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TOXICOLOGY OF NANOMATERIALS AND ENVIRONMENT

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- ABSTRACT: Manufactured nanoparticles demonstrated a variety of biological activities, including toxicity. The goal of this review articles is to summarize some evidence of a variety of toxic effects produced by manufactured nanoparticles, including both the data from literature and the new data of the authors (e.g., [42]). The toxicity of nanomaterials was shown both to prokaryotic and eukaryotic organisms. As for eukaryotic organisms, toxicity was found in bioassays with both animal and plant test systems. In further studies, it is necessary to continue the studies of various aspects of toxicity of nanoparticles, and to extend the range of organisms and test systems that are being used for assessing the biological effects of nanomaterials. The methods that were previously developed to study phytotoxicity of chemicals [15-28, 48] will be useful to generate new data on toxicology of nanomaterials.

INTRODUCTION.

Manufactured nanoparticles (NP) and nanomaterials (nanometer materials) are a new type of man-made chemical products that are produced in significant amounts and finally may enter the environment [1]. Their toxic and ecotoxicological characteristics should be studied in detail.

The goal of this review is to summarize some evidence of a variety of toxic effects produced by manufactured nanoparticles and nanomaterials.

In this paper, we used many publications in the international scientific literature (cited in the list of references), and our own data on plants. Among many sources, especially useful was the paper by Jiang et al. (2009) [2].

TOXICITY OF NANOPARTICLES.

There are many publications that reported various types of toxicity produced by nanoparticles. Some of the examples are summarized in Table 1. The results are commented below in the text following table 1.

Table 1. Toxicity of nanoparticles to eukaryotic and prokaryotic organisms and cells (examples)

Biological objects	Type of NP	Comments	References
Rat liver cell (BRL 3A rat liver cells)	Ag NP	In vitro toxicity: mitochondrial function (MTT assay): mitochondrial function decreased significantly in cells exposed to Ag nanoparticles at 5–50 μg/ml; significant depletion of GSH level, reduced mitochondrial membrane potential and increase in ROS levels;.	Hussain et al., 2005 [3]
Rat liver cell	NP: Fe_3O_4, Al, MoO_3 and TiO_2	NP Fe_3O_4, Al, MoO_3 and TiO_2 had no measurable effect at lower doses (10–50 μg/ml)	[3]
Rat liver cell	NP: Fe_3O_4, Al, MoO_3 and TiO_2	NP Fe_3O_4, Al, MoO_3 and TiO_2: there was a significant effect at higher levels (100–250 μg/ml)	[3]
Rat liver cell		membrane leakage of lactate dehydrogenase (LDH assay): LDH leakage significantly increased in cells exposed to Ag nanoparticles (10–50 μg/ml)	[3]
Rat liver cell	NP: Fe_3O_4, Al, MoO_3 and TiO_2	the other nanoparticles tested displayed LDH leakage only at higher doses (100–250 μg/ml)	[3]
Mammalian cell lines	Oxide NPs	In vitro toxicity; comparison to asbestos, silica; effect of particle	Brunner et al., 2006 [4]

		solubility was studied;	
Mammalian cell lines	Oxide NP	silica nanoparticles; in vitro cytotoxicity was studied;	Chang et al. , 2007 [5]
Algae	Oxide NP	ZnO NP, also bulk ZnO, and $ZnCl_2$; Freshwater microalgae Pseudokirchneriella subcapitata; BP (bulk particles) were also toxic	Franklin et al. 2007 [6]
Higher plants	Oxide NP, metal NP	50% inhibitory concentrations (IC50) of nano-Zn and nano-ZnO were estimated to be near 50 mg/L for radish, and about 20 mg/L for rape and ryegrass	Lin, Xing, 2007 [7];
	Oxide NP	ZnO NP	Lin, Xing , 2008 [8];
	Oxide NP	NP of CuO	Ostroumov S.A., Xing B. New data on toxicity to plant seedlings of Lens culinaris Medik. (Ostroumov, Xing , in preparation)
Crustaceans	Oxide NP, organic NP	titanium dioxide, nano-C60 and C60HxC70Hx; behavioral and physiological changes in Daphnia magna	Lovern, Strickler, 2007 [9];
Crustaceans	Oxide NP	ZnO, CuO, TiO_2 (nanosized and bulk), Daphnia magna and Thamnocephalus platyurus	Heinlaan et al.,2008 [10];
bacteria	Oxide NP	MgO	Stoimenov et al. 2002 [11];
bacteria	Oxide NP	ZnO, Escherichia coli in ultrafine ZnO nanoparticles colloidal medium	Brayner et al. 2006 [12];
bacteria	Oxide NP	TiO_2, SiO_2, and ZnO (comparative eco-toxicity of	Adams et al., 2006 [13];

