

Genotoxicity of metal oxide nanomaterials: Review of Recent data and discussion of possible mechanisms

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Abstract

Nanotechnology has rapidly entered into human society, revolutionized many areas, including technology, medicine and cosmetics. This progress is due to the many valuable and unique properties that nanomaterials possess. In turn, these properties might become an issue of concern when considering potentially uncontrolled release to the environment. The rapid development of new nanomaterials thus raises questions about their impact on the environment and human health. This review focuses on the potential of nanomaterials to cause genotoxicity and summarizes recent genotoxicity studies on metal oxide nanomaterials. Though the number of genotoxicity studies on metal oxide/silica nanomaterials is still limited, this endpoint has recently received more attention for nanomaterials and the number of related publications has increased. Analysis of these peer reviewed publications over nearly two decades shows that the test most employed to evaluate the genotoxicity of these nanomaterials is comet assay, followed by Micronucleous, Ames and Chromosome aberration tests.

Analysis of the literature shows an increasing number of genotoxicity studies for nanomaterials every year. Based on the data studied we concluded that experimental data for genotoxicity for nanomaterials with the same core chemical composition, may vary to some extent due to: 1) variation in size of the nanoparticles; 2) variations in size distribution; 3) varying purity of nanomaterials ; 4) variation in surface areas for nanomaterials with the same average size; 5) differences in coatings; 6)

differences in crystal structures of the same types of nanomaterials; 7) differences in sizes of aggregates in solution/media; 8) differences in assays; 9) different concentrations of nanomaterials in assay tests; 10) variation in concentration of analytes in assays. As a result, due to the considerable inconsistencies in the recent literature and the lack of standardized test methods - reliable genotoxicity assessment of NPs is still challenging.

Keywords: comet assay, micronucleus test, Ames test, nanoparticles, nanomaterials, metal oxides, silica

1. Introduction

Nanotechnology is currently utilized in many areas of industry, medicine, and military applications [1, 2]. Nanomaterials (NMs) form the basis of nanotechnology and may be described as materials “with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale”, where the “nanoscale” may be considered to be 1-100 nm [3]. However, it should be noted that variations of this definition exist [3]. For example, the recently proposed definition from the European Commission takes account of the fact that NMs will typically be composed of particles with a distribution across different sizes [4] and particles with larger sizes, up to 1000 nm (to include aggregates and agglomerates), may also be considered to be NMs [5]. Nanoparticles (NPs), as a sub-category of NMs, may be defined as particles with all three external dimensions in the range 1-100 nm although, again, variations on this definition exist [3]. The special physicochemical properties of NMs due to their small size and structure confer novel capabilities to these materials, suitable for a wide range of applications [6-12]. The development of NMs is also driven by hope that these chemicals will offer improved performances and new functionalities leading, e.g., to smart drugs and to their aiding in achieving sustainable development, e.g., by reducing the consumption of energy and materials and reducing environmental contamination [13]. At the same time, despite the huge benefits of nanotechnology, there is current concern regarding NMs’ potential hazardous effects on biological systems [14-20].

The same properties that make these particles exciting for technological research and development may also make them problematic from a toxicological perspective: NMs are relatively unexplored with regard to long term, low dose, exposure [1, 15, 21-25]. However, it should be noted that the question of whether or not NMs exhibit novel mechanisms of toxic action is currently a subject of considerable debate, as noted in the recent paper of Donaldson and Poland [26] and elsewhere within the literature [1, 27-30]. Even NMs that have the same chemical composition differ in their toxicological properties; the differences in toxicity depend upon NMs’ size, shape, and surface charge, type of coating material and reactivity [16, 31]. The potential toxicity and mechanisms of toxic action of NMs are still topics of particular interest due to the lack of sufficient toxicity data and mechanistic understanding. Indeed, uncertainties around the safe use of NMs are considered a major obstacle to innovations and investment in nanotechnology [32].

As well as hazard considerations, exposure is also a critical factor which affects the risk, to the environment and human health, associated with the use of NMs. As for conventional chemicals, a thorough risk assessment would require “effects assessment” (i.e. determination of the toxicity associated with a given dose, exposure duration and exposure route) in a toxicology study followed by consideration of realistic exposure estimates for the environment and human populations [33]. In addition to challenges associated with environmental/human exposure estimation [34], “effects assessment” is complicated for NMs, as compared to conventional small molecule chemicals, by problems with the toxicity data as well as the challenge of determining appropriate dose metrics [35] [26].

Since the available data on NMs’ toxicity (the focus of this review) and environmental/human exposure [34] are unfortunately limited, they do not allow for significant quantitative risk assessment of the safety of synthesized NMs to be made. Moreover, the problem of the lack of data becomes even more complicated by the questionable suitability of tests used for NMs’ toxicity evaluation, including the common genotoxicity tests which are the focus of this review. For example, some inconsistencies in data from different tests are found in the supporting literature and the validity of some OECD genotoxicity Test Guidelines for NMs has been called into question [36, 37]. Indeed, the OECD Working Party on Manufactured Nanomaterials is, at the time of writing, currently reviewing possible modifications or additions to existing OECD Test Guidelines and/or OECD Guidance Documents for a number of different (eco)toxicological and physicochemical endpoints which may be required for NMs [38-40] [40].

It should be noted, when considering the toxicity of NMs, that a variety of different kinds of NMs exist. NMs may initially be differentiated based upon their chemical composition. For example, Stone *et al.* [41] suggested NMs might be categorized as carbon based (e.g. carbon black, carbon nanotubes and fullerenes), mineral based (e.g. metals, metal oxides), organic (e.g. polymers, dendrimers and surfactant coatings), composites/hybrids (e.g. multicomponent NMs, such as quantum dots, or doped metal/metal oxides) with nanoclays suggested to be difficult to assign.

In this article, we concentrate on metal oxide and silica NMs for which experimental investigations were reported in the literature and summarize the *in vivo* and *in vitro* studies of genotoxic effects that these NMs exhibit. Metal oxide NMs are an important group of engineered NMs, as they are used in various areas of human life such as cosmetics, sunscreens, self-cleaning coatings, textiles and paints. Other applications include their use as water-treatment agents, as materials for solar batteries and, more recently, automobile catalytic converters [42]. Silica (silicon dioxide) based NMs are also of significant commercial relevance, as recognized by the Organisation for Economic Co-operation and Development (OECD) [43], and concerns regarding their use in cosmetics were recently raised by the European Commission, which requested a safety assessment of “nano silica” from the Scientific Committee on Consumer Safety (SCCS) in October 2013 [44]. Whilst there is precedence in the nanotoxicology literature for considering silica to be a metal oxide [10, 45], silicon is technically a metalloid [46]. It is included in our review due to its various industrial applications.

The importance of metal oxide/silica NMs is demonstrated by their large use in consumer products. Indeed, according to The Project on Emerging Nanotechnologies online database [47], at the time of writing 1809 different products containing NMs (including metal oxides) are currently marketed. Moreover, it is expected that the nano-market will grow exponentially and will reach an annual turnover of \$2.6 trillion in 2014. As far as metal oxide NMs are concerned, their widespread use is highlighted by the fact that five classes of this specific category of NMs are represented in the repository. To be more precise, 180 out of 1809 (i.e. 10% of the total number) unique consumer products found in the aforementioned online database are metal oxide/silica NMs, including titanium dioxide (91 products), silicon dioxide (41 products), zinc oxide (38 products), aluminium oxide (8 products) and cerium oxide (2 products).

