Mutation Research 676 (2009) 41–47

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Evaluation of genotoxic effects of oral exposure to Aluminum oxide nanomaterials in rat bone marrow

A. Balasubramanyam[a], N. Sailaja[a], M. Mahboob[a], M.F. Rahman[a], S. Misra[a], Saber M. Hussain[b], Paramjit Grover[a,*]

[a] Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Hyderabad 500 607, Andhra Pradesh, India
[b] Applied Biotechnology, Air Force Research Laboratory/HEPB, Wright-Patterson, AFB, OH, USA

ARTICLE INFO

Article history:
Received 7 October 2008
Received in revised form 2 March 2009
Accepted 18 March 2009
Available online 31 March 2009

Keywords:
Micronucleus test
Chromosomal aberrations assay
Aluminum oxide
Nanomaterials
Rat
Genotoxicity

ABSTRACT

Nanomaterials have novel properties and functions because of their small size. The unique nature of nanomaterials may be associated with potentially toxic effects. The aim of this study was to evaluate the *in vivo* genotoxicity of rats exposed with Aluminum oxide nanomaterials. Hence in the present study, the genotoxicity of Aluminum oxide nanomaterials (30 and 40 nm) and its bulk material was studied in bone marrow of female Wistar rats using chromosomal aberration and micronucleus assays. The rats were administered orally with the doses of 500, 1000 and 2000 mg/kg bw. Statistically significant genotoxicity was observed with Aluminum oxide 30 and 40 nm with micronucleus as well as chromosomal aberration assays. Significantly ($p < 0.05$ or $p < 0.001$) increased frequency of MN was observed with 1000 and 2000 mg/kg bw dose levels of Aluminum oxide 30 nm (9.4 ± 1.87 and 15.2 ± 2.3, respectively) and Aluminum oxide 40 nm (8.1 ± 1.8 and 13.9 ± 2.21, respectively) over control (2.5 ± 0.7) at 30 h. Likewise, at 48 h sampling time a significant ($p < 0.05$ or $p < 0.001$) increase in frequency of MN was evident at 1000 and 2000 mg/kg bw dose levels of Aluminum oxide 30 nm (10.6 ± 1.68 and 16.6 ± 2.66, respectively) and Aluminum oxide 40 nm (9.0 ± 1.38 and 14.7 ± 1.68, respectively) compared to control (1.8 ± 0.75). Significantly increased frequencies ($p < 0.05$ or $p < 0.001$) of chromosomal aberrations were observed with Aluminum oxide 30 nm (1000 and 2000 mg/kg bw) and Aluminum oxide 40 nm (2000 mg/kg bw) in comparison to control at 18 and 24 h. Further, since there is need for information on the toxicokinetics of nanomaterials, determination of these properties of the nanomaterials was carried out in different tissues, urine and feces using inductively coupled plasma mass spectrometry (ICP–MS). A significant size dependent accumulation of Aluminum oxide nanomaterials occurred in different tissues, urine and feces of rats as shown by ICP–MS data. The results of our study suggest that exposure to Aluminum oxide nanomaterials has the potential to cause genetic damage.

1. Introduction

Nanotechnologies are among the fastest growing areas of scientific research and have important applications in a wide variety of fields. Nanomaterials (NM) are engineered structures whose size is 100 nanometers (nm) or less. NM such as nanotubes, nanowires, nanocrystals, quantum dots, spherical and dendritic aggregated NM are gaining importance in commercial use due to their unique physical and chemical properties. The same unique properties of NM that make them so attractive, may lead to nanostructure dependent biological activity that differs from and is not directly predicted by the bulk properties of the constituent chemicals and compounds [1]. Like most new technologies, there is a rising debate concerning the possible harmful effects derived from the use of NM at the nano level [2]. There are a few toxicological studies that have addressed the effects of NM in different systems and environments, which raise concerns about the adverse effects on biological systems [3–8]. These studies suggest, that NM are not inherently benign and they affect biological behaviors at cellular, subcellular and protein levels [9]. Hence there is need to evaluate the toxicity of NM.

Aluminum (Al) and Al compounds have a wide range of uses in industrial, domestic, consumer and medicinal products [10]. Nanosized Al is being used in energetic systems to increase the specific impulse per weight of composite propellants for possible use in solid rocket fuels [11]. They are being utilized to replace lead primers in artillery and wear resistant coatings on propeller shafts of ships [12]. Al NM of different sizes have also been proposed as drug delivery systems to increase solubility [13]. Concerns over risks of inducing cancer are universal and as a result genotoxicity studies are an important feature of safety assessment of drugs

* Corresponding author. Tel.: +91 40 27193135; fax: +91 40 27193227.
E-mail addresses: param_g@yahoo.com, grover@iict.res.in (P. Grover).

1383-5718/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrgentox.2009.03.004

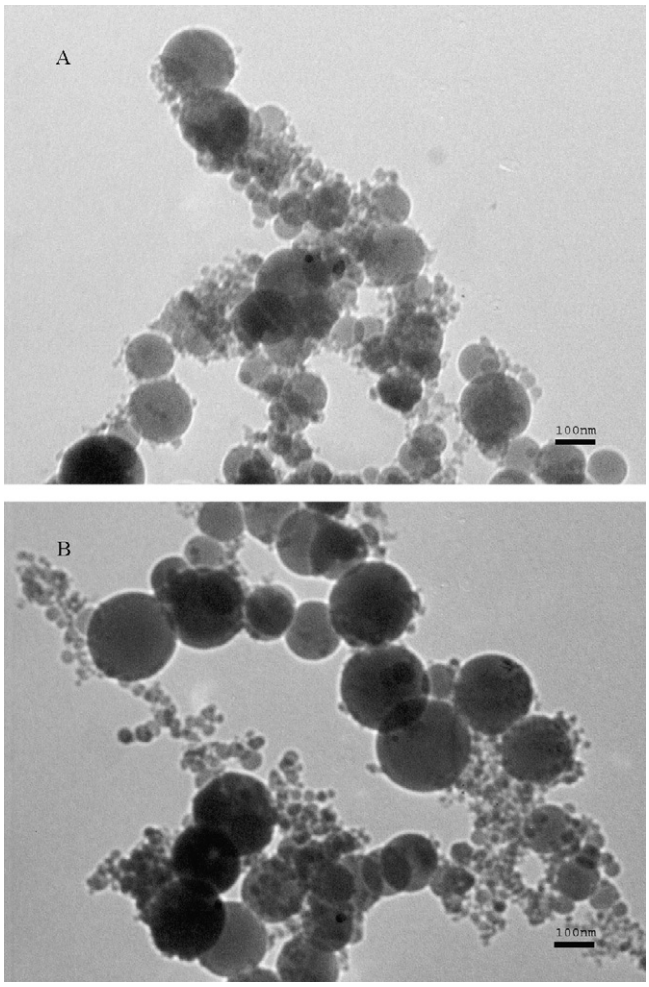

Fig. 1. (A) TEM image for Al_2O_3-30 nm; (B) TEM image for Al_2O_3-40 nm.

