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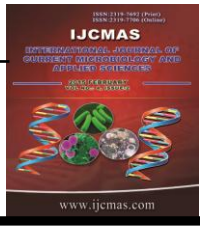


**Original Research Article**

# Kinetics of biodistribution of ZnO-NPs in *Caenorhabditis elegans*

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

The information about the possible impact of manufacture nanoparticles on human health implications and ecological receptors is limited. The objective of the present study was to evaluate to uptake and kinetics of bio-distribution of nanoparticles using invertebrate models *Caenorhabditis elegans*. Despite of the broad spectrum of studies in *C.elegans*, very limited is known about their kinetics of translocation of NPs. We tracked ZnO-NPs in *C. elegans* and correlate particle distribution after 2-10 hrs of exposure. Labeled NPs in living worms was analyzed with help of fluorescence microscope. The uptake of ZnO-NPs was examined *via* the pharynx to the intestinal system. Screening of two different sized nanoparticles (10-50nm) exhibits high rate (60-70%) of kinetics of distribution at initial hrs (2-4 hrs) of exposure however, after 4 hrs the particles either accumulated or excreted out from the body, if worms survived.

**Keywords**

*C.elegans*, ZnO-NPs, biodistribution

## Introduction

Particles of nano-sized have been on the earth for millions of years and have been used by mankind long back. Recently, nanoparticles have attracted a lot of attention because of our increasing ability to synthesize and manipulate the nano-materials. Nanoscale materials are now being used in variety of different uses like electronic, biomedical, pharmaceutical, cosmetic, energy, environment, catalytic and material applications. Because of the wide application nanotechnology promises to bring major advances in various areas including medicine, manufacturing, electronics, energy production and bioremediation.

Engineered nanoparticles represents particle with one or more dimensions of less than 100nm that display unique mechanical, thermal, optical, electrical and medicinal property. The production of manufactured nanoparticles is expected to increase in the near future because of its anticipated utilization in occupational and public exposure. With the increase in production of manufactured nanoparticles, it is anticipated that significant portion of these nanoparticles will be released into environment either through waste products or accidental discharge. Among various manufactured nanoparticles, zinc oxide nanoparticles (ZnO-NPs) are most exploited at nano-dimension level. They are

abundantly used nanomaterials in cosmetics and sunscreens as they exhibit high catalytic efficiency, as well as strong absorption ability for UV light. Colloidal solutions of ZnO-NPs as nano-fertilizer (500–1000 ppm) have potential to boost yield and growth of crops. Prasad *et al.*(2012) recorded application of ZnO-NPs (25 nm @ 1000 ppm) enhanced seed germination, seedling vigor, plant and root growth in peanuts. They are also being used in the food industry as additives and packaging due to their antimicrobial properties (Gerloff, 2009; Jin et al., 2009). Hence is an essential to understand human health implications and consequences of exposure of these nanoparticles The information regarding the fate and transport of these nanoparticles in biological system is limited. The current study highlighted the kinetics of distribution of nanoparticles in *C.elegans* at different time interval.

## Materials and Methods

ZnO-NPs (50 and 10 nm) were purchased from Sigma Aldrich Chemical (St Louis, MO, USA). 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 three different-sized ZnO-NPs (10, 50 and 100nm). The test consisted series of seven ZnO-NP concentrations (0.1 and 2.0 g/l). 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. Each 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 by using fluorescence microscope equipped with a peltier cooled charge-coupled camera. Both differential interference contrast (DIC) and epi-fluorescence images were taken. Filter set with maxima of 460 nm was used for visualization of fluorescence. Results are the means of three replicates. Two-way analysis of variance (ANOVA) was performed by using the SPSS 10.5 software. The objective of statistical analysis was to determine any significant differences among the kinetics of distribution of nanoparticles at different interval of time.

## Result and discussion

The present study revealed the movement and absorption of 10 and 50nm ZnO-NPs during 2-10 hrs of exposure. On exposure of 10nm NPs, the distribution of percentage of particles was recorded 70 ± 1.34 and was little low (60 ± 2.34) with 50nm sized. After 4 hrs, absorption of 10nm sized particles increased by 10 (±1.13) percent and remains constant for 50nm (60±1.34). But after next 2 hrs worms checked to intake food material and excreted out fortified food material gradually, finally at 10 hrs worms either died or don't have NPs spiked food

materials in their gut. The fraction of nanoparticles spiked food materials at different time interval is presented in fig1-3. Both sized nanoparticles presented top-kinetics, with a short period of exposure relatively at fast surface sorption followed by a short period of redistribution, through to form strongly bound forms within the nanoparticles spiked food materials. The relative distribution of both particles not varied at large. The absorption of particles was found slow after 4 hrs of exposure while it was fast during the initial stage of exposure *i.e.*, 1-2 hrs. This difference in percentage of distribution is presented in fig 4. At 6 hrs of exposure the sorption of kinetics was observed low by for 40 and 50 percent for both sized particles. However, the initial uptake was upto 70 and 60 % for 10 to 50 nm particles respectively. The auto-catalytic effect of nutrient medium may also be not ignored during sorption and distribution of these particles. As higher the particle loading represents higher auto-catalysis per unit volume in biological system (Hatje *et al*., 2003). Slow kinetics of nanoparticles after 4 hrs may explain as a result of the colloidal pumps *i.e.* the use of colloid as an intermediary in particle spiked ingested food materials.

