



Study of quantum dots (CdS, ZnS) toxicity in *Danio rerio*: preliminary results

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Ethics Committee of Egas Moniz. Inclusion criteria were: children of both sexes aged 5–18 years, having clinical record at EMDC and with the correspondent informed consent signed by the parents. Exclusion criteria were: children who had past orthodontic treatment and having craniofacial anomalies or syndromes. The method used to assess breathing and swallowing patterns was adapted by Marchesan [2]. Data were analysed by using descriptive and inferential methodologies (chi-square test). A significance level of 5% was established in the latter case.

Results: A higher prevalence of mouth-breathers was found in women $n=49$ (56.5%) than in men $n=37$ (43.5%). Atypical swallowing, was also more prevalent in women $n=48$ (56.1%) than in men $n=38$ (43.9%), however, in both cases, the differences were not found to be statistically significant ($p=.594$, $p=.570$). $n=52$ (83.9%) of the mouth-breathers were identified as having atypical swallowing, conversely to $n=14$ (58.3%) of the normal (nasal) breathers. Overall, there was a statistically significant association between mouth-breathing and patients with atypical swallowing ($p=.012$).

Discussion and conclusions: The results show that mouth-breathing and atypical swallowing were found to be significantly associated $n=52$ (83.9%) in this study, being closed to Lemos et al. who found higher results (97.2%) [1]. Other studies reported that is not statistically significant the association between mouth-breathing and atypical swallowing [6]. The results of this study are clinically relevant and encouraging the search for early diagnosis and intervention.

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Study of quantum dots (CdS, ZnS) toxicity in *Danio rerio*: preliminary results

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ABSTRACT

Introduction: Quantum Dots (QDs) are nanoparticles with potential applications in many industrial and biomedical areas (e.g. fluorescent dyes, LED). QDs have unique and remarkable properties, however, little is known about their toxicity [1]. Zebrafish (*Danio rerio*) has been the first vertebrate model in many research areas and is recognised as a good model in nanotoxicology [2]. This work aims to evaluate the toxicity of QDs (CdS and ZnS) in *D. rerio*, singly and combined, by determining a set of biomarkers (e.g. antioxidant enzymes).

Materials and methods: Adult zebrafish ($n=50$ ($\times 2$); 0.3 ± 0.1 g weight; 2.7 ± 0.4 cm) were distributed by 4 glass containers (1 L) and exposed to different concentrations of QDs (0 ppb, 10 ppb, 100 ppb and 1000 ppb), for 7 days. Trials were performed in duplicate using 3 different groups exposed to: (1) ZnS-QDs, (2) CdS-QDs, (3) (ZnS-QDs + CdS-QDs). Oxidative stress was assessed by measuring catalase (CAT), glutathione-S-transferase (GST), lipid peroxidation (LPO), superoxide dismutase (SOD) and Total Antioxidant Capacity (TAC), as described previously [3,4]. Trials followed the 3Rs and animal welfare and were authorised by national authorities. Statistics was carried out using the Kruskal-Wallis test (STATISTICA 8.0, USA).

Results and Discussion: Results show no significant differences between the 3 groups of experiments. Regarding enzyme activities (nmol/min/mg tot. protein), CAT the highest levels in fish exposed to 100 ppb Zn-QDs (45 ± 2) and the lowest levels were measured in controls (26 ± 4). GST showed the highest GST levels in fish exposed to 10 ppb Zn-QDs (50 ± 1) and lowest levels in fish exposed to 1000 ppb Cd-QDs (27 ± 16). SOD results (as % inhibition/mg tot. protein) showed highest values in fish exposed to 10 ppb QDs combined ($89 \pm 3\%$) and lowest levels in fish exposed to 100 ppb of Zn-QDs (35 ± 9). CAT, GST and SOD showed higher levels in fish exposed to 10 ppb and 100 ppb QDs. LPO showed non-significant differences TAC showed a trend to decrease in all groups exposed to different concentrations of QDs.

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 ± 0.06 g; 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 μ g NPs/L and 100 μ g 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 $10,000 \times g$ (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 μ g NPs/L. Likewise, the highest TAC levels were determined in samples of fish exposed to 100 μ g 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 μ g NPs/L, for all biomarkers. However, significant differences ($p < .05$) were detected between controls and fish exposed for 14 days to 100 μ g NPs/L, for all biomarkers analysed.

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