and chemicals. Significant genotoxicity of Al and Al compounds was revealed from limited studies found in literature. Aluminum chloride ($AlCl_3$) significantly increased the number of micronuclei (MN) examined with human peripheral blood lymphocytes (PBL) [14]. The level of DNA damage assessed by utilizing the comet assay in PBL treated with $AlCl_3$ was significantly high [15]. Similarly, $AlCl_3$ evaluated with the comet assay and chromosomal aberrations (CA) analysis, induced significant DNA damage and CA in PBL [16]. *In vivo* studies with rats revealed that aluminum sulfate and potassium aluminum sulfate induced dose dependent increase in CA [17]. Investigations that have examined the genotoxic effects of NM of Al are limited. Hence, the scarcity of *in vivo* studies on the genotoxicity of NM of Al, led us to conduct a study on the genotoxic effects of NM of Aluminum oxide (Al_2O_3-30 and Al_2O_3-40 nm) and bulk (Al_2O_3-B) using the rat bone marrow CA and micronucleus test (MNT). The *in vivo* MNT is one of the most common genotoxicity screening tests. The assay detects clastogenicity because of chromosome breakage and also aneugenicity, due to chromosome lagging resulting from dysfunction of mitotic apparatus. CA are due to failure or mistakes in repair processes such that breaks either do not rejoin or rejoin in abnormal configurations. These *in vivo* tests are especially relevant for genotoxicity, because they allow consideration of factors of *in vivo* metabolism, pharmacokinetics and DNA repair process [18].

To properly ascribe reasons for toxic effects of NM information on the absorption, distribution and excretion of NM is warranted [19]. Therefore another aim of this study was to evaluate the biodistribution of the NM after acute treatment in different tissues, urine and feces using ICP–MS.

The purpose of the present work was to determine whether Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B administered to rats by oral route were capable of causing genotoxic effects in bone marrow using MN formation and CA as genetic endpoints. The study was also aimed to evaluate the dose dependent genotoxicity of Al_2O_3 NM. Further, the study tested the hypothesis that the genotoxicity of Al_2O_3 NM was size dependent.

2. Materials and methods

Al_2O_3-B (CAS number 1344-28-1, purchased from Sigma–Aldrich Inc., USA; size: 70–290 mesh (50–200 μm); purity: >90%). Al_2O_3-30 and Al_2O_3-40 nm (product code: M1056, M1049-D, a gift from Dr Karl Martin of NovaCentrix, Austin, TX, USA; purity: >90%). The size of the nanoscale Al_2O_3-30 and 40 nm particles was determined by transmission electron microscopy (TEM, H-7600). A total of 100 particles per sample were measured (mean ± standard deviation) and sizes were found to be 39.85 ± 31.33 nm and 47.33 ± 36.13 nm diameter, respectively (Fig. 1A and B). The genotoxic effects of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B were investigated in bone marrow cells of inbred female albino Wistar rats (W NIN), using the MN and CA assays. The mitotic index (MI) was also determined to detect the cytostatic effect of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B. An acute oral toxicity study based on OECD Guideline 420, updated and adopted on 17 December 2001 [20] of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B was the basis for determination of doses. A single rat was dosed first with 5 mg/kg bw dose. If no mortality and symptoms were found, the second rat received 50 mg/kg bw dose, then 300 mg/kg bw and finally, 2000 mg/kg bw dose in sequence in the sighting study. Since no mortality and toxic symptoms were found at any dose level in the sighting study, the main study with five rats was done at 2000 mg/kg bw dose level for Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B (Table 1). Therefore, three dose levels of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B: 500, 1000, 2000 mg/kg bw were used for the rat bone marrow MN and CA assays.

Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B do not dissolve in water. To prepare suspended solutions of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B, it is recommended to use 1% Tween 80, a surfactant in double distilled water (DDW) and dispersed by ultrasonic vibration for 10 min. The suspensions are mixed thoroughly prior to use.

The experimental design for both *in vivo* tests included three groups: the positive control, the control, and the experimental groups. The experimental groups were divided into three subgroups based on the selected doses of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B. All groups had equivalent numbers of animals per test. Thus, for the MN and CA assays five animals were used per dose. We used 4- to 5-week-old Wistar rats with an average weight of 90–100 g. Animals were kept under uniform conditions and housed under 12/12-h light–dark period at constant temperature (22 ± 3 °C) with free access to standard laboratory feed (composition: wheat flour, 2.5%; roasted Bengal gram flour, 60%; skimmed milk powder, 5%; casein, 4%; refined ground oil, 4%; salt mixture, 4% and vitamin mixture, 0.5%) and water. The laboratory feed and rats for the experiment were purchased from the animal house of National Institute of Nutrition, Hyderabad, India. The rats were kept for one week of acclimatization before use. The present study was conducted in strict accordance with directive 86/609/EEC on the protection of laboratory animals. Institutional

Table 1
Summary of acute oral toxicity study of Al_2O_3 nanomaterials and bulk.

Treatment	Sighting study 1 rat/dose (mg/kg bw)				Main study 5 rats/dose (mg/kg bw)	LD_{50} (mg/kg bw)	Hazard category ranking by GHS[a]
	5	50	300	2000	2000		
Control	–	–	–	–	No mortality	–	–
Al_2O_3-30 nm	No mortality	No mortality	No mortality	No mortality	No mortality	>2000	5
Al_2O_3-40 nm	No mortality	No mortality	No mortality	No mortality	No mortality	>2000	5
Al_2O_3 bulk	No mortality	No mortality	No mortality	No mortality	No mortality	>2000	5

Data tabulated as per OECD test guideline 420.

[a] Globally Harmonized System (GHS) ranking of category 5 indicates relatively low acute toxicity hazard.

Table 2
The frequency of MN–PCEs and percent PCEs in rat bone marrow cells treated with Al_2O_3 nanomaterials and bulk at 30 and 48 h.

