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Review

# Genotoxicity of metal based engineered nanoparticles in aquatic organisms: A review

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ABSTRACT

Engineered nanoparticles (ENPs) are an emerging class of environmental contaminants, but are generally found in very low concentrations and are therefore likely to exert sub-lethal effects on aquatic organisms. In this review, we: (i) highlight key mechanisms of metal-based ENP-induced genotoxicity, (ii) identify key nanoparticle and environmental factors which influence the observed genotoxic effects, and (iii) highlight the challenges involved in interpreting reported data and provide recommendations on how these challenges might be addressed. We review the application of eight different genotoxicity assays, where the Comet Assay is generally preferred due to its capacity to detect low levels of DNA damage. Most ENPs have been shown to cause genotoxic responses; e.g., DNA or/and chromosomal fragmentation, or DNA strand breakage, but at unrealistic high concentrations. The genotoxicity of the ENPs was dependent on the inherent physico-chemical properties (e.g. size, coating, surface chemistry, e.tc.), and the presence of co-pollutants. To enhance the value of published genotoxicity data, the role of environmental processes; e.g., dissolution, aggregation and agglomeration, and adsorption of ENPs when released in aquatic systems, should be included, and assay protocols must be standardized. Such data could be used to model ENP genotoxicity processes in open environmental systems.



**Contents**

1. Introduction . . . 135
2. Mechanisms of ENP-induced genotoxicity . . . 136
3. Methods for testing genotoxicity of ENPs . . . 136
   3.1. The comet assay . . . 150
   3.2. The micronucleus test . . . 152
   3.3. Chromosomal aberration assay . . . 152
   3.4. The Ames test . . . 152
   3.5. DNA laddering assay . . . 152
4. Factors influencing genotoxicity of ENPs in aquatic systems . . . 152
   4.1. Physicochemical properties of ENPs . . . 152
      4.1.1. ENP size . . . 152

*Abbreviations:* n, prefix to denominate "nano"; μ, prefix to denominate "micro"; ENPs, engineered nanoparticles; ZVI, zero-valent iron; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; RAPD, random amplified polymorphic DNA; RT-PCR, real time polymerase chain reaction; DMSO, dimethyl sulphoxide; ROS, reactive oxygen species; $H_2O_2$, hydrogen peroxide; NOM, natural organic matter; TEM, transmission electron microscope; $IC_{50}$, inhibition concentration response reduced by half; TCDD, 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin; MN, micronucleus; CA, chromosome aberrations; OECD, Organisation for Economic Cooperation and Development; H2AX, histone H2AX phosphorylation; 8-OHdG, 8-hydroxy-deoxyguanosine; SCGE, single-cell gel electrophoresis; SSB, single-strand breaks; ALSs, alkali-labile sites; ENA, erythrocyte nuclear abnormalities; UVA, ultraviolet A; $nFe_3O_4$, iron oxide ENPs; $nTiO_2$, Titanium dioxide ENPs; ENPs, $nSiO_2$, silica ENPs; $nCeO_2$, cerium oxide ENPs; ZnO, zinc oxide ENPs; nCuO, copper oxide ENPs; nAg, silver ENPs; nAu, gold ENPs; SOD, superoxide dismutase.

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4.1.2. Crystal structure . . . 153
4.1.3. Surface charge . . . 153
4.1.4. Morphology . . . 153
4.1.5. Surface area . . . 153
4.1.6. Surface coating . . . 153
4.2. The influence of co-pollutants . . . 153
4.3. Concentration and time dependent effects . . . 154
4.4. Illumination effects . . . 154
4.5. Particulate vs ionic species effects . . . 154
5. Conclusions . . . 155
Conflict of interest . . . 155
Acknowledgements . . . 155
References . . . 155

## 1. Introduction

The dramatic growth in the commercialization of nano-enabled products is driven by recent advances in the precision tuning of the functionality of engineered nanoparticles (ENPs) to meet stringent specifications and performance expectations [1,2]. For example, ENPs finds applications in cosmetics and sunscreens [3], bioimaging probes [4,5], photovoltaic cells [6], therapeutics [7], drug delivery [8], and catalysis [9,10], with the global nanotechnology market projected at a compound annual growth rate of about 17.5% from 2016 to 2022 [11]. Metal-based ENPs are most widely used in consumer products and applications [12–15], and these uses are summarised in Table 1. The increasing use of ENPs has led to their increasing release into the environment [25–27] at different product lifecycle stages [28], in wastewater treatment plants [29,20,30] and river systems [29,31,22,32].

