iCare | Integrated Assessment and Advanced Characterisation of Neuro-Nanotoxicity

Freshwater planarians as bioindicators for nanoparticles toxicity assessment

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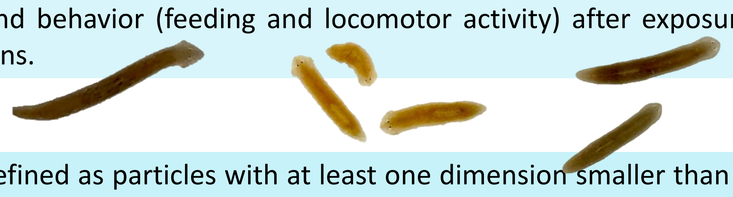
INTRODUCTION & METHODS

With their ability to repair and/or regenerate any damaged or missing part of their body, freshwater planarians have gained more and more attention over the years as potential sentinel species and, eventually, as bridging models. They can be used to study regeneration, developmental biology, and behavior (feeding and locomotor activity) after exposure under strictly controlled conditions.



Nanoparticles (NPs) are defined as particles with at least one dimension smaller than 100 nm (Biswas and Wu, 2005)[1]. Due to their small size and unique properties, they are widely used in various commercial products and industrial sectors. The distinctive properties of NPs, and by extension nanomaterials (NMs), do not necessarily confer only beneficial effects. On the contrary, their high reactivity—stemming from a large surface-to-volume ratio—can potentially exert harmful impacts on biological systems, ranging from the cellular to the organismal level.

- Specimens of S.mediterranea and *D.japonica* were exposed for 7 days, in artificial freshwater, to increasing concentrations of several types of NPs.
- For the detection of the primary DNA damage, Comet assay and Diffusion assay have been performed on isolated cells according to Tice et al., 2000[2], with slight modifications
- Neurobehavioral changes were assessed according to Raffa et al., 2001[3].

SURVIVAL RATE ASSESSMENT

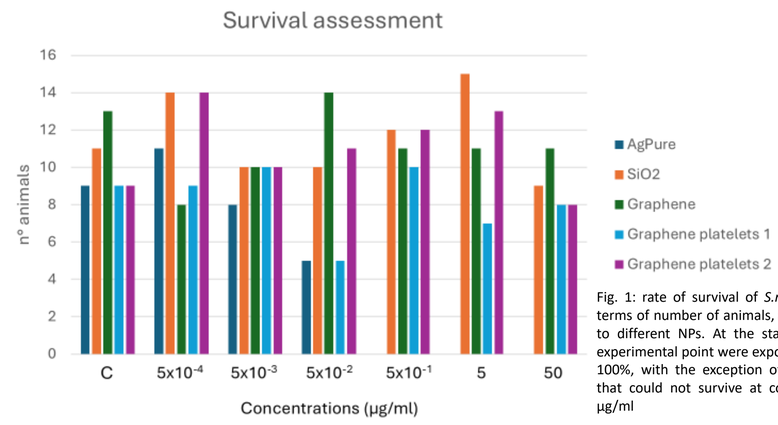


Fig. 1: rate of survival of *S.mediterranea* specimens, in terms of number of animals, after one week of exposure to different NPs. At the starting point, 5 animals *per* experimental point were exposed. The overall rate was of 100%, with the exception of AgPure® treated animals, that could not survive at concentrations above $5x10^{-2}$ µg/ml

- After one week of exposure, the overall survival rate of *S.mediterranea* specimens was 100%, with the only exception of AgPure®;
- In all the concentrations tested, control included, there was an enhancement of the division rate of the animals. The highest division rates was observed after treatment with SiO_2 and Graphene NPs, eventually as a consequence of an increase in ROS production (hypotesis validation in progress).

