2010

Exposure to Nanoparticles and Hormesis

Ivo Iavicoli
Edward C. Calabrese, Ph.D., *University of Massachusetts - Amherst*
Marc A. Nascarella, Ph.D.

Dose-Response, 8:501–517, 2010
Formerly Nonlinearity in Biology, Toxicology, and Medicine

ISSN: 1559-3258
DOI: 10.2203/dose-response.10-016.Iavicoli

International Dose-Response Society
www.Dose-Response.org

EXPOSURE TO NANOPARTICLES AND HORMESIS

Ivo Iavicoli □ Institute of Occupational Health, Catholic University of Sacred Heart, School of Medicine, Italy

Edward J. Calabrese □ University of Massachusetts Amherst, Department of Public Health, USA

Marc A. Nascarella □ Gradient, USA

□ Nanoparticles are particles with lengths that range from 1 to 100 nm. They are increasingly being manufactured and used for commercial purpose because of their novel and unique physicochemical properties. Although nanotechnology-based products are generally thought to be at a pre-competitive stage, an increasing number of products and materials are becoming commercially available. Human exposure to nanoparticles is therefore inevitable as they become more widely used and, as a result, nanotoxicology research is now gaining attention. However, there are many uncertainties as to whether the unique properties of nanoparticles also pose occupational health risks. These uncertainties arise because of gaps in knowledge about the factors that are essential for predicting health risks such as routes of exposure, distribution, accumulation, excretion and dose-response relationship of the nanoparticles. In particular, uncertainty remains with regard to the nature of the dose-response curve at low level exposures below the toxic threshold. In fact, in the literature, some studies that investigated the biological effects of nanoparticles, observed a hormetic dose-response. However, currently available data regarding this topic are extremely limited and fragmentary. It therefore seems clear that future studies need to focus on this issue by studying the potential adverse health effects caused by low-level exposures to nanoparticles.

Key words: nanoparticles, hormesis, health effects.

INTRODUCTION

Nanotechnology is an emerging multidisciplinary science that involves applications based upon the synthesis of molecules in the nanoscale (10^{-9} m) size range. This technology has the ability to manipulate matter on a near-atomic scale to produce new structures, materials and devices with unique physical and chemical properties (NIOSH 2009). These characteristics enhance versatility and efficacy in product development, resulting in more effective industrial and medical applications, concomitant with the production of more versatile and efficacious products (Colvin 2003). Consequently, nanotechnology has the potential to dramatically improve the effectiveness of a number of existing consumer

Address correspondence to Ivo Iavicoli, Institute of Occupational Health, Catholic University of Sacred Heart, School of Medicine, Largo Francesco Vito, 100168 Roma, Italy; Tel: +39 06 3015 4486; Fax: +39 06 305 3612. Email: iavicoli.ivo@rm.unicatt.it

and industrial products and could have a substantial impact on the development of new products in all sectors, ranging from disease diagnosis and treatment to environmental remediation (NIOSH 2009). In fact, research in nanoscale technologies is growing rapidly worldwide and the National Science Foundation (NSF 2001) estimates that, by 2015, nanotechnology will have a $1 trillion impact on the global economy and will employ 2 million workers, 1 million of whom may be in the United States while Lux Research (2007) predicts that new emerging nanotechnology applications will affect nearly every type of manufactured product through the middle of the next decade, becoming incorporated into 15 % of global manufacturing output and totalling $2.6 trillion in 2014.

According to ISO/TS 27687 (ISO 2008), a nano-object is defined as material with one, two, or three external dimensions in the size range from approximately 1–100 nm. Subcategories of nano-objects are nanoplate, a nano-object with one external dimension at the nanoscale, nanofiber, a nano-object with two external dimensions at the nanoscale with a nanotube defined as a hollow nanofiber and a nanorod as a solid nanofiber and nanoparticle, a nano-object with all three external dimensions at the nanoscale. Nano-objects are commonly incorporated in a larger matrix or substrate referred to as a nanomaterial.

Reducing particle size increases surface area and modifies unique physicochemical properties such as high conductivity, strength, durability, and chemical reactivity (Nel *et al.* 2006). For these reasons, the industrial applications of nanomaterials are very wide-ranging and include those that may lead to more efficient water purification, stronger and lighter building materials, increased computing power and speed, improved generation and conservation of energy and new tools for the diagnosis and treatment of diseases (Card *et al.* 2008). The latest update of the nanotechnology consumer product inventory, maintained by the Project on Emerging Nanotechnologies (PEN), includes over 1,000 products, and has grown nearly 4-fold since it was established in March 2006 (PEN 2009). However, the PEN database does not evaluate whether a product truly contains nanoparticles, it simply reports manufacturer claims, hence the number of nanoenabled products is probably lower. Nonetheless, new nanotechnology consumer products are coming on the market at the rate of three to four per week (PEN 2008). In fact, silica (SiO_2) nanoparticles are used as biomarkers for leukaemia cell identification (Santra *et al.* 2001), cancer therapy (Hirsch *et al.* 2003), drug delivery (Venkatesan *et al.* 2005) and they also find extensive applications in chemical mechanical polishing and as additives to drugs, cosmetics, printer toners, varnishes and food (Lin *et al.* 2006). Metal nanoparticles such as cerium oxide nanoparticles have wide-ranging applications for solar and fuel cells, gas sensors, abrasives for chemical mechanical planarizations, oxygen pumps, metallurgic, glass and ceramic applications (Zheng

et al. 2005; Gao *et al.* 2006); titanium dioxide, in nanoparticle form, is one of the most important materials for photocatalysts, paints, sterilization, bio-medical ceramic and implanted biomaterials, cosmetics and pharmaceuticals (Gelis *et al.* 2003; Sun *et al.* 2004); silver nanoparticles are used in bedding, washers, water purification, toothpaste, shampoo and rinse, infant nipples and nursing bottles, fabrics, deodorants, filters, kitchen utensils, toys and humidifiers (Maynard 2006). Finally, carbon nanotubes (CNTs) are currently of interest for a variety of applications in electronics, reinforced rods, micro-fabricating conjugated polymer activators, biosensors and enhanced electron/scanning microscopy imaging techniques (Shvedova *et al.* 2009).