NMs represent high tonnage materials. For instance, Hendren and colleagues [48] estimated upper and lower bounds for annual U.S. production volumes of five classes of NMs, including cerium oxide and titanium dioxide. The results of this investigation showed that titanium dioxide NMs was estimated to reach the greatest annual production among the considered NMs, ranging between 7,800 and 38,000 tons/year. Furthermore, a study of the Dutch National Institute for Public Health and the Environment (RIVM) estimated the amount of NMs used in consumer products on the market at the time of their analysis (2009) as well as the amount of NMs which were expected to be used in consumer products in the near future [49]. In order to assess the most relevant “exposure characteristics”, i.e. factors of most relevance to estimated exposure, within the considered categories of NMs, a working group of seven RIVM experts on NM consumer exposure was consulted. The individual estimations from the seven experts were combined with the ranking of NMs in consumer products, based on the amount used within all considered products, as well as data from product inventories to identify high priority NMs for future exposure studies. As a result, product categories with a high priority for future exposure studies were as follows: sun screens (which often contain zinc oxide and titanium dioxide NMs), coatings and adhesives. In addition, cerium oxide (motor vehicles consumer category) was labelled as high priority as well as titanium dioxide and alumina contained in cleaning products.

Many industrial chemicals are capable of causing genetic damage to living organisms [50]. The potential for NMs to exhibit genotoxicity has been discussed in several reviews [17, 51-55]. Among them, metal oxide/silica NMs were found to cause genotoxicity in some, but by no means all studies [17, 19]. Various kinds of features can influence the mechanism(s) of metal oxide/silica NMs’ genotoxicity - for example, their size, surface charge (and other surface properties), composition, shape, solubility, aggregation and agglomeration [41, 56]. All these properties can affect both primary and secondary genotoxicity [57]. (Primary and secondary genotoxicity mechanisms are discussed in section 5 of the current review.) A key genotoxicity mechanism that is often described is ability of the particles to cause oxidative stress, a term that can be described as an imbalance in the oxidative and antioxidative status of a cell in favor of the former [11]. However, there is a need for a more detailed understanding of NM toxicity mechanisms, including genotoxicity, and an appreciation of how the physico-chemical properties of NMs are responsible for interactions with cells. Therefore, there is an urgent need for as many toxicity data as possible to ultimately allow for the risk assessment of metal oxide NPs to be undertaken.

Despite the need, obtaining reliable genotoxicity data for NMs, including metal oxide/silica NMs, is a challenging task as there are many various complications associated with their testing. A number of short term test systems, which were originally designed for conventional chemical compounds and have subsequently been applied to NMs, are available for the assessment of genetic hazard [2, 13, 17, 19, 51-54, 58-61]. These systems are often characterized by the endpoints that they measure: gene mutation, chromosome damage, or deoxyribonucleic acid (DNA) damage [13, 17, 52, 54, 58, 60]. At the same time, none of these tests are ideal for the estimation of NMs' genotoxicity: some show low reproducibility, some need specifically adjusted protocols for NMs and discussions on this are ongoing as indicated above [2, 13, 58].

In this paper, we have gathered and discussed the latest experimental data on metal oxide/silica NMs' genotoxicity. In updating this fast-changing research area, we concentrate in particular on the discussion of genotoxicity study calls among these metal oxide/silica NMs, methods of investigation and possible mechanisms of genotoxicity. The genotoxicity profiles considered in this paper are based on common test systems used for genotoxicity studies: the comet assay [62], micronucleous test (MN) [63], Ames test [64], and chromosome aberration test [65]. When considering the data from these assays, the potential limitations of these test systems for NMs must be remembered [37].

2. Metal oxide structures and key physical properties of their NM counterparts.

A metal oxide is a chemical compound that contains at least one metal atom and one or more oxygen atoms. The metal oxides can adopt a vast number of structural geometries with an electronic structure that can exhibit metallic, semiconductor or insulator characteristics [66, 67]. Oxides of most metals adopt polymeric structures with M-O-M crosslinks. Moreover, because these crosslinks are characterized by strong interactions, the solids tend to be insoluble in solvents, though they are attacked by acids and bases. In metal oxides, the coordination number of the oxide ligand is two for most electronegative elements and 3–6 for most metals [66]. (WHAT ABOUT METALS FROM THE FIRST AND SECOND GROUP?) A selection of representative structures of metal oxides are shown in Figure 1. Some metal oxides are composed of oxygen atoms bound to transition metals (for example, titanium oxide, Figure 1a). These metal oxides are commonly utilized for their catalytic activity and semi-conductive properties [68, 69]. Transition metal oxides are also frequently used as pigments in paints and plastics, most notably - titanium dioxide [7, 70, 71]. Transition metal oxides have a wide variety of surface structures which affect the surface energy of these compounds and influence their chemical properties. Interestingly, there is very little known about the surface structures of metal oxides (transition metal oxides), however their bulk crystal structures are well researched.

Metal oxide NPs mostly have similar crystal structures to bulk-sized metal oxides but with particles sizes between 1 and 100 nm [72]. A metal oxide of NM size can have a very large surface size, which affects its reactivity and other physico-chemical properties. In order to display mechanical or structural stability, a NM must have a low surface free energy. Due to this feature, even phases that have a low stability in bulk materials can become very stable in nanostructure materials. For example, this structural

phenomenon has been detected in TiO_2 , VOx , Al_2O_3 or MoOx oxides [67, 73-76]. Size-induced structural distortions associated with changes in cell parameters have been observed, for example, in NMs of Al_2O_3 , NiO , Fe_2O_3 , ZrO_2 , MoO_3 , CeO_2 , and Y_2O_3 [76]. The second important effect of size is related to the electronic properties of the oxide. In any material, the nanostructure produces so-called quantum size or confinement effects which essentially arise from the presence of discrete, atom-like electronic states [67, 76]. Thus, in their bulk state, many oxides have wide band gaps and low reactivity [77]. A decrease in the average size of an oxide particle does, in fact, change the size of the band gap, with a strong influence on conductivity and chemical reactivity [78, 79]. This can dramatically affect the behavior of metal oxide NMs and their interactions, including interactions with biomolecules of cell systems [80].

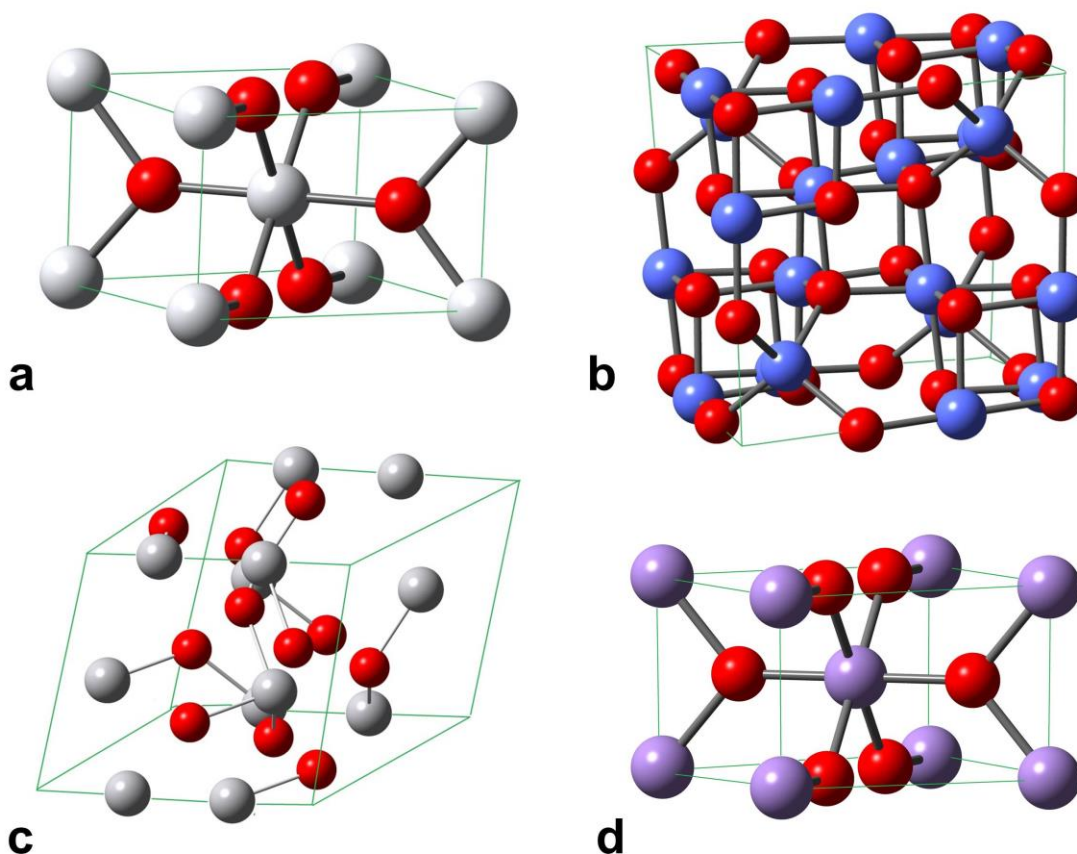


Figure 1. The crystal structures of selected metal oxides: (a) TiO_2 , (b) Cr_2O_3 , (c) V_2O_5 , (d) MnO_2 .

