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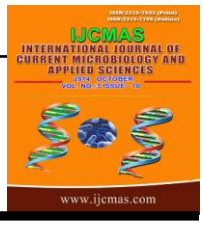


**Original Research Article**

# Retention of Particles in *Caenorhabditis elegans* after exposure of Zinc Oxide Nanoparticles

**Shweta Yadav***

Department of Zoology, Dr H S Gour Vishwavidyalaya, (A Central University), Sagar MP, India
**Corresponding author*

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Nanotoxicity, Zinc oxide nanoparticles, *C.elegans*, metal oxide nanoparticles.

**A B S T R A C T**

It is known that the rapid increase in manufacturing and use of nanoparticles is expected to elevate levels of exposure to humans and other organisms. There is little understanding of the potential toxic effects of nanoparticles exposure. Nano-sized metal oxides including zinc oxide nanoparticles, may adversely affect biological systems due to their unique physiochemical properties. The present study is largely inconclusive to clarify the complete retention even after the complete egestion. The purpose of this study is to understand how the nanoparticles sized retained in body and deposited in vital organs? The current study is aimed to fill gaps metal oxide nanoparticle toxicity and to provide insight cumulative effect of their retention using the soil nematode *Caenorhabditis elegans*.

## Introduction

In current and upcoming decades nanotechnology is one of the key technologies as creating an enormous number of novel marketing potential. Especially, engineered metal nanoparticles offers great industrial opportunities due to their unique properties. Among all manufactured metal oxide nanoparticles, zinc oxide are produced in huge amount and utilized in several commercial products. It has excellent UV-absorbing properties and used in UV-protectors cosmetics like sunscreens as well as in paints or finishing materials of buildings. It is also used as antibacterial agents in ointments, lotions, mouthwashes and surface coatings to prevent microorganism growth. ZnO-NPs have also been used as a dietary supplement in human and livestock because zinc can stimulate the immune system and act as an anti-inflammatory way (Rincker *et al*., 2005; Horie *et al*., 2009). Due to wide application of ZnO-NPs (zinc oxide nanoparticles) it implies, human exposures *via* different entrance routes, including inhalation and ingestion. During the use of these commercial products NPs may also be released into environment and became a threat to ecosystems including soil organisms. Aruoja *et al*., (2009) reported

that ZnO-NPs inhibit the growth of microalgae *Pseudokirchneriella subcaptata*, crustacean *Daphnia magna* and *Thamnocephalus platyurus* and bacteria *Vibrio fischeri* (Heinlaan *et al*., 2008). Several *in vitro* studies demonstrated that ZnO-NPs are toxic to mammalian cells and even more toxic than other nanoscale structures of metallic oxide (Horie *et al*., 2009;Jeng and Swanson, 2006).In combination of UV exposure, ZnO-NPs are known to generate reactive oxygen species (ROS) like hydroxyl radicals or hydrogen peroxide in aqueous solutions leading to efficient decomposition of organic compounds (Li and Haneda, 2003). Brunner *et al.* (2006) showed that a three day exposure of human mesothelioma and rodent fibroblast cell to ZnO-NPs (19 nm) caused DNA and mitrochondrial changes.

Hence, it is essential to understand their human health implications and ecological consequences of exposure to before the commercial benefits. Several researchers (Daniels *et al*., 2006;Johansen *et al*., 2008;Kim *et al*., 2008;Scott-Fordsmand *et al*., 2008;Petersen *et al*., 2009) studied the toxicity of other NPs like silver, platinum and carbon tubes. However, research on this nanoparticle is far from being completed and the studies of potential adverse effects and related mechanisms exerted by ZnO-NPs in human and soil ecosystem are even more limited (Lin and Xing, 2007;Warheir *et al*.,2007; Mortimer *et al*., 2008; Jemer *et al*., 2008; Hu *et al*., 2010; Sahu *et al*., 2013; Gupta *et al*., 2014). Therefore, the present study aimed to evaluate the retention of ZnO-NPs especially in reproductive structures using soil nematode *Caenorhabditis elagans* as model organism.

