Journal of Toxicology and Health
ISSN 2056-3779 | Volume 4 | Article 4

HOAJ
Herbert Open Access Journals

Original Research Open Access

Toxicity of *N*-Nitrosodimethylamine and SiO_2 nanoparticles to HaCaT and caco-2 cell lines found in waste water treatment

Abdulmajeed G. Almutary* and Barbara J. S. Sanderson

*Correspondence: Almu0047@flinders.edu.au

CrossMark
Click for updates

Flinders University, School of Medicine, Nursing and Health Sciences Building, South Australia.

Abstract

Nanoparticles (NPs) offer the possibility of safe removal of pollutants and microbes in water treatment and purification. Nowadays, NPs are used in the detection and purification of water from chemicals and biological substance such as metals (cadmium, copper, lead and zinc) nutrients (nitrate, nitrite ammonia and phosphate) bacteria, viruses, parasite and antibiotics. Metal containing NPs are among the four classes of particles used commonly in water treatment. Membrane technologies such as Ultrafiltration (UF), Nanofiltration (NF) and Reverse Osmosis (RO) have been widely used all over the world especially for water treatment and desalination. NPs after have been incorporated into membranes gained attention due to its ability to enhance membrane permeability, mechanical properties and selectivity in some cases. However, these membranes are suspected to fouling causing NPs and other contaminate to reach waterways. In this study, we tested the toxicity of SiO_2 NPs synthesised by Stöber method and *N*-Nitrosodimethylamine (NDMA) as potent known carcinogens forms during chlorination on HaCaT and caco-2 cell lines for 4, 24 and 48h using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays also the morphology and size of SiO_2 determined by Scanning electron microscopy (SEM). After exposure to SiO_2 NPs (concentrations between 0.05-2 mg/mL), the concentration of 2 mg/mL inactivated LDH in both cell lines; however, it did not reduce the metabolic activity of both cell lines when MTT assay used. SEM revealed spherical and uniformed SiO_2 particles with size 200 nm in diameter. NDMA (concentrations between 0.1-1000 µg/mL) inactivated LDH leakage in HaCaT and caco-2 also reduced the metabolic activity of HaCaT cell line at 48 hours exposure. The outcome of this study suggest that a concentration of SiO_2<2 mg/mL used in water treatment can reduce the risk of nanmaterials toxicity to human and possibly the ecosystem. Our results urgefor more studies on the effect of nanomaterials to the aquatic environment and human exposure to NPs.

Keywords: Water contamination, nanoparticles, SiO_2, *N*-Nitrosodimethylamine, ultrafiltration, reverse osmosis, toxicity, MTT assay, LDH assay

Background

The rapid development of nanoparticles (NPs) applicationshave resulted in enhancing the process of products manufacturing, developed products that reduce the concentrations of toxic compounds and assisted in the attainment of water quality standards and health advisories [1]. Therefore, it is necessary to investigate the possible risks related to these small particles to human in environmental exposure. Microfiltration (MF) and Ultrafiltration (UF) are one of the most effective methods for raw water treatment [2]. The majority of UF membranes are formed by hydrophobic polymers such as polysulfone (PS), polyethersulfone (PES), polypropylene (PP), polyethylene (PE) and polyvinylidenefluoride (PVDF) [2]. PES is the most used as UF membrane due to its excellent thermal, mechanical properties and chemical resistance. However, the PES is hydrophobic in nature and is suspected to fouling [3]. The main interest in the clearing efficiency of nanoparticles within sewage plant is to prevent nanomaterials from reaching the waterways [4]. Recently, possible toxic effect of carbon nanotubes to aquatic species such as *larval Zebrafish* and *DaphinaMagna* has been studied [5,6]. Another study investigated the physiological changes and toxicity to *DaphinaMagna* exposed to Titanium

