Journal of Multidisciplinary Engineering Science Studies (JMESS)
ISSN: 2912-1309
Vol. 1 Issue 2, December - 2015

Human Toxicology Of Engineered Nanoparticles

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***Abstract*—The increasing use of Engineered Nanoparticles in medical applications as well as other industries warrants the assessment of toxicity that can be induced by these Nanoparticles in humans. This mini-review focuses on studies published from 2013-2015 that report toxicity of Magnetic, Ag and ZnO Nanoparticles in humans and human cell lines. This review will look at recent evidence to determine what factors greatly influence the toxicity profile of engineered Nanoparticles and which Nanoparticles can be considered safe for use in medical applications involving humans. In addition to it the review will discuss briefly the mechanisms through which Nanoparticles cause toxicity focusing on generation of Reactive Oxygen Species (ROS) and induction of Oxidative stress within cells leading to DNA damage. This review also presents a two-way simple classification system for NP-induced toxicity in human cell lines.**

Keywords—cancer cells; DNA Damage; Engineered Nanoparticles; Toxicology

I. INTRODUCTION

The increasing use of Nanoparticles in medical applications and industrial products warrants the assessment of their toxicity to ensure their safe use in humans. This review will be focusing on recent research on toxicity of Iron Oxide based Magnetic Nanoparticles (MNPs), Silver (Ag) and Zinc Oxide (ZnO) NPs in human cells and organs. Iron oxide NPs have been selected for this review as they have a potential use as diagnostic contrast agents, in hyperthermia and in targeted drug delivery. Silver Nanoparticles are widely used in a number of commercialized products due to their anti-microbial activities, high electrical and thermal conductivity, chemical stability, surface enhanced Raman Scattering, catalytic activity and non-linear optical behaviour making them the fastest growing class of NPs in consumer products applications [1]-[2]. In the category of metal containing NPs, ZnO have the third highest global production volume after SiO_2 and TiO_2 NPs [3]. This warrants the assessment of their toxicity in humans.

II. MATERIALS AND METHODS

A. Selection of articles

First, In this literature review relevant articles in the fields of toxicology especially experimentally acquired data concerning in-vitro toxicity in humans and human cell lines were found online using Google scholar, Science direct and Pubmed.

B. Search criteria

Only open-accessed articles from 2013-2015 were included in this study. Keywords were used to locate relevant articles such as Nanoparticle toxicity in Humans, magnetic Nanoparticles and Human Toxicity. The search articles were further refined by excluding studies involving animal models. At the end of this selection process only 13 studies were used for this review.

III. TOXICOLOGY OF NANOPARTICLES TO HUMANS AND HUMAN CELL LINES

This section will discuss toxicity of three metal based Nanoparticles i.e. iron oxide, silver and Zinc Oxide.

A. Toxicity of MNPs

Magnetic Nanoparticles such as Iron oxide based SPIOs and USPIOs are widely investigated in different medical applications. They are used in diagnostic imaging as contrast Agents. They are also used as anti tumour drug delivery vehicles and in inducing magnetic hyperthermia.

Hence it is important to determine the toxicity of these Nanoparticles in humans. Generally iron oxide based Nanoparticles are considered safe due to their biocompatibility, storage and clearance of excess iron in body by liver and spleen. However Iron oxide Nanoparticles can cause carcinogenesis and can be cytotoxic. A study has shown that when human MCF 7 breast cancer cells are subjected to different concentrations of Iron oxide based Nanoparticles they undergo cell membrane damage [4]. The extent of damage is time and concentration dependent. The membrane damage was indicated by release of LDH (Lactate Dehydrogenase). As the concentration of Nanoparticles increased and duration of exposure to NP was increased more cell membrane damage was

observed. The same study also showed that iron oxide based NPs has induced intracellular oxidative stress in MCF7 cells by enhancing the production of ROS in dose and time dependent manner. At the same time activities of antioxidant defenses such as GSH, Superoxide, dismutase, and Catalase were reduced. Caspase 3, which plays a vital role in apoptotic cell death, activity was also found to be increased in concentration and time dependent manner. The study suggest that Iron oxide NPs caused imbalance between ROS generation and antioxidant defense system thereby inducing oxidative stress that caused DNA damage and apoptosis of cells.

Amin and colleagues [5] studied the toxicity of bare and Au-coated Fe_3O_4 NPs on human skin cell lines (Fibroblasts, SCC cells and epidermal keratinocytes, HaCaT) with Fe_3O_4 Nanoparticles in the size ranges of about 54 nm and in concentration range from 10 ug/ml to 500 ug/ml concentration for upto 24 hrs and 48 hrs.

HaCaT cells either showed min toxicity (i.e. less than 5% cell kill) or cell proliferation (at 10, 100, 300, 500 ug/ml) when exposed to bare Fe_3O_4 NPs for 24hrs. After 48rs of incubation time HaCaT cells showed no more than 10% of cell kill with greatest loss in cell survival observed at 500 ug/mL concentration. -------no marked effect at 24 and moderate toxicity after 48 hrs at 500 ug/ml concentration.

Overall the, normal Human fibroblasts showed either no reduction in cell survival or minimal reduction in cell survival (i.e. about 10% reduction in cell survival) after 24hrs exposure to bare Fe_3O_4 Nanoparticles. Similarly normal Human fibroblasts did not show significant reduction (i.e less than 5% cell killed) in cell survival after being exposed to bare Fe_3O_4 NPs after 48 hrs exposure time. On the contrary normal Human fibroblasts showed sign of proliferation after 48hrs when treated with bare Fe_3O_4 NPs at 50 and 500ug/ml concentration. In short bare Fe_3O_4 NPs had almost no significant or marked effect on human fibroblasts survival after 24hrs and 48 hrs.

In case of malignant cells (SCC-A431), the bare Fe_3O_4 Nanoparticles either showed minimum reduction in cell survival (15% or less cell killed) or sign of repair after 24hrs of exposure time. After 48hrs of exposure time no significant effect on cell survival was observed i.e. either min reduction (5% or less) in cell survival or cell proliferation was observed.

Human Skin cell lines incubated with Au coated Fe_3O_4 Nanoparticles in the size ranges of about 22-55nm for 24hrs showed reduced cell survival at higher concentrations. These skin cells were able to recover after an incubation period of 48 hrs as there was no sign of decreased cell viability.

