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Study of the effects of nanoplastics ingestion in a freshwater fish (*Danio rerio*)

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Conclusions: These results suggest that QDs can induce moderate or low oxidative stress. The higher results observed in fish exposed to 10 ppb and 100 ppb of QDs can be due to QDs aggregation occurring at higher QDs concentrations, which can also affect the bioavailability of toxic ions released. Therefore, in this preliminary study, the lower concentrations of QDs seem to be more hazardous to Zebrafish.

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Study of the effects of nanoplastics ingestion in a freshwater fish (*Danio rerio*)

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ABSTRACT

Introduction: The pollution by nanoplastics (NPs) has been shown in several ecosystems and aquatic biota, worldwide [1,2]. It is known that NPs can contaminate the food chain [1,2]. However, the toxicity of NPs in aquatic animals, especially freshwater fish, has been less studied [2]. Thus, this work intended to answer the question: how exposure to NPs affects the activities of antioxidant enzymes and the total antioxidant capacity in a freshwater fish species (*Danio rerio*). It also aimed to assess the vulnerability of this species to environmental contamination by NPs.

Materials and methods: The fish (*D. rerio*), were randomly distributed by three aquaria of 15 L ($n = 36$; weight: 0.21 ± 0.06g; length: 2.7 ± 0.3 cm) and exposed during 7 and 14 days to different concentrations of NPs *via* food ingestion (fed daily). Thus, food pellets were previously embedded in a suspension of NPs (Sigma-Aldrich) containing 50 μg NPs/L and 100 μg NPs/L, respectively. Fish were sampled at the end of the exposure periods to assess oxidative stress biomarkers (antioxidant enzymes and TAC levels). In brief, samples (whole fish) were homogenised in a buffer solution (PBS), centrifuged at 10,000 × *g* (15 min at 4 °C), transferred to 1.5 mL microtubes and stored at −80 °C until further analyses. The tissues were assessed for: superoxide dismutase (SOD) determined following the method described by Sun et al. [3], catalase (CAT) measured following Johansson and Borg [4], glutathione S-transferase (GST) was determined according to Habig et al. [5], and total antioxidant capacity (TAC) levels were determined as described in Madeira et al. [6]. The fish assays were approved by the competent national authorities (DGAV). Statistics were performed using the non-parametric Kruskal–Wallis test to compare differences between exposed and control fish, with a significance level of 5%, using the software Statistica 8.0 (USA).

Results: The highest SOD, GST and CAT activities were found in fish samples, after 14 days of exposure to 100 μg NPs/L. Likewise, the highest TAC levels were determined in samples of fish exposed to 100 μg NPs/L, after 14 days of exposure *via* food. The statistical results showed no significant differences ($p > .05$) between the controls and the fish exposed for 7 days to 50 and 100 μg NPs/L, for all biomarkers. However, significant differences ($p < .05$) were detected between controls and fish exposed for 14 days to 100 μg NPs/L, for all biomarkers analysed.

Discussion and conclusions: Preliminary results show that exposure *via* ingestion of 50 μg NPs/L did not cause significant effects on fish during the experimental period (7 and 14 days), while fish exposed to 100 μg NPs/L showed a

significant increase in enzyme activities (SOD, GST, CAT) and TAC levels, after 14 days of exposure suggesting that this concentration of NPs may cause oxidative stress in fish. In addition, the variability found in some results may be due to fish not ingesting the same amount of food containing NPs, as they compete for food. Overall, the present study contributes to a better understanding of the risk of exposure to NPs for aquatic biota.

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2-Phenoxyethanol derivatization in ink dating determination

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ABSTRACT

Introduction: 2-Phenoxyethanol (PE) is a volatile compound present in the composition of inks. After the deposition in a document, it starts to evaporate over time. This ageing process potentially allow to estimate the date of an ink in a document [1], however this is a complex system where the PE derivatization with different derivatization agents and methods can contribute to improve ink dating validation [2]. The aim of this work is to determine if PE derivatization with MSTFA:TMCS, will increase the sensitivity of the method contributing to the estimation of the ink age for a longer period of time.

Materials and methods: In order to compare peak resolution between PE and derivatized PE, a solution of 50 μg/ml of PE was prepared in hexane. The derivatized sample was prepared using chemical derivatization with MSTFA:TMCS (95:5) at a 80°C during 30 min. The samples were analysed using GC/MS with an Agilent Technologies 5973 – 6890 N. GC MEGA-5 MS; 0.25μm, 0.32mm, 30 m capillary column was used. Chromatographic analysis was carried out under the following conditions: injection volume 2 μl, split ratio of 2:1 and injection temperature at 280 °C. The oven temperature program starting at 90 °C during 8 min, then ramped at 10 °C min^{-1} to 100 °C, and increased to 240 °C at a rate of 30 °C min^{-1} during 4.67 min. The MS analysis was carried out in SIM mode, which *m/z* of PE was 77, 94, 138 and PE-TMS was 151, 195, 210.

Results: The chromatogram results (Figure 1) showed that PE-TMS has a longer retention time (23.2 min) and a better resolution than PE (retention time of 13.2 min).

Discussion and conclusions: This preliminary study showed an increase of the sensitivity of the PE-TMS. Our results revealed that PE derivatization with MSTFA:TMCS, might be useful in ink dating determination and can contribute to the decreasing of LOQ. However, more work has to be done. In conclusion, derivatization proves to be promising in the field of ink ageing, allowing getting sensitivity to estimate the age of an ink, in a long period of time.

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