© 2017 Almutary et al; licensee Herbert Publications Ltd. This is an Open Access article distributed under the terms of Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0). This permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

doi: 10.7243/2056-3779-4-4

dioxide (TiO_2) NPs which is one of the most important metal oxide nanoparticles [7]. These studies urge for clarifying the entrances of nanomaterials into aquatic environment. So far, little is known about the nanomaterials reaching the waterways [8]. Some studies criticised the standard wastewater treatment as it poorly suited to capture Nanomaterials [8]. Moreover, Wiesner et al. (2008) reported that waterways contaminated with nanomaterials water desalination plants may become problematic [9]. Due to the lack of experimental data, the method of nanomaterials disposal is not understood clearly [9,10]. Obviously, this will be a problematic because there is no sufficient data explaining the risk of inorganic nanoparticles dispersed in the environment or public sewage treatment plants. *N*-nitrosodimethylamine (NDMA) is well known for its carcinogenic properties [11,12]. For many years, NDMA was used in pesticide (nematicide), plasticizer for rubber, battery component, antioxidant, lubricant additive, and in polymers [13]. Recently, some studies revealed that water treatment polymers such as poly (epichlorohydrin dimethylamine) (polyamine) and poly (diallyldimethylammonium chloride) (polyDADMAC) may form NDMA if contacted with chloramine water [14]. In addition, NDMA is mainly used in research to induce cancer in mice [15]. Thus, the health concerns of this compound has been the focus for some industries after recognised as disinfection of drinking and wastewater containing chlorine and chloramines [16]. Recently, successful attempts have been made to add nanoparticles to polymers in membrane synthesis for removing water contaminants [17,18].

Ceramic membranes have been made with catalytic nanoparticles for synergistic effects on the membrane enhancement [17]. However, other studies suggest that silica polymerisation in the presence of polyvalent cations and anions in Reverse Osmosis (RO) systems are the cause of membranes fouling [19]. RO is by far the most effective method in removing dissolved silica from brackish water. The present study investigates the toxicity of NDMA as a known water contaminate and silica NPs as newly NPs used in membranes separationand found in high concentrations in brackish water [20]. NDMA and silica NPs exposed to human skin keratinocytes (HaCaT) and human colon carcinoma (caco-2), the toxicity determined using3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and Lactate dehydrogenase (LDH) assays also the particle morphology was identified by Scanning Electron Microscope (SEM).

Materials and methods

Reagents

All reagents were from Sigma Aldrich, Australia unless otherwise stated. These included, [3-(4,5-dimethylthiazol-2yl)-diphenyltetrazolium bromide] MTT, β-nicotinamide adenine dinucleotide hydrate (NADH), β-nicotinamide adenine dinucleotide (NAD), phenazinemethosulfate (PMS), Trizma base, TrizmaHCl, lithium lactate, iodonitrotetrazolium chloride (INT). The culture mediums used were Dulbecco's Modified Eagle Medium (DMEM) for caco-2 cell line and Roswell Park Memorial Institute (RPMI) for HaCaT cell line with 10% heat-inactivated fetal bovine serum (FBS) (HYQ®, Hyclone, Utah, USA), penicillin and streptomycin and Triton™ X-100 (positive control). Millipore Milli-Q water with resistivity=18.2 MΩ.cm was used for all the experiments. *N*-Nitrosodimethylamine solution was provided by Flinders University School of Chemical and Physical.

Preparation of SiO_2 Nanoparticles

Silica NPs were synthesized by Stöber method [21]. A silica precursor of TEOS (1 mL) was added rapidly to a mixture of 95% ethyl alcohol (10 mL) and Milli-Q water (3 mL). The reaction was allowed to proceed for 30 min with mild stirring at 50°C to obtain a homogeneous mixture. Subsequently, ammonia solution (30%) (1 mL) was injected drop-wise over an 8 min period with stirring. The mixture gradually changed from transparent to milky as the nanoparticles formed. The mixture was then stirred for a further 2 h. The resultant precipitate was then centrifuged at 101890 G and washed with Milli-Q water four times. Finally, the silica particles were dried overnight at 75°C.

Nanoparticle characterization

Scanning electron microscopy (SEM) was performed Silica NPs. The samples were sputter coated with a 10 nm layer of platinum using a Quorumtech K757X Sputter coater. The images of the sample were collected using a CAMScan MX2500 SEM with a field emission source at an accelerating voltage of 10 kV.

Cell culture

HaCaT and caco-2 cell lines were obtained from American Type Culture Collection (ATCC). HaCaT cells were grown in RPMI and caco-2 cells in DMEM medium with 10% fetal bovine serum (FBS) and incubated at 37°C, 5% CO_2 in a humidified incubator. Cells growth started from $2x10^6$ cells/ml and subcultured when confluence reached 60-70% every 2-3 days.