Analysis of cytotoxicity of Gold coated Fe_3O_4 Nanoparticles on human SCC cells (A431) showed reduction in cell viability in concentration dependent manner. At a concentration of 500ug/ml the SCC cell survival reduced by 32%. These cells partly recovered after subjecting them to 48 hrs incubation period. However 26% of cells still remained unviable at highest concentration (i.e. at 500ug/ml). Gold coated Fe_3O_4 Nanoparticles had no effect on HaCaT cells even at a concentration of 500ug/mL when incubated for 24 hrs. At 48 hrs incubation time 18% of cells were killed at highest concentration.

Thus Fe_3O_4 Nanoparticles coated with Au showed moderate toxicity (18% HaCaT and 26% SCC cells killed) at higher concentration starting at 100ug/mL on p53 lacking HaCaT cells and on malignant Squamous cell carcinoma lines (A431) but not on normal skin cells. The authors suggest that HaCaT cells which have a defect in their p53 repair system tend to accumulate the damage as there was no sign of repair or cell proliferation in HaCaT cells after 48 hrs of incubation period whereas skin cells (fibroblasts) and malignant cells(A431) seem to have an adaptive repair mechanism as they showed cell proliferation after 48 hrs of incubation period to compensate for loss of cells thereby reducing the number of cells killed from 23% to 0% reduction of cell viability and from 32% to 26% respectively. This study concluded that the application of Fe_3O_4 Nanoparticles within the examined range (10-500ug/mL) is safe whereas the use of Gold coated Fe_3O_4 Nanoparticles is moderately toxic.

A study investigated the toxicity of Fe_3O_4 Magnetic Nanoparticles of different sizes (10nm, 100-150nm) having different functional groups (Amine, Hydroxyl) on Human fibroblasts and Fibrosarcoma cells (HT-1080) [6]. Some of the NPs were coated with silicon dioxide and therefore they had a shell/core structure. Four different types of Nanoparticles were produced and tested in this study i.e. bare NPs, TEOS, APTMS, TEOS/APTMS (Tetraethyorthosilicate / aminopropyltrimethoxysilane). This study found that all MNP produced about 5% or less cytotoxicity and genotoxicity in fibrosarcoma cells at lower than 500 ug/mL, APTMS-coated NPs resulted in higher than 10% toxicity against normal cells.

In case of HT-1080 cells again positively charged APTMS-coated MNPs at dose concentration of 1000 ug/mL adversely affected cell viability. Positively charged MNPs (APTMS-Coated and TEOS/APTMS coated) showed highest release of LDH (112%, 111%) in fibroblasts when exposed to highest dose concentration (1000ug/mL) for 24 hrs whereas negatively charged MNPs caused about 109% LDH release from cells. HT-1080 cells showed similar LDH release pattern

Genotoxicity induced by MNPs in cells was reliant on dose, charge and size of the MNP indicating that small and positively charged MNPs (APTMS-coated)

caused more severe toxicity in normal cells compared to malignant cells [6]. In fibrosarcoma cells positively charged MNP induced significant DNA injury only at 1000ug/ml. This study concluded that MNP rarely demonstrate genotoxicity below 100ug/ml and normal cells are more vulnerable to internalized MNPs than cancer cells.

A Study of Human endothelial Cells treated with 10nm diameter sized magnetic iron oxide Nanoparticles coated with Dimercaptosuccinic acid (DMSA) was investigated for cellular toxicity, cell injury, angiogenesis (tube formation), endocrine function and expression of genes related to apoptosis cascade, endoplasmic reticulum stress, oxidative stress, adhesion molecules and calcium handling proteins [7].

Graded concentration of DMSA-Fe2O3 from 0.001 - 0.2mg/ml was applied to HAECs for 24hours [7]. To study time dependent effect, 0.05mg/ml of DMSA Fe_2O_3 was applied to cells for 4, 24, 48 and 72 hours.

The cytotoxicity was found to be concentration dependent with concentrations less than 0.02 mg/ml exhibiting little toxic effect. DMSA- Fe_2O_3 concentration greater than 0.05 mg/ml resulted in substantial cell loss thereby showing cellular toxicity whereas concentrations less than 0.05mg/ml did not caused any cell loss. At 0.02mg/ml no demonstrable cellular injury in terms of LDH release was observed. At 0.2mg/ml DMSA Fe_2O_3 concentration, endocrine function of HAEC remained unchanged with respect to release of NO (a vasodilator) whereas another vasodilator PG1-2 and a vasoconstrictor ET-1 was significantly decreased in HAEC cells when treated for 24hours. This means that endocrine function is sensitive to treatment with DMSA Fe_2O_3 Nanoparticles and these functions may be hampered before serious cell injuries occur. DMSA Fe_2O_3 Nanoparticles showed differential effect on gene expression at 0.2mg / ml concentration thereby resulting in no change in cell viability. All ER genes were down regulated by 50% whereas expression of oxidative stress genes increased with expression of COX-2 and superoxide raised to 2.44 and 1.96 respectively. This in turn means that oxidative stress and not ER stress is sensitive to DMSA- Fe_2O_3 Nanoparticles. Urea concentration in HAEC was found higher when treated with 0.02mg/ml DMSA Fe_2O_3 indicating decrease of Urea transporter. Small amounts of DMSA- Fe_2O_3 Nanoparticles were found to be harmful to angiogenesis in normal cells. Overall the study concluded that DMSA- Fe_2O_3 Nanoparticles have some toxicity that can result in side effects in normal endothelial cells.

The characterization and in-vitro-cytotoxicity of cobalt Zinc Ferrite MNP (CZF-MNP) and Cobalt Zinc Ferrite –NP coated with biocompatible DMSA (Dimercaptosuccinic acid) on Human Prostate Cancer cells (PC3, DU145) was examined in a study carried out by Shahbazi-Gahrouei and colleagues [8]. The MNPs were of spherical shape with mean particle size of CZF-MNP 16NM and CZF-MNP @DMSA 40 nm. Fe concentration in CZF-MNP and CZF-MNP @DMSA was 225ppm.

Different concentrations of Fe in CZF-MNP and CZF-MNP @DMSA (0.3, 0.6, 0.9, 1.2, 1.5mM) were used to investigate cytotoxicity on Human prostate cancer cell lines (HPC). The results of this study showed that CZF-MNP coated with DMSA exhibited higher toxicity at high concentrations (1.2 and 1.5mM of Fe) than that of pure NPs.