Treatments	Dose (mg/kg bw)	30 h		48 h	
		MN–PCEs/2000 PCEs	%PCEs	MN–PCEs/2000 PCEs	%PCEs
Control[a]	1% (v/v)	2.5 ± 0.70	42.1 ± 3.47	1.8 ± 0.75	40.9 ± 5.45
Al_2O_3-30 nm	500	4.3 ± 1.03	39.1 ± 2.97	5.0 ± 1.05	39.9 ± 2.27
	1000	9.4 ± 1.87**	40.2 ± 2.47	10.6 ± 1.68**	33.7 ± 3.32
	2000	15.2 ± 2.30***	35.1 ± 3.19	16.6 ± 2.66***	36.7 ± 3.56
Al_2O_3-40 nm	500	3.8 ± 1.05	43.1 ± 2.13	3.9 ± 1.08	39.1 ± 2.91
	1000	8.1 ± 1.80*	33.9 ± 4.53	9.0 ± 1.38*	37.1 ± 3.21
	2000	13.9 ± 2.21***	35.5 ± 3.27	14.7 ± 1.68***	33.2 ± 4.12
Al_2O_3 bulk	500	1.9 ± 0.73	38.2 ± 3.21	2.0 ± 0.64	42.1 ± 3.01
	1000	3.3 ± 1.16	32.1 ± 3.98	4.2 ± 1.07	41.5 ± 3.31
	2000	5.9 ± 1.71	33.8 ± 3.63	6.6 ± 1.68	37.5 ± 3.26
CP[b]	40	33.2 ± 3.46***	27.3 ± 2.66**	30.2 ± 4.16***	25.3 ± 2.92**

Data represented as mean ± S.D.
[a] Tween 80.
[b] Cyclophosphamide.
* Significantly different from control at $p < 0.05$.
** Significantly different from control at $p < 0.01$.
*** Significantly different from control at $p < 0.001$.

Animal Ethics Committee approved the study. Experimental doses of all the three groups was obtained by suspending 500, 1000 and 2000 mg/kg bw of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B in DDW–Tween 80 mixture and administered to the rats by oral gavage. The control group was treated with DDW–Tween 80 mixture. A known mutagen, cyclophosphamide at a dose of 40 mg/kg bw was used for the positive control group. It was given intraperitoneally (i.p.), and the volume injected was 0.01 ml/g b w. All animals received a single dose i.p. All treated animals were sacrificed by cervical dislocation.

The method described by Schmid [21] was used for analysis of MN in polychromatic erythrocytes (PCEs) of rat bone marrow. At least four slides were made for each animal, allowed to dry overnight and then stained with Giemsa (Sigma Chemical Co., St. Louis, MO) for conventional assessment of the MN frequency. All slides were coded for microscope analysis at 1000× magnification. Per animal, 2000 PCEs from all the four randomly selected slides were scored for the presence of MN. The study was performed at 30 and 48 h of sampling times and was in accordance with OECD Guideline 474, updated and adopted on 21 July 1997 [22].

Cytogenetic analysis was performed by the direct method of rinsing marrow of long bones (femur and tibia) according to Adler [23]. Slides were made by the flame-dried technique and later stained with Giemsa. CA were identified on the basis of criteria established by the OECD Guideline 475, updated and adopted on 21 July 1997 [24]. We analyzed 500 well-spread metaphases for each treatment at 18 and 24 h of sampling times to detect the presence of CA, whereas the mitotic index (MI) was determined on 1000 or more cells at both the sampling times. The slides were randomly selected and coded prior to scoring.

The animals were sacrificed after 14 days of acute oral treatment with 500, 1000 and 2000 mg/kg bw of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B for tissues viz., liver, kidneys, heart, brain, spleen and blood. Urine and feces samples were collected 48 h after the dosing. About 0.1–0.3 g of fresh liver, kidneys, heart, brain, spleen, blood and feces samples from the treated rats were predigested in nitric acid (ultrapure grade) for overnight. Samples were then heated at 80 °C for 10 h followed by additional heating at 130–150 °C for 30 min. Finally, in the presence of 0.5 ml of 70% perchloric acid, the samples were again heated for 4 h, and evaporated nearly to dryness [25]. Subsequently, solutions and the urine (0.5 ml) samples were made up to 5 ml with deionized water and filtered. ICP–MS (ELAN DRC II, PerkinElmer Sclex.) was used to analyze the Al_2O_3 concentration in the samples. Rhodium at 20 ng/ml was used as an internal standard element.

Statistical analyses were carried out using the Analysis of Variance (ANOVA), the Student's *t*-test, and the LSD-test.

3. Results

The effects of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B were evaluated in bone marrow cells of Wistar rats by monitoring the MI, MN, and CA.

Administration of Al_2O_3-30 and Al_2O_3-40 nm resulted in the induction of MN in PCEs of bone marrow of Wistar rats (Table 2). Statistical analysis demonstrated a significant increase ($p < 0.05$) in MN induction at 1000 and 2000 mg/kg bw experimental groups of Al_2O_3-30 (9.4 ± 1.87 and 15.2 ± 2.3, respectively) and Al_2O_3-40 nm (8.1 ± 1.8 and13.9 ± 2.21 respectively) compared with the control (2.5 ± 0.7) at 30 h. Similarly, the frequency of MN was increased in 1000 and 2000 mg/kg bw Al_2O_3-30 (10.6 ± 1.68 and 16.6 ± 2.66, respectively) and Al_2O_3-40 nm (9.0 ± 1.38 and 14.7 ± 1.68, respectively) treated groups at 48 h post treatment in comparison to control (1.8 ± 0.75). Al_2O_3-B did not induce a significant increase in the frequency of MN–PCEs at all dose groups compared with the control at both treatment periods. However, Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B did not induce a significant decrease in the percent PCEs compared to the control group.

The results show (Tables 3 and 4) that all experimental groups of Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B (500, 1000, 2000 mg/kg bw) did not cause any significant reduction in mitotic index at both the sampling times 18 and 24 h in comparison with the control.