The increase in nanoparticle applications has led to an increase in concern about their potential human toxicity and their environmental impact. Indeed, as a result of their small size and unique physicochemical properties, the toxicological profiles of nanoparticles may differ considerably from those of larger particles composed of the same materials (Borm *et al.* 2006; Nel *et al.* 2006). Consequently, in the last few years, nanotoxicology has emerged in order to clarify the relationship between the physical and chemical properties (eg. size, shape, surface chemistry, composition, aggregation and surface area) of nanomaterials and the induction of toxic biological responses (Lynch *et al.* 2006). In the literature, most of the studies, that have addressed this topic have focused their attention on the adverse effects of nanoparticles on the respiratory system (Mitchell *et al.* 2007; Park *et al.* 2008, 2009; Wang *et al.* 2008; Sung *et al.* 2009). However, when inhaled nanoparticles are deposited in lung cells and translocated through epithelial and endothelial cells into the blood and lymph circulation, they may reach potentially sensitive target sites including bone marrow, lymph nodes, spleen, heart and central nervous system. Several studies have also investigated the toxic effects of nanoparticles on other organs and systems (Lai *et al.* 2008; Belyanskaya *et al.* 2009; Legramante *et al.* 2009; Murray *et al.* 2009; Sharma *et al.* 2009; Simeonova and Erdely 2009). Nevertheless, there is a general lack of information concerning the effects of manufactured nanomaterials on human health and the environment and, in particular, uncertainty remains with regard to the nature of the dose-response curve for low level exposures. Furthermore, since these studies investigated the toxic effects of different nanoparticles of the same compound, the results reported for a particular type of nanomaterial cannot be considered representative of the whole class. In fact, nanoparticles of the same compound can have different toxicological profiles because of differences in the chemical composition, in the aggregation and surface area, in the shape or size. All of these factors can affect the dose-response relationship.

Hormesis is a dose-response relationship characterized by a low-dose stimulation and a high-dose inhibition (Eaton and Klaassen 2001). The

hormetic dose response has been typically represented in graphs as an inverted U- or J-shaped dose response, depending on the endpoint measured. For example, in the cases of growth, cell proliferation, memory and longevity, hormetic responses have typically been graphed as an inverted U-shaped dose response. In the case of endpoints such as disease incidence (e.g., tumour formation, cardiovascular disease, genotoxicity, birth defects), hormetic effects are typically graphed as a J-shaped dose response. However, this broad range of inverted U- and J-shaped dose response relationships are all considered examples of hormesis (Calabrese 2009).

In this review we have explored the possible presence of hormesis in the studies that have investigated the adverse health effects of nanoparticles.

IN VITRO STUDIES

In the literature there are some *in vitro* toxicological studies (Table 1) that reported a hormetic dose-response following exposure to nanoparticles, in particular carbon nanotubes, quantum dots, metal nanoparticles.

The molecular mechanisms underlying the pathological behaviour of carbon nanotubes (CNTs) were investigated and observed in several *in vivo* and *in vitro* studies. The results obtained by Pulskamp *et al.* (2007a) are particularly interesting in relation to the purpose of our review. In this study, human alveolar epithelial cells A549 were exposed at various concentrations (5-100 μg/ml) of different CNT preparations (NT1-AP, the most impure form; NT2-AT, free of carbonaceous materials and markedly reduced in metal content; NT2-DMF, free of carbonaceous materials but still with high levels of metals) to elicit oxidative stress response. The NT1-AP were commercially purchased (Nanostructured and Amorphous Materials Inc.) and were synthesized using chemical vapour deposition. The NT2-DMF were obtained from NT2 (synthesized by means of the laser vaporization method using graphite targets doped with equal parts of Ni and Co) after purification and separation from the amorphous carbon-containing preparations using DMF (N, N-dimethyl formamide). Finally, NT2-AT were obtained from NT2-DMF after acid treatment with HNO_3 to yield a purified version with reduced catalyst concentrations. Before use in the experiment, the CNTs were precipitated in acetone and resuspended in bidistilled water, sedimented again by centrifugation, and finally added to growth media and diluted to the final concentrations. Prior to use, the samples were sonicated and vigorously mixed to break up the nanotube bundles and sediments. To keep the sample preparations free from other disturbing chemical additives like DMSO or SDS (which facilitate CNT dispersion), a certain degree of CNT aggregation was accepted in the sample fluid. In order to determine oxidative stress, cultured cells were assayed for short-term incubation of 10 minutes as

TABLE 1. *In vitro* studies that showed a hormetic dose - response

Aim of the study	Type of nanoparticles	Type of cells	Range of concentrations	Hormetic response	References
To assess CNTs toxicity and pathology	Single Walled Carbon Nano Tubes	Human alveolar epithelial cell line A549	5 – 100 µg/ml	Biphasic oxidative burst	Pulskamp *et al.* (2007a)
To assess CNTs toxicity and pathology	Single Walled Carbon Nano Tubes	Rat alveolar macrophage cell line NR8383	5 – 100 µg/ml	Low dose stimulation and high dose inhibition of cell viability	Pulskamp *et al.* (2007b)
To explore the possibility of building a high – content, high – throughput cytotoxicity assay platform based on high – content screening technology to meet the demands for nanotoxicity studies.	(TGA)-capped CdTe Quantum Dots and TGA-capped CdTe Quantum Dots produced in the presence of gelatin	NG108-15 murine neuroblastoma cells	0 – 100 nM	Increase in total neurite length at lower concentrations and inhibition of neurite outgrowth at higher concentrations	Jan *et al.* (2008)
To assess the cytotoxicity of different types quantum dots	Quantum Dots, with a core material of indium gallium phosphide	Porcine renal proximal tubule cell line LLC-PK1	4 – 1000 nM	Low dose stimulation and high dose inhibition of cell viability	Stern *et al.* (2008)
To assess the suitability of a spermatogonial stem cell line as a model for the assessment of nanotoxicity	Silver, molybdenum and aluminium nanoparticles	Spermatogonia l stem cell line C18-4	5 – 100 µg/ml	Low dose stimulation and high dose inhibition of mitochondrial function and cell viability	Braydich-Stolle *et al.* (2005)
To study the potential cytotoxic effects of silver nanoparticles	Silver nanoparticles	Peripheral blood mononuclear cells PBMCs	1 – 30 ppm	Low dose stimulation and high dose inhibition of cell proliferation	Shin *et al.* (2007)