The cell viability of DMSA-coated MNP (PC3 and DU145) at 0.3 and 0.6 mM concentration of Fe was about 100%, at 0.9mM concentration of Fe was approximately 120 % (indicating cell proliferation) and at 1.2 and 1.5 Mm concentration of Fe was less than 50% [8].

Uncoated NPs showed high viability against PC3 cells i.e. no decrease in cell viability vs control group was observed by increasing Fe concentration of CZF-MNPs except at 0.3Mm concentration of Fe. Actually the graph showed more than 100% cell viability at all concentrations of Fe except 0.3mM. This is indicative of cell proliferation. At 0.3mM concentration of Fe, negligible toxicity in the form of 3% reduction in cell viability was observed.

Viability of DU145 cells against different concentrations of uncoated MNPs is greater than 75%. This means that DU145 cell lines exhibited greater toxicity when treated with uncoated MNPs. The authors concluded that Human prostate Cancer cell viability is concentration dependent and 14nm uncoated CZF MNPs did not exhibit toxicity in PC3 cell lines. Infact increasing concentrations of CZF-MNP (uncoated) resulted in cell proliferation [8].

This effect of DMSA-Coated MNP showing cell proliferation and no toxicity at certain concentrations was also observed in the study conducted by Ge and colleagues [7]. Fe_2O_3@DMSA NPs showed cell proliferation at 0.01mg/ml concentration.

A study investigating the effects of Human-like collagen protein (HLC) coated Fe_3O_4 NPs on heat induction and cell toxicity showed that HLC coated Fe_3O_4 NPs are less toxic to fibroblasts (NIH3T3) cells than their counter parts (i.e. Fe_3O_4 NPs without HLC coating) especially at higher concentrations [9]. At 100-250 ug/mL concentration of Fe, HLC coated Fe_3O_4 NPs did not produce noticeable cell toxicity whereas Fe_3O_4 NPs without HLC coating produced cytotoxic effects in fibroblasts at concentration of 250 ug/mL of Fe. Both Fe_3O_4 NPs with and without HLC coating showed no reduction in viability of NIH3T3 cells at 25-100 ug/mL concentration of Fe.

The study also showed that the rate of temperature rise of HLC coated Fe_3O_4 NPs after 100s was faster compared to the sample of Fe_3O_4 NPs without HLC coating due to probably better NP/Water interface interactions in transferring heat. Good dispersion after

coating with HLC perhaps also contributed to improved Brownian motion resulting in improved heat transfer and magnetic hypothermic performance of HLC coated Fe_3O_4 NPs.

A study examined the nanotoxicity of Gold Magnetic Nanoparticles (GMNPs) on Human Umbilical Vein Endothelia cells (HUVECs) and determined optimal size, concentration and exposure time for MRI imaging [10].

The uptake of particles as well as nanotoxicity in terms of tube formation (angiogenesis), ROS generation, apoptotic cell death, cytoskeleton structure and cell doubling time were depended on size, concentration and exposure time. Overall 50nm GMNPs exhibited higher nanotoxicity than 30nm GMNP with similar concentration and exposure time.

The study also showed that more of 50nm GMNPs were internalized than 30nm GMNPs which in turn resulted in higher concentration or number of 50nm GMNPs within HUVEC cells. This higher presence of 50nm GMNPs in the cells is probably responsible for increased production of ROS in cells and greater harmful effects on DNA, proteins, cell membrane, cytoskeleton causing higher nanotoxicity.

In general 50nm GMNPs showed nanotoxicity at a concentration of 25ug/mL where as notable toxicity were observed at concentrations of 50ug/ml for 30nm GMNPs.

Substantial Increase in ROS generation, higher inhabitation of tube formation ability (angiogenesis), significant increase in the number of apoptotic cells and increased cytoskeleton damage was observed at concentration of 25ug/ml and 50ug/ml for 50nm and 30nm GMNPs when exposed for 24 hours.

The 50nm GMNPs exhibited greater hypo-intensities at the same concentration compared with that of 30nm GMNPs. This is because 50nm GMNPs showed 1.23 times higher relaxivity (r2= 98.65 $Mm^{-1}S^{-1}$) than 30nm GMNPs (r2 = 80.18 $Mm^{-1}S^{-1}$).

The authors concluded that the 50nm GMNPs are more apt for HUVEC labelling and MRI and the optimal concentrations were 25ug/mL and 12 hours.

abbreviations and acronyms the first time they are used in the text, even after they have been defined in the abstract. Abbreviations such as IEEE, SI, MKS, CGS, sc, dc, and rms do not have to be defined. Do not use abbreviations in the title or heads unless they are unavoidable.

B. Toxicity of Silver NPs

Use A study conducted by Holder and Marr [11] investigated the size dependent toxicity of silver Nanoparticles free of aggregation on Human Alveolar Epithelial Cell line (A549). Silver NPs in suspension had a size of 30-50nm and were coated with PVP (Polyvinyl Pyrolidone) whereas Ag NPs in aerosol form exhibited a geometric mean diameter of 37nm and volume- weighted geometric mean diameter of 169nm. Nickel oxide NPs acted as positive control in this study and exhibited particle size of 10-20nm.

In this study silver NPs in suspension were responsible for causing weak cytotoxic and pro-inflammatory response and only at high dose of 50ug/mL (13.2ug/cm) whereas Aerosoled cells showed no substantial toxicity to any dose (from 0.005 to 0.7 ug/cm^2). The authors conclude that these doses are well above the maximum estimated alveolar dose limits [11]. With increasing dose of AgNPs cell metabolism as measured by MTT Assay diminished.

Interestingly LDH release in cells exposed to Ag NPs suspensions was comparatively less than the control value (i.e. Nickel suspensions). Further investigation revealed that Ag NPs inactivated LDH protein thereby inhibiting its measurement resulting in lower than expected LDH release.

Ag NPs in aerosol showed a weak cytotoxic effect by high LDH release and increased metabolic rate but increased IL-8 secretion that indicated increased pro-inflammatory response. On the other hand a strong cytotoxic effect was shown by Nickel oxide aerosol with decrease of cellular metabolism (MTT) and membrane integrity.

Another effect observed by this study was size dependent toxicity. It was noted that same number of 75nm diameter particles resulted in greater response than either the 50nm or 100nm diameter particles.

Ag NPs with 100nm size showed lowest response for the mass and surface area dose metrics again proposing that there could be a size threshold for the response to silver NP. The authors acknowledge the limitations this study deals with only single acute dosing whereas multiple repeated exposures needed to be studied as they are more likely to happen in real world.