It was established by cytogenetic analysis that there was an increase in the frequency of abnormal metaphases (aneuploid and polyploid type) and total CA in rat bone marrow cells after 18 and 24 h of treatment with oral dose of Al_2O_3-30 at 1000 and 2000 mg/kg bw where as Al_2O_3-40 nm showed only at 2000 mg/kg bw in comparison with the control. Al_2O_3-30 and Al_2O_3-40 nm showed the ability to induce abnormal metaphases and structural changes such as chromatid breaks, isochromatid breaks, acentric fragments, minutes, and translocations as the total cytogenetic changes excluding gaps. Significantly increased frequencies ($p < 0.05$) of numerical CA (aneuploidies and polyploidies) were observed at the experimental doses 1000 and 2000 mg/kg bw of Al_2O_3-30 (8.6 ± 0.77 and 12.2 ± 1.62, respectively) and 2000 mg/kg bw (10.1 ± 1.46) of Al_2O_3-40 nm compared to control (0.5 ± 0.16) at 18 h. Furthermore, significant increased frequencies in numerical CA were observed with 1000 and 2000 mg/kg bw of Al_2O_3-30 nm (7.8 ± 1.06 and 12.8 ± 2.49, respectively) and 2000 mg/kg bw of Al_2O_3-40 nm (11.3 ± 2.05) treated groups compared with the control (0.3 ± 0.19) at 24 h (Tables 3 and 4).

Regarding total cytogenetic changes (Tables 3 and 4), an 18 and 24 h post treatment with 1000 (6.9 ± 0.61 and 7.3 ± 0.59, respectively) and 2000 mg/kg bw (11.8 ± 1.10 and 10.5 ± 1.31, respectively) of Al_2O_3-30 nm showed significant increase in total cytogenetic changes ($p < 0.05$) compared with the control (0.6 ± 0.32 and 0.5 ± 0.42, respectively). Similarly, at both the sampling times 2000 mg/kg bw of Al_2O_3-40 nm significantly ($p < 0.05$) increased the total cytogenetic changes (9.6 ± 1.84 and 9.9 ± 1.05, respectively) over control. However, Al_2O_3-B (500, 1000, 2000 mg/kg bw) experimental doses did not show any significant increases of

Table 3
Mitotic index, distribution of the different types of chromosomal aberrations observed in rats bone marrow cells at 18 h.

Treatments	Dose (mg/kg bw)	M.I. (%) $M \pm$ S.D.	Aneuploidy	Polyploidy	Chromosomal aberrations		Minutes	Acentric fragments	Reciprocal translocations	TA + gaps $M \pm$ S.D.	TA – gaps $M \pm$ S.D.
					Gaps	Breaks					
Control[a]	1% (v/v)	2.97 ± 0.12	0.5 ± 0.16	0.00 ± 0.00	1.2 ± 0.24	0.8 ± 0.2	0.4 ± 0.16	0.3 ± 0.15	0.00 ± 0.00	1.2 ± 0.9	0.6 ± 0.32
Al_2O_3-30 nm	500	3.28 ± 0.16	2.0 ± 0.85	0.00 ± 0.00	1.3 ± 0.7	1.1 ± 0.52	1.0 ± 0.49	0.9 ± 0.54	0.00 ± 0.00	3.3 ± 0.72	2.3 ± 0.52
	1000	3.08 ± 0.08	8.6 ± 0.77*	0.00 ± 0.00	2.6 ± 0.93	2.0 ± 0.63	1.3 ± 0.24	1.6 ± 0.67	0.00 ± 0.00	8.1 ± 0.91*	6.9 ± 0.61*
	2000	3.20 ± 0.10	12.2 ± 1.62***	0.00 ± 0.00	3.9 ± 0.91	3.4 ± 0.7	2.2 ± 0.44	3.2 ± 0.59	0.00 ± 0.00	12.9 ± 1.51***	11.8 ± 1.10***
Al_2O_3-40 nm	500	2.83 ± 0.14	1.7 ± 0.86	0.00 ± 0.00	1.5 ± 0.71	1.2 ± 0.61	0.7 ± 0.59	0.7 ± 0.30	0.00 ± 0.00	3.8 ± 0.46	1.8 ± 0.36
	1000	2.96 ± 0.08	5.2 ± 0.71	0.00 ± 0.00	2.1 ± 0.82	1.7 ± 0.36	1.1 ± 0.44	1.3 ± 0.61	0.00 ± 0.00	5.5 ± 1.43	4.5 ± 0.53
	2000	3.23 ± 0.14	10.1 ± 1.46***	0.00 ± 0.00	2.7 ± 0.36	2.8 ± 0.55	1.9 ± 0.34	2.1 ± 0.40	0.00 ± 0.00	11.4 ± 1.01***	9.6 ± 1.84***
Al_2O_3 bulk	500	3.04 ± 0.13	0.7 ± 0.39	0.00 ± 0.00	0.9 ± 0.41	0.5 ± 0.22	0.3 ± 0.21	0.2 ± 0.13	0.00 ± 0.00	1.6 ± 0.42	0.6 ± 0.22
	1000	2.72 ± 0.10	2.7 ± 0.68	0.00 ± 0.00	1.2 ± 0.48	0.9 ± 0.44	0.7 ± 0.19	0.7 ± 0.33	0.00 ± 0.00	3.2 ± 0.99	2.2 ± 0.79
	2000	3.09 ± 0.12	6.9 ± 1.15	0.00 ± 0.00	1.9 ± 0.31	1.2 ± 0.35	1.3 ± 0.39	1.4 ± 0.54	0.00 ± 0.00	5.7 ± 1.02	4.3 ± 1.01
CP[b]	40	2.23 ± 0.13*	38.9 ± 2.07***	3.21 ± 0.99***	9.8 ± 0.71***	12.7 ± 1.09***	14.3 ± 1.76***	13.7 ± 1.09**	1.30 ± 1.25***	53.9 ± 2.21***	42.6 ± 2.32***

Hundred metaphases were analyzed per animal ($n = 5$), number of animals per group *Abbreviations*: MI, mitotic index; $M \pm$ S.D., mean ± standard deviation; TA, total aberrations.
[a] Tween 80.
[b] Cyclophosphamide.
* Significantly different from control at $p < 0.05$.
*** Significantly different from control at $p < 0.001$.

Table 4
Mitotic index, distribution of the different types of chromosomal aberrations observed in rats bone marrow cells at 24 h.