GENOTOXICITY & NEUROBEHAVIOR ASSESSMENT

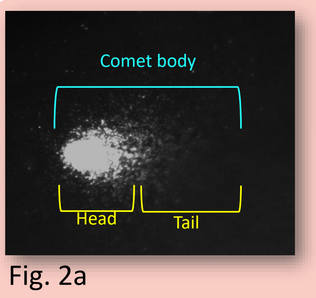


Fig. 2a

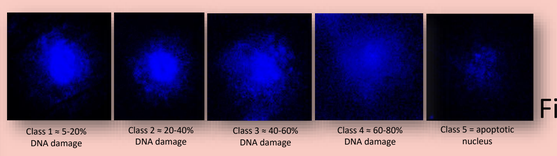


Fig. 2b

Fig 2a: comet-shaped nucleus highlighting moderate DNA fragmentations. Fig 2b: different levels of DNA damage assessed with Diffusion assay. Preliminary results showed that SiO_2 NPs do induce an increase of DNA primary damage (Fig 2c).

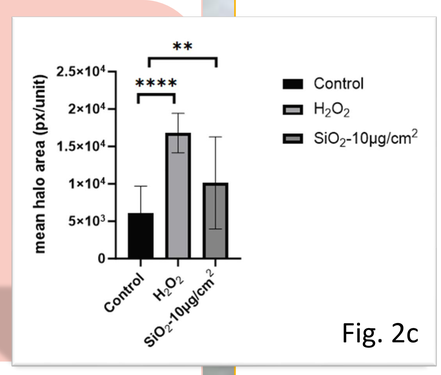


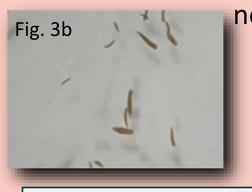
Fig. 2c

Fig. 3a

Fig. 3: set up for neurobehavioral analysis. Fig. 3a: tracking system developed by Phylumtech (https://www.phylumtech.com/); Fig. 3b: specimens of *D.japonica*; Fig. 3c: exemplary output of data after recording the animals exposed to AgPure. A reduction of motility was observed at the highest doses.

Behavioral assays (still ongoing) do evaluate how exposure to nanomaterials impacts the nervous system of flatworms. This could include changes in locomotion, feeding behavior, and other neurobehavioral parameters

Fig. 3b



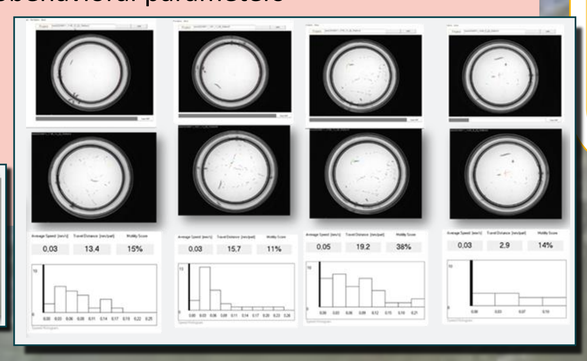


Fig. 3c

DISCUSSION & CONCLUSIONS

Our findings, although preliminar, have shown how versatile the flatworm *S.mediterranea* can be in terms of biological responses following an exposure;

The overall high survival rate recorded for the NPs, with the exception of the silver ones, demonstrates that these organisms are sensitive enough to highlight different levels of toxicity.

Putting an eye on the preliminary results of the genotoxicity assays, they showed how a high survival rate is not necessarily linked with a good "health status": as shown, the nuclei of the treated animals showed an increase in DNA damage statistically higher than the one recorded for the control ones.

Last but not least, the preliminary results concerning neurobehavior analysis demonstrated that there was a decrease in the locomotor velocity of the animals after treatment with silver NPs.

So, as a final consideration… → This model can be used to assess the effects of NM exposure on the nervous system. Indeed, they represent an easy and quick way of assessing toxicity and provide direct information on induced neurobehavioral and developmental effects.

References:

[1] Biswas and Wu, 2005 DOI: 10.1080/10473289.2005.10464656

[2] Tice et al., 2000 DOI: 10.1002/(sici)1098-2280(2000)35:3<206::aid-em8>3.0.co;2-j

[3] Raffa et al., 2001 DOI: 10.1016/S1056-8719(01)00152-6

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