A study conducted by Coman and colleagues [12] investigated the toxicity of Ag NPs on Human RBCs utilizing an in-vitro system. The study showed non-**statistically** significant dose dependent cytotoxicity with low metabolic activity with treatment doses up to 20ug/mL. The Silver NP diameter ranged from 0.5 to 0.65nm where as concentrations of colloidal silver solution used were 5, 10, 20 ug/ml. This study concluded that treatment doses of up to 20ug/ml are well beneath the threshold level of cytotoxicity [12].

A study evaluated toxicity of chemically synthesized and biologically synthesized Ag NPs from garlic on human gut flora (Bacillus Subtilis) [13]. This study showed that toxicity of chemically synthesized Ag NPs (85.45%) is comparatively higher than toxicity of biologically synthesized Ag NPs (46.35%) thereby suggesting that biosynthesized Ag NPs are more biocompatible and less toxic to cellular micro-

environment and normal gut micro flora inside the human body [13]. Bacillus Subtilis were exposed to Ag NPs concentration that ranged from 25-50ug/ml for duration of 4 hrs [13]. The study does not provide any information regarding the size and shape of synthesized Ag NPs.

Size and coating dependent toxicity of Silver NPs was investigated by Gliga and colleagues [14] in Human Lung cells (BEAS-2B cells). Cells were treated with Citrate coated AgNPs of various sizes, PVP coated AgNP (10nm) and uncoated AgNP (50nm). Small 10nm sized AgNPs were found to be toxic in human lung cells. This study failed to show any evidence for increased intracellular production of ROS by AgNPs in Human lung cells.

C. ZnO NP Toxicity

A study explored the response of primary human hepatic stellate cells (hHSC) to treatment with four types of ZnO NPs consisting of two uncoated, (Z-Cote, Nanosun) and two coated (triethoxycaprylysilane HP1, dimethoxydihenysilane MAX) NPs [15]. The uncoated NPs of different particle size whereas coated NPs had similar particle size. The study also examined $ZnSO_4$ as a source of ionic zinc using a systems biology approach including assessments of cell function and viability, the commencement of cell signalling pathways and whole genome transcriptional profiling. The tested concentration in the present study was 30ug/mL. This study also had a negative control i.e. medium containing no Nanoparticles and a positive control i.e. medium containing 5% DMSO (dimethylsulfoxide).

The three ZnO NPs (Z-COTE, HP1, MAX) were longer and narrower than z-cote giving them not only larger aspect ratio than Z-COTE but also producing a mix of heterogenous rectangular shaped particles. Nanosun NPs were smaller homogenous spherical particles with mean diameter of 25nm [15].

The study showed that surface coated NPs did not have a striking effect on cell signalling, function, viability or transcriptional profile of human hepatic Stellate cells whereas uncoated ZnO NPs selectively activated pathways known to regulate cellular responses to abiotic stresses and mechanisms known to control cell survival or apoptosis and decrease cell viability and deregulate transcription [15]. Ionic zinc did not show similar stress responses in hHSC cells to that of induced by uncoated NPs. The study concluded that coated NPs exerted clear protective effect against ZnO NP cytotoxicity [15].

Another study examined the toxic effects of 50nm ZnO-NPs in human lung epithelial cells (L-132) and also tried to clarify the involvement of oxidative stress therein [16]. The concentrations used in this study were 5, 25, 50, 100 ug/mL and duration of exposure was 24hrs. Human epithelial cells exposed to ZnO-NP at concentrations between 5-100 ug/mL showed reduced cell viability in a concentration dependent manner with non-significant reduction in cell viability at lower concentrations of 5ug/mL. Concentrations ranging from 25-100ug/mL caused significant reduction in cell viability from 55%-25%.

Reduction in GSH level was observed in concentration dependent manner with significant difference observed at 50-100ug/mL with remaining GSH of about 14% and 4% respectively. ZnO-NPs produced substantial formation of ROS FROM 16% TO 25% at concentrations of 25-100ug/mL after 24hrs of exposure [16]. This depletion in GSH level and increase in ROS levels indicate generation of oxidative stress [16].

DNA fragmentation indicating apoptotic cell death was caused by ZnO-NP exposure after 48hrs in concentration dependent manner where as no DNA damage was observed after 24 hours [16]. ZnO-NPs enhanced the expression of metallothionein gene from concentrations of 5-50 ug/mL, which is considered as a biomarker in metal-induced toxicity by scavenging free radicals. The gene expression decreased at 100ug/ml concentration [16]. In short, 50nm sized ZnO-NPs induced cytotoxicity in human epithelial lung cells by raising oxidative stress in concentration-dependent manner.

A study compared the cytotoxicity of ZnO NP with various thicknesses of silica shell (thin SiO2/ZnO, thick SiO2/ZnO) with bare ZnO NPs in vitro in Human skin dermal fibroblasts neonatal (HDFn) cells [17]. The study showed that thicker SiO2/ZnO caused decreased enzyme leakage, decreased peroxide production and less oxidative stress than bare ZnO NPs or thinner SiO2/ZnO NPs by limiting free radical formation and the release of zinc ions as well as by reducing surface contact with cells [17]. Nevertheless core of ZnO NP showed cytotoxicity over time irrespective of shell thickness [17].

