.1007/S11356-012-1359-1/TABLES/2.

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Results STX Contro STXControl Control

Figure 1. Antioxidant enzymes activity (A to E); and TBARS levels (F) in *D. magna* exposed to 30 μg L⁻¹ of saxitoxin (STX, in black) and control organisms (Control, in grey). Error bars correspond to standard error of the mean values; * represents statistically differences (Independent samples T-test or Mann-Whitney U test, p < 0.05), ** represents statistically differences (Independent samples T-test or Mann-Whitney U test, p < 0.001) between the exposed and

STX

Control

STX

Control

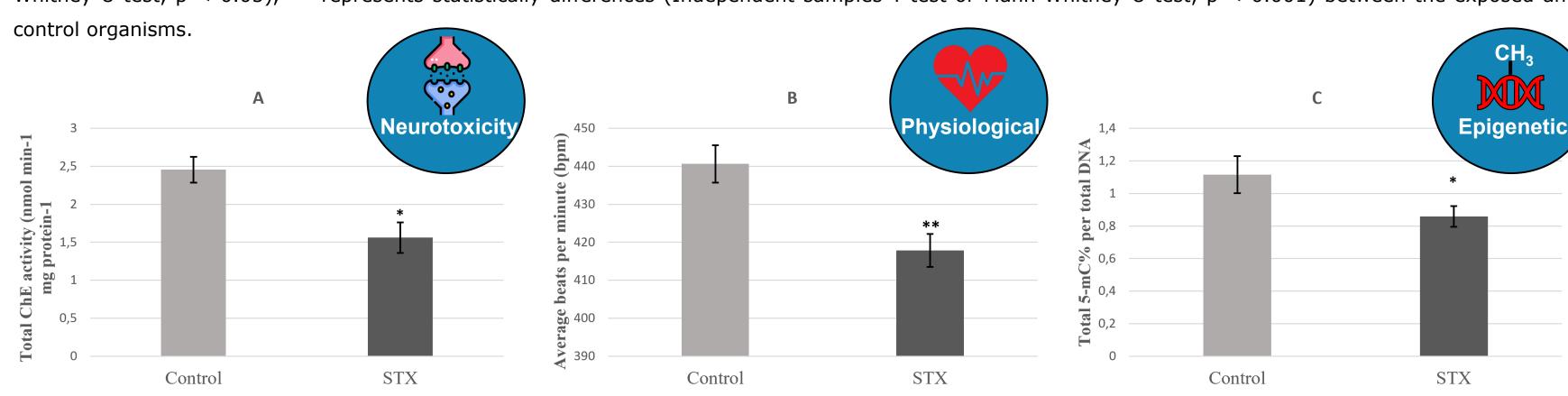


Figure 2. Total cholinesterases (ChE) activity (A); heart rate (B); and total 5-methylcytosine DNA methylation levels (C) in D. magna exposed to 30 μg L-1 of saxitoxin (STX, in black) and control organisms (Control, in grey). Error bars correspond to standard error of the mean values; * represents statistically differences (Independent samples T-test or Mann-Whitney U test, p < 0.05), ** represents statistically differences (Independent samples T-test or Mann-Whitney U test, p < 0.001) between the exposed and control organisms.

- > Exposure to STX inhibited CAT activity, possibly leaving organisms with an impaired antioxidant metabolism
- \triangleright The glutathione detoxification system (particularly <u>GSTs</u> and <u>Se-dependent GPx</u>) seems to play a crucial role in STX detoxification
- > The increase in GSTs and GPx activity was not sufficient to prevent lipid peroxidation
- > STX-induced oxidative damage can lead to the oxidation and loss of cholinesterases activity
- > The decrease in the heart rate in STX-exposed individuals could be the consequence of acetylcholine accumulation
- > Organisms exposed to STX showed a significant decrease in total 5-mC DNA methylation levels, an important epigenetic mark

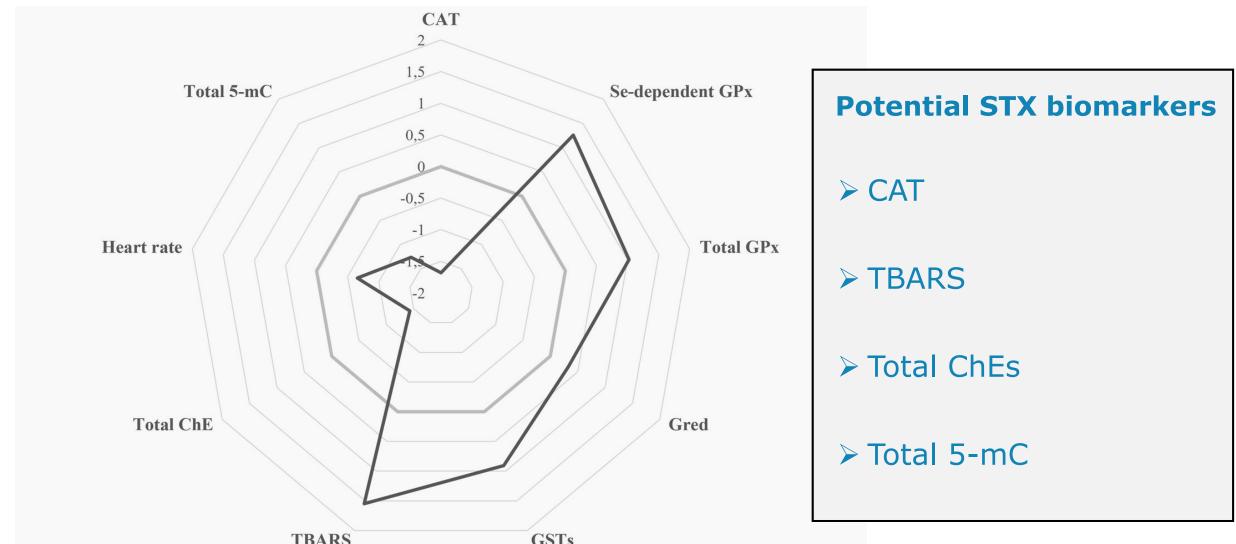


Figure 3. In the star plot, the black line represents the IBRv2 index [2] of STX-exposed organisms that is presented relatively to the control organisms (grey line; 0). Values above 0 indicate biomarker induction, and values below 0 indicate biomarker inhibition.

Takeaway

- A STX concentration deemed safe for humans by WHO constitutes a challenge to aquatic biota.
- STX exposure induces important alterations in *D. magna*, from the epigenetic landscape up to physiological function.
- The occurrence of HABs and aquatic toxins in ecosystems are predicted to rise, reinforcing the need for further studies involving aquatic neurotoxins.