For toxicity assays

HaCaT and caco-2 cells were seeded at 10,000 cells/well in a 96-flat plate then incubated for 18h to allow adherence. NDMA and SiO_2 were diluted in fresh medium. After incubation, cells were treated with 6 concentrations of silica nanoparticles (0.05-2 mg/mL) and five concentration of NDMA (0.1-1000 µg/mL) for 4, 24 and 48h. The solution was removed and the cells were washed twice with phosphate buffered saline (PBS) twice to remove excess SiO_2 residue.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay Experimental Procedure

The toxicity of SiO_2 and NDMA was determined by MTT assay as described in literature [22,23]. $1x10^4$ cells were seeded in volume of 100 µl into 96-well flat bottom plate. MTT was

doi: 10.7243/2056-3779-4-4

added to each well at 0.5 mg/mL, and then plates were incubated at 37°C for 4h, then 80 µl of 20% SDS in 0.02 M HCl was added to each well. The plates were kept in the dark at room temperature for overnight.Absorbance was read at a primary wavelength of 570 nm and a reference wavelength of 630 nm by ELISA reader. In each experiment a standard curve was run to convert the OD values to cells/well.

Lactate Dehydrogenase leakage assay

The leakage of LDH in HaCaT and caco-2 cells was detected by LDH assay. Cells were plated into the 96- well plates and exposed to concentrations of SiO2 and NDMA as described previously. At the end of exposure time, 50 µl of aliquot cell medium was used for LDH activity analysis. The absorption was measured using ELISA reader at primary wavelength 490 nm and 630 nm as a reference wavelength. LDH assay experiments were programed in triplicate.

Statistical analysis

The data were expressed as mean +/-SD of at least three independent experiments using one-way analysis of variance (ANOVA) and Tukey–Kramer multiple comparisons test using SPSS software to compare exposure groups. All comparisons were considered significant level $p<0.05$.

Result and discussion

Exposure of monodisperse spherical SiO_2 (size 200 nm in diameter) to HaCaT and caco-2 cell lines for 4, 24 and 48h did not interrupt the cells growth. LDH leakage was clearly low at concentrations <2mg/mL in both cell lines. Similarly, all concentrations did not reduce the cell growth using MTT assay in both cell lines compared to positive control **Figure 1**. Until now, researchers cannot decide with each other on dose and physiochemical properties of NPs that could cause biological respond [**24**]. Some related the toxicity of NPs to the particle total weight, others to the number of particle per volume [**25**]. Recently, some studies suggested that the logical way to define particles toxicity is to calculate the dose based on the NPs total surface area also other studies on SiO2 NP_s found that the shape of particle is the main cause of particle pathogenesis of lung diseases [**26-29**]. The ecotoxicological impact of SiO_2 NPs on freshwater fish has not been completely understood [**30**]. Therefore, the haematological, ionoregulatory and enzymological toxicity effect of silica NPs concentrations 1, 5 and 25 mg L^{-1} were evaluated on freshwater teleost fish *Labeorohita* [**30**]. The exposure to Silica NPs concentrations has altered the values of haematological parameters such as haemoglobin (Hb) red blood cells, white blood cells, and plasma electrolytes. The plasma electrolytes such as potassium, sodium, chloride levels and Na^+/K^+ ATPase activity were shifted by all Silica NPs concentrations through all study exposure times. Moreover, the effect of the Silica NPs was found to be time and concentration dependent [**30**]. In another study, twelve fish cell lines taken from six species which is *rainbow*