TABLE I
IOMNP Induced Toxicity in Human cell lines

Cell lines	Coated	Uncoated	NP Size	Conc	Exp Time	Major Outcomes	HC	Ref
Human MCF 7 breast cancer cells		Fe2O3	<=20 to >50nm	0, 10, 30, 60, and 120 µg/ml	24, 48hrs	IO NP induced sig cytotoxicity at 60ug/mL *	Toxic	[4]
Human skin cell linesa		Fe3O4 NP	54	10-500 ug/mL	24, 48hrs	No effect on cell viability	Harmless/ safe	[5]
	Au coated Fe3O4 Nanoparticles					moderately toxic	Harmful/ mod toxicity	
SCC	Au coated Fe3O4 Nanoparticles		22-54			26% of Cells remain unviable at highest conc after 48hrs	Mod toxic	
HaCaT	Au coated Fe3O4 Nanoparticles					At 48 hrs 18% of cells were killed at highest concentration	Mod toxic	
Human Fibroblasts and Fibrosarcoma cells (HT-1080).	SiO2		100-150nm,	200-1000ug/mL		All MNP produced about 5% or less toxicity in HT-1080 cells at < 500ug/ml c	Not harmful at 100ug/ml or less conc.	[6]
		Fe3O4	10nm b					
Human Endothelial cells	DMSA		10nm	0.001 - 0.2mg/ml		DMSA- Fe2O3 Nanoparticles have some toxicity. Conc less than 0.02 mg/ml are harmless.	Toxic	[7]
Human Prostate Cancer cells (PC3, DU145)	CZF-MNP @DMSA		40nm	0.3, 0.6, 0.9, 1.2, 1.5mM		Coated NPs exhibited higher toxicity at high conc than that of pure NPs	Toxic	[8]
		CZF-MNP	16nm			High viability against PC3 and High toxicity against DU145 cells	Moderately toxic to DU145	
Fibroblasts (NIH3T3) cells	HLC coated super paramagnetic Fe3O4		35.5nm HS	25-250 ug/ml	24hrs	25-100ug/ml w/o coating had no effect on CV	Safe in 25-250ug/ml range.	[9]
		Bare Fe3O4 NPs	Mean size=8.2 nm, 24.8nm HS				Toxic at 250ug/ml	
Human Umbilical Vein Endothelial cells	Au-coated Fe3O4 (SPIO core)		30nm, 50nm	Various(0, 5, 10, 25, 50, 100 ug/ml)	Variable for different tests (3, 6, 12, 24, 48)	50nm more toxic than 30nm. Not toxic up to 25ug/ml and 50ug/ml and 12hrs and 24 hrs for 50nm and 30nm resp.	Toxic @ 25ug/ml and 48hrs for 50nm and @ 50ug/ml and 72hrs for 30nmd	[10]

Note: Conc= concentration, Mod=moderate, HC= Hazard Category,CV=Cell viability, resp=respectively, [a] Fibroblasts, SCC, Epidermal Keratinocytes-HaCaT, [b] Bare MNP, [c] APTMs-coated NP showed 10% toxicity, *Cell viability was 46.9% and 39% after 24 and 48hrs respectively at 120ug/mL concentration. Highest DNA damage was at 60ug/mL and caspase3 activity increased to 230% at 60ug/mL., [d] noticeable cell necrosis

TABLE II
Toxicity of Silver NPs to humans and human cell lines

Cell lines	Coated	Uncoated	NP Size	Conc	Exp Time	Major Outcomes	HC	Ref
Human Alveolar Epithelial Cell line (A549	Ag NP coated with PVP		30-50nm. 50, 70, 100nm (PS tested)	Suspension;2.6, 6.6, 13.2ug/cm^2 (10, 25, 50ug/ml). Aerosol: 0.7ug/cm^2.	3h	Aerosol: 50nm and 75nm NPs more toxic than 100nm Suspension: min toxicity & only at high con-50ug/ml (13.2ug/cm^2). No toxicity@ 0.005-0.7ug/cm^2	PVP AgNPs are mildly Cytotoxic in suspension and nontoxic at ALI to A549 cells.	[11]
Human RBCs		Ag NP	0.5 to 0.65nm	5, 10, 20 ug/ml		doses of up to 20ug/ml are below threshold level for cytotoxicity		[12]
Human Gut Flora (Bacillus Subtilis)	Bio Syn		Not available	25-50ug/ml	4hrs	BioS AgNPs less toxic (46.35%) than ComS AgNPs (85.45%)	Toxic	[13]
	Com Syn							
Human Lung cells (Beas-2B)	Citrate coated AgNPs		10, 40, 75nm	5-50ug/mL	4, 24hrs	Significant toxicity shown by 10nm citrate and PVP coated AgNPs at 20 & 50ug/ml after 24hrs.	Toxic	[14]
	PVP coated AgNPs		10nm					
		Uncoated AgNPs	50nm					

Note: PVP= Polyvinyl Pyrolidone, PS= Particle size), con=concentration, ALI= Air liquid Interface, BioS= Biologically synthesized, ComS=Commercially synthesized, HC= Hazard C

Table III

Toxicity of Zinc Oxide NPs to humans and human cell lines

Cell lines	Coated	Uncoated	NP Size	Conc	Exp Time	Major Outcomes	HC	Ref
hHSC	HP1		240nm*	30, µg/mL	24hrs	Coated NPs provide protection against NP induced toxicity	Coated ZnO are almost non-toxic.	[15]
	MAX		Broad*					
		Z-Cote, Nanosun	410nm* 600nm*					
L132		ZnO	50nm	5, 25, 50, 100 ug/mL	24hrs.	25-100ug/mL caused sig loss of cell viability. Generation of OS		[16]
HDFn	Thin SiO_2 shell		105.3nm*	Various. 0-50ug/mL	MTT:12,24,48hrs.LDH, OS,LPO=48hrs	Thicker SiO2/ZnO less toxic than thin and bare ZnO NPs	Toxic	[17]
	Thick SiO_2 shell		158.1nm*					
		ZnO	76.8nm*					

Note: hHSC=human hepatic stellate cells, MAX=dimethoxydihenysilane, HP1=triethoxycaprylysilane , L-132= human lung epithelial cells, OS=Oxidative stress, (HDFn) =Human skin dermal fibroblasts neonatal cells, MTT Assay, LDH Assay, OS=Oxidative stress Assay, LPO assay, *= Hydrodynamic particle size measured by DLS in water

IV. Results

Main findings of the review are summarized are given below

- Small sized NPs 10nm-50nm are generally more toxic than their larger counter parts.

- Cationic NPs can be more toxic due to electrostatic attraction towards negatively charged cellular membrane and DNA.

- Small and positively charged NP can be more toxic in normal human cell lines e.g. fibroblasts than in malignant cells.

- Concentrations less than 100ug/ml are generally safe for MNP use although there are exceptions.

- Au coated and DMSA coated MNPs can induce higher toxicity than bare MNPs. DMSA coated MNPs with Concentrations less than 20ug/ml are harmless in human aortic endothelial cells. Similarly DMSA coated CZF-MNPs with concentration of 16.7 and 33.5 ug/mL produce almost 100% cell viability in prostate cancer cells (DU145 and PC3).

- Au@Fe_3O_4 Nanoparticles show higher toxicity against malignant (SCC) skin cells than against normal skin cells.

- At 100-250 ug/mL concentration of Fe, HLC coated Fe_3O_4 NPs did not produce noticeable cell toxicity whereas Fe_3O_4 NPs without HLC coating produced cytotoxic effects in fibroblasts at concentration of 250 ug/mL of Fe.