3. Methods used for *in vitro* genotoxicity studies

This review considers experimental genotoxicity data for NMs. Most of the data obtained were from three methods used to test primary genotoxicity (as opposed to secondary genotoxicity, which is discussed in section 5): the comet assay, micronucleus and Ames tests. The chromosome aberration test

is also discussed briefly, but limited data were obtained from this test and so it was not considered in detail.

The data obtained for the comet assay, micronucleus and Ames tests are summarized in tables 1, 2 and 3 respectively. In these tables, each row contains data for all nano metal oxides (or silica), reported in a given publication, with a single core chemical composition. If a paper comprised results for NMs with more than one core chemical composition, these data were spread over different rows. However, one single row of the table may summarize data for multiple NMs, reported in the same publication, corresponding to the same core composition but with differences in other characteristics such as size, surface functional groups etc. Hence, the column “Result” indicates whether any of the NMs, corresponding to a given core composition and reported in the corresponding publication, produced a single positive result in the relevant assay (i.e. Comet, Micronucleus or Ames depending upon the table). In other words, if a row contains data for multiple NMs with, say, different sizes or surface coatings, but the same core chemical composition, the “Result” column will report a positive overall result (i.e. “+”) if at least one positive result was reported for one of the tested NMs described in the current row. On the other hand, we reported a negative overall result (i.e. “-“) whenever all the data points included in a single row are negative. We undertook this approach considering that in the majority of the publications analysed in this review, the metal oxide (or silica) nanoparticles of the same core chemical composition did not show different genotoxicity study calls (i.e. positive or negative) in the same test. For instance, Balasubramanyam et al tested two aluminium oxide NMs with nominal diameters of 30 nm and 40 nm. The results from the comet assay showed a statistically significant increase in percentage tail DNA in comparison to the control group, i.e. a positive result, at 1000 and 2000 mg/kg dose levels after 4, 24 and 48 hours with both aluminium oxide NMs studied [81].

In a few cases, the data were not clear, so the “Result” column reports “+/-“. For instance, Downs et al. [82] tested two amorphous silica NMs with different sizes in an *in vitro* micronucleus assay for 24 hours of exposure, at four concentration levels: 31.6, 100, 316, 1000 µg/mL. In this publication, no increase was observed in the percentage of *micronuclei* at the lowest concentrations, for both of the NMs described above. This outcome was also observed for the larger silica NM when tested at the highest concentration. However, for the highest concentration tested (i.e. 1000 µg/mL), the author was unable to score the percentage of *micronuclei* for the smallest silica nanomaterial reported in the paper, since the test material excessively precipitated on the slides. In such a case, we labelled the results for the corresponding set of nanomaterials as equivocal i.e. the “Result” column reports “+/-“.

Here it should be noted that, in addition to *in vitro* tests, *in vivo* versions of the comet assay and micronucleus tests also exist [37]. *In vivo* data are also available [51, 59, 60, 63, 81, 83-89], but they are quite limited and where available, they were reported in table 1. Below, we give some general information on the genotoxicity tests which are the focus of this review as well as a brief overview of the chromosome aberration test. This test is not the focus of the review and therefore not further discussed.

3.1. The Comet Assay

The comet assay (also known as the single-cell gel electrophoresis assay) is a method for measuring deoxyribonucleic acid (DNA) strand breaks in eukaryotic cells [90, 91]. Cells are embedded in agarose on a microscope slide and lysed with detergent, containing a high concentration of salt, to form nucleoids which contain supercoiled loops of DNA linked to the nuclear matrix. Electrophoresis at high pH gives rise to structures resembling comets, as observed by fluorescence microscopy; the number of DNA breaks is reflected in the intensity of the comet tail relative to the head. The probable basis for this phenomenon is that DNA loops lose their supercoiling upon breaking and are able to extend toward the anode [58]. One way of quantifying DNA damage using this assay is via the so-called “Olive Tail Moment (OTM)” which is equal to the percentage of DNA in the comet tail multiplied by the length of the tail [92]. The assay has found a number of applications: assessing novel chemicals for genotoxicity, monitoring genotoxin contamination of the environment, human biomonitoring and molecular epidemiology, and fundamental research in DNA damage and repair [90, 91]. The specificity and sensitivity of the assay are considerably increased if the nucleoids are incubated with bacterial repair endonucleases which recognize specific kinds of DNA damage and convert DNA lesions to breaks, increasing the DNA content of the comet tail. As well as detecting DNA strand breaks caused by a chemical of interest (for example, a NM), the repair activity in a cell extract can be determined via either incubating cells after treatment with damaging agent and measuring the damage remaining at intervals or by incubating the cell extract with nucleoids containing specific damage [58]. Tice and colleagues reported that modifications to the traditional comet assay can be suitable to investigate specific categories of DNA damage. In greater detail, oxidized pyrimidines can be detected using the endonuclease III enzyme, whereas 8-OH guanine as well as other damaged purines can be detected by using the formamidopyrimidine DNA glycosylase (Fpg) enzyme [91].

3.2. The Micronucleus Test

Micronucleus (MN) assays are one of the preferred methods for assessing chromosome damage for conventional chemicals, as they enable both chromosome breakage and chromosome loss to be measured reliably [93]. Since micronuclei can only be expressed in cells which undergo complete nuclear division, a version of the micronucleus test was developed that identifies cells which have undergone nuclear division by their binucleate appearance when blocked from performing cytokinesis (cell division) by cytochalasin-B, a microfilament-assembly inhibitor [93, 94]. The cytokinesis-block micronucleus (CBMN) assay enables better precision because it prevents the data obtained from being confounded by altered cell division kinetics due to, possible, cytotoxicity of tested agents or suboptimal cell culture conditions [95].

As discussed above, the standard MN assay (OECD Test Guideline No. 487) [96] often (but not always) employs cytochalasin B (CB) to detect micronucleus frequency in binucleate cells formed after mitosis. However, it is known that CB also inhibits endocytosis, and thus might prevent NM cellular uptake. Hence, a modified protocol needs to be used for testing NMs: incubating with NM before adding CB.

This example illustrates that, with certain precautions, standard tests for DNA and chromosome damage may be applied to NM [37, 62].

3.3. The Ames Test

This is a test for identifying mutagens by studying the frequency with which they cause mutations inducing production of an essential amino acid in bacterial colonies initially lacking the ability to synthesize this amino acid [64, 97]. Those bacterial colonies for which mutations occur, giving rise to the ability to produce the essential amino acid, are termed “revertant colonies” [97]. Typically, as recommended in OECD Test Guideline No. 471 [97], one or more strains of *Salmonella* (*S. typhimurium*) and/or *Escherichia coli* are used e.g. the *S. typhimurium* strains TA97a, TA98, TA100, TA102, TA1535 and TA1537 or the *E. coli* strain WP2u-vrA⁻referred to in Table 3. It can also be used with or without metabolic activation i.e. typically with or without “S9- mix” [97]. OECD Test Guideline No. 471 [97] recommends that a “positive” result for a single strain, with or without metabolic activation, should be identified based on identifying a concentration related increase in the number of revertant colonies and/or a reproducible increase at a single concentration. A “positive” Ames test result would then be assigned if a “positive” result was observed with any strain with or without metabolic activation. It is widely used for the assessment of organic molecules, such as prospective pharmaceutical active ingredients [98], and there are considerable Ames test data for these chemicals in the public domain [99, 100]. However, it has been suggested that the Ames test is one of the least appropriate genotoxicity tests for NMs due to poor uptake of NMs by bacterial cells [37, 101].