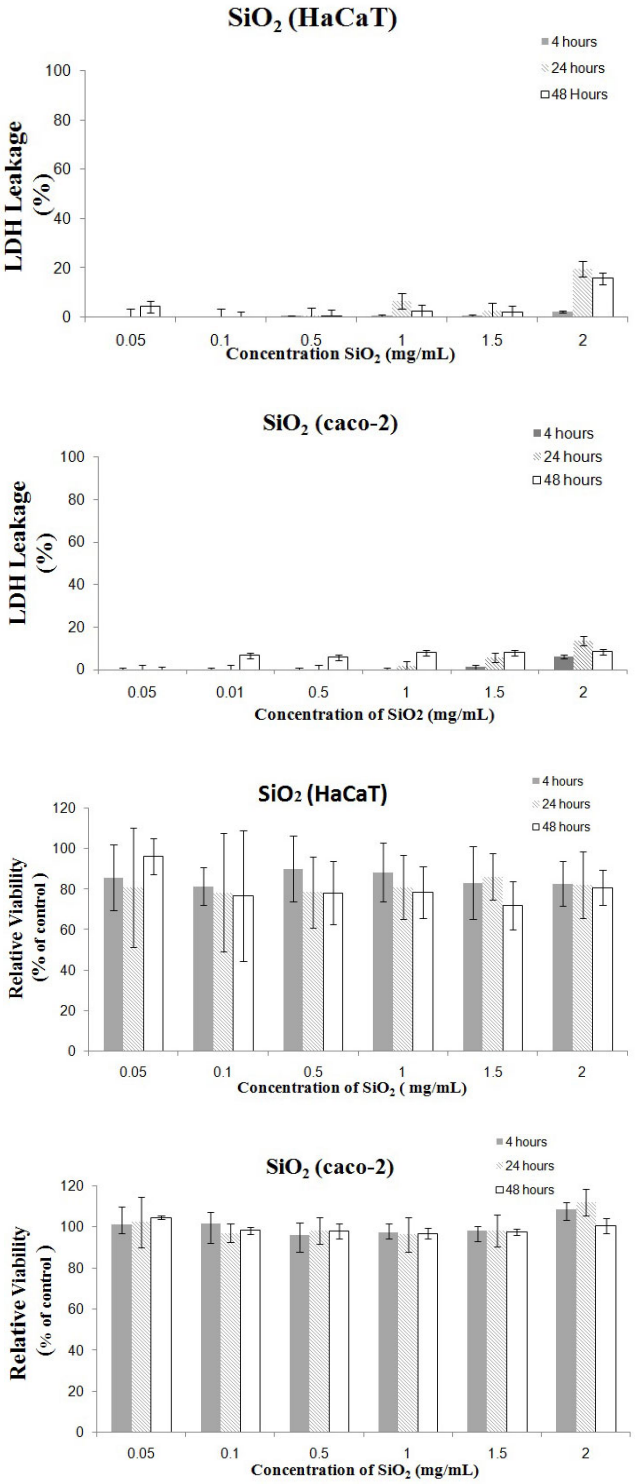

Figure 1. The Effect of SiO_2 NPs on HaCaT and caco-2 cell viability using MTT assay also LDH leakage assessed by LDH assay.
HaCaT and caco-2 cells were exposed to six concentrations of SiO_2. Data are expressed as means±S.D. from three independent experiments. *p<0.05 compared with control group.

doi: 10.7243/2056-3779-4-4

trout, *zebrafish*, *fathead minnow*, *haddock* and *American eel* tested for metabolic activity using Alamar Blue assay [31]. The toxicity of SiO_2NPs (16, 24, and 44 nm in diameter)was related to physiochemical properties such as the size and temperature also dose dependent and tissue specific. SiO_2 NPs (size 44 nmin diameter) concentrations greater than 100 µg/mL for 24h exposure reduced the fish cell lines viability by 50% [31]. Smaller NPs (size 16 nm) were found to induce more toxicity than larger NPs. In addition to size related toxicity, cells derived from external lining epithelial tissues (skin, gills) were more effected by SiO_2NPs than cells taken from internal tissues (brain, liver, intestine, gonads) [31]. SEM image of the SiO_2 synthesised by Stöber method showeda monodisperse spherical silica particle with a size 200 nm in diameter **Figures 2** and **3**

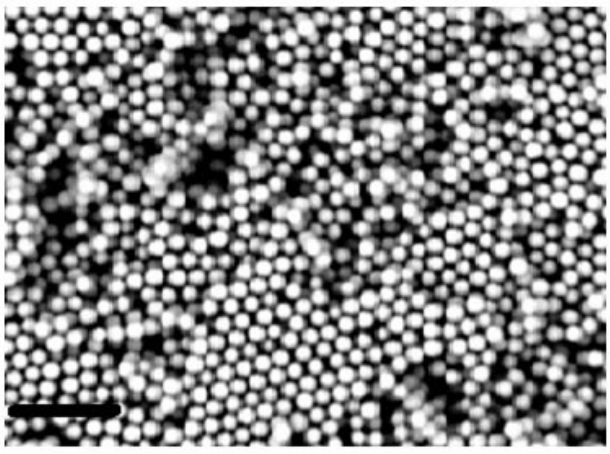

Figure 2. SEM image of monodisperse SiO_2 NPs, the scale bar represent 200 nm in diameter.
The final NPs formed were spherical and uniform.