Treatments	Dose (mg/kg bw)	M.I. (%) $M \pm$ S.D.	Aneuploidy	Polyploidy	Chromosomal aberrations		Minutes	Acentric fragments	Reciprocal translocations	TA + gaps $M \pm$ S.D.	TA – gaps $M \pm$ S.D.
					Gaps	Breaks					
Control[a]	1% (v/v)	3.25 ± 0.10	0.3 ± 0.19	0.00 ± 0.00	1.6 ± 0.49	0.9 ± 0.27	0.3 ± 0.22	0.4 ± 0.16	0.00 ± 0.00	0.9 ± 0.42	0.5 ± 0.42
Al_2O_3-30 nm	500	2.91 ± 0.08	1.4 ± 0.49	0.00 ± 0.00	1.8 ± 0.64	1.0 ± 0.47	0.9 ± 0.48	0.8 ± 0.29	0.00 ± 0.00	1.9 ± 0.33	1.2 ± 0.23
	1000	2.74 ± 0.06	7.8 ± 1.06*	0.00 ± 0.00	3.4 ± 0.85	2.4 ± 0.73	1.6 ± 0.68	2.1 ± 0.65	0.00 ± 0.00	8.7 ± 0.98*	7.3 ± 0.59*
	2000	2.77 ± 0.07	12.8 ± 2.49***	0.00 ± 0.00	4.5 ± 1.19	3.5 ± 0.96	3.2 ± 0.92	3.5 ± 0.92	0.00 ± 0.00	12.5 ± 1.71***	10.5 ± 1.31***
Al_2O_3-40 nm	500	2.87 ± 0.12	1.0 ± 0.49	0.00 ± 0.00	1.5 ± 0.63	0.8 ± 0.32	0.6 ± 0.23	0.6 ± 0.4	0.00 ± 0.00	1.5 ± 0.29	1.2 ± 0.29
	1000	2.69 ± 0.04	5.2 ± 1.54	0.00 ± 0.00	2.2 ± 0.59	1.8 ± 0.53	1.3 ± 0.74	1.8 ± 0.77	0.00 ± 0.00	5.8 ± 0.87	4.3 ± 0.37
	2000	2.72 ± 0.13	11.3 ± 2.05***	0.00 ± 0.00	3.8 ± 1.39	2.8 ± 0.80	2.6 ± 0.53	2.6 ± 0.83	0.00 ± 0.00	10.4 ± 1.45***	9.9 ± 1.05***
Al_2O_3 bulk	500	2.73 ± 0.13	0.6 ± 0.30	0.00 ± 0.00	2.7 ± 0.87	0.4 ± 0.16	0.4 ± 0.16	0.3 ± 0.15	0.00 ± 0.00	0.8 ± 0.22	0.5 ± 0.31
	1000	2.67 ± 0.07	2.0 ± 0.44	0.00 ± 0.00	1.3 ± 0.55	1.1 ± 0.48	0.8 ± 0.35	1.0 ± 0.49	0.00 ± 0.00	2.2 ± 0.76	1.2 ± 0.51
	2000	2.83 ± 0.14	8.8 ± 1.89	0.00 ± 0.00	1.3 ± 0.66	1.8 ± 0.72	1.1 ± 0.58	2.1 ± 0.43	0.00 ± 0.00	6.9 ± 0.99	5.5 ± 0.31
CP[b]	40	2.41 ± 0.13	43.6 ± 2.61***	3.47 ± 0.19***	11.5 ± 0.96***	14.3 ± 1.74***	16.3 ± 2.2***	15.5 ± 1.49***	2.1 ± 0.39***	57.1 ± 2.08***	46.7 ± 1.88***

Hundred metaphases were analyzed per animal ($n = 5$), number of animals per group Abbreviations: MI, mitotic index; $M \pm$ S.D., mean ± standard deviation; TA, total aberrations.
[a] Tween 80.
[b] Cyclophosphamide.
* Significantly different from control at $p < 0.05$.
*** Significantly different from control at $p < 0.001$.

Table 5
Al content in different tissues, urine and feces of rats treated with Al_2O_3 nanomaterials and bulk.

Samples analyzed for Al content	Conc. of Al in tissues, urine and feces of treatment groups									
	Control	Al_2O_3-30 nm dose (mg/kg bw)			Al_2O_3-40 nm dose (mg/kg bw)			Al_2O_3 bulk dose (mg/kg bw)		
		500	1000	2000	500	1000	2000	500	1000	2000
Whole blood[a]	3 ± 1	47 ± 16	79 ± 25**	156 ± 21***	45 ± 14	73 ± 26*	129 ± 17***	12 ± 4	32 ± 9	76 ± 9
Liver[a]	6 ± 2	56 ± 18	84 ± 12*	158 ± 23***	42 ± 11	78 ± 18**	119 ± 21***	22 ± 18	52 ± 15	71 ± 11
Spleen[a]	2 ± 1	28 ± 21	59 ± 22*	153 ± 17***	37 ± 16	60 ± 13**	116 ± 11***	15 ± 7	28 ± 7	51 ± 10
Heart[a]	6 ± 3	37 ± 11	69 ± 29*	121 ± 13***	35 ± 21	66 ± 14*	102 ± 18***	14 ± 6	25 ± 9	57 ± 10
Kidneys[a]	9 ± 4	62 ± 19*	122 ± 20**	231 ± 29***	48 ± 15*	92 ± 18**	156 ± 22***	21 ± 7	34 ± 16	63 ± 20
Brain[a]	2 ± 3	50 ± 21	109 ± 22**	187 ± 19***	35 ± 17	90 ± 23*	151 ± 13***	14 ± 3	23 ± 6	59 ± 11
Urine[b]	5 ± 2	48 ± 15*	95 ± 17**	191 ± 16***	44 ± 9	80 ± 17**	183 ± 18***	9 ± 5	19 ± 8	54 ± 12
Feces[c]	1 ± 1	4 ± 3	7 ± 3	21 ± 5	5 ± 1	8 ± 2	27 ± 6	16 ± 7	26 ± 7	37 ± 9**

Data represented as mean ± S.D.
[a] Values represented as μg/g tissues.
[b] Values represented as μg/ml urine.
[c] Values represented as mg/g feces.
* Significantly different from control at $p < 0.05$.
** Significantly different from control at $p < 0.01$.
*** Significantly different from control at $p < 0.001$.

numerical CA and total CA in comparison with the control at both the sampling times respectively.

The contents of Al in each tissue, urine and feces of rats after 14 days of exposure to different sized Al_2O_3 by oral route are shown in Table 5. The experimental doses (500, 1000, 2000 mg/kg bw) of all the three groups Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B accumulated in all tissues viz., liver, heart, kidneys, brain, spleen and blood. In all the tissues, the Al concentrations in the Al_2O_3-30 and 40 nm groups were significantly higher than those of control at 1000 and 2000 mg/kg bw dose levels ($p < 0.05$). In case of Al_2O_3-B treated group, the Al concentrations were found in all tissues. However, there was no significant increase in the Al content in Al_2O_3-B treated rats at any of the dose levels compared to the control. Al accumulation was highest in the kidneys at the experimental doses 500, 1000 and 2000 mg/kg bw (62 ± 19, 122 ± 20 and 231 ± 29 μg/g, respectively) of the 30 nm group followed by (48 ± 15, 92 ± 18 and 156 ± 22 μg/g, respectively) in the 40 nm group where as Al content was 21 ± 7, 34 ± 16 and 63 ± 20 μg/g, respectively, in the bulk group. Al content was lowest in the heart (121 ± 13 μg/g) of the 30 nm group and (102 ± 18 μg/g) in the 40 nm group dosed with 2000 mg/kg bw concentration but the bulk group showed lowest (51 ± 10 μg/g) in spleen at the same dose level.