3.4. Chromosome aberration

Chromosome aberrations result from failures in repair processes such that breaks either do not rejoin or rejoin in abnormal configurations [102]. The purpose of the *in vitro* chromosomal aberration test is to identify agents that cause structural chromosome aberrations in cultured mammalian cells.

However, it is considered sub-optimal, compared to the micronucleus test, as it is substantially slower to perform and cannot detect the same kinds of chromosomal abnormalities i.e. it cannot detect aneugens as well as clastogens [37].

Table 1. Current review of comet assay results on metal oxide/silica NMs (+positive; -negative; +/-equivocal). As explained in section 3, each row in this table summarizes all genotoxicity data found for this test for all nanomaterials with a given core chemical composition reported in a given publication.

Nanomaterial core chemical composition	Physical characteristics (size and etc)	Cells	Exposure	Results	Summary	Reference
Al ₂ O ₃	Two aluminium oxide NMs were studied. Nominal diameters: 30 nm and 40 nm. TEM analysis: 39.85 ± 31.33 nm and 47.33 ± 36.13nm. DLS analysis: average diameters 212.0 and 226.1 nm in water.	<i>In vivo</i> female Wistar rat peripheral blood cells	Three dose levels (via gavage): 500, 1000 and 2000 mg/kg body weight. Whole blood was collected at 4, 24, 48 and 72 h	Results showed statistically significant increase in % Tail DNA at 1000 and 2000 mg/kg dose levels after 4, 24 and 48 h with both aluminium oxide NMs studied in comparison to the control group.	+	[81]
Al ₂ O ₃	Nominal size: 16.7 nm. DLS analysis: hydrodynamic diameter 16.7 ± 1.3 nm.	<i>In vitro</i> human embryonic kidney (HEK293) and peripheral blood lymphocytes cells	Three concentration levels: 1, 10, and 100 µg/ml. Exposure duration: 3 h	Results showed that all concentration levels of Al ₂ O ₃ did not induce any marked genotoxicity	-	[103]
Al ₂ O ₃	Nominal size < 50 nm.	<i>In vitro</i> mouse lymphoma (L5178Y) cells and human bronchial epithelial (BEAS-	Three concentration levels for L5178Y cells: 1250, 2500, 5000 µg/mL. Three concentration levels for	Al ₂ O ₃ NM induced DNA damage in L5178Y at 1250 to 5000 µg/ml with S9 mix (+S9) and induced DNA damage at 2500	+	[104]

		2B) cells	BEAS-2B cells: 68.36, 136.72, 273.44 µg/mL (+S-9); 97.66, 195.32, 390.63 µg/ml (-S-9). Exposure duration: 2 h	µg/mL without S9 mix (-S9). A significant increase in DNA tail was observed in BEAS-2B cells at all concentrations tested under both +S9 and -S9 conditions.		
Bi ₂ O ₃	Nominal size between 90 and 210 nm.	<i>In vitro Allium cepa</i> root cells	Five concentration levels: 12.5, 25, 50, 75, and 100 ppm. Exposure duration: 4 h	Results showed a dose-dependent increase in the DNA damage at all concentrations except 12.5 ppm compared to negative control.	+	[105]
CeO ₂	Nominal size < 25 nm. TEM analysis: 25 ± 1.512 nm. DLS analysis: hydrodynamic diameter 269.7 ± 27.398 nm	<i>In vitro</i> human neuroblastoma (IMR32) cells	Five concentration levels: 10, 20, 50, 100, and 200 mg/ml. Exposure duration: 24 h	A significant increase in the percentage of tail DNA was observed only at the highest dose of 200 mg/ml	+	[106]
CeO ₂	Nominal size: 7 nm. DLS analysis: hydrodynamic diameter 15 nm	<i>In vitro</i> human dermal fibroblasts	Seven concentration levels: 0.006, 0.06, 0.6, 6, 60, 600, 1200 mg/l. Exposure duration: 2 h	A dose-response increase in the olive tail moment at very low doses (0.006 mg/l) was observed	+	[107]
CeO ₂	TEM analysis: average size 5.5 nm. XRD analysis: 6.3 nm	<i>In vitro</i> human lens epithelial cells	Two concentration levels: 5 and 10 µg/ml. Exposure duration: 24 h	Results showed that nano-CeO ₂ , at either tested dose, did not cause any damage to the DNA in cultured eye lens	-	[108]

				epithelial cells.		
CeO ₂	Nominal size < 25 nm. TEM analysis: longest dimension between 4 and 25 nm. DLS analysis: 225 nm.	<i>In vitro</i> human alveolar Type II-like epithelial (A549) and bronchial epithelium (BEAS-2B) cells	Two concentration levels: 40 and 80 µg/ml. Exposure duration: 4 h	In A549 cells, CeO ₂ significantly increased the amounts of DNA breaks compared to control group at both tested concentrations in a dose-dependent manner. In BEAS-2B cells, CeO ₂ caused significantly increased levels of DNA breaks only at 80 µg/ml.	+	[109]
CeO ₂	SEM analysis: average diameter between 16 and 22 nm.	<i>In vitro</i> human alveolar adenocarcinoma (A549), colorectal adenocarcinoma (CaCo2) and hepatic carcinoma (HepG ₂) cells	Three concentration levels: 0.5, 50, 500 µg/ml. Exposure duration: 24 h		+	[110]
CeO ₂	Two different types of CeO ₂ were studied. Nominal size: 30 and 15 nm	<i>In vivo Daphnia magna</i> and <i>Chironomus riparius</i>	Single dose level: 1 mg/l. Exposure duration: 24 h	Tail and olive tail moments increased in both tested species	+	[111]
CeO ₂	Nominal size: 3 nm. DLS analysis: hydrodynamic diameter 350 nm	<i>In vitro</i> female mice oocytes and follicular cells	Four concentration levels: 2, 5, 10 and 100 mg/l. Exposure duration: 2 h	A significant and dose-dependent increase in DNA damage was shown in follicular cells exposed to CeO ₂ NMs at all concentration levels. In oocytes surrounded by	+	[92]

				zona pellucida, DNA damage was observed only at 10 and 100 mg/l		
Co ₃ O ₄	TEM analysis: 21 nm. DLS analysis: average hydrodynamic size 264.8 nm (water)	<i>In vitro</i> human hepatocarcinoma (HepG2) cells	Three concentration levels: 5, 10 and 15 µg/ml. Cells were treated for 24 and 48 h	Cells exhibited a significant increase in DNA damage at almost all concentration levels, after 24 and 48 h, except for 5 µg/ml after 24 h.	+	[112]
Co ₃ O ₄	Nominal size < 50 nm. TEM analysis: longest dimension between 9 and 62 nm. DLS analysis: 222 nm.	<i>In vitro</i> human alveolar Type II-like epithelial (A549) and bronchial epithelium (BEAS-2B) cells	Two concentration levels: 40 and 80 µg/ml. Exposure duration: 4 h	In A549 cells, Co ₃ O ₄ significantly increased the amounts of DNA breaks compared to control group only at 40 µg/ml. In BEAS-2B cells, Co ₃ O ₄ caused significantly increased levels of DNA breaks only at 80 µg/ml.	+	[109]
CuO	Nominal size: 28 nm. SEM analysis: primary particle size 50 nm	<i>In vitro</i> human lung type II epithelial (A549) cells	One concentration level: 80 µg/ml. Exposure duration: 4 h	CuO NM showed significant levels of DNA damage at test conditions	+	[113]
CuO	Nominal size: 42 nm. TEM analysis: average size between 20 and 40 nm. DLS analysis: 220 nm	<i>In vitro</i> human lung type II epithelial (A549) cells	Three concentration levels: 2, 40 and 80 µg/ml. Exposure duration: 4 h	A significant increase in DNA damage was observed at concentrations of 40 and 80 µg/ml	+	[114]
CuO	Nominal size: 42 nm. TEM	<i>In vitro</i> human lung type II	Two concentration levels: 40 and 80 µg/ml.	A significant damage was found at 80 µg/ml.	+	[31]