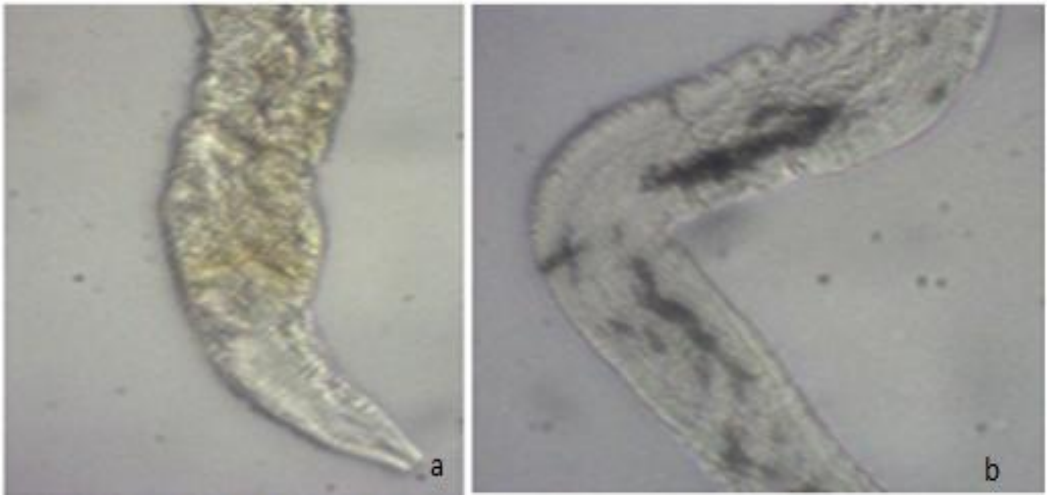


**Fig.1** Biodistribution of ZnO-NPs after exposure of 2 hrs: a, 10nm; b, 50 nm

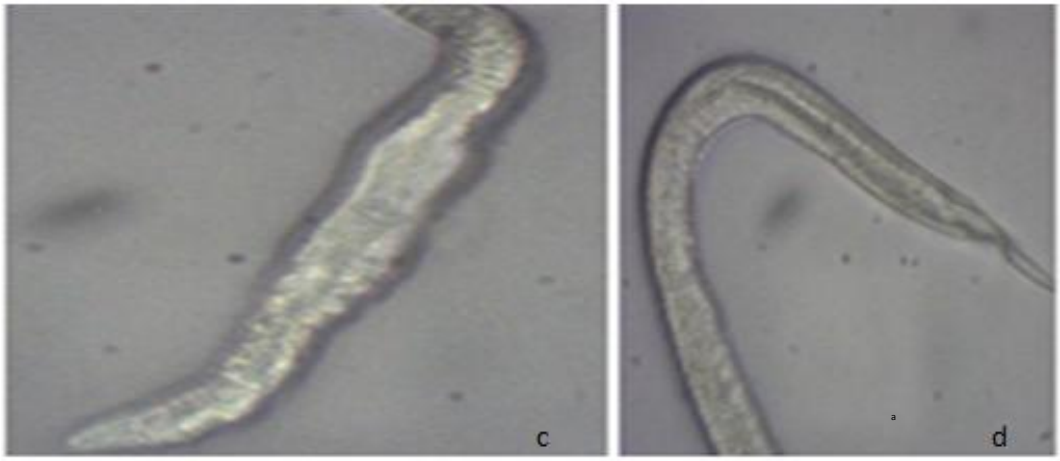

**Fig.2** Biodistribution of ZnO-NPs after exposure of 6 hrs: a, 10nm; b, 50 nm

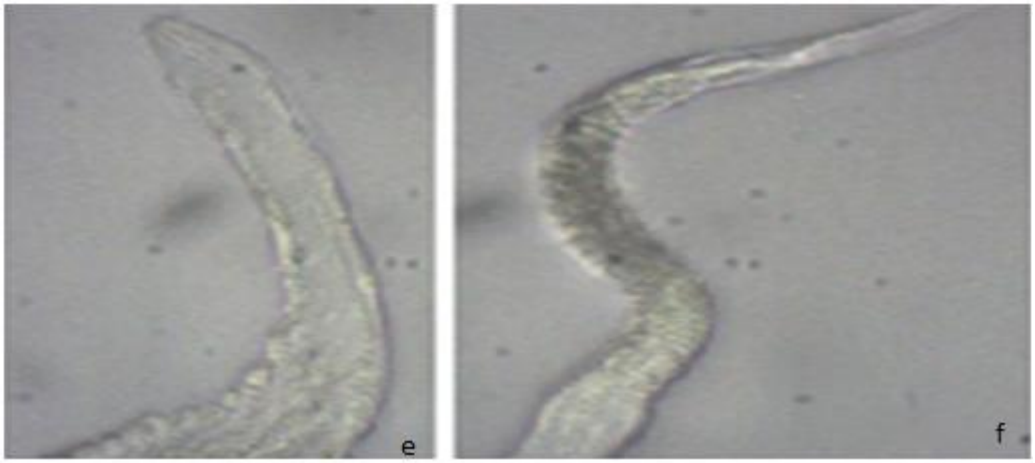


**Fig.3** Biodistribution of ZnO-NPs after exposure of 8hrs: a, 10nm; b, 50 nm

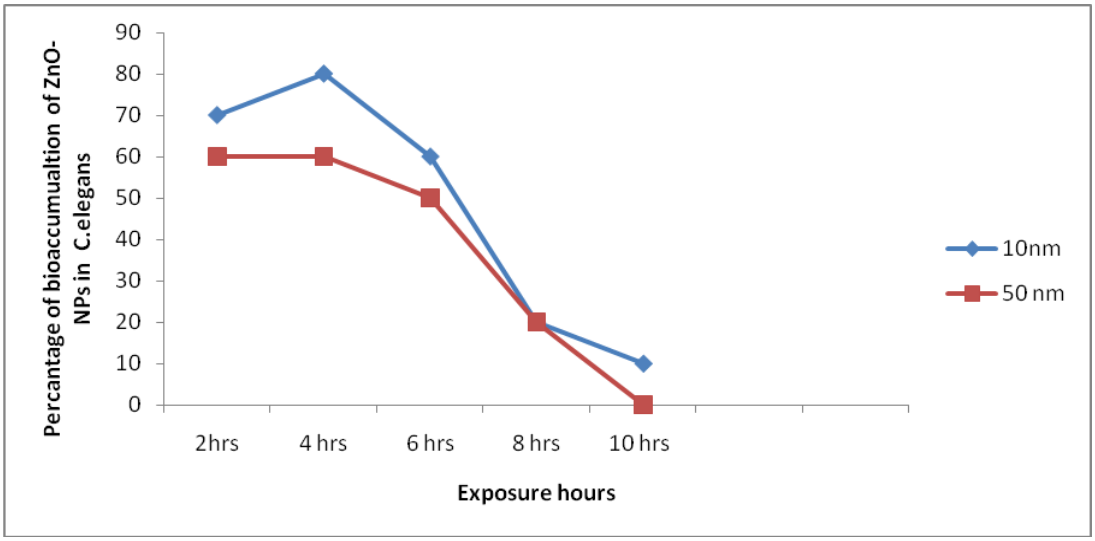


**Fig.4** Kinetics of bio-distribution of ZnO-NPs in *C.elegans* at period of different intervals.

The fast processes of uptake at initial hours may generally attribute in the formation of surface complexes. The slow processes can be attributed to diffusion into micropores (Hatje *et al*., 2003) to show kinetics of axillary reactions which perturb the equilibrium of the sorption reaction or in case of surface precipitation. The present study employed a kinetic approach to determine ZnO –NPs uptake rate in *C.elegans*. The short term exposure provided a high bio-distribution of particles in intestinal region. In addition to being sub-choronic toxicity effects of NPs, long term exposures due to decreased filtration activity (starvation stress) or complexation with metabolites/exudates or mucus biased high kinetics of distribution.

Fowler *et al*. (1975) demonstrated that the excretion kinetics of Zn in the shrimp *Lysmata seticauda* was dependent on the route of Zn exposure, due to different equilibration times of Zn with stable Zn