## Materials and Methods

ZnO-NPs (35 nm) were purchased from Sigma-Aldrich. Particles were labelled as suggested by Tachikawa *et. al.* (2011) with fluorescent polymer. The size of the particles was measured in 20-µl particle suspension from the test medium on 400 mesh carbon-coated copper grid and observed using a transmission electron microscope (40-100KV) at Sophisticated Analytical Instrumentation Facility of Electron Microscopy, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.

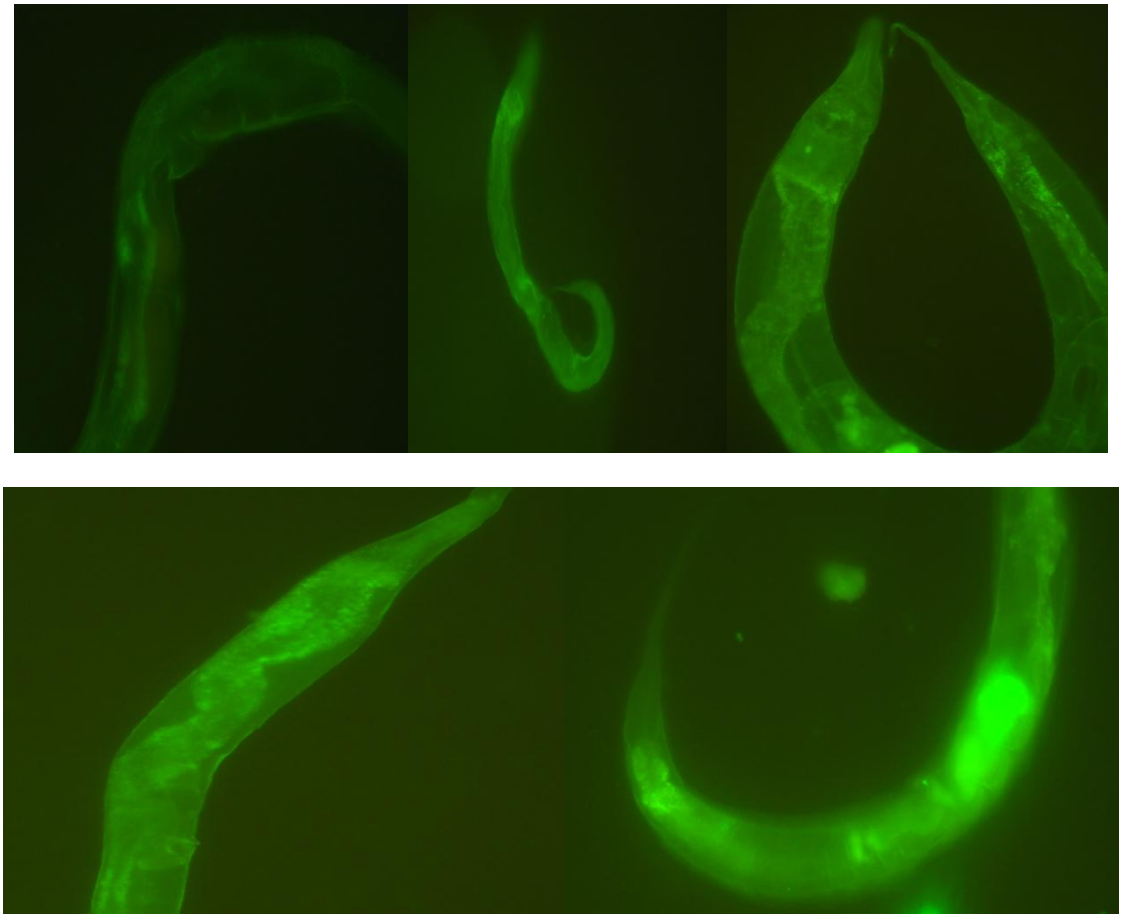
The wild-type *C. elegans* Bristol strain N2 was obtained from Caenorhabditis Genetic Centre (CGC), USA, and culture was maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* strain OP50 at 20°C, using the standard method (Brenner,1974). Young adult (3 days old) synchronized culture were used in all the experiments. Worms were incubated at 20°C for 24 h without a food source and were then subjected to the analysis (Van der Ploeg *et al*., 2011). Nematodes were exposed to 35 nm sized ZnO-NPs (7 ml/l) for 24 hrs. NPs were diluted in K-medium (32 mM KCl, 51 mM NaCl) following Williams and Dusenbery (1990) buffered in 140 mM sodium acetate (pH 6.0) to avoid aggregation. The treatment was replicated for three times, and control (K-medium + buffer) was maintained for the entire test. After exposure of ZnO-NPs, fluorescence distribution images were observed at 6 hrs, 8 hrs, 10 hrs, 12 hrs and 24 hrs by using fluorescence microscope equipped with a peltier cooled charge-coupled camera. Both differential interference contrast (DIC) and epi-fluorescence images were taken.