	analysis: average size between 20 and 40 nm. DLS analysis: 200 nm	epithelial (A549) cells	Exposure duration: 4 h	However, an increased (non-significant) DNA damage was also observed at 40 µg/ml		
CuO	Four different CuO nanoparticles were studied, with the following size measurements and shapes determined by TEM: (1) 10-100 nm (unspecified shape); (2) 7±1 nm (spheres); (3) 7±1×40±10 nm (rods); (4) 1200±250×270± 50×30±10 nm (spindles)	<i>In vitro</i> human murine macrophages RAW 264.7 cells and peripheral blood lymphocytes (PBL).	Three concentration levels: 0.1, 1 and 10 µg/ml. Exposure duration: 2 and 24 h	For RAW264.7 cells, all four tested nanoparticles caused statistically significant increase in primary DNA damage after 2 and 24h treatments at all concentrations. For PBL cells, statistically significant primary DNA damage was also detected for all tested CuO samples except for the following results: spheres (0.1 µg/mL , 24h; 1 µg/mL, 2h) and spindles (0.1 µg/mL , 24h; 0.1 µg/mL, 2h).	+	[115]
CuO	Nominal size: 10 nm. TEM analysis: size between 20 and 40 nm. DLS analysis: hydrodynamic diameter 276.4 nm (water)	<i>In vitro</i> human lung epithelial (A549) cells	Two concentration levels: 5 and 15 mg/l. Exposure duration: 2, 4, 8, 16 and 24 h	A time-dependent increase in DNA damage was observed for the 15 mg/l concentration level at 8, 16 and 24 h of exposure. For the 5 mg/l concentration level, a significant increase in DNA damage was shown only at 24 h of exposure	+	[116]

CuO	SEM analysis: diameter ranging from 20 to 200 nm. DLS analysis: average size 500 ± 20 nm after sonication	<i>In vitro</i> rainbow trout (<i>O. mykiss</i>) red blood cells; <i>in vivo</i> rainbow trout (<i>O. mykiss</i>) erythrocytes	<i>In vitro</i> : One concentration level = 7.5 µg/ml; Exposure duration: 1 h. <i>In vivo</i> (intraperitoneal injection): One dose level of CuO NM expressed in terms of the equivalent mass of Cu = 1 µg/g body weight. Exposure duration: 38 h	The percentage of tail DNA significantly increased in the presence of Cu compared to the control only in the <i>in vitro</i> study	+	[117]
CuO	Nominal size < 50 nm. TEM analysis: 31 ± 10 nm. DLS analysis: 284.0 ± 21.2 nm	<i>In vivo</i> <i>Mytilus galloprovincialis</i> hemolymph cells	One dose level: 10 µg/l. Exposure duration: 3, 7 and 15 days	An increase both in the olive tail moment and in the percentage of tail DNA was observed following 7 days of exposure.	+	[118]
CuO	Nominal size < 100 nm. Hydrodynamic size of 204 nm	<i>In vivo</i> <i>Macoma balthica</i> soft tissue cells	One concentration level of CuO expressed in terms of the equivalent mass of Cu = 10 mg/l. Exposure duration: 35 days	No significant genotoxic effects were observed	-	[119]
CuO	Nominal size: 10–100 nm. TEM analysis: 29.5 nm. Hydrodynamic size: 197 nm (deionized water) and 810	<i>In vivo</i> worms <i>H. diversicolor</i> coelomocytes cells and clams <i>S. plana</i> hemocytes cells	One dose level: 10 µg/l. Exposure duration: 21 days	In both species, percentages of tail DNA were significantly increased	+	[120]

	nm (seawater)					
CuO	Nominal size: < 50 nm. DLS analysis: 1511 ± 468 nm (water) 3475 ± 357 nm (medium)	<i>In vitro S. cerevisiae</i> cells	One concentration level: 31.25 mg/l. No information about exposure duration	Cells exposed to CuO NMs showed a significant amount of DNA damage compared to control.	+	[121]
CuO	Nominal average particle size: 50 nm. TEM analysis: 55.80 ± 8.70 nm. DLS analysis: 68.5 ± 5 nm	<i>In vitro</i> human skin epidermal (HaCaT) cells	Three concentration levels: 5, 10 and 20 mg/ml. Cells were treated for 24 and 48 h	A significantly high DNA damage in treated cells was observed	+	[122]
Fe ₂ O ₃	TEM analysis: 35 ± 14 nm. DLS analysis: Z-average hydrodynamic diameter 900 nm	<i>In vitro</i> Syrian hamster embryo (SHE) cells	Three concentration levels: 10, 25 and 50 µg/cm ² . Cells were treated for 24 h	No significant DNA damage was found with Fe ₂ O ₃ particles, at any of the tested concentrations	-	[123]
Fe ₂ O ₃	Nominal size: 90 nm. TEM analysis: average particle diameter 93 nm. DLS analysis:	<i>In vitro</i> human lung epithelial cells (A549) and murine alveolar macrophages (MH-S)	One concentration level: 40 µg/cm ² . Exposure duration: 24 h	Tail DNA was not modified following incubation with Fe ₂ O ₃ NM	-	[124]

	average particle hydrodynamic diameter 68 nm					
Fe ₂ O ₃	Nominal size < 100 nm. DLS analysis: hydrodynamic diameter 50 nm	<i>In vitro</i> human lung fibroblasts (IMR-90) and human bronchial epithelial cells (BEAS-2B)	Four concentration levels: 2, 5, 10, 50 µg/cm ² . Exposure duration: 24 h	DNA damage was showed at concentrations of 10 and 50 µg/cm ² in IMR-90 cells and at 50 µg/cm ² in BEAS-2B cells	+	[125]
Fe ₂ O ₃	Nominal size: 29 nm. TEM analysis: average size between 30 and 60 nm. DLS analysis: 1580 nm.	<i>In vitro</i> human lung type II epithelial (A549) cells	Three concentration levels: 2, 40 and 80 µg/ml. Exposure duration: 4 h	No significant DNA damage was observed at tested concentration levels	-	[114]
Fe ₂ O ₃	Nominal size: 29 nm. TEM analysis: average size between 30 and 60 nm. DLS analysis: 1.6 µm.	<i>In vitro</i> human lung type II epithelial (A549) cells	Two concentration levels: 40 and 80 µg/ml. Exposure duration: 4 h	No significant DNA damage was showed at tested concentration levels	-	[31]
Fe ₂ O ₃	Nominal size < 50 nm. TEM analysis: mean size 29.75 ± 1.87 nm. DLS analysis: hydrodynamic	<i>In vivo</i> albino Wistar female rat peripheral blood cells	Three dose levels (oral administration): 500, 1000 and 2000 mg/kg body weight. Exposure duration: 6, 24, 48 and 72 h	No statistically significant damage was observed at any sampling time in comparison to control.	-	[126]