- Both Fe_3O_4 NPs with and without HLC coating showed no reduction in viability of NIH3T3 cells at 25-100 ug/mL concentration of Fe.

- Iron oxide NP concentrations at 60ug/ml and above are responsible for inducing significant toxicity in human breast cancer cells. Almost 61% of cells become non-viable at 120ug/mL concentration after 48hrs. This could be due to absence of surface coating.

- At certain concentrations MNPs can induce cell proliferation (e.g. CZF-MNPs @ DMSA at concentration of 0.9 mM Fe which is equivalent to 50.22 ug/mL in prostate cancer cells and Fe2O3 @ DMSA with human aortic endothelial cells at concentration of 0.01 mg/mL which is equivalent to 10ug/mL).

- Biologically synthesized AgNPs are less toxic than commercially synthesized AgNPs.

- Surface coating has a protective effect against ZnO induced Toxicity. HP1 (Triethoxycaprylylsilane), MAX(Dimethoxydiphenylsilane/triehoxycaprylyisilane cross polymer) and SiO_2 coated ZnO NPs showed either no or less toxicity at 30ug/ml (with Hydrodynamic Size of 240nm in water) and 0-50ug/ml concentration range (with 158.1nm) respectively.

- Bare AgNPs with concentrations under 20ug/ml are biocompatible in human RBCs.

- PVP coated and citrate coated AgNPs having 10nm particle size are toxic at 20 and 50 ug/ml after 24hrs compared to bare AgNPs of 50nm particle size in human lung cells.

- Present literature review did not show clear evidence of ROS generation in tested human cell lines by AgNPs.

Iron Oxide based MNP and ZnO NPs can cause cytoxicity and DNA damage by increasing the production of intracellular ROS, by reducing antioxidant defenses and by enhancing the expression of apoptotic and oxidative stress related genes.

V. Analysis and Discussion

The study by Alarifi and colleagues [4] did not clearly mention Iron oxide based Nanoparticles size and hydrodynamic diameter. The study does not tell if the oxidative stress induced by Iron oxide NPs in Human breast cancer cells were of long term duration. It seems the oxidative stress was of short-term duration. Similarly other toxicities observed could be of short-term duration and could be reversible. It seems NP concentrations at 60ug/ml and above are responsible for inducing significant toxicity. The greatest reduction in cell viability was observed when exposed to IONP concentration of 120ug/ml for 48 hrs. Almost 61% of cells become non-viable at this concentration. Greatest DNA damage was reported at 60 ug/ml IONP concentration. Caspase 3 activity was increased to about 230% of the level of the control group at 60ug/ml concentration after 48hr exposure. Cell membrane damage, increased Caspase activity, intracellular oxidative stress and depletion of anti-oxidant defenses were all time and concentration dependent.

The toxicity observed in this study could be attributed to the lack of Iron oxide Nanoparticle surface coating. There is no mention of surface coating and it seems that Iron oxide NPs were bare particles without any surface coating. Presence of appropriate surface coating can result in reduced toxicity. Iron oxide Nanoparticles when used in medical applications for diagnosis and therapy they normal have some sort of surface coating. Hence they are likely to exhibit less damage to host cells.

In the study conducted by Amin and colleagues [5] bare Fe_3O_4 NPs had almost no effect on all human skin cell lines after 24 hrs and 48hrs except HaCaT that showed moderate toxicity (about 10% cell loss) at 500ug/ml after 48 hrs exp time. From this study it seems that if the aim is to kill malignant cells then best concentration to use is 500ug/ml as it shows about 5% reduction in cell survival at 24 hrs and 48hrs and no sign of proliferation after 48hrs. reduction in cell survival was observed at 100ug/ml after 24hrs of exposure with bare Fe_3O_4 NPs but this loss of cells was partly recovered/compensated after incubation period of 48hrs i.e. cell viability improved from 15%-2%.

Au coated Fe_3O_4 Nanoparticles showed higher toxicity for malignant cells (SCC) than for normal cells after 4hrs and this toxicity is still considered moderate. For SCC cells 50ug/ml concentration showed least cell loss after 24 hrs (i.e. 20% cell loss at 10ug/ml to 5% cell loss at 50ug/ml) whereas 500ug/ml concentration showed greatest reduction in cell survival (32%). At 48hrs all malignant cells showed cell recovery-only partly. This means Au Thus Au@Fe_3O_4 Nanoparticles can be used for skin cancer treatment by exploiting their higher toxicity for malignant cells than for normal cells(fibroblasts).

The study examining the toxicity of Fe_3O_4 Magnetic Nanoparticles of different sizes on human fibroblasts and Fibrosarcoma cells (HT-1080) uses high concentrations of MNPs [6]. High NP doses and concentrations are not normally utilized in diagnostic and therapeutic applications as mentioned by authors of the study. Above all suitable surface coating can help reduce the toxicity of Fe_3O_4 Magnetic Nanoparticles. In this case APTMS coated NPs small and positively charged NPs remained toxic.

In the study by Gong and colleagues [10] the 50nm GMNPs exhibited greater hypo-intensities at the same concentration compared with that of 30nm GMNPs as they showed higher relaxivity than 30nm GMNPs. Higher relaxivity means higher relaxation of protons because relaxivity is a measure of relaxation efficiency of an MRI contrast agent [18].

We know that $R_2 \propto 1/T_2$ [18] and we also know that T2 Time measures how fast an MRI signal fades after excitation. Thus higher R_2 relaxivity (for 50nm GMNPs) means faster T_2 relaxation time i.e. short T_2 time. The short T_2 relaxation time means quick loss of MRI signal. Consequently the tissue appears dark on T_2 weighted image. In other words 50nm GMNP contrast agent has high R_2 relaxivity which in turn means 50nm GMNPs have short T_2 time and therefore tissues containing 50nm GMNPs rapidly loses signal and become dark. 50nm produced higher negative enhancement compared with 30nm GMNPs at same concentration because R_2, relaxivity is proportional to particle size.

Main findings of the studies are summarized in Table I-111

A. Concentration Effect

Some studies have shown that MNP coated with DMSA and gold as well as bare MNPs in certain concentrations not only are not toxic but also cause proliferation of cancer cells [5], [7]-[8]. CZF-MNP@DMSA at concentration of 0.9mM Fe causes proliferation of PC3 and DU145 cells after 24 hrs incubation period i.e. more than 100% cell viability [8]. Similarly at 0.01mg/ml concentration of DMSA-Fe_2O_3 Human aortic endothelial cells showed cell proliferation [7].