4. Discussion

Nanotechnology has the potential to dramatically improve the effectiveness of a number of existing consumer and industrial products and could have a substantial impact on the development of new products. A continuing evaluation of the human health implications of exposure to NM is essential before the commercial benefits of these materials can be fully realized [26]. Among toxic effects, particular concern is raised by the possibility of irreversible adverse effects subsequent to genetic damage, hence in this study the genotoxic effects of oral exposure to Al_2O_3 NM was studied.

In the present investigation characterization of NM studied using TEM showed a good correlation of the mean size distribution of Al_2O_3 NM with the manufacturer specified sizes (30 and 40 nm) as the calculated mean sizes of Al_2O_3-30 and 40 nm ranged at 30 and 40 nm, respectively. However, both the sizes did not show any significant difference from each other. Nevertheless, we have considered them as two different treatment groups since our concern was to study if the two different sizes were capable of producing a prominent change in genotoxic effects independently. Likewise, recent studies on *in vitro* toxic effects of Al_2O_3-30 and Al_2O_3-40 nm have also regarded these two sizes as different treatment groups with similar mean size measurements [8,27]. The results obtained in the current study, revealed a significant increase in the mean MN at 1000 and 2000 mg/kg bw in rat bone marrow indicating genotoxicity with the Al_2O_3-30 and 40 nm at their respective time intervals in comparison to the bulk and control groups. Sampling times of 30 and 48 h for bone marrow MNT, when compared, showed no difference in the mean value of MN suggesting its effectiveness was the same at respective sampling times. However, the percent PCEs in the bone marrow of different treatment groups did not show any significant reduction than the control, which suggested that cell death had not occurred in any of the treatment groups. The results of the bone marrow CA analysis with Al_2O_3-30 and Al_2O_3-40 nm in rats indicated that these compounds were positive for the induction of total CA including and excluding gaps in bone marrow but not Al_2O_3-B. Regardless, percent mitotic index suggested that Al_2O_3-30, Al_2O_3-40 nm and Al_2O_3-B were not cytotoxic. The data from this study demonstrated that Al_2O_3 NM induced dose dependent genotoxicity. The combined results from the *in vivo* genotoxicity study suggested that the oral administration of Al_2O_3-30, Al_2O_3-40 nm NM and Al_2O_3-B show that Al_2O_3-30 was most genotoxic and the

bulk was the least. Similarly, Rahman et al. [28] found ultra fine titanium dioxide (TiO_2) to induce MN significantly in Syrian hamster embryo cells. In contrast fine TiO_2 did not induce MN to a significant extent. The genotoxicity observed with Al_2O_3 NM may be due to pro-inflammatory effects through a reactive oxygen species mediated mechanism [29]. Based on the current findings we hypothesize that the size of the Al_2O_3 NM may be the cause of the significant genotoxicity.

Our results are in agreement with an *in vitro* study of TiO_2, Al_2O_3 and gold NM, investigated in A549, HEPG2 and NRK-52E cells with MNT, comet and gamma-H2AX histone foci quantification [30]. Our study is also in accordance with the investigations of TiO_2 NM using cultured human MRC-5 fibroblasts and WIL2-NS lymphoblastoid cells which showed pronounced genotoxicity with cytokinesis block MNT, the comet assay and the hypoxanthine–guanine phosphoribosyl transferase gene mutation assay [31,7]. Likewise, genotoxicity investigated with magnetite nanoparticles using MNT in mice revealed a genotoxic effect [32]. In contrast, results obtained with NM of TiO_2–P25 and T805 elucidated no significant differences between control and the NM in amount of 8-oxoGua as a marker of DNA damage when analyzed in single cells in lung tissue of rats exposed by instillation. Probably the instillation of low doses (0.15, 0.3, 0.6 and 1.2 mg) of TiO_2–P25 or TiO_2–T805 was not sufficient to produce a persist inflammation in the rat lung [33]. Similarly magnetic NM was evaluated for genotoxicity using the Ames test and CA assay with the Chinese hamster lung fibroblast cells. The NM increased the number of revertants in Ames assay, but the mutation pattern was neither reproducible nor concentration dependent. However, the NM did not induce any significant CA. Their findings suggest *Salmonella* assay data may not be relevant to predict the toxicity of magnetic NM. These results can be further supported by the finding that the predictive relationship between the mutagenic potential determined by the *Salmonella* assay and genotoxicity was at best weak [4].

In the present study significant biodistribution of Al_2O_3-30 and Al_2O_3-40 nm occurred in treated rats different tissues, urine and feces. Our data revealed that maximum accumulation in the tissues was of Al_2O_3-30 NM. Al_2O_3-30 nm showed increased amount of retention in kidneys followed by Al_2O_3-40 nm. This could be attributed to the Al_2O_3 NM being entrapped in the reticular endothelial system and excreted by the kidneys *in vivo*. Al_2O_3-30 and Al_2O_3-40 nm also mainly accumulated in the brain and it may be due to the presence of sites in the brain that circumvent blood brain barrier (BBB) and could allow the test compounds to reach the brain. At all the dose levels of Al_2O_3-B treated rats did not show significant biodistribution in any of the tissues where as it showed higher amounts in the feces than Al_2O_3-30 and Al_2O_3-40 nm indicating that size was the barrier in absorption of Al_2O_3 into the rat system.

Our results are supported by a few studies. A study on magnetic NM revealed tissue distribution in various organs including brain of mice and showed that circumventricular organs within the brain are not protected by the BBB [4]. Similar kinetic studies in mice with different nanosized TiO_2 particles demonstrated its retention in the liver, spleen, kidneys and lung tissues [34]. Likewise, mice treated with nano-copper (23.5 nm), ion–copper and micro-copper (17 μm) were evaluated for distribution in different organs after a single dose oral exposure by ICP–MS. In nano-copper treated group, the contents of copper were significantly high in liver, kidneys and blood. However, in micro-copper exposure group, the distribution of copper was most similar to that in the control group [35]. It can be concluded that characteristics of NM could influence the toxicokinetic properties of the particles. However, it is unclear to what extent the different NM characteristics contribute to their kinetics [36].