Continued

TABLE 1. *Continued*

Aim of the study	Type of nanoparticles	Type of cells	Range of concentrations	Hormetic response	References
To study the cellular responses of two different cell lines exposed to silver nanoparticles	Silver nanoparticles	Human skin carcinoma cell line A431 and human fibrosarcoma cell line HT-1080	0.39 – 25 μg/ml	In A431 cells induction of caspase-3 production at concentrations in the range of 1.56 – 6.25 μg/ml and inhibition, at concentrations ≤ 0.78 and ≥ 12.5 μg/ml. In HT-1080 cells induction of caspase-3 production at concentrations in the range of 0.78 – 6.25 μg/ml ml and inhibition, at concentrations ≤ 0.39 and ≥ 12.5 μg/	Arora *et al.* (2008)
To investigate the potential toxic effects of silver nanoparticles	Silver nanoparticles	Human hepatoma derived cell line HepG2	0.1 – 3 mg/L	Low dose stimulation and high dose inhibition of cell viability	Kawata *et al.* (2009).
To evaluate the cytotoxicity and genotoxicity of graphite nanofibers	Graphite nanofibers	Human bronchial epithelial cells BEAS 2B	1 – 100 μg/cm^2	Low dose stimulation and high dose inhibition of cell viability	Lindberg *et al.* (2009).
To assess efficacy of paclitaxel-loaded in P-SSMM and P-DMSO in circumventing the P-glycoprotein-mediated paclitaxel resistance	Paclitaxel-loaded in sterically stabilized, biocompatible and biodegradable sterically stabilized mixed phospholipid nanomicelles (P-SSMM)	Human breast cancer cell line BC19/3	0.128 – 2000 ng/ml	Low dose stimulation and high dose inhibition of cell viability	Önyüksel *et al.* (2009)

well as for an extended period of 24 hours. Data provided by this work showed that the NT2-AT did not elicit reactive oxygen species (ROS), while NT2-DMF caused a significant increase in ROS production after only 24 hours and finally the most impure form of CNTs induced an increase in ROS after 10 minutes and 24 hours. Moreover, NT1-AP showed a stimulation of ROS production at 5 and 10 μg/ml, while the ROS levels decreased at 50 and 100 μg/ml.

The same author (Pulskamp *et al.* 2007b) studied, in the rat alveolar macrophage cell line NR8383, the cytotoxic effects caused by exposure to different types of commercially available CNTs. Viability of the cells treated with 5, 10, 50 and 100 μg/ml of nanoparticles was evaluated using the 3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl -2H- tetrazolium bromide (MTT) test and the 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium (WST-1) assay. Results of the MTT test showed a dose-dependent decrease in cell viability but, surprisingly, data of the WST-1 test did not agree with the MTT test and they did not indicate a loss of viability due to exposure to all kinds of CNTs. Furthermore, at lower concentrations, in the WST-1 assay, an increase in viability was observed, which rather suggested an increase in proliferation upon particle stimulation. These findings revealed a low-dose stimulation and a high-dose inhibition that could be explained by the presence of the hormetic phenomenon.

Jan *et al.* (2008) explored the possibility of building a high-content, high-throughput cytotoxicity assay platform based on high-content screening technology to meet the demands for nanotoxicity studies. In this attempt they carried out several experiments using differentiated and undifferentiated NG108-15 murine neuroblastoma cells and HepG2 human hepatocyte carcinoma cells exposed to cadmium telluride quantum dots (CdTe QDs) and to gold nanoparticles, respectively. In particular, NG108-15 murine neuroblastoma cells were exposed to thioglycolic acid (TGA)-capped CdTe QDs (TGA-QD) and to a TGA-capped CdTe QD produced in the presence of gelatin (Gelatin-QD). Both of these QDs had a diameter of 3 nm. To investigate the different toxicological effects of TGA-QD and of Gelatin-QD, a neurite outgrowth assay was used. The results showed that a 6-hour treatment with 25 nM of TGA-QDs prior to neuronal differentiation reduced the total neurite length by approximately 50 %. However, treatment with 25 nM of Gelatin QDs led to a slight increase in total neurite length. This difference was most evident at the 50 nM dose of exposure, where all TGA-QD treated cells were killed while Gelatin-QD treated cells still exhibited some viability and a moderate level of neurite outgrowth. The authors suggested that this contradictory effect at low dose could be a hormetic response, as hormesis is frequently observed as a result of low-dose stimulation in toxicological studies. In fact, the mechanisms involved in countering the cytotoxic effect of

a short, non lethal low-dose treatment might have stimulated the formation of neurites.

Stern *et al.* (2008) studied the toxic effects of another type of QDs, with a core material of indium gallium phosphide (InGaP-QDs), on porcine renal proximal tubule cell line (LLC-PK1) treated for 24 and 48 h with concentrations of InGaP-QDs in the range of 4 – 1000 nM. Cytotoxicity of this nanomaterial was determined by the MTT assay and, in the 24h experiment, the results showed an important loss of cell viability at concentrations exceeding 100 nM. Surprisingly, exposure of LLC-PK1 to doses of InGaP-QDs in the range of 30 – 80 nM caused a significant increase in cell viability. Therefore, these results showed the presence of a hormetic dose-response.

In 2005, Braydich-Stolle *et al.* (2005) assessed the toxicity of metal nanoparticles in the male germ line. The authors exposed the spermatogonial stem cell line C18-4 to silver (Ag-NPs, 15 nm in diameter), molybdenum (Mo-NPs, 30 nm in diameter) and aluminium nanoparticles (30 nm in diameter), dispersed in phosphate buffered saline, at concentrations in the range of 5 – 100 μg/ml. The evaluation of the germline stem cell mitochondrial function and viability, after treatment with Mo-NPs, showed the presence of a hormetic-like biphasic dose response. In fact, the results of this study indicated that, in the C18-4 cell line, Mo-NPs exert toxic effects on cellular metabolic activity at concentrations of 50 μg/ml and above whereas, at low doses (5 – 25 μg/ml), they stimulate the mitochondrial function.