		nanoscale TiO_2, SiO_2, and ZnO water suspensions)	
bacteria	Oxide NP	ZnO	Huang et al. 2008 [14];
bacteria	Oxide NP	ZnO, Cuo, TiO_2 , Vibrio fisheri (Toxicity of nanosized and bulk ZnO, Cuo, and TiO_2 to bacteria, and crustaceans)	Heinlaan et al., 2008 [10]
bacteria	Oxide NP	Toxicity of NP (ZnO, Al_2O_3, SiO_2) to Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens	Jiang et al., 2009 [2];

The results presented in the table could be commented in the following way, with special attention to performing bioassays using three types of biological objects as test-systems: mammalian cells, higher plants, and bacteria.

TOXICITY OF NANOPARTICLES (NP) TO MAMMALIAN CELLS.

Many authors studied effects produced by NP on mammalian cells, especially in culture.

E.g., Hussain et al. (2005) evaluated the acute toxic effects of metal/metal oxide nanoparticles proposed for future use in industrial production methods using the in vitro rat liver derived cell line (BRL 3A) [3]. Different sizes of nanoparticles such as silver (Ag; 100 nm), molybdenum (MoO_3; 150 nm), aluminum (Al; 103 nm), iron oxide (Fe_3O_4; 47 nm), and titanium dioxide (TiO_2; 40 nm) were evaluated for their potential toxicity. Also, the toxicity was assessed of relatively larger particles of cadmium oxide (CdO; 1 μm), manganese oxide (MnO_2; 1–2 μm), and tungsten (W; 27 μm). For toxicity evaluations, cellular morphology, mitochondrial function (MTT assay), membrane leakage of lactate dehydrogenase (LDH assay), reduced glutathione (GSH) levels, reactive oxygen species (ROS), and mitochondrial membrane potential (MMP) were assessed. The exposure was 24 h [3].

As a result, in the paper by Hussain et al. (2005) it was showed that mitochondrial function decreased significantly in the cells exposed to Ag nanoparticles at 5–50 μg/ml. However, Fe_3O_4, Al, MoO_3 and TiO_2 had no measurable effect at lower doses (10–50 μg/ml), while there was a significant effect at higher levels (100–250 μg/ml). LDH leakage significantly increased in cells exposed to Ag nanoparticles (10–50 μg/ml). The other nanoparticles tested displayed LDH leakage only at higher doses (100–250 μg/ml) [3].

Hussain et al. (2005) concluded that the Ag was highly toxic whereas, MoO_3 moderately toxic and Fe_3O_4, Al, MnO_2 and W displayed less or no toxicity at the doses tested. The microscopic studies demonstrated that nanoparticle-exposed cells at higher doses became abnormal in size, displaying cellular shrinkage, and an acquisition of an irregular shape [3].

The finding of a higher level of toxicity of NP of silver made it necessary to further study the mechanism of toxicity. The results exhibited a significant depletion of GSH level, reduced mitochondrial membrane potential and increase in ROS levels. On the basis of those finding, it was suggested that cytotoxicity of NP of Ag (100 nm) in liver cells is likely to be mediated through oxidative stress (Hussain et al., 2005) [3].

Toxicity of NP to mammalian cells was studied by many other authors as well.

Not only mammalian cells but also higher plants were productively used in bioassay of nanoparticles, which generated lots of new data (see below in the next section of the review).

TOXICITY OF NANOPARTICLES (NP) TO PLANTS.

Earlier, a series of studies of phytotoxicity of various chemicals was published (e.g. [15 - 28]). In those and other studies, the methodology of using plants in bioassay of chemicals was developed and applied with generation a large amount of data.

Plant seedlings were found to be a very efficient and useful tool in bioassay of potentially hazardous chemicals and materials.

This method was applied to studying manufactured NP. Toxicity was found in many, but not all, studies that used plant seedlings.

In addition to the studies made by other authors, NP of metal oxides (TiO_2 , CuO , Al_2O_3) were tested using plant seedling of lentils *Lens culinaris* Medik. (Ostroumov, Xing , in preparation). The new data have shown that most toxic were NP of CuO, and less toxic were NP of Al_2O_3.

Additional data on phytotoxicity of NP are presented in Table 2.

It sould be noted that the data on phytotoxicity of NP are sometimes contradictory. In some studies, no visible signs of phytotoxicity were detected.

In some studies, it was shown that carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth (e.g., the research made by Khodakovskaya et al., 2009 [34]).