On the other side, exposure of NDMA (concentrations between 0.1-1000 µg/mL) to HaCaT and caco-2 cell lines activated LDH leakage in all concentrations but mostly observed at 4h exposure. The LDH leakage by NDMA reduced after 24 and almost 5% at 48h. Comparably, NDMA interacted the metabolic activity of HaCaT cell line at concentration 1µg/mL and higher after 48h exposure, which show clearly the toxic impact. NDMA formation potential (FP) test is a fast and simple method to detect NDMA precursor concentrations in wastewater [32]. This method was applied on a tertiary wastewater treatment plants using an Ultrafiltration-Reverse Osmosis membranes. The results revealed that the NDMA FP from a variety of water samples were at the ranges between 350 to 1020±20 ng/L [32]. Furthermore, the fate of NDMA precursors was also investigated over two different points of advanced water treatment plants. The final results showed that more than 98.5±0.5% of NDMA precursors were removed by RO [32]. In California, one of the most used herbicides are phenylurea herbicide diuron has been continuously found in California's water sources, and

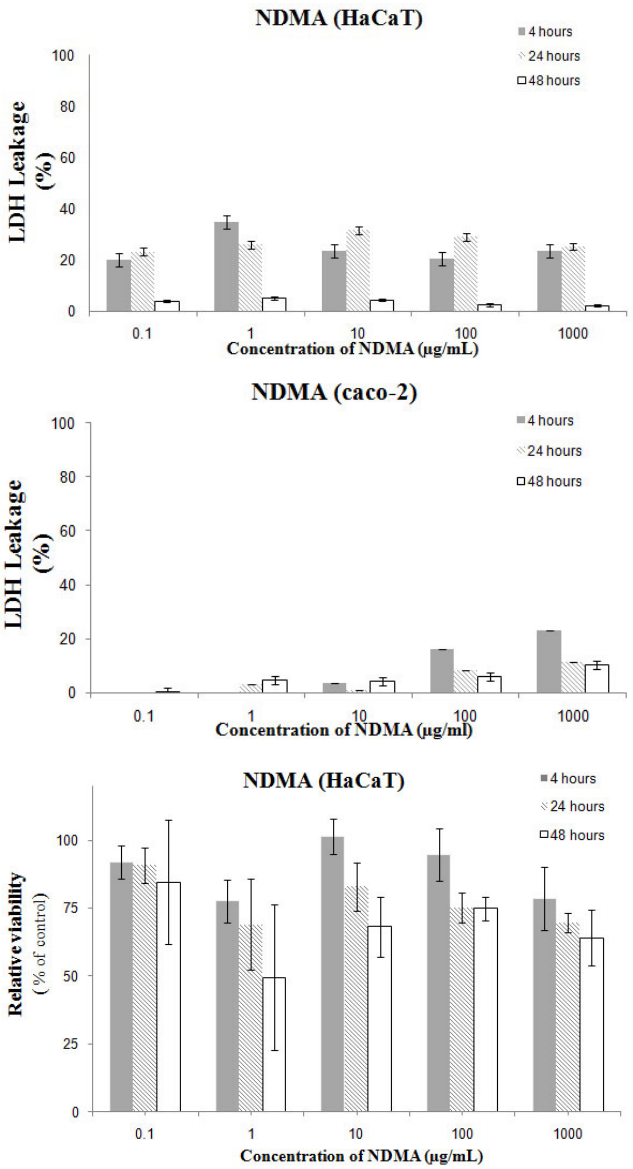

Figure 3. Toxic effect of NDMA on HaCaT and caco-2 cell lines assessed by MTT and LDH assays.
Both assays showed reduction in cell viability and increase in LDH leakage compared to positive control. Data are expressed as means±S.D. from three independent experiments.
*p<0.05 compared with control group.

ithad a structure that might be an NDMA precursor. NDMA precursors then consistently were found even in the absence of added ammonia which is a cause of nitro-nitrogen formation during chloramination. This is due to the both nitrogen atoms were donated by diuron during chlorination even where there is no ammonia exist.