Another example of hormesis was observed in a subsequent study that investigated the potential cytotoxicity, the effects on the production of cytokines by and on the proliferation of peripheral blood mononuclear cells (PBMCs) exposed for 72 h to 1, 3, 5, 10, 20 and 30 ppm of Ag-NPs (Shin *et al.* 2007). The cytotoxic effects of these nanoparticles were evaluated using an aqueous cell proliferation assay and a 5-chloromethylfluorescein diacetate (CMFDA) assay**.** Results showed a dose-response relationship characterized by a low-dose stimulation and a high-dose inhibition. In fact, cell proliferation was found to be significantly decreased at Ag-NPs concentrations exceeding 15 ppm whereas, at lower doses an important stimulatory effect was observed.

Spherical silver nanoparticles of 7 – 20 nm in diameter were also used, as colloidal aqueous suspension, by Arora *et al.* (2008) to study the cellular responses of the cell lines A431 (human skin carcinoma) and HT-1080 (human fibrosarcoma) exposed to different concentrations of Ag-NPs. Particularly interesting was the effect of these nanoparticles on caspase-3 activity. In fact, in A431 cells, Ag-NPs induce caspase-3 production at concentrations in the range of 1.56 – 6.25 μg/ml but, at concentrations ≤ 0.78 and ≥ 12.5 μg/ml, data showed a lack of caspase-3 activity. Similar results were obtained in HT-1080 cells where Ag-NPs induce caspase-3

production at concentrations in the range of 0.78 – 6.25 μg/ml whereas, at concentrations ≤ 0.39 and ≥ 12.5 μg/ml caspase-3 activity was not detected. Therefore, these findings could be explained by the presence of a hormetic response.

Similar results were obtained by investigating the toxic effects of low exposure levels of Ag-NPs (7 – 10 nm in diameter), stabilized with polyethylenimine, in human hepatoma derived cell line HepG2 (Kawata *et al.* 2009). To evaluate the cytotoxicity of Ag-NPs, cells were exposed for 24 hours to 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l of the nanoparticles. At high exposure levels, the tested material exhibited a marked cytotoxicity. In fact, the cell viability of the HepG2 cells drastically decreased for Ag-NP concentrations > 1 mg/l. Surprisingly, non-cytotoxic doses (0.1 – 0.5 mg/l) of the nanoparticles significantly increased the viability of HepG2 cells. Hence, the low-dose stimulation and the high-dose inhibition of cell viability showed by the results of this study was interpreted by the authors as being due to hormesis.

A study carried out in human bronchial epithelial BEAS 2B cells to evaluate the cytotoxicity and genotoxicity of graphite nanofibers obtained similar results (Lindberg *et al.* 2009). The nanomaterials were dispersed in bronchial epithelial growth medium cell culture and subjected to ultrasonication for 20 min prior to addition to the cell cultures. The viability of cells, exposed for 24, 48 and 72 h to eight doses (1, 5, 10, 20, 40, 60, 80 and 100 μg/cm^2) of nanofibers was determined using the Trypan blue dye exclusion technique by counting the number of living cells under phase-contrast microscopy. In general, the number of viable cells decreased with the incubation time, dropping to 50 % of the respective control value at about 10 – 40 μg/cm^2 in the 24-h treatment and 40 – 60 μg/cm^2 in the 48-h and 72-h treatment. However, interestingly, in the 24-h treatment a significant increase in the number of living cells was observed at the lowest dose (1 μg/cm^2). However, in the evaluation of viability of cell cultures exposed to CNTs or to carbon nanofibers, it is important to note that these nanoparticles may show unexpected behaviour, such as aggregation or interference with optical measurements, when routine *in vitro* assays are performed (Pfaller *et al.* 2010). These interferences represent an important confounding factor because they can be misinterpreted as cell death.

Finally, in a study performed to assess the efficacy of paclitaxel-loaded in sterically stabilized, biocompatible and biodegradable sterically stabilized mixed phospholipid nanomicelles (P-SSMM) and of paclitaxel dissolved in dimethyl sulfoxide (P-DMSO) in circumventing the P-glycoprotein-mediated paclitaxel resistance in the human breast cancer cell line BC19/3 revealed also the presence of a biphasic dose – response (Önyüksel *et al.* 2009). In fact, exposure of BC19/3 to P-SSMM and to P-DMSO at concentrations in the range of 0.128 – 2000 ng/ml showed a slight

increase in cell survival at the lower doses and a significant inhibition of viability at concentrations exceeding 15 ng/ml.

IN VIVO STUDIES

A hormetic-like response has been also observed in diverse *in vivo* studies (Table 2). In 2007, Roberts *et al.* (2007), while determining the behaviour of a water-soluble, lysophophatidylcholine-coated single-walled carbon nanotube (LPC-SWCNTs) in the presence of *Daphnia magna,* an aquatic invertebrate, observed a typical biphasic dose-response. The assessment of survival of *Daphnia magna*, exposed to 0.1, 0.25, 0.5, 1 and 2.5 mg/L of LPC-SWCNTs showed that the peak survival was reached at an LPC-SWCNT concentration of 0.5 mg/L. At higher test concentrations, survival decreased in a dose-dependent manner as opposed to survival at concentrations below 0.5 mg/L in which survival increased in a dose-dependent manner. On the basis of these findings, the authors suggested the presence of a hormetic response in which the organism gains some benefit from low-level exposure, until a threshold concentration is reached and toxicity occurs.