pools in the animals. Neither exposure time nor route of uptake (dissolved vs food) had a significant effect on distribution of metals. However, the proportion of radioisotope in the slowest compartment increased with duration of exposure was observed in many other studies (Cutshall, 1974; Nugegoda and Rainbow 1989).

Pluskota *et al*.(2009) observed silica-NPs induces reduction of progeny production in the nematode worm *C. elegans. Elder et al., (2006) observed* NP-mediated interference with neural function and translocation of nano-sized manganese oxide particles to the central nervous system *via* the olfactory bulb in rodent animal models. However, observations in species such as *Drosophila* and zebra fish cannot be modulated in *C. elegans* by environmental factors. Huang (2004) also reported post larval stage application of NPs, responsible for the development of reproductive organs and proposed that silica-NPs mediate an age-related degeneration of the interaction between neural and reproductive systems. Scott-Fordsmand (2008) observed shows abnormalities in reproduction in *Eisenia veneta* earthworms fed on double-walled nanotubes and C60 fullerenes, while survival or mortality remained unchanged.

Thus observation of translocation 10 and 50nm ZnO- NPs efficiently distinct in *C. elegans* organs and tissues exhibits an ecotoxic aspect, since nematodes of the *Caenorhabditis* species are abundant inhabitants of composts and soils and may thus participate in the food chain.

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