The result of Al_2O_3-B content analysis indicated that it accumulated the least, possibility as a consequence of its poor bioavailability. It has been reported that Al is poorly absorbed following oral exposure. Approximately 0.1% of ingested Al is usually absorbed, although absorption of more bioavailable forms can be on the order of 1%. The unabsorbed Al is excreted in the feces. The 10-fold range in absorption of Al is largely due to differences in bioavailability related to the form of ingested Al and the presence of dietary constituents which can complex with Al and there by enhance or inhibit its absorption. The mechanism of absorption is probably passive diffusion through paracellular pathways. Al binds to various ligands in the blood and distributes to every organ, with highest concentrations found in bone and lung tissues. Absorbed Al is excreted principally in the urine and, to a lesser extent, in the bile [10].

Over all our data indicates bioaccumulation of orally administered Al_2O_3 NM for the significant genotoxic potential *in vivo*. The mechanisms of genotoxic activity of Al are not well understood. It can be hypothesized that Al could induce DNA damage through three mechanisms: modification of chromatin structure, induction of reactive oxygen species and liberation of DNase from the lysosomes [14]. The first mechanism is supported by the findings that Al can influence the structure of chromatin [37,38]. In addition, many metals including Al are known to inhibit DNA repair mechanisms [39]. These mechanisms would lead to DNA damage. The second mechanism is substantiated by the observations that interaction of cells with Al can lead to the formation of reactive oxygen species [40,41]. The last mechanism is evident from the finding that Al can change the permeability of hepatic lysosomal membrane [42] and that it also inhibits the lysosomal proton pump [43]. Enhanced permeability of the lysosomal membrane could lead to DNase being liberated into the cytoplasm and its passage into the nucleus, where it would cut DNA. It has been shown that DNase introduced into the cytoplasm by electroporation is a potent inducer of cytogenetic damage [44]. It is conceivable that Al induces genotoxicity by either or all three mechanisms. Further studies are necessary before any firm conclusion can be drawn.

To our knowledge, this is the first study showing that Al_2O_3 NM induced genotoxicity in rats. However, the exact mechanism is still unknown. Therefore, considering the wide spread use of Al and the increasing commercial applications of NM, these compounds should be regarded with concern.

Conflict of interest statement

The authors declare that there are no conflicts of interests.

Acknowledgements

We thank financial support from the Asian Office of Aerospace Research and Development Japan (# FA5209-05-P-0540-AOARD-05-21, supported by AFOSR-AOARD). We acknowledge Indian Council of Medical Research and Council of Scientific and Industrial Research, New Delhi for providing fellowships to Mr. A. Balasubramanyam and Mrs. N. Sailaja. The authors express their sincere thanks to Dr. J.S. Yadav, Director, Indian Institute of Chemical Technology, Hyderabad for providing facilities and his encouragement during the study.

References

[1] G. Oberdorster, E. Oberdorster, J. Oberdorster, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, Environ. Health Perspect. 113 (2005) 823–839.
[2] C. Medina, M.J. Santos-Martinez, A. Radomski, O.I. Corrigan, M.W. Radomski, Nanoparticles: pharmacological and toxicological significance, Brit. J. Pharmacol. 150 (2007) 552–558.