To evaluate the toxicity of nanosized titanium dioxide (TiO_2), Drobne *et al.* (2009) performed a laboratory single-species toxicity test with the terrestrial arthropod (*Porcellio scaber*, Isopoda, Crustacea). The isopods were exposed for 14 days to 10, 100 and 1000 μg/g dry food of two different TiO_2 nanoparticles (< 25 nm in diameter and < 75 nm in diameter). The results of this study showed that there was a threshold-like dose-dependent pattern for feeding parameters when animals were fed on small nanosized TiO2, while when animals were exposed to larger nano-TiO_2 , no recognizable dose–response relationship pattern was observed for feeding parameters. On the basis of previous work (Drobne and Hopkin 1995), a reduction in feeding rate was expected as recorded many times upon exposure to metal-dosed food but, contrary to expectations, nanosized TiO_2 enhanced feeding rate. The authors suggested that the increase in feeding parameters could be a hormetic-like response which can have complex time response dynamics.

TiO_2 nanoparticles of different sizes (10 and 30) were also used to assess ecotoxicity to the freshwater green alga *Pseudokirchneriella subcapitata* (Hartmann *et al.* 2010). In particular, the authors assessed the growth rate inhibition of algae by exposing them for 72 h to 16 concentrations (0.6 – 250 mg/L) of several TiO_2 nanoparticles. Nanoparticles stock solutions were prepared by suspending the TiO_2 nanoparticles in algal test medium in a concentration of 250 mg/L followed by 10 min sonication in a water bath. These suspensions were sonicated again 10 min prior to preparation of test suspensions. The results of this study showed a tendency of the smallest (10 nm) nanoparticles to induce higher inhibition at lower concentrations. However, an extra data analysis, carried out

TABLE 2. *In vivo* studies that showed a hormetic dose - response

Aim of the study	Type of nanoparticles	Type of living organism	Range of concentrations	Hormetic response	References
To study the interactions between an acquatic invertebrate and a water-soluble, lysophophatidylcholine coated single-walled carbon nanotube	Water – soluble, lysophophatidylcholine coated single – walled carbon nanotube (LPC-SWCNTs)	Acquatic invertebrate *Daphnia magna*	0.1 – 2.5 mg/L	Low dose stimulation and high dose inhibition of survival	Roberts *et al.* (2007)
To investigate the hazard of titanium dioxide nanoparticles	Titanium dioxide nanoparticles	Terrestrial arthropod *Porcellio scaber*, Isopoda, Crustacea	10 – 1000 µg/g of dry food	Enhancement of the feeding rate	Drobne *et al.* (2009)
To evaluate the ecotoxicity of three different sizes of titanium dioxide particles	Titanium dioxide nanoparticles	Freshwater green alga *Pseudokirchneriella subcapitata*	0.6 – 250 mg/L	Significant stimulation of the algal growth rate at lower concentrations and inhibition at higher doses.	Hartmann *et al.* (2010)
To evaluate the toxic effects of two different types of metal nanoparticles	Copper and zinc nanoparticles	Ciliated protozoa *Tetrahymena thermophila*	Copper nanoparticles: 31.25 – 500 mg/L Zinc nanoparticles: 1.85 – 25 mg/L	At the lowest and sub-toxic concentrations tested the nanoparticles had a stimulatory effect on ATP concentration of *Tetrahymena thermophila*	Mortimer *et al.* (2010),
To assess the phytotoxicity of zinc and zinc oxide nanoparticles	Zinc and zinc oxide nanoparticles	Radish, rape and ryegrass	0 – 2000 mg/L	Exposure to lower doses of nanoparticles caused a slight increase of root length whereas, root growth was clearly restricted with increasing concentration	Lin and Xing (2007)
To evaluate the effects of rare earth oxide nanoparticles on root elongation of plants	Cerium, lanthanum, gadolinium and ytterbium oxide nanoparticles	Rape	0.2 – 2000 mg/L	At low concentrations (< 0.8 mg/L) lanthanum and ytterbium nanoparticles had positive effects on root elongation but negative effects at higher concentrations	Ma *et al.* (2010)

using a three parameter log logistic curve fitting with a hormesis parameter added as described by Brain and Cousens (1989) and with a lack of fit model as described by Cedergreen *et al.* (2005), revealed, for the larger nanoparticles (30 nm), a biphasic relationship with a statistically significant stimulation of the algal growth rate at lower concentrations and an inhibition at higher doses.

Recently, a study by Mortimer *et al.* (2010), conducted on the ciliated protozoa *Tetrahymena thermophila* exposed to 31.25, 62.5, 125, 250 and 500 mg/L of copper nanoparticles (Cu-NPs) and to 1.85, 5.55, 8.33, 12,5 and 25 of zinc nanoparticles (Zn-NPs) showed the presence of a hormetic response. The toxic effects of these nanoparticles to protozoa were evaluated at two exposure times (4 and 24 h), using propidium iodide staining and cellular ATP concentration that are both correlated to the cell viability. Interestingly, at the lowest and sub-toxic concentrations tested (31.25 mg/L and 1.85 mg/L for Cu-NPs and Zn-NPs, respectively), the nanoparticles had a stimulatory effect on ATP concentration of *Tetrahymena thermophila*. The hormetic phenomenon was detected only by the ATP measurements and not in the propidium iodide staining assay.

The assessment of the phytotoxicity of Zn-NPs (35 nm in diameter) and zinc oxide nanoparticles (ZnO-NPs, 20 ± 5 nm in diameter) on seed germination and root growth of radish, rape and ryegrass showed a hormetic dose-response (Lin and Xing 2007). No significant root growth inhibition was observed under low concentrations (less than 10 mg/L for rape and ryegrass and 20 mg/L for radish). In particular, the exposure of radish and rape to lower doses of ZnO-NPs and the exposure of ryegrass to lower doses of Zn-NPs caused a slight increase in root length whereas, root growth of these plant species was clearly restricted with increasing concentration and was almost terminated at 200 mg/L.