It was found that ZnO nanoparticles greatly adhered on to the rootsurface of ryegrass (*Lolium perenne*) [8]. Individual ZnO nanoparticles were observed present in apoplast and protoplast of the root endodermis and stele. However, translocation of Zn from root to shoot remained very low under ZnO nanoparticle treatments, and were much lower than that under

Zn2+ treatments, implying that little if any ZnO nanoparticles could translocate up in the ryegrass under the conditions of that study [8].

Table 2. Studying phytotoxicity of NP using phytotests with higher plants (examples).

Types of NP	Plant species, Latin name	Plant species, Common name	Phytotoxicity observed: + No noticeable phytotoxicity found: - Results are ambiguous: ±	References
multi-walled carbon nanotube, aluminum, alumina, zinc, and zinc oxide	*Raphanus sativus, Brassica napus, Lolium perenne, Lactuca sativa* L., *Zea mays* L., *Cucumis sativus*	six higher plant species (radish, rape, ryegrass, lettuce, corn, and cucumber)	+ (root elongation;)	Lin, Xing, 2007 [7];
ZnO	*Lolium perenne*	ryegrass	+	Lin, Xing, 2008 [8]
multiwalled carbon nanotubes [MWCNTs], Ag, Cu, ZnO, Si	*Cucurbita pepo*	zucchini	- (seed germination)	Stampoulis et al. , 2009 [29]
Cu	*Cucurbita pepo*	zucchini	+ (root elongation)	Stampoulis et al. , 2009 [29]
MWCNTs; Ag; Cu	*Cucurbita pepo*	zucchini	+ (biomass)	Stampoulis et al. , 2009 [29]
Cu	*Phaseolus radiatus*	mung bean	+	Lee et al., 2008 [30]
Cu	*Triticum aestivum*	wheat	+	Lee et al., 2008 [30]
Cu	*Phaseolus radiates; Triticum aestivum*	mung bean; wheat	+	Lee et al., 2008 [30 old]
SWCNTs	*Solanum lycopersicum; Brassica oleracea L., Daucus carota L.* subsp. *sativus (Hoffm.) Arcang., Lactuca sativa L.*	tomato, cabbage, carrot and lettuce	+	Canas et al., 2008 [31]
SWCNTs	*Allium cepa, Cucumis sativus*	onion and cucumber	- (root elongation)	Canas et al., 2008 [31]
AgNPs	*Arabidopsis thaliana*	thale cress	+	Ma et al., 2010a [32]
Al2O3	*Arabidopsis*	Mouse-ear cress	- (root	Lee et al., 2010 [33]

nanoparticles	*thaliana*		elongation)	
ZnO	*Arabidopsis*			Lee et al., 2010 [33]
TiO2	*Spinacia oleracea L.*	spinach	-	Yang et al., 2007; [35].
mixture of SiO2 and TiO2 nanoparticles	*Glycine max*	soybean	-	Lu et al., 2002; [36]
SiO2 nanoparticles (nanostructured silicon dioxide)	*Larix olgensis*	Changbai larch (seedlings)	-	Lin et al., 2004 [37]
aluminum nanoparticles (Nano-aluminum)	*Phaseolus vulgaris*	kidney bean	-	Doshi et al., 2008; [38]
aluminum nanoparticles	*Lolium perrene*	rye grass	-	Doshi et al., 2008 [38]
CeO2	*Brassica oleracea, Triticum aestivum, Cucumis sativus, Raphanus sativus, Lycopersicon esculentum, Lactuca sativa, Brassica napus*	Cabbage, wheat, cucumber, radish, tomato, lettuce, rape (root elongation)	-	Ma et al., 2010b [39]
bentonite and TiO_2 (Colloidal suspensions of clay or titanium dioxide nanoparticles)	*Zea mays L.*	Maize (inhibition of leaf growth, transpiration, root water transport)	+	Asli and Neumann, 2009; [40]
Ag NPs	*Allium cepa*	onion	+ (Genotoxicity)	Kumari et al., 2010; [41]
rare earth oxide nanoparticles La2O3, Gd2O3, Yb2O3	*Brassica oleracea, Triticum aestivum, Cucumis sativus, Raphanus sativus, Lycopersicon esculentum, Lactuca sativa, Brassica napus*	Cabbage, wheat, cucumber, radish, tomato, lettuce, rape (effects on root elongation of plants)	+	
Au	*Ceratophyllum demersum*	Aquatic macrophyte	+	Ostroumov, Poklonov 2009 [42]
CeO2	*Lactuca sativa , Cucumis sativus , Solanum lycopersicum , Spinacia oleracea*	Lettuce, cucumber, tomato, spinach	+	García et al., in press [43]
titanium dioxide, iron oxide	*Lactuca sativa , Cucumis sativus ,*	Lettuce, cucumber, tomato, spinach	±	García et al., in press [43]

	Solanum lycopersicum , *Spinacia oleracea*			
TiO_2; CuO; Al_2O_3	*Lens culinaris*	lentils	+	New data; this study

The data considered above demonstrated toxicity of NP to eukaryotic organisms. It was shown that nanomaterials produce toxic effects on some prokaryotic organisms (bacteria) as well. Some examples are discussed below.