[3] S.M. Hussain, K.L. Hess, J.M. Gearhart, K.T. Geiss, J.J. Schlager, *In vitro* toxicity of nanoparticles in BRL 3A rat liver cells, Toxicol. In vitro 19 (2005) 975–983.
[4] J.S. Kim, T.J. Yoon, K.N. Yu, B.G. Kim, S.J. Park, H.W. Kim, K.H. Lee, S.B. Park, J.K. Lee, M.H. Cho, Toxicity and tissue distribution of magnetic nanoparticles in mice, Toxicol. Sci. 89 (2006) 338–347.
[5] Z. Chen, H. Meng, G. Xing, C. Chen, Y. Zhao, G. Jia, T. Wang, H. Yuan, C. Ye, F. Zhao, Z. Chai, C. Zhu, X. Fang, B. Ma, L. Wan, Acute toxicological effects of copper nanoparticles *in vivo*, Toxicol. Lett. 163 (2006) 109–120.
[6] B. Wang, W.Y. Feng, T.C. Wang, G. Jia, M. Wang, J.W. Shi, F. Zhang, Y.L. Zhao, Z.F. Chai, Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice, Toxicol. Lett. 161 (2006) 115–123.
[7] J.J. Wang, B.J.S. Sanderson, H. Wang, Cyto- and genotoxicity of ultrafine TiO_2 particles in cultured human lymphoblastoid cells, Mutat. Res. 628 (2007) 99–106.
[8] A.J. Wagner, C.A. Bleckmann, R.C. Murdock, A.M. Schrand, J.J. Schlager, S.M. Hussain, Cellular interaction of different forms of aluminum nanoparticles in rat alveolar macrophages, J. Phys. Chem. Biol. 111 (2007) 7353–7359.
[9] A. Nel, T. Xia, L. Madler, N. Li, Toxic potential of materials at the nanolevel, Science 311 (2006) 622–627.
[10] ATSDR—Agency for toxic substances and disease registry toxicological profile of aluminium, U.S. Department of health and human services, public health service. (June 1999).
[11] A. Miziolek, Nanoenergetics: an emerging technology area of national importance, AMPTIAC Q. 6 (2002) 43–48.
[12] Department of Defense Director, (2005). Defense Research and Engineering, (2005). Defense Nanotechnology Research and Development. (4 January 2006). http://www.nano.gov/html/res/DefenseNano2005.pdf.
[13] K.M. Tyner, S.R. Schiffman, E.P. Giannelis, Nanobiohybrids as delivery vehicles for camptothecin, J. Control. Rel. 95 (2004) 501–514.
[14] A. Banasik, A. Lankoff, A. Piskulak, K. Adamowska, H. Lisowska, A. Wojcik, Aluminium-induced micronuclei and apoptosis in human peripheral blood lymphocytes treated during different phases of the cell cycle, Environ. Toxicol. 20 (2005) 402–406.
[15] A. Lankoff, A. Banasik, A. Duma, E. Ochniak, H. Lisowska, T. Kuszewski, S. Góźdź, A. Wojcik, A comet assay study reveals that aluminium induces DNA damage and inhibits the repair of radiation-induced lesions in human peripheral blood lymphocytes, Toxicol. Lett. 161 (2006) 27–36.
[16] P.D.L. Lima, D.S. Leite, M.C. Vasconcellos, B.C. Cavalcanti, R.A. Santos, L.V. Costa-Lotufo, C. Pessoa, M.O. Moraes, R.R. Burbano, Genotoxic effects of aluminum chloride in cultured human lymphocytes treated in different phases of cell cycle, Food Chem. Toxicol. 45 (2007) 1154–1159.
[17] A.K. Roy, A. Sharma, G. Talukder, Effects of aluminum salts on bone marrow chromosomes in rats *in vivo*, Cytobios 66 (1991) 105–111.
[18] G. Krishna, M. Hayashi, *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation, Mutat. Res. 455 (2000) 155–166.
[19] W.I. Hagens, A.G. Oomen, W.H. de Jong, F.R. Cassee, A.J. Sips, What do we (need to) know about the kinetic properties of nanoparticles in the body? Regul. Toxicol. Pharmacol. 49 (2007) 217–229.
[20] OECD Guidelines 420 acute oral toxicity: fixed dosed procedure. (December 2001).
[21] W. Schmid, The micronucleus test, Mutat. Res. 31 (1975) 9–15.
[22] OECD Guidelines 474 genetic toxicology: micronucleus test. (July 1997).
[23] I.D. Adler, Cytogenetic tests in mammals, in: S. Venitt, J. Parry (Eds.), Mutagenicity Testing: A Practical Approach, IRL Press, Oxford, 1984, pp. 273–306.
[24] OECD Guidelines 475 genetic toxicology: *in vivo* mammalian bone marrow cytogenetic test-chromosome analysis. (July 1997).
[25] M. Gómez, D.J. Sánchez, J.M. Llobet, J. Corbella, J.L. Domingo, The effect of age on aluminum retention in rats, Toxicology 116 (1997) 1–8.
[26] K. Thomas, P. Sayre, Research strategies for safety evaluation of nanomaterials. Part I: evaluating the human health implications of exposure to nanoscale materials, Toxicol. Sci. 87 (2005) 316–321.
[27] R.C. Murdock, L. Braydich-Stolle, A.M. Schrand, J.J. Schlager, S.M. Hussain, Characterization of nanomaterial dispersion in solution prior to *in vitro* exposure using dynamic light scattering technique, Toxicol. Sci. 101 (2008) 239–253.
[28] Q. Rahman, M. Lohani, E. Dopp, H. Pemsel, L. Jonas, D.G. Weiss, D. Schiffmann, Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts, Environ. Health Perspect. 110 (2002) 797–800.
[29] D.M. Brown, K. Donaldson, P.J. Borm, R.P. Schins, M. Dehnhardt, P. Gilmour, L.A. Jimenez, V. Stone, Calcium and ROS-mediated activation of transcription factors and TNF-alpha cytokine gene expression in macrophages exposed to ultrafine particles, Am. J. Physiol. Lung Cell Mol. Physiol. 286 (2004) L344–L353.
[30] A. Simon, B. Gouget, M. Mayne, N. Herlin, C. Reynaud, J. Degrouard, M. Carriere, *In vitro* investigation of TiO_2, Al_2O_3, Au nanoparticles and multi-walled carbon nanotubes cyto- and genotoxicity on lung, kidney cells and hepatocytes, Toxicol. Lett. 172 (Suppl. 1) (2007) 36.
[31] R. Dunford, A. Salinaro, L. Cai, N. Serpone, S. Horikoshi, H. Hidaka, J. Knowland, Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients, FEBS Lett. 418 (1997) 87–90.
[32] N. Sadeghiani, L.S. Barbosa, L.P. Silva, R.B. Azevedo, P.C. Morais, Z.G.M. Lacavaa, Genotoxicity and inflammatory investigation in mice treated with magnetite nanoparticles surface coated with polyaspartic acid, J. Magn. Magn. Mater. 289 (2005) 466–468.
[33] B. Rehn, F. Seiler, S. Rehn, J. Bruch, M. Maier, Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: untreated and surface treated, Toxicol. Appl. Pharmacol. 189 (2003) 84–95.
[34] J. Wang, G. Zhou, C. Chen, H. Yu, T. Wang, Y. Ma, G. Jia, Y. Gao, B. Li, J. Sun, Y. Li, F. Jiao, Y. Zhao, Z. Chai, Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration, Toxicol. Lett. 168 (2007) 176–185.
[35] Z. Chen, H. Meng, H. Yuan, G. Xing, C. Chen, F. Zhao, Y. Wang, C. Zhang, Y. Zhao, Identification of target organs of copper nanoparticles with ICP–MS technique, J. Radioanal. Nucl. Chem. 272 (2007) 599–603.
[36] K.W. Powers, S.C. Brown, V.B. Krishna, S.C. Wasdo, B.M. Moudgil, S.M. Roberts, Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation, Toxicol. Sci. 90 (2006) 296–303.
[37] J.S. Hanas, C.G. Gunn, Inhibition of transcription factor IIIA–DNA interactions by xenobiotic metal ions, Nucleic Acids Res. 24 (1996) 924–930.
[38] K.S. Bharathi, J. Rao, R. Stein, First evidence on induced topological changes in supercoiled DNA by an aluminium D-aspartate complex, J. Biol. Inorg. Chem. 8 (2003) 823–830.
[39] A. Hartwig, Carcinogenicity of metal compounds: possible role of DNA repair inhibition, Toxicol. Lett. 102–103 (1998) 235–239.
[40] R. Anane, E.E. Creppy, Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase + catalase and vitamins E and C, Hum. Exp. Toxicol. 20 (2001) 477–481.
[41] R. Moumen, N. Ait-Oukhatar, C. Fleury, D. Bougle, P. Arhan, D. Neuville, F. Viader, Aluminium increases xanthine oxidase activity and disturbs antioxidant status in the rat, J. Trace Elements Med. Biol. 15 (2001) 89–93.
[42] G.B. van der Voet, A.E. Brandsma, E. Heijink, F.A. de Wolff, Accumulation of aluminium in rat liver: association with constituents of the cytosol, Pharmacol. Toxicol. 70 (1992) 173–176.
[43] P. Zatta, A. Taylor, P. Zambenedetti, R. Milacic, P. dell'Antone, Aluminum inhibits the lysosomal proton pump from rat liver, Life Sci. 66 (2000) 2261–2266.
[44] M. Sayed Aly, A. Wojcik, C. Schunck, G. Obe, Correlation of chromosomal aberrations and sister chromatid exchanges in individual CHO cells pre-labelled with BrdU and treated with DNaseI or X-rays, Int. J. Radiat. Biol. 78 (2002) 1037–1044.