Recently, a similar study, carried out by Ma *et al.* (2010), showed the ability of other types of nanoparticles to induce a biphasic dose-response relationship. As in the previous study, the phytotoxicity of cerium (CeO_2-NPs), lanthanum (La_2O_3-NPs), gadolinium (Gd_2O_3-NPs) and ytterbium (Yb_2O_3-NPs) oxide nanoparticles was evaluated in several higher plant species by means of root elongation experiments. The effects of exposure to different concentrations (0.2 – 2000 mg/L) of rare earth oxide NPs on root elongation of rape were particularly interesting. In fact, after exposure to La_2O_3-NPs and Yb_2O_3-NPs, root elongation in this plant was enhanced at less than 0.8 mg/L but, as the concentration increased, root growth was restricted and almost halted at 200 and 2000 mg/L. Therefore, La_2O_3-NPs and Yb_2O_3-NPs had positive effects on root elongation at low concentrations (< 0.8 mg/L), but negative effects at higher concentrations. The authors suggested that these findings could be explained by "hormesis effects".

DISCUSSION AND CONCLUSION

The hormetic dose-response may be reliably described by a stimulation in the low dose zone and an inhibitory response at higher doses (Calabrese 2009) The last three decades have witnessed growing interdisciplinary evidence of hormetic - biphasic dose-responses that are characterized by remarkably similar quantitative features of the dose-response and similar underlying mechanistic explanatory strategies. It is the emergence and integration of these findings from diverse biomedical fields that has led to consolidation of the hormesis dose- response concept (Calabrese 2008a).

According to Stebbing's theory, the key factor in the hormesis concept is not the chemical but, rather, the organism. In fact, the examples gathered from the literature reviewed by Stebbing led him to suggest that hormesis is not a specific effect of the agent that induces it, since it can be induced by such a wide variety of agents of different kinds. Furthermore, the ubiquity of hormesis that follows a similar pattern (beta curve) in many taxa, suggests a common explanation. Therefore the hypothesis tested was that hormesis is a consequence of an adaptive response common to biological systems to the inhibitory effect that different agents have in common at higher concentrations (Stebbing, 1998). In other words, the hormetic response is found in the organism's overcompensation to a disruption in homeostasis (Stebbing 1987, 1998). If this is the case, then any agent - even in nanoparticle form - that can disrupt homeostasis (i.e. cause toxicity) would be expected to induce a hormetic response to the damage induced. The Stebbing theory does not infer that all chemicals will be hormetic for all endpoints. It does, however, imply that biological systems respond in a hormetic manner to signals that indicate stress, toxicity or disruptions in homeostasis (Calabrese 2008b).

In fact, the occurrence of hormetic dose-response in the toxicological literature is extremely high and, to date, approximately 8000 dose-responses have been reported in the hormesis database (Calabrese and Blain 2005). Nevertheless, to our knowledge, this review represents the first attempt to summarize current data regarding the possible induction of hormesis by nanoparticles.

In recent years, the number of studies that have investigated the adverse health effects of nanoparticles has increased significantly because the massive development of nanotechnologies has led to considerable concern regarding the potential biological effects and human toxicity of these materials. However, the toxicity of nanoparticles has not been fully evaluated and, at present, there are many uncertainties as to whether the unique properties of engineered nanoparticles also pose a health risk for humans. These uncertainties arise because of gaps in knowledge about the factors that are essential for predicting health risks such as the nature

of the dose-response curve at low level exposures below the toxic threshold. In fact, most of the *in vitro* and *in vivo* studies that investigated and identified several toxicological effects of CNTs, metal nanoparticles or other nanomaterials used high doses of these chemicals, at exposure levels in excess of those encountered in the different environmental matrices or workplaces. Obviously, this feature represents a serious problem when trying to detect the presence of a hormetic response. Toxicological assessments that include either too few doses, too high doses or inadequate dose spacing are not capable of accurately assessing the nature of the dose-response relationship. Consequently, using only high doses of exposure, these studies might underestimate the occurrence of hormetic responses.

Although, the Stebbing theory suggests that all chemicals have the capacity to induce a hormetic response in some experimental settings, a clear structural specificity exists in the induction of hormetic-like biphasic dose responses for specific endpoints and experimental conditions (Calabrese 2008b). If this is true, then the contribution of the chemical structure and composition of nanoparticles to the induction of hormesis must be addressed very carefully since these materials are manipulated on a near-atomic scale and their toxicological profiles may be quite different from those of larger particles with the same chemical composition (NIOSH 2009). Furthermore, the toxicity of nanoparticles is not only related to their chemical composition but there are several other parameters that must be taken into account. In fact, current evidence suggests that the biological impact and the biokinetics of nanoparticles are dependent on their small size (surface area and size distribution), chemical composition (purity, crystallinity, electronic properties, etc.), surface structure (surface reactivity, surface groups, inorganic or organic coatings, etc.), solubility, shape, and aggregation (Nel *et al.* 2006). Hence, a correct evaluation of the induction of a hormetic response by nanoparticles is not possible without a preliminary, accurate and precise characterization of these materials.

The hormetic dose-response must also be seen within a temporal context, that is, as a dose - time - response relationship. The reason for incorporating a temporal feature in hormesis is that it may also be described as a modest overcompensation response following an initial disruption in homeostasis, i.e. a type of rebound effect. The hormetic dose response therefore represents the effects of a repair process that slightly or modestly overshoots the original homeostatic set point, resulting in the low-dose stimulatory response (Calabrese 1999, 2001). The assessment of the dose response is therefore a dynamic process. Whereas harmful agents may induce toxicity in affected biological systems, the organism or biological system is not a passive entity but will respond to damage signals with a coordinated series of temporally- mediated repair processes. This

dynamic aspect of toxicological assessment requires the inclusion of not only a broad range of doses but also a series of temporal evaluations (i.e. repeated measurements). Only by assessing the dose-response process over time can an accurate assessment of the dose-response relationship be determined, within which the hormetic dose response is best revealed (Calabrese 2008b). Unfortunately, also in the studies that we have reported in this review the presence of a hormetic response was not evaluated over time, therefore it would be of great interest to repeat these experiments using a series of temporal evaluations to better define the occurrence of hormesis.