TOXICITY TO BACTERIA.

In some studies, it was shown that NP may produce toxic effects on bacteria. Some facts concerning the representatives of the most common bacteria, Bacillus subtilis , Escherichia coli, and Pseudomonas fluorescence , are presented in Table 3 (below).

Table 3. Relative toxicity of some NP to bacteria Bacillus subtilis , Escherichia coli, and Pseudomonas fluorescence (on the basis of data of Jiang et al., 2009) [2]

Species of bacteria	Types of nanoparticles, all at concentration 20 mg/L			
	ZnO	SiO_2	Al_2O_3	TiO_2
Bacillus subtilis	ZnO NP were more toxic than SiO_2 and Al_2O_3; Amount of CFU decreased 100% as compared to control (no NP)	SiO_2 NP were less toxic than ZnO NP; Amount of CFU decreased ca. 40% as compared to control (no NP)	Al_2O_3 NP were less toxic than ZnO NP; Amount of CFU decreased ca. 57 % as compared to control (no NP)	No visible toxicity
Escherichia coli	ZnO NP were more toxic than SiO_2 and Al_2O_3; Amount of CFU decreased 100% as compared to control (no	SiO_2 NP were less toxic than ZnO NP; Amount of CFU decreased 58% as compared to control (no	Al_2O_3 NP were less toxic than ZnO NP; Amount of CFU decreased 36 % as compared to control (no	No visible toxicity

	NP)	NP)	NP)	
Pseudomonas fluorescence	ZnO NP were more toxic than SiO_2 and Al_2O_3; Amount of CFU decreased 100% as compared to control (no NP)	SiO_2 NP were less toxic than ZnO NP; Amount of CFU decreased 70% as compared to control (no NP)	Al_2O_3 NP were less toxic than ZnO NP; Amount of CFU decreased 70 % as compared to control (no NP)	No toxicity

Among important conclusions of the paper by Jiang et al., 2009 [2], the following should be underlined:

(1) Transmission electron microscopy (TEM) images showed attachment of oxide nanoparticles to the bacteria, suggesting that the toxicity was affected by bacterial attachment.

(2) Bacterial responses to nanoparticles were different from their bulk counterparts; Oxide nanoparticles show higher toxicity than their bulk counterparts. Hence, nanoparticle toxicity mechanisms need to be studied thoroughly.

Other authors demonstrated toxicity of TiO_2 NP when they used much higher concentrations of NP and tested toxicity in the presence of light (the studies by Fu et al., 2005 [44] ; and by Adams et al., 2006 [13]). In some studies it was shown that in the presence of light reactive oxygen species (ROS) generate, which is one of possible mechanisms of TiO_2 NP toxicity (Neal, 2008) [45].

Among other interesting studies of toxicity of NP to bacteria, the paper by Sotiriou and Pratsinis (2010) [36] may be mentioned. In that paper, the combined nanoparticles (Ag/SiO_2) were studied. The antibacterial activity of nanosilver against Gram negative *Escherichia coli* bacteria was investigated by Sotiriou and Pratsinis (2010) by immobilizing nanosilver on nanostructured silica particles and closely controlling Ag content and size [46].

The material presented above provides some examples of toxicity of manufactured NP. The toxicity was shown both to prokaryotic and eukaryotic organisms. As for eukaryotic organisms, toxicity was found in bioassays with both animal and plant test systems. It is necessary to continue the studies of various aspects of toxicity of nanoparticles, and to extend the range of organisms and test systems that are being used for assessing the biological effects of nanomaterials. The methods that were previously developed to study phytotoxicity of chemicals [15-28, 48] will be useful to generate new data on toxicology of nanomaterials.

The new data considered and summarized above provide additional insight into the role of nanomaterials in the context of the issues environmental risks and concerns that arise from the current and future pollution of environment [47-50], which makes necessary to further study all aspects of toxicity from that new class of manufactured chemical products [1, 47].

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