	diameter 363 nm (water)					
Fe ₂ O ₃	Nominal mean size: 6 nm. TEM and XRD mean analysis coherent diameter: 6 nm.	<i>In vitro</i> human fibroblast cells	Six concentration levels: 0.001, 0.01, 0.1, 1, 10 and 100 µg/ml. Exposure duration: 2 and 24 h	No DNA damage was observed at any tested concentration levels	-	[127]
Fe ₂ O ₃	Nominal size < 50 nm. TEM analysis: 29.75 ± 1.87 nm. DLS analysis: 363 nm	<i>In vivo</i> female Wistar rats peripheral blood leucocytes	Three dose levels (oral administration): 500, 1000 and 2000 mg/kg body weight. Exposure duration: 6, 24, 48 and 72 h	No statistically significant damage was observed at 6, 24, 48, 72 h sampling time in comparison to control	-	[128]
Fe ₃ O ₄	TEM analysis: average diameter 10 nm (naked particles) increased up to 150 nm upon surface modifications	<i>In vitro</i> murine fibroblast cell line (L-929 cells from mouse subcutaneous connective tissue)	Three concentration levels: 100, 200 and 1000 ppm. Exposure duration: 24 h	NMs tested showed dose-dependent genotoxic effects on the cells, which varied with surface modifications, although not all surface variations gave statistically significant results versus the non-treated control	+	[129]
Fe ₃ O ₄	TEM analysis: 27 ± 8 nm. DLS analysis: hydrodynamic diameter between 700 and 800 nm	<i>In vitro</i> Syrian hamster embryo (SHE) cells	Three concentration levels: 10, 25 and 50 µg/cm ² . Cells were treated for 24h	No significant DNA damage was found with iron oxide particles at any concentration level.	-	[123]
Fe ₃ O ₄	TEM analysis: 24.83 nm. DLS analysis:	<i>In vitro</i> human skin epithelial	Three concentration levels: 25, 50 and 100 µg/ml. Cells were	The cells exposed to different concentrations, exhibited significantly	+	[130]

	average hydrodynamic size was 247 nm (water) and 213 nm (cell culture medium)	(A431) cells	treated for 24h	higher DNA damage in cells than those of the controls.		
Fe ₃ O ₄	TEM analysis: 24.83 nm. DLS analysis: average hydrodynamic size was 247 nm (water) and 213 nm (cell culture medium)	<i>In vitro</i> human lung epithelial (A549) cells	Three concentration levels: 25, 50 and 100 µg/ml. Cells were treated for 24h	The cells exposed to different concentrations, exhibited significantly higher DNA damage in cells than those of the controls.	+	[130]
Fe ₃ O ₄	Nominal size: from 20 nm to 60 nm. Photon correlation spectroscopy (PCS) analysis: mean diameter of 311 nm	<i>In vitro</i> human lung adenocarcinoma type-II alveolar epithelial cells A549	Four dose levels: 1, 10, 50 and 100 µg/cm ² . Cells were exposed for 4 h	DNA damage was observed in a concentration-dependent manner	+	[131]
Fe ₃ O ₄	Nominal size: 29 nm. TEM analysis: average size between 30 and 60 nm. DLS analysis: 1.6 µm	<i>In vitro</i> human lung type II epithelial (A549) cells	Two concentration levels: 40 and 80 µg/ml. Exposure duration: 4 h	No DNA damage was showed at the two tested concentration levels	-	[31]
Fe ₃ O ₄	Nominal size between 20 and 30 nm. TEM	<i>In vitro</i> human lung type II epithelial (A549)	Three concentration levels: 2, 40 and 80 µg/ml. Exposure	No DNA damage was observed at the tested concentration levels	-	[114]

	analysis: average size between 20 and 40 nm. DLS analysis: 200 nm	cells	duration: 4 h			
Fe ₃ O ₄	TEM analysis: average size 8.0 ± 2.0 nm	<i>In vitro</i> human embryonic kidney (HEK-293) and peripheral blood lymphocytes (HPL) cells	Three concentration levels: 10, 30 and 70 µg/ml. Exposure duration: 30 min and 1 h	A significant increase in DNA damage was observed at all tested concentrations after 1 h exposure with both types of cells	+	[132]
Fe ₃ O ₄	Nominal size: 30 nm. TEM analysis: : longest dimension between 20 and 40 nm. DLS: 200 nm	<i>In vitro</i> human alveolar Type II- like epithelial (A549) and bronchial epithelium (BEAS-2B) cells	Two concentration levels: 40 and 80 µg/ml. Exposure duration: 4 h	Fe ₃ O ₄ NM caused significant DNA damage only In BEAS-2B cells, at 40 µg/ml	+	[109]
MgO	Nominal size: 8 nm	<i>In vitro</i> human colon epithelium (CaCo-2) cells	One concentration level: 20 mg/cm ² . Exposure duration: 4 h	No significant change compared to the control was observed.	-	[133]
MnO ₂	Nominal size: 45 nm. TEM analysis: 45 ± 17 nm. DLS analysis: 334.4 nm.	<i>In vivo</i> female albino Wistar rat peripheral blood leucocytes (PBL)	Three dose levels: 100, 500 and 1000 mg/kg body weight. Exposure duration: 6, 24, 48 and 72 h	A significant increase in the percentage of tail DNA was observed in the at the highest dose of 1000 mg/kg body weight at 24 and 48 h sampling times; however, no significant DNA damage was observed at 6 and 72 h. An increase in the percentage of tail DNA was observed after	+	[128]

				treatment with lower doses of 100 mg/kg body weight and 500 mg/kg body weight, but these results were not statistically significant at all-time intervals compared with the control groups.		
MnO ₂	TEM average mean size diameter: 42.63 ± 23 nm. DLS size in water: 324.8nm. (The result of DLS showed larger values than NPs size measured by TEM, indicating NPs formed larger agglomerates in water suspension. Surface area: 52.21 m ² /g.)	<i>In vivo</i> wistar rat leucocytes and bone marrow cells	After 28-day repeated oral dosing in male and female Wistar rats at various doses (30, 300, 1000 mg/kg/body weight per day) for 28 days.	A statistically significant ($P < 0.01$) increase in the DNA damage (percentage of tail DNA) with the highest and medium doses. No significant increase was found with the lowest dose.	+	[134]
NiO	Nominal size: <50 nm. TEM analysis: 2-67 nm. DLS: 167 nm.	<i>In vitro</i> human alveolar type II like epithelial A549 and bronchial	40-80 µg/mL for 4h	In A549 cells DNA in tail 31.6% at 40 µg/mL increased amounts of DNA breaks, significantly increased at 80 µg/mL. In	+	[109]

		epithelium BEAS-2B cells		BEAS-2B cells NiO (29.0%) at the low dose and 28.5% at the higher dose.		
SiO ₂	Nominal size: 15 nm. TEM analysis: 11-27 nm. DLS: 8.7 nm. The particle size was calculated from the Brownian motion of the particles using the Stokes–Einstein equation.	<i>In vitro</i> human alveolar type II like epithelial A549 and bronchial epithelium BEAS-2B cells	40-80 µg/mL for 4h	No significant change compared to the control was observed	-	[109]
SiO ₂	Two different types of SiO ₂ were used; Nominal size: 10 and 7 nm. BET surface area: 644.44 and 349.71 m ² /g.	<i>In vivo</i> in the freshwater crustacean Daphnia magna and the larva of the aquatic midge Chironomus riparius	The fish were collected 24 h from the control and experimental tanks after exposure to 1 mg/L of NPs.	No genotoxic effect on either species as no significant increase both in the tail and in the Olive Tail Moment was observed	-	[111]
SiO ₂ (amorphous)	Two silica NMs were tested. Nominal sizes: 15 nm and 55 nm. DLS analysis: z-average particle	<i>In vivo</i> Wistar rat peripheral blood cells	Single dose for 15 nm SiO ₂ : 50 mg/kg; two dose levels for 55 nm SiO ₂ : 25 mg/kg and 125 mg/kg. Rats injected i.v. at 48h, 24h, and 4h prior to tissue collection	The percentage of DNA damage was increased compared to vehicle-treated rats by 1.4–1.6-fold in all three tissues at the maximum tolerated dose (50 mg/kg)	+	[82]

	diameters (pH 7.5): 31.6 nm and 105.1 nm			of the 15 nm silica NPs, which was significant in the liver.		
SiO ₂ (with three types of functionalization: unmodified, vinyl and aminopropyl groups)	SEM average diameter: 10 to 50 nm. DLS average hydrodynamic diameter: 4 ± 4.6 , 176.7 ± 5.1 and 256.3 ± 7.2 nm respectively.	<i>In vitro</i> human peripheral blood lymphocytes	10, 25, 50 and 100 µg/mL after 2 and 24 h	Results revealed no significant increase of basal DNA strand breaks in cells treated with all three types of silica.	-	[135]
SiO ₂ (amorphous, fumed)	Nominal size: 14 nm. BET surface area: 200 m ² /g.	<i>In vitro</i> human colon epithelium cell line (CaCo-2)	Cells were treated 4h with 20 mg/cm ² particles (in MEM without serum)	FPG comet assay results found no significant effect in DNA strand breakage and slight effects in oxidative DNA damage study.	-	[133]
SiO ₂	Nominal diameter: 50 nm 50 ± 3 nm. TMR- and RuBpy-doped luminescent silica NP (laboratory synthesised)	<i>In vitro</i> A549 cells	0.1–500 µg/mL in DMEM with serum for 48 and 72 h	Low genotoxicity was found in alkaline comet assay. Tail length was not significantly different from the control after 48h. Results showed a slight increase in DNA single-strand breaks after 72 h. The comet assay result was further verified using PFGE indicating no additional DNA damage as compared to the untreated control.	-	[136]