Conclusion

In summary, SiO_2 NPs synthesised by Stöber method resulted in a monodisperse spherical particle with size 200 nm in diam-

doi: 10.7243/2056-3779-4-4

eter analysed by SEM. Due to multiple factors the membrane can foul and nanoparticles can reach the waterways causing adverse effect on ecosystem. In this study, we tested the toxicity of SiO_2 NPs used in water filtration and NDMA as a member of a family of extremely potent carcinogens found in drinking water contaminant resulting from reactions occurring during chlorination. After 4, 24 and 48h exposure of SiO_2 (concentrations range between 0.05-2 mg/mL) to HaCaT and caco-2 cell lines using MTT and LDH assays only 2 mg/mL increased LDH leakage compared to positive control. NDMA activated LDH leakage in both cells and interrupted the metabolic activity of HaCaT cell line at most concentrations. Our finding suggests that SiO_2 imposed less environmental risk to humans and animals at the mentioned concentrations.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	AGA	BJSS
Research concept and design	✓	✓
Collection and/or assembly of data	✓	--
Data analysis and interpretation	✓	✓
Writing the article	✓	--
Critical revision of the article	✓	✓
Final approval of article	✓	✓
Statistical analysis	✓	--

Acknowledgement

The authors would like to thank the Ministry of Higher Education, Saudi Arabia, for partial support of this project.

Publication history

Editor: David Han, University of Texas at San Antonio, USA.
Received: 26-Mar-2017 Final Revised: 24-Apr-2017
Accepted: 02-May-2017 Published: 17-May-2017

References

1. Salata O. **Applications of nanoparticles in biology and medicine**. *J Nanobiotechnology*. 2004; **2**:3. | Article | PubMed Abstract | PubMed FullText
2. Shen Jn et al. **Preparation and characterization of PES–SiO 2 organic–inorganic composite ultrafiltration membrane for raw water pretreatment**. *Chemical engineering journal*. 2011; **168**:1272-1278. | Article
3. Darracq G et al. **Clarification: Impact on Ultrafiltration Membrane Fouling in Drinking Water Treatment**. *Journal Awwa*. 2015; **107**:12. | Article
4. Dahle JT and Arai Y. **Environmental geochemistry of cerium: applications and toxicology of cerium oxide nanoparticles**. *Int J Environ Res Public Health*. 2015; **12**:1253-78. | Article | PubMed Abstract | PubMed FullText
5. Farre M, Gajda-Schrantz K, Kantiani L and Barcelo D. **Ecotoxicity and analysis of nanomaterials in the aquatic environment**. *Anal Bioanal Chem*. 2009; **393**:81-95. | Article | PubMed
6. Baun A, Hartmann NB, Grieger K and Kusk KO. **Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing**. *Ecotoxicology*. 2008; **17**:387-95. | Article | PubMed
7. Lovern SB, Strickler JR and Klaper R. **Behavioral and physiological changes in Daphnia magna when exposed to nanoparticle suspensions (titanium dioxide, nano-C60, and C60HxC70Hx)**. *Environ Sci Technol*. 2007; **41**:4465-70. | PubMed Abstract | PubMed FullText
8. Lynch I. **Water governance challenges presented by nanotechnologies: tracking, identifying and quantifying nanomaterials (the ultimate disparate source) in our waterways**. *Hydrology Research*. 2016; 107. | Article
9. Wiesner MR, Hotze EM, Brant JA and Espinasse B. **Nanomaterials as possible contaminants: the fullerene example**. *Water Sci Technol*. 2008; **57**:305-10. | Article | PubMed
10. Thomas K, Aguar P, Kawasaki H, Morris J, Nakanishi J and Savage N. **Research strategies for safety evaluation of nanomaterials, part VIII: International efforts to develop risk-based safety evaluations for nanomaterials**. *Toxicol Sci*. 2006; **92**:23-32. | Article | PubMed
11. Halden RU. **Epistemology of contaminants of emerging concern and literature meta-analysis**. *J Hazard Mater*. 2015; **282**:2-9. | Article | PubMed Abstract | PubMed FullText
12. Sanagi M.M et al. **Nano iron porphyrinated poly (amidoamine) dendrimer mobil composition matter-41 for extraction of N-nitrosodiphenylamine nitrosamine from water samples**. *Microporous and Mesoporous Materials*. 2015; **213**:68-77. | Article
13. Dimethylnitrosamine, S., C. No, and S. No, *N-Nitrosodimethylamine (NDMA)*.
14. Zhang T, Ge J, Hu Y, Zhang Q, Aloni S and Yin Y. **Formation of hollow silica colloids through a spontaneous dissolution-regrowth process**. *Angew Chem Int Ed Engl*. 2008; **47**:5806-11. | Article | PubMed
15. Bogovski P and S. Bogovski. **Special report animal species in which n-nitroso compounds induce cancer**. *International Journal of Cancer*. 1981; **27**:471-474. | Article
16. Mitch W.A et al. **N-nitrosodimethylamine (NDMA) as a drinking water contaminant: a review**. *Environmental Engineering Science*. 2003; **20**:389-404. | Pdf
17. Kim J and Van der Bruggen B. **The use of nanoparticles in polymeric and ceramic membrane structures: review of manufacturing procedures and performance improvement for water treatment**. *Environ Pollut*. 2010; **158**:2335-49. | Article | PubMed
18. Mahapatra S.S and N. Karak. **Silver nanoparticle in hyperbranched polyamine: synthesis, characterization and antibacterial activity**. *Materials Chemistry and Physics*. 2008; **112**:1114-1119. | Article
19. Koo T, Y. Lee and R. Sheikholeslami. **Silica fouling and cleaning of reverse osmosis membranes**. *Desalination*. 2001; **139**:43-56.
20. Patil S.S et al. **Nanoparticles for environmental clean-up: a review of potential risks and emerging solutions**. *Environmental Technology & Innovation*. 2016; **5**:10-21. | Article
21. Ibrahim I.A, A. Zikry and M.A. Sharaf. **Preparation of spherical silica nanoparticles: Stober silica**. *J. Am. Sci*. 2010; **6**:985-989.
22. Mosmann T. **Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays**. *J Immunol Methods*. 1983; **65**:55-63. | Article | PubMed
23. Young FM, Phungtamdet W and Sanderson BJ. **Modification of MTT assay conditions to examine the cytotoxic effects of amitraz on the human lymphoblastoid cell line, WIL2NS**. *Toxicol In Vitro*. 2005; **19**:1051-9. | Article | PubMed
24. Park EJ, Choi J, Park YK and Park K. **Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells**. *Toxicology*. 2008; **245**:90-100. | Article | PubMed
25. Peyron MA, Mishellany A and Woda A. **Particle size distribution of food boluses after mastication of six natural foods**. *J Dent Res*. 2004; **83**:578-82. | Article | PubMed
26. Liu Y et al. **Nanoparticles in wastewaters: hazards, fate and remediation**. *Powder Technology*. 2014; **255**:149-156. | Article
27. Bowman CR, Bailey FC, Elrod-Erickson M, Neigh AM and Otter RR. **Effects of silver nanoparticles on zebrafish (Danio rerio) and Escherichia coli (ATCC 25922): a comparison of toxicity based on total surface area**

