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Supplementary material

A micro-sized model for the in vivo study of nanoparticle toxicity: what has *Caenorhabditis elegans* taught us?

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Table S1. Published nanotoxicity studies in *C. elegans* related to oxidative stress (OS) as mechanism of toxicity

Nanosize	*C. elegans* strains (life stage of the exposure)	Exposure condition	Mechanism		Finding	Ref.
			OS related endpoints	OS consequences		
Pt nanoparticles (NPs) (2.4 ± 0.7 nm)	N2 and *mev-1* (L4)	0.1 to 1 mM for PtNPt in S-medium	Measurement of reactive oxygen species (ROS) and lipofuscin accumulation, SOD, catalase activity	Antioxidant effect	Pt NPs are superoxide dismutase (SOD) and catalase mimetic scavenge superoxide and hydrogen peroxide. Oxidative stress resistance; antioxidant effect	[1]
Pt NPs[A]	N2 and *nuo-1* (L4)	1 to 25 µM in S-medium	Measurement of ROS and life span, NAD^+ and NADH assays	5 µM conjugated Pt-NPs decreased cytosolic and mitochondrial ROS by inducing NADH oxidase activity	Pt NPs have NADH oxidase activity and rescue the $[NAD^+]$/[NADH] ratio in LB25, leading to effective extension of the lifespan of LB25	[2]
Pt NPs (2.5 ± 1 nm)	N2 (L4 and young adult)	0, 2, 5 or 10 ppb in electrolysed reduced (ERW) medium	Life span assay and ROS detection	Antioxidant effect	Pt NPs in ERW medium extend the longevity of nematode, at least partly by scavenging ROS	[3]
Ag NPs (<100 nm)	N2, *sod-*, *daf-12 and mtl-2*	0.1 mg L^{-1} of AgNPs (microarray) 0.05, 0.1 and 0.5 mg L^{-1}, (quantitative reverse-transcriptase real-time PCR, qRT–PCR, lethality, growth, reproduction) In K-medium	Gene expression using microarray followed by qRT–PCR	reproductive failure and toxicity	Changes in *sod-3* and *daf-12* gene expressions	[4]

Nanosize	*C. elegans* strains (life stage of the exposure)	Exposure condition	Mechanism		Finding	Ref.
			OS related endpoints	OS consequences		
Ag NPs (uncoated, citrate and polyvinyl-pyrrolidone (PVP) coated)	N2, *nth-1*, *sod-2*, *mtl-2*, *xpa-1* and *mev-1* (All developmental stages started from L1)	0.5, 5, 25 and 50 mg L^{-1} In K-medium	Growth measurement of variety of oxidative stress related mutant strains	Growth inhibition	The result does not support that Ag NP-mediated toxicity can be attributed to oxidative stress	[5]
Ag NP citrate-coated (CTL_7-7 nm), PVP_8 (8 nm) and PVP_{38} (38 nm), gum arabic (GA)-coated GA_{22} (22 nm)	N2, *sod-3*, *Pcs-1* and *mev-1*	20 to 46 µM of Ag in EPA water	Pharmacological (*N*-acetylcysteine (NAC) and Trolox) rescue of Ag NP toxicity (mortality and growth inhibition)		NAC completely rescued the growth inhibition of all the tested AgNPs. In contrast, partial rescue by trolox was observed in the following order of effect: CIT 7 > PVP_{38} > GA_{22}, and no rescue by trolox was observed for PVP_8 or GA_{22}	[6]
Ag NPs (20–30 nm)	N2, *pmk-1* and *pmk-1::gfp*	0.1, 0.5 and 1 mg L^{-1} in K-medium	ROS formation, glutathione-S-transferase (GST) enzyme activity	increased ROS formation and decreased reproduction potential	PMK-1 dependent oxidative stress response. Decreased reproductive potential and increased ROS formation induced by AgNPs in wild type (N2) *C. elegans* were rescued in the *pmk-1* (km25) mutant.	[7]

Nanosize	*C. elegans* strains (life stage of the exposure)	Exposure condition	Mechanism		Finding	Ref.
			OS related endpoints	OS consequences		
ZnO NP (40–100 nm)	N2	4, 8, 20, 40, 60, 80 mg L^{-1} Zn for 24 h	measuring photocatalytic activity, ROS generation by methylene blue (MB), Lipid peroxidation (MDA)	mortality	Phototoxicity was closely related to photocatalytic ROS generated by the ZnO particles when exposed to natural sunlight for 2 h. In contrast, under artificial laboratory light, 24 h exposure caused toxicity by a mechanism other than photocatalytic ROS generation	[8]
Al_2O_3 NP (60 nm)	N2 and *phsp-16.2::gfp* (L1, L4, young adult)	51–203.9 mg L^{-1} (acute toxicity – 24 h) 8.1–30.6 mg L^{-1} (chronic toxicity – 10 day)	Intestinal auto-fluorescence, ROS production measurement	accumulation of intestinal auto-fluorescence as well as stress response in intestine	Adverse effects on intestinal lipofuscin accumulation	[9]
CeO_2 NP (8.5 nm)	N2 (L1)	1 to 100 nM	Life span, lipofuscin accumulation, ROS production	ROS accumulation and oxidative damage may the cause of cyto- and genotoxicity	Decreased lifespan	[10]

Nanosize	*C. elegans* strains (life stage of the exposure)	Exposure condition	Mechanism		Finding	Ref.
			OS related endpoints	OS consequences		
TiO_2 NPs (10 nm)	N2 and *mtl-1*, *mtl-2*, *sod-1*, *sod-2*, *sod-3*, *sod-4*, *sod-5*, *mev-1*, *aak-2*, *xpa-1*, *pcm-1*, *hsp-16.48*, *hsp-16.2*, *gst-4*, *gst-8*, *gst-24*, *gst-5*, *gst-42* and *isp-1* mutants (Yong adult stage)	20 g^{-1}	Lethality, intestinal auto-fluorescence, ROS production and gene expression (qPCR)	Comparison of all mentioned endpoints in wild-type and oxidative stress related mutant nematodes	Oxidative stress-related genes such as *sod-2* and *sod-3*, might be responsible for TiO_2-NP toxicity	[11]
Fluorescent nanodiamond (FND) (120 nm)	N2, *daf-16::gfp* and *gcs-1::gfp* (L4, young adults)	1 mg L^{-1} for 2–3 h and moved to bacteria seeded nematode growth agar medium (NGM) plates. Feeding and microinjection of FND into gonads of the worm	ROS measurement (young adults)	FND does not induce ROS generation	FNDs are stable and nontoxic and do not cause any detectable stress, change in longevity, or reproductive potential. FND is expected to become one of the most prominent fluorescent nanoprobes for long-term tracking and imaging	[12]

[A]Pt NPs were functionalised by conjugation with this fusion protein at a 1 : 1 ratio of TAT-platinum binding peptide (PtBP) to Pt atoms

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