SiO ₂	Size distribution measured with High Performance Particle Sizer (HPPS): by volume 7.21 nm (100%); by intensity 9.08 nm (71.4%) and 123.21 (28.6%).	<i>In vitro</i> WIL2-NS human B-cell lymphoblastoid cells	60 and 120 µg/mL for 24 h	No significant levels of DNA tail percentage in alkaline comet assay	-	[137]
SiO ₂	TEM analysis: 20.2 ± 6.4 nm	<i>In vitro</i> primary mouse embryo fibroblasts (PMEF)	5 and 10 µg/mL particles in DMEM for 24 h	There were significant increases in tail length, percentage of DNA in tail, tail moment and Olive Tail Moment after PMEF cells were treated with at both examined concentrations (6.8% tail DNA, <i>p</i> < 0.05) in alkaline comet assay.	+	[55]
SiO ₂	Particle size distribution in the suspension as measured by the high performance particle sizer (Z-Average size): 12.2 nm.	<i>in vitro</i> WIL2-NS human B cell lymphoblastoid cell line	0, 60, 120 µg/mL in 10h	There is no significant increment in DNA damage observed when measured by the comet assay.	-	[138]
SiO ₂ (amorphous, alumina-coated)	Five different samples of nominal size:	<i>In vitro</i> human lymphoblastoid fibroblasts (T3T-	4 and 40 µg/mL for 3, 6, and 24h	In the comet assay, DNA damage of human cells was assessed via	-	[139]

with a positive charge)	30–400 nm	L1)		measuring tail length, percentage of tail DNA, and Olive Tail Moment. There were no significant differences between the control and tested NM		
SiO ₂	Nominal powder diameter: 10 nm	<i>In vitro</i> mouse lymphoma cell line (L5178Y thymidine kinase (tk)+/-3.7.2C cells) and human bronchial epithelial cells (BEAS-2B)	Concentrations from 629.88 µg/mL to 2,519.53 µg/mL with S-9 and from 610.36 µg/mL to 2,441.41 µg/mL without S-9 in L5178Y cells	Nano-silica significantly induced DNA damage at all concentrations compared to control (<i>P</i> < 0.05)	+	[140]
SiO ₂	TEM analysis: 16.4 ± 2.5 nm and 60.4 ± 8.3 nm	<i>In vitro</i> human lung carcinoma (A549) cells	Two concentration levels: 46 and 60 µg/ml. Exposure duration: 15 min and 4h	Treatment with 16 nm or 60 nm SiO ₂ showed no increase of DNA strand breaks after 15 min or 4 h as compared to the controls.	-	[141]
SiO ₂	Nominal size: 15, 30, 100 nm. Hydrodynamic sizes in MEM suspension after 24 hour were 14.6 ± 0.3, 20.4 ± 1.7, 169.2 ± 3.1 nm	<i>In vitro</i> human epidermal keratinocyte (HaCaT) cells	Three concentration levels: 2.5, 5 and 10 µg/ml. Cells were treated for 24h	Three SiO ₂ NMs caused more DNA damage and more percentage of DNA in the tail than untreated groups	+	[142]

SiO ₂	Nominal size: 12 nm and 40 nm. TEM analysis: "12-nmNPs" ranged between 16 and 40 nm; "40-nm NPs" varied between 50 and 100 nm. DLS analysis (water): 165 nm (12-nm NP); 271 nm (40-nm NP)	<i>In vitro</i> human colon carcinoma cells (HT29)	Seven concentration levels: 0.03, 0.3, 3.1, 15.6, 31.3, 93.8, 156.3 mg/cm ² . Exposure duration: 3 and 24h	After 3h as well as after 24h of incubation, none of the tested particles really affected the integrity of the DNA of HT29 cells in the investigated concentration range. However, for some scattered concentration steps, significant differences compared with the medium control were apparent. The appearance of these significances seems to be more random and did not follow a recognisable trend	-	[143]
SiO ₂	Nominal size: 2-5 nm. Agglomeration: 1-5-μ granules. No further characterization reported in the paper	<i>In vivo</i> F1(CBA×C57Bl/6) mice bone marrow and brain cells	Two dose levels: 5 and 50 mg/kg Exposure duration: 3, 24h and 7 days	A significant increase in the levels of DNA damage in the bone marrow cells of animals injected with 5 mg/kg nc-Si was observed after 24h exposure.	+	[144]
SnO ₂	No characterization of the NM is reported in the paper	<i>In vitro</i> lymphocyte cells	Two concentration levels: 50 and 100 μg/mL. No exposure duration information	No significant changes in the tail length were showed at both tested concentration levels	-	[145]
TiO ₂	Nominal size: 25	<i>In vitro</i> murine	Five dose levels: 10, 20,	Results showed that TiO ₂	-	[146]

	nm. DLS analysis: mean particle size 300 nm Manufacturer data: SEM analysis: aggregates. DLS analysis: 220 nm. Ramon spectroscopy: a mixture of rutile and anatase forms. BET specific surface area: 27.1 m ² /g.	fibroblasts BALB/3T3 clone A31	60, 100 and 250 µg/mL. Cells were exposed for 3 and 24h	NM caused only a slight (however with a clear concentration-effect relationship) genotoxic effect in BALB/3T3 fibroblasts at the highest concentrations used.		
TiO ₂	Nominal size: 21 nm. DLS hydrodynamic diameter: 129.50 ± 2.6 nm	<i>In vivo</i> Adult male Wistar rats bone marrow cells	Single dose of 5 mg/kg body weight. Animals were sacrificed at 24h, 1 week and 4 weeks after the injections	The values for exposed animals were sometimes slightly enhanced as compared to control, but results were not statistically significant	-	[147]
TiO ₂	Nominal size < 25 nm. DLS hydrodynamic size (Z-average): 1611 ± 21 nm after 24h	<i>In vivo</i> P. mesopotamicus (pacu caranha) erythrocytes cells	The fish were exposed (with visible light or ultraviolet and visible light) to the following concentrations of nano-TiO ₂ during a 96 h period: 0 (control), 1, 10, and 100 mg/l.	No statistically significant differences between the groups were observed.	-	[148]
TiO ₂	Nominal average size: 75 ± 15 nm. ZetaSizer Nano	<i>In vitro</i> chinese hamster lung fibroblasts (V79 cells)	Three dose levels: 5, 20 and 100 µg/mL. Exposure duration: 6h and 24h	However, TiO ₂ NPs exposure only increased the percentage of DNA in the tail (% Tail DNA) at	-	[149]