versus mass concentration of particles in a model eukaryotic and prokaryotic system. *Environ Toxicol Chem*. 2012; **31**:1793-800. | Article | PubMed

28. Huang S.M et al. **Metabolomics Studies Show Dose-Dependent Toxicity Induced by SiO2 Nanoparticles in MRC-5 Human Fetal Lung Fibroblasts**. *Advanced healthcare materials*. 2012; **1**:779-784. | Article
29. Brown S.C. et al. **Influence of shape, adhesion and simulated lung mechanics on amorphous silica nanoparticle toxicity**. *Advanced Powder Technology*. 2007; **18**:69-79. | Article
30. Krishna Priya K, Ramesh M, Saravanan M and Ponpandian N. **Ecological risk assessment of silicon dioxide nanoparticles in a freshwater fish Labeo rohita: Hematology, ionoregulation and gill Na(+)/K(+) ATPase activity**. *Ecotoxicol Environ Saf*. 2015; **120**:295-302. | Article | PubMed
31. Vo NT, Bufalino MR, Hartlen KD, Kitaev V and Lee LE. **Cytotoxicity evaluation of silica nanoparticles using fish cell lines**. *In Vitro Cell Dev Biol Anim*. 2014; **50**:427-38. | Article | PubMed
32. Farre MJ, Keller J, Holling N, Poussade Y and Gernjak W. **Occurrence of N-nitrosodimethylamine precursors in wastewater treatment plant effluent and their fate during ultrafiltration-reverse osmosis membrane treatment**. *Water Sci Technol*. 2011; **63**:605-12. | Article | PubMed

Citation:
Almutary AG and Sanderson BJS. **Toxicity of *N*-Nitrosodimethylamine and SiO_2 nanoparticles to HaCaT and caco-2 cell lines found in waste water treatment**. *J Toxicol Health*. 2017; **4**:4.
http://dx.doi.org/10.7243/2056-3779-4-4