In conclusion, the results of some studies that have investigated the toxicological effects of the nanoparticles suggest that these chemicals may be able to induce, in some experimental settings, a hormetic response for specific endpoints. Nevertheless, the data currently available on this topic are extremely limited and fragmentary and for this reason, at the present time, it is not possible to reach comprehensive conclusions or a broad consensus. Consequently, more research is needed on the occurrence of the hormetic dose-response elicited by exposure to nanoparticles. However, to be able to detect the real presence of hormesis, future studies should focus on deep and accurate characterization of nanoparticles, they should use a broad range of exposure doses and also include a series of temporal evaluations.

REFERENCES

Arora S, Jain J, Rajwade JM, Paknikar KM. 2008. Cellular responses induced by silver nanoparticles: In vitro studies. Toxicol Lett 179:93-100.

Belyanskaya L, Weigel S, Hirsch C, Tobler U, Krug HF, Wick P. 2009. Effects of carbon nanotubes on primary neurons and glial cells. Neurotoxicology 30:702-711.

Borm PJ, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdorster E. 2006. The potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol 3:11.

Brain P and Cousens R. 1989. An equation to describe dose responses where there is stimulation of growth at low doses. Weed Res 29:93-96

Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann MC. 2005. In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicol Sci 88:412-419.

Calabrese EJ. 1999. Evidence that hormesis represents an ''overcompensation'' response to a disruption in homeostasis. Ecotoxicol Environ Saf 42:135–137.

Calabrese EJ. 2001. Overcompensation stimulation: A mechanism for hormetic effects. Crit Rev Toxicol 31:425–470.

Calabrese EJ and Blain R. 2005. The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: an overview. Toxicol Appl Pharmacol 202:289–301.

Calabrese EJ. 2008a. Hormesis and medicine. Br J Clin Pharmacol 66:594-617.

Calabrese EJ. 2008b. Hormesis: why it is important to toxicology and toxicologists. Environ Toxicol Chem 27:1451-1474.

Calabrese EJ. 2009. Getting the dose-response wrong: why hormesis became marginalized and the threshold model accepted. Arch. Toxicol 83:227-247.

Card JW, Zeldin DC, Bonner JC, Nestmann ER. 2008. Pulmonary applications and toxicity of engineered nanoparticles. Am J Physiol Lung Cell Mol Physiol 295:L400-L411.

Cedergreen N, Ritz C, Streibig JC. 2005. Improved empirical models describing hormesis. Environ Toxicol Chem 24:3166-3172.

Colvin VL. 2003. The potential environmental impact of engineered nanomaterials. Nat Biotechnol 1:1166-1170.

Drobne D and Hopkin SP. 1995. The toxicity of zinc to terrestrial isopods in a standard laboratory test. Ecotoxicol Environ Saf 31:1-6.

Drobne D, Jemec A, Pipan Tkalec Z. 2009. In vivo screening to determine hazards of nanoparticles: nanosized TiO_2. Environ Pollut 157:1157-1164.

Eaton DL and Klaassen CD. 2001. Principles of toxicology, In: Klaassen CD (ed), Casarett and Doull's Toxicology, The Basic Science of Poisons, pp 11-34. McGraw-Hill, New York.

Gao F, Lu Q, Komarneni S. 2006. Fast synthesis of cerium oxide nanoparticles and nanorods. J Nanosci Nanotechnol 6:3812-3819.

Gelis C, Girard S, Mavon A, Delverdier M, Pailous N, Vicendo P. 2003. Assessment of the skin photoprotective capacities of an organo-mineral broadspectrum sunblock on two ex vivo skin models. Photodermatol Photoimmunol Photomed 19:242–253.

Hartmann NB, Von der Kammer F, Hofmann T, Baalousha M, Ottofuelling S, Baun A. 2010. Algal testing of titanium dioxide nanoparticles—testing considerations, inhibitory effects and modification of cadmium bioavailability. Toxicology 269:190-197.

Hirsch LR, Stafford RJ, Bankson JA, Sershen SR, Rivera B, Price RE, Hazle JD, Halas NJ, West JL. 2003. Nanoshell-mediated nearinfrared thermal therapy of tumors under magnetic resonance guidance. Proc Natl Acad Sci U. S. A. 100:13549–13554.

ISO (International Organization for Standardization). 2008. Nanotechnologies — Terminology and definitions for nano-objects — Nanoparticle, nanofibre and nanoplate. ISO/TS 27687. Geneva, Switzerland.

Jan E, Byrne SJ, Cuddihy M, Davies AM, Volkov Y, Gun'ko YK, Kotov NA. 2008. High-content screening as a universal tool for fingerprinting of cytotoxicity of nanoparticles. ACS Nano 2: 928-938.

Kawata K, Osawa M, Okabe S. 2009. In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. Environ Sci Technol 43:6046-6051.

Lai JC, Lai MB, Jandhyam S, Dukhande VV, Bhushan A, Daniels CK, Leung SW. 2008. Exposure to titanium dioxide and other metallic oxide nanoparticles induces cytotoxicity on human neural cells and fibroblasts. Int J Nanomedicine 3:533-545.

Legramante JM, Valentini F, Magrini A, Palleschi G, Sacco S, Iavicoli I, Pallante M, Moscone D, Galante A, Bergamaschi E, Bergamaschi A, Pietroiusti A. 2009. Cardiac autonomic regulation after lung exposure to carbon nanotubes. Hum Exp Toxicol 28:369-375.

Lin D, Xing B. 2007. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environ Pollut 150:243-250.

Lin W, Huang Y, Zhou XD, Ma Y. 2006. In vitro toxicity of silica nanoparticles in human lung cancer cells. Toxicol Appl Pharmacol 217:252-259.

Lindberg HK, Falck GC, Suhonen S, Vippola M, Vanhala E, Catalán J, Savolainen K, Norppa H. 2009. Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanofibres in human bronchial epithelial cells in vitro. Toxicol Lett 186:166-173.

Lux Research. 2007. The Nanotech Report, 5th Edition. New York.

Lynch I, Dawson KA, Linse S. 2006. Detecting cryptic epitopes created by nanoparticles. Sci STKE 327:pe14.

Ma Y, Kuang L, He X, Bai W, Ding Y, Zhang Z, Zhao Y, Chai Z. 2010. Effects of rare earth oxide nanoparticles on root elongation of plants. Chemosphere 78:273-279.

