

***In vivo* evaluation of three differently charged gold nanoparticles using the zebrafish embryo model**

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Due to their unique physicochemical properties, gold nanoparticles (AuNP) are widely used for numerous applications in different fields, and in recent years they have gained considerable interest in biomedical research. Their small size enables them to cross biological barriers such as the blood-brain barrier, which makes them promising candidates for diagnosis and treatment of neurological disorders. In this context, evaluating their potential toxicity is essential to ensure their biocompatibility and safe use in biomedical applications. On this basis, the main objective of the present study was to assess the biological effects of three differently charged AuNP (i.e. anionic, cationic and neutral) over a wide range of concentrations (6.25–100 µg/mL) using zebrafish (*Danio rerio*) embryos as an *in vivo* model. Embryos were exposed for 96 hours, and toxicity was assessed by the Fish Embryo Acute Toxicity (FET) test according to the established OECD Test Guideline No. 236. Several developmental endpoints were analyzed, including embryonic viability, hatching rate and morphological alterations. Additionally, heart rate and blood flow were measured to detect sublethal effects. Under the experimental conditions tested, no significant alterations were observed for any of the AuNP tested. To gain deeper insight into their mechanism of action, targeted gene expression analysis using microfluidic qPCR on chips (Fluidigm) was performed, focusing on genes associated with oxidative stress, DNA damage response, and neuronal development. Findings from this study provide a better understanding of AuNP biological behaviour and support their possible use in nervous system-targeted applications.

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