The increasing production and utilisation of ENPs has also triggered concerns relating to their potential environmental health implications [33] with respect to aquatic organisms, including bacteria [34,35], invertebrates [36–38] and fish [39–42]. To date, most nanotoxicity assessments have focused on phenotypic end point-based cytotoxicity [43]. Studies have demonstrated that low concentrations of ENPs, as are typically found in environmental systems, may not cause gross cytotoxic effects but may have effects at the molecular level [44,45]. For example Lee et al. [44] found that the cytotoxic effects of titanium dioxide ($nTiO_2$), silicon dioxide or silica ($nSiO_2$) and cerium oxide ($nCeO_2$) nanoparticles on daphnids and chironomids were not apparent at the organism level for end-points such as mortality, growth, or reproduction, but adverse effects were observed at the genetic level. Also, $nTiO_2$ did not induce mortality in fish, *Piaractus mesopotamicus* under UV and visible light conditions, but induced sub-lethal effects that were influenced by illumination conditions [46].

To date, both field experimental [47,16,30] and modelling [31,22,48,20,32] studies have reported very low concentrations of ENPs in various environments, including wastewater, freshwater systems and agricultural soils (Table 1). Such data, therefore, suggest that the most likely impacts of ENPs in environmental systems may be restricted to sub-lethal effects, at the molecular level rather than as organismal effects as previously observed for conventional chemical pollutants [49,50]. Review findings of [51] indicated the measured or modelled ENPs environmental concentrations ranged from a low $\leq$ 0.001 ppm to a high >1000 ppm. The lowest concentrations were $\leq$0.001 ppm in surface water, wastewater treatment plant (WWTP) effluent and solid media (soil, sediments, and biosolids). The highest concentrations were in WWTP effluent (0.11 to 1 ppm) and biosolids (>1000 ppm). In the European Union, genotoxicity is recognised as an important biomarker for the regulation of chemical usage and disposal, especially in undertaking risk assessments in the context of regulatory toxicology [52]. The presence of ENPs at very low concentrations in the aquatic systems (Table 1) highlights the importance of acquiring genotoxicity data to support decision-making with the aim of protecting the health of aquatic systems (as recently recognized by the Organization for Economic Co-operation and Development (OECD) [53,54].

Genotoxicity biomarkers are regarded as useful tools for the assessment of chemical hazards in aquatic ecosystems [55]. This is because chemicals which damage DNA, even at very low concentrations, can significantly alter the functioning of ecological systems [56]. The genotoxicity of a chemical entity can be assessed through a number of changes to the structure of DNA such as:

**Table 1**
Quantities, applications and likely concentrations of ENPs in different environmental systems.

| ENPs | Global production (tons/year) | Applications | Concentrations in environmental systems (modelled values) | | |
|---|---|---|---|---|---|
| | | | WWTP effluent (μg/L) | WWTP sludge (μg/g) | Solid waste (μg/g) |
| $TiO_2$ | 3000 [16] 88,000 [17] | Paint [18], sunscreen [19] | 16 [20] | 170 [20] | 12 [20] |
| Ag | 55 [16]; 452 [17] | Personal care products, laundry additives, paints and textiles [21] | 0.00017 [20]; 0.05–0.2 [22] | 0.02 [20] | 0.06 [20] |
| $CeO_2$ | 55 [16]; 10,000 [17] | Fuel catalyst [16] | 0.00001 [22]; <0.0001 [23] | <0.01 [23] | <0.01 [23] |
| $SiO_2$ | 5500 [16]; 95,000 [17] | UV-protection, ceramics, electronics, food, plastics, sunscreen [16] | 0.0074 [23] | 0.21 [23] | 0.31 [23] |
| $Fe_3O_4$ | 55 [16] | Biochemical assays, removal of contaminants, bio-manipulation [16] | – | – | – |
| ZnO | 550 [16]; 34,000 [17] | Skin care products, sunscreens [24] | 2.3 [20]; 0.5–1.5 [22] | 24 [20] | 0.89 [20] |
| $Al_2O_3$ | 55 [16]; 35,000 [17] | Batteries, grinding, fire protection, metal- and bio-sorption, paints [16] | 0.0025 [23] | 0.07 [23] | 0.10 [23] |
| Au | No data | Drug delivery [8], and catalysis [9,10] | 0.10 [23] | 2.90 [23] | 4.26 [23] |

References: [16], [17], [18], [19], [20], [21], [22], [23], [24], [8], [9], [10]. Acronym: WWTP: wastewater treatment plant.