## Results and Discussion

The labeled ZnO-NPs were tracked during 6 -24 hrs after exposure. It was found ZnO-NPs were readily absorbed through the gut and retained even after egestion (figure 1).

At 6 hrs of exposure the particles were detected at the margin of gut and immediate after 2 hrs, the clear deposition matrix for 35 % were visible at florescence microscope. At 10 hrs of exposure particles were uniformly distributed in gut and reproductive structures even in eggs and in next two hrs fluroscence matrix showed 56 % that reflects to dark deposition of nanoparticles. Finally at interval of 24 hrs 78 % of particles were retained within the body of *C.elegans.* The study revealed *C.elegans* retains major partition of the particles retained as ingested with food even after the egestion. The ZnO-NPs readily transported to distinct organs and tissues immediate after ingestion.

**Fig.1** Retention of ZnO-NPs in *C.elegans* after 6 hrs; 8 hrs; 10 hrs; 12 hrs and 24 hrs of exposure



However, the real time of translocation of particles needs to be further investigated. Since nematodes of the *Caenorhab-ditis* species are abundant inhabitants' soils and may thus participate in the food chain. The progeny production in *C. elegans* may be effected with permeated NPs. The used small sized particles (35 nm) rapidly permeated throughout the body even at low concentration (7ml/l) and shown high retention end points. Several abnormalities on exposure of NPs including inflammation, absence of egg laying organs or defects in reproductive organs were recorded after 12

hrs of exposure. ZnO-NPs may induce defective physiology of *C. elegans* due to rapid permeation and retention of particles.

The study revealed that when *C. elegans* exposed to ZnO-NPs they immediately absorbed and retained in their vital organs. Such retention of particles increases with time of exposure. Eventually may causes more toxicity compared to microparticles. It is known that ZnO nanoparticle exposure can have a severe adverse effect in *C. elegans*, however, it has yet to be tested a possible mechanism of toxicity (Horie *et al*., 2009; Jeng and Swanson, 2006). ZnO nanoparticles were observed *in vivo* in the worms using fluorescence microscopy, suggesting that the worms can possibly absorb the nanoparticles *via* various routes.

However, the real impact of nanoparticles and their mechanism of reactions against biological system are still not known. Researchers have provided evidence for NP-mediated production of ROS and generation of oxidative stress as a possible mechanism of toxicity (Oberdorster, 2005, Pickering and Wiesner, 2005, Zeng *et al.* 2015) especially for carbonanceous nanoparticles *(i.e.* fullerenes, fullerols and carbon nanotubes) and nanoparticulate like titanium dioxide and zinc oxide (Reeves *et al.* 2008).

The present study need to be further investigated in terms of impact of continuation retention of particles in biological system. Responses of nanoparticles with life forms in the environment can take several routes and their response on organisms against NPs may depends on various factors like cellular uptake, receptors and degree of absorbance, aggregation and cellular interaction.

However, specific response to an organism at cellular level may be in terms of effect of biotic uptake. The retention of NPs may depends on their size, shape and surface charge that interacts with the organism or the as well as type of organism.

Their responses on cellular endocytosis (depend on acceptor activation) or direct membrane penetration (as reported in nanotubes and fullerenes) and extracellular medium (protein and lipid adsorption pattern) may not ignore in present study. It may concluded particles may joined together at the corner or edges and/or aggregates when uptake by living organism, get retained in biological system.

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