Maynard AD. 2006. Nanotechnology: A Research Strategy for Addressing Risk. Woodrow Wilson International Center for Scholars, Washington, DC.

Mitchell LA, Gao J, Wal RV, Gigliotti A, Burchiel SW, McDonald JD. 2007. Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. Toxicol Sci 100:203-214.

Mortimer M, Kasemets K, Kahru A. 2010. Toxicity of ZnO and CuO nanoparticles to ciliated protozoa Tetrahymena thermophila. Toxicology 269:182-189.

Murray AR, Kisin E, Leonard SS, Young SH, Kommineni C, Kagan VE, Castranova V, Shvedova AA. 2009. Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes. Toxicology 257:161-171.

Nel A, Xia T, Madler L, Li N. 2006. Toxic potential of materials at the nanolevel. Science311:622-627.

NIOSH (National Institute for Occupational Safety and Health). 2009. Approaches to Safe Nanotechnology: managing the health and safety concerns associated with engineered nanomaterials. Centers for Disease Control and Prevention, Atlanta.

NSF (National Science Foundation). 2001. Societal implications of nanoscience and nanotechnology. Nanoscale Science, Engineering, and Technology (NSET) Workshop Report, Arlington, VA.

Önyüksel H, Jeon E, Rubinstein I. 2009. Nanomicellar paclitaxel increases cytotoxicity of multidrug resistant breast cancer cells. Cancer Lett 274:327-330.

Park EJ, Choi J, Park YK, Park K. 2008. Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. Toxicology 245:90-100.

Park EJ, Yoon J, Choi K, Yi J, Park K. 2009. Induction of chronic inflammation in mice treated with titanium dioxide nanoparticles by intratracheal instillation. Toxicology. 260:37-46.

PEN (Project on Emerging Nanotechnologies). 2008. New Nanotech Products Hitting the Market at the Rate of 3-4 Per Week. Project News of April 24, 2008. Available at http://www.nanotechproject.org/news/archive/6697/

PEN (Project on Emerging Nanotechnologies). 2009. Consumer products: An inventory of nanotechnology - based consumer products currently on the market. Available at http://www.nanotechproject.org/inventories/consumer/updates/

Pfaller T, Colognato R, Nelissen I, Favilli F, Casals E, Ooms D, Leppens H, Ponti J, Stritzinger R, Puntes V, Boraschi D, Duschl A, Oostingh GJ. 2010. The suitability of different cellular *in vitro* immunotoxicity and genotoxicity methods for the analysis of nanoparticle-induced events. Nanotoxicology 4:52-72

Pulskamp K, Wörle-Knirsch JM, Hennrich F, Kern K, Krug HF. 2007a. Human lung epithelial cells show biphasic oxidative burst after single-walled carbon nanotube contact. Carbon 45:2241-2249.

Pulskamp K, Diabaté S, Krug HF. 2007b. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. Toxicol Lett 168:58-74.

Roberts AP, Mount AS, Seda B, Souther J, Qiao R, Lin S, Ke PC, Rao AM, Klaine SJ. 2007. In vivo biomodification of lipid-coated carbon nanotubes by Daphnia magna. Environ Sci Technol 41:3025-3029.

Santra S, Zhang P, Wang K, Tapec R, Tan W. 2001. Conjugation of biomolecules with luminophore - doped silica nanoparticles for photostable biomarkers. Anal Chem 73:4988–4993.

Sharma HS, Ali SF, Hussain SM, Schlager JJ, Sharma A. 2009. Influence of engineered nanoparticles from metals on the blood-brain barrier permeability, cerebral blood flow, brain edema and neurotoxicity. An experimental study in rat and mice using biochemical and morphological approaches. J Nanosci Nanotechnol 9:5055-5072.

Shin SH, Ye MK, Kim HS, Kang HS. 2007. The effects of nano-silver on the proliferation and cytokine expression by peripheral blood mononuclear cells. Int Immunopharmacol 7:1813-1818.

Shvedova AA, Kisin ER, Porter D, Sculte P, Kagan VE, Fadeel B, Castranova V. 2009. Mechanisms of pulmonary toxicity and medical applications of carbon nanotubes: Two faces of Janus? Pharmacol Ther 121:192-204.

Simeonova PP and Erdely A. 2009. Engineered nanoparticle respiratory exposure and potential risks for cardiovascular toxicity: predictive tests and biomarkers. Inhal Toxicol 21 Suppl 1:68-73.

Stebbing ARD. 1987. Growth hormesis—A by-product of control. Health Phys 52:543–547.

Stebbing ARD. 1998. A theory for growth hormesis. Mutat Res 403:249–258.

Stern ST, Zolnik BS, McLeland CB, Clogston J, Zheng J, McNeil SE. 2008. Induction of autophagy in porcine kidney cells by quantum dots: a common cellular response to nanomaterials? Toxicol Sci 106:140-152.

Sun D, Meng TT, Loong H, Hwa TJ. 2004. Removal of natural organic matter from water using a nano-structured photocatalyst coupled with filtrating membrane. Water Sci Technol 49:103–110.

Sung JH, Ji JH, Park JD, Yoon JU, Kim DS, Jeon KS, Song MY, Jeong J, Han BS, Han JH, Chung YH, Chang HK, Lee JH, Cho MH, Kelman BJ, Yu IJ. 2009. Subchronic inhalation toxicity of silver nanoparticles. Toxicol Sci 108:452-461.

Venkatesan N, Yoshimitsu J, Ito Y, Shibata N, Takada K. 2005. Liquid filled nanoparticles as a drug delivery tool for protein therapeutics. Biomaterials 26:7154–7163.

Zheng X, Zhang X, Wang X, Wang S, Wu S. 2005. Preparation and characterization of Cu/CeO_2 catalysts and their applications in low-temperature CO oxidation. Appl Catal A: Gen 295:142-149.

Wang L, Castranova V, Rojanasakul Y, Lu Y, Scabilloni J, Mercer RR. 2008. Direct fibrogenic effects of dispersed single walled carbon nanotubes on human lung fibroblasts. Toxicologist 102:A1499.