Both Au coated Fe_3O_4 NPs and bare Fe_3O_4 NPs did not show concentration dependent reduction in cell viability at 50ug/ml after 24hrs exposure. At 10ug/ml Au-coated Fe_3O_4 NPs exhibited 18% reduction in cell viability where as at 50ug/ml these cells exhibited about 5% reduction in cell viability i.e. about 13% increase in cell survival.

Bare Fe_3O_4 NPs at concentration of 50ug/ml caused 5% reduction in cell viability where as at 50ug/ml these cells caused cell proliferation i.e. more than 100% cell viability [5].

B. Coating

It is commonly believed that suitable surface coatings can reduce Nanoparticle toxicity. In the study by Li and colleagues [9] HLC-Coated Fe_3O_4 NPs do not produce noticeable cytotoxic effects on fibroblasts (NIH3T3) cells at higher concentrations (250ug/ml) which suggests that these NPs have improved biocompatibility than uncoated Fe_3O_4 NPs. HLC-Coating not only reduced NP toxicity but also turned them into better hyperthermia mediators by showing efficient heat conduction. Similarly surface coated ZNO NPs (HP1, MAX) conferred almost complete protection against ZnO NP-induced cytotoxicity in human hepatic Stellate cells [15]. In another study thick silica coated SiO_2/ZnO NPs produced less severe toxicological responses (e.g. more viable cells and less dead cells) than thin and bare ZnO NPs in HDFn cells especially at 50ug/mL concentration and for longer exposure times (48hrs) suggesting that coatings on ZnO surface could remain stable for longer periods of time [17].

C. Surface Charge

This review found that cationic NPs were more toxic due to their ability to interact strongly with negatively charged plasma membrane. Cationic bare ZnO NPs carried a zeta potential of +33mv whereas thick and thin Silica coated SiO_2/ZnO had a zeta potential of -41.5mv and -20.7mv respectively [17]. Consequently cationic NPs showed reduced cell viability and therefore were found to be more toxic.

However positively charged HLC-coated Superparamagnetic Fe_2O_3 NPs (carrying a zeta potential of +1.5mv) exhibited reduced toxicity and better biocompatibility than negatively charged bare Fe_2O_3 NPs (zeta potential -24.7mv) [9]. These results are in contradiction to the study by Ramasamy and colleagues [17]. The authors do not provide any explanation of the effect of zeta potential or surface charge of HLC coated NPs with respect to its reduced toxicity. In both studies the toxicological response of NPs was studied in Human fibroblast cells of different origins (Fibroblasts NIH3T3 cells, HDFn = Human skin dermal fibroblasts neonatal cells). It could be due to the magnitude of zeta potential e.g. in the study conducted by Ramasamy and colleague [17], positively charged bare ZnO NPs carried +33mv which is much larger than +1.5mv carried by positively charged HLC-coated Superparamagnetic Fe_2O_3 NPs. Another explanation could be that coating irrespective of the charge it carries manages to shield cells from toxic Iron oxide core.

In Ramasaya study bare NPs were more toxic than thin and thick coated silica coated NPs because of high positive surface charge (+33mv zeta potential) and also because of small particle size (76.8nm vs. 105 vs. 158.1nm) compared to thin and thick silica coated NPs.

However HLC coated MNPs [9] despite having a positive surface charge(+1.5mv) did not show increased toxicity compared to uncoated and negatively charged MNPs perhaps because of their large hydrodynamic size after coating (35.5nm vs. 24.8nm). Above all surface coating reduced cellular contact with NP core. Hence the effect of surface charge was offset by particle size and by advantages of surface coating (e.g. less leaching of metallic ions into cellular environment and by shielding toxic core from coming into direct contact with cellular structures.

D. Particle Size and Exposure Time

Nanotoxicity of AuMNPs was found to be dependent on size, concentration and time [10]. AuMNPs with 50nm size were found to be more toxic than 30nm AuMNPs and the suitable concentration and time for endothelial cell labeling and MRI were 25ug/ml for 12hrs for 50nm AuMNPs. No toxicity was observed at 50ug/ml and 24hrs for 30nm AuMNPs making it a safe concentration and time range for 30nm AuMNPs [10].

Likewise PVP AgNP study found particle-size dependent toxicity towards Human Alveolar Epithelial Cell line (A549) i.e. 100nm PVP coated AgNPs caused least toxicity whereas 75nm diameter PVP coated AgNPs caused greatest cytotoxic response suggesting a size threshold for the cytotoxic response [11].

Another study showed size dependent toxicity of AgNPs i.e. only 10nm AgNPs were toxic to Human lung BEAS-B2 Cells irrespective of coatings (i.e. both PVP and citrate coated 10nm sized AgNPs showed similar toxicity) [14].

The studies examined in this review did not show that AgNP induced toxicity in humans and human cell lines is coating dependent. However a study by Nguyen et al, 2012 involving murine macrophage and

human colonic epithelial cells showed PVP-coated AgNPs caused greater reduction in cell viability than citrate coated NPs (for same size AgNPs) suggesting surface coating dependent toxicity. The study also found that uncoated AgNPs were more toxic than coated AgNPs and uncoated AgNPs seem to enhance oxidative stress while suppressing inflammatory response. On the other hand coated NP induced toxicity was mainly due to up regulation of cytokines [19]. This study was not included in this review as it contains not only human but also animal cells lines.

The study conducted by Alarifi and colleagues [4], showed that at concentration of 60ug/ml IOMNP induced significant toxicity in breast cancer cells – MCF-7 i.e. about 32.6% and 42.2% loss of cell viability was observed after 24hrs and 48hrs respectively. Cell viability, ROS generation, cell membrane damage and DNA damage was time and concentration dependent [4] i.e. as the dose and time exposure increased more cytotoxicity occurred.

E. NP Induced Oxidative stress

Oxidative stress is considered one of the mechanisms behind NP-induced cytotoxicity. This in turn can lead to cell death by either apoptosis or necrosis [20]. Serious oxidative stress results in necrosis whereas moderate oxidative stress results in apoptosis [20]. Presence of large DNA fragments and apoptotic body formation are distinctive features of apoptosis.

Various studies have shown that ZnO NPs are able to induce intracellular ROS, increased LPO levels and reduced Glutathione (GSH) levels in concentration and time dependent manner leading to oxidative stress induced cell death by apoptosis [15-17]. It was observed that coated ZnO NPs produced less ROS than uncoated or bare ZnO NPs [15], [17]. Studies investigating toxicity of AgNPs in this review did not show clear evidence of ROS generation in tested human cell lines [11-12], [14]. However other studies have shown that AgNP induced toxicity is based on generation of ROS as well as on ROS-Independent pathways in human lung cell lines (e.g. A549) and human tumoral cell lines (i.e. hepatoma and leukemia) [21]-[22]. Avalos and colleagues [22] showed that AgNPs of 4.2nm and 42nm sizes caused ROS generation, glutathione depletion and statistically non significant inhibition of SOD (super oxide dismutase) in human tumoral cell lines. The study concluded that oxidative stress was mainly responsible for the cytotoxicity of AgNPs and smaller AgNPs were more toxic than larger ones.

Studies have shown that Iron oxide based MNP can induce intracellular oxidative stress cells by enhancing ROS production, by reducing the activities of antioxidant defenses [4] and by increased expression of genes related to oxidative stress (e.g. SOD2, COX-2, Caspase 3) [7] in size, dose and time dependent manner which in turn leads to DNA damage and apoptosis of cells) in MCF cells [4], Human endothelial cells [7] and Human Umbilical Vein Endothelial cells [10].

Therefore it seems that Iron Oxide based MNP, AgNPs and ZnO NPs can cause cytoxicity and DNA damage by increasing the production of intracellular ROS, by reducing antioxidant defenses and by enhancing the expression of apoptotic and oxidative stress related genes.

VI. PROPOSED CLASSIFICATION SYSTEM

Author of this review could not find any practical and workable classification system to classify toxicity or hazard of Metallic Nanoparticles to humans and human cell lines. Hence the author has proposed her own classification system that categories NP into various hazard or toxicity categories according to the following:

- Loss of cell viability
- NP dose or concentration in ug/ml

Mahmoudi and colleagues [23] consider less than 20% reduction in cell viability as being biocompatible. Fratoddi and colleagues [24] consider NPs toxic if particle concentration brings about 50% reduction in growth in cell culture. Hence in the proposed classification system (Table 1V) 20% or less reduction in cell viability induced by NPs is considered biocompatible.

Moreover NPs inducing more than 20% toxicity at low concentrations are considered toxic. Similarly NP inducing toxicity only at higher concentrations are considered less toxic at low concentrations. We know that low concentrations of NP are usually used in commercial processes e.g. commercialized SPIONS in MRI imaging are used at 5-25ug/ml depending on patient's weight [25]-[26]. Therefore generally in practical or clinical work we are concerned with low doses or concentrations. However in research work high concentrations are also used. Toxicity is further categorized into: moderately toxic, very toxic and extremely toxic categories.

This is no way a comprehensive classification system as it does not consider particle size, surface charge, functional groups, surface coating and NP target (e.g. human cell lines, various human organs, bacteria, viruses, fungus) all of which may alter NP induced toxicity.

However to keep things simple and to initiate the process of developing NP toxicity classification system the author has proposed this 2-way simple classification system. Other researchers, scientists and Nanoparticle experts are welcomed to express their opinions about how to improve this system. This will help modify this proposed classification system a comprehensive classification system based on experimental studies as well as expert opinion will emerge.

VII. LIMITATIONS OF THE REVIEW

Toxicity data in humans and human cell lines is limited. Above all there were shortage of open accessed articles discussing metal NP induced toxicity in humans and human cell lines. Hence one of the limitations of this literature review is availability and inclusion of fewer studies.

In-vivo assessment of Cytotoxicity and genotoxicity of metal NPs needs to be done in future studies to reduce the hazard potential for humans. Toxicity of metal NPs in various human cancer cell lines need to investigated. Also an easy to use but efficient classification system for toxicity categorization needs to be developed. Standardization of experimental studies is required so that toxicity data can be compared among various studies.

VIII. CONCLUSION

Toxic impact of Metal based NPs is multi-factorial and difficult to predict. Surface charge, particle size, concentration, method of production, surface coating and charged surface functional groups can influence cytotoxic and genotoxic responses of NPs in human cell lines.

This review of recently published literature concerning metal based NP induced toxicity in humans and human cell lines has shown that smaller NPs are more toxic than larger NPs [9], [14]. Nature of surface coating can cause reduction in toxic effects of NPs. In case of ZnO NPs surface coatings provided protection against NP induced cytotoxicity [15], [17].

This review has found that the cytotoxicity of Ag NPs is Size [11], [14] and concentration [12] dependent. There is contradictory data on coating dependent cytotoxicity of AgNPs and there is limited data on coating dependent cytotoxic effect on human cell lines.

Data on MNP coatings is mixed. DMSA and Au coated MNPs were more toxic than their uncoated or bare counterparts [5]. APTMS coated MNPs were more toxic at 600ug/ml concentration than other MNPs [6]. Small and positively charged MNPs (e.g. APTMS-coated 10nm size) were significantly genotoxic at 200 and 1000 ug/ml concentrations (dose dependent genotoxicity) in human fibroblast cells and were more prone to enter nucleus via nuclear pores and interact directly with DNA than large MNPs [6]. Size, concentration and time dependent genotoxicity was also observed by Gold MNPS in Human umbilical venous endothelial cells [10] with 50nm and 30nm GMNPs exhibiting significant increase in intracellular ROS levels at 25ug/ml and 24hrs and 50ug/ml and 24hrs respectively. HLC coated super paramagnetic Fe_3O_4 NPs were less toxic than bare Fe_3O_4 NPs [9].

Iron oxide based NPs and ZnO based NPs are able to produce ROS and induce oxidative stress. This review could not find evidence for AgNP induced oxidative stress. ROS generation in case of MNPs could be of short-term duration. Data on use of various concentrations of NPs is conflicting. MNPs are usually biocompatible below 100ug/ml concentration. Biosynthesized AgNPs are less toxic than commercially synthesized AgNPs. Effect of different toxicity including oxidative stress could be of short term duration and some of the cytotoxic effects such as loss of cell viability could be partly reversible i.e. repair mechanism may take over and cell proliferation may occur.

IX. CONCLUSION AUTHOR'S DISCLOSURE

No financial support was obtained to conduct this review.

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