	ZS90 hydrodynamic diameter: 473.6 nm and 486.8 nm size when suspended in H2O and FBS-free DMEM			the concentration of 100 µg/mL and at the time point 24h.		
TiO ₂	Nominal size: 2.3 nm. TEM analysis: average diameter 2.3 ± 0.5 nm	<i>In vitro</i> human embryonic kidney (HEK293) cells and human peripheral blood lymphocytes	Concentrations of 1, 10, or 100 µg/mL	Results demonstrated that at all concentration NP did not induce any marked genotoxicity, except for 100 µg/mL. Produced significant genotoxicity and appeared more effective in the comet assay with and without the FPG and Endo III enzymes in both human peripheral blood lymphocytes and HEK293 cell. In contrast ionic type did not show any positive result in the cells	+	[103]
TiO ₂	Nominal size: <25 nm	<i>In vitro</i> leukocytes from dolphins	20, 50, and 100 µg/mL for 4, 24, and 48 h in RPMI with serum	Positive in alkaline comet assay at 24 and 48 h	+	[150]
TiO ₂	Nominal size: <100 nm. BET surface area: 49.71 ± 0.19 m ² /g. DLS average particle	Human lung fibroblasts (IMR-90) and human bronchial epithelial cells (BEAS-2B)	Concentrations of 2, 5, 10, 50 µg/cm ² for 24 h in MEM with serum and in keratinocyte serum-free medium, respectively.	TiO ₂ -NPs did not induce DNA-breakage measured by the comet-assay in both human cell lines.	+	[125]

	hydrodynamic diameter: 91 nm.					
TiO ₂	Nominal size: 20-50 nm. Particles were extracted from sunscreens. The precise composition of the samples (particle size, surface area per unit weight, presence/absence of coatings).	<i>In vitro</i> MRC-5 fibroblasts with or without irradiation from a solar simulator	0.025% w/v particles	Positive in alkaline comet assay after combined treatment with sunscreen extract and irradiation.	+	[151]
TiO ₂	Two crystalline forms (phases) of TiO ₂ . Nominal size: nanosized rutile (>95%, <5% amorphous SiO ₂ coating; 10 × 40 nm), nanosized anatase (99.7%; size <25 nm). BET analysis: rutile 132 and anatase 222 m ² /g. The particles were also characterized by	<i>In vitro</i> human bronchial epithelial cell line (BEAS-2B)	Eight doses of 3.8-380 µg/mL for 24, 48, or 72 h	In alkaline comet assay results showed induction of DNA damage by all the TiO ₂ forms examined, with SiO ₂ -coated nanosized rutile having the lowest effects. Anatase was more active than (coated) rutile.	+	[152]

	TEM and XRD.					
TiO ₂	<p>TEM analysis: TiO₂ particles consisted of agglomerates of 10–60 nm crystallites with an average primary particle size of 21 nm. XRD analyses: the particles comprised of two phases of TiO₂, anatase (74%, v/v) and brookite (26%, v/v), with respective crystallite sizes of 41 nm and 7 nm.</p>	<p><i>In vivo</i> alveolar type II/Clara cells collected immediately after the last exposure of male C57BL/6J mice.</p>	<p>The mice were exposed repeatedly, 4 h per day during 5 consecutive days, to three different concentrations of nanosized TiO₂ (0.8, 7.2 and 28.5 mg/m³).</p>	<p>No significant induction of DNA damage was seen in the comet assay at any of the three doses of nanosized TiO₂, when the exposed mice were compared with the corresponding negative controls. However, the ethylene oxide-treated mice (the positive control group) showed a statistically significant 1.7-fold increase in the mean percentage of DNA in tail in comparison with the concurrent negative control group, despite the high inter-individual variation in DNA damage levels seen in the concurrent control animals.</p>	-	[153]
TiO ₂	<p>Nominal diameter: 5 nm. TEM primary particle size: 4.9 nm. BET surface area: 316 m²/g. DLS analysis: 19.0 (13.5–31.3) (Anatase form)</p>	<p><i>In vivo</i> lung cells of rat (after a single or repeated intratracheal instillation in rats)</p>	<p>The NPs were instilled intratracheally at a dosage of 1.0 or 5.0 mg/kg body weight (single instillation group) and 0.2 or 1.0 mg/kg body weight once a week for 5 weeks (repeated instillation group) into male</p>	<p>In the comet assay, there was no increase in % tail DNA in any of the TiO₂ groups. In the EMS group, there was a significant increase in % tail DNA compared with the negative control group. TiO₂ NPs in the anatase crystal phase are not</p>	-	[85]

			Sprague-Dawley rats. A positive control, ethyl methanesulfonate (EMS) at 500mg/kg, was administered orally 3h prior to dissection.	genotoxic following intratracheal instillation in rats.		
TiO ₂	The UV-Titan L181 (NanoTiO ₂) was a rutile coated with Si, Al, Zr and polyalcohol. The average crystallite size was determined to be 20.6 nm and the powder had a specific surface area of 107.7 m ² /g.	<i>In vivo</i> bronchoalveolar lavage cells of mice	Mice received a single intratracheal instillation of 18, 54 and 162 µg of NanoTiO ₂ or 54, 162 and 486 µg of the sanding dust from paint with and without NanoTiO ₂ (evaluated 1, 3 and 28 days after intratracheal instillation).	Pulmonary inflammation and DNA damage and hepatic histopathology were not changed in mice instilled with sanding dust from Nano TiO ₂ paint compared to paint without Nano TiO ₂ .	-	[154]
TiO ₂	Nominal size: 20-80 nm. BET surface area: 50 m ² /g (mixture of anatase and rutile)	<i>In vitro</i> human colon epithelium cell line (CaCo-2)	Cells were treated 4h with 20 mg/cm ² particles (in MEM without serum)	No significant change comparing to the control in FPG-modified comet assay was observed.	+	[133]
TiO ₂	SEM analysis: 40–70 nm. (anatase form)	<i>In vitro</i> lymphocytes and sperm cells	4 concentration levels: 3.73, 14.92, 29.85 and 59.7 µg/ml in PBS for 30 min in the dark, preirradiated and simultaneous irradiation with UV	Positive in alkaline comet assay with both cell types. The ZnO particles are capable of inducing genotoxic effects on human sperm and	+	[155]

				lymphocytes Stronger effects with TiO ₂ in lymphocytes with UV treatment.		
TiO ₂	Nominal size: 7 nm (NM 101), 10 nm (NRCWE 001, NRCWE 002, NRCWE 003) and 94 nm (NRCWE 004). TEM analysis: 4-8/50-100 nm, 80-400 nm and 1-4/10-100/100-200/1000-2000 nm. DLS analysis (MEM): 185, 742 nm; 203-1487 nm; 339 nm	<i>In vitro</i> human hepatoblastoma C3A cells	Three concentration levels: 5, 10 and 20 µg/cm ² or 2.5, 5 and 10 µg/cm ² . Exposure duration: 4h	DNA damage was most evident following exposure to NM 101 (TiO ₂ - 7 nm) and NRCWE 002 (TiO ₂ - 10 nm positively charged). NRCWE 003 – negatively charged TiO ₂ 10 nm is the only exception.	+	[156]
TiO ₂	Nominal size: 12 nm, 20 nm, 25 nm. TEM analysis: 12 nm, 21 nm, 24 nm	<i>In vitro</i> A549 human lung carcinoma cells (CCL-185)	One concentration level: 100 µg/ml. Exposure duration: 4h, 24h, 48h	After 4h of exposure a significant increase in the level of DNA breaks was observed. This increase in the level of breaks further increased after 24h of exposure.	+	[157]
TiO ₂	Nominal size: 17 nm. No further characterization reported in the	<i>In vivo</i> nulliparous time-mated mice (C57BL/6BomTac) bronchoalveolar	One dose level (inhalation during the gestational period): 42 mg UV-Titan/m ³ .	Inhalation of UV-Titan did not affect the levels of DNA strand breaks in BAL or liver cells in the non-	-	[158]

	paper	lavage (BAL) and liver cells	Exposure duration: 1h/day X 11 days	pregnant females and dams compared with their controls. Prenatal exposure to UV-titan did not affect the levels of DNA strand breaks in the livers of newborn or weaned offspring compared with their controls		
TiO ₂	Nominal size: 10-20 nm. No further characterization reported in the paper	<i>In vivo</i> earthworm <i>Eisenia fetida</i> (Savigny, 1826)	Four dose levels: 0.1, 0.5, 1.0, 5.0 g/kg dry soil. Exposure duration: 7 days	Earthworms exhibited DNA damage when exposed to ZnO at 1 and 5 g/kg. At 5 g/kg the degrees of DNA damage were significant when compared to controls.	+	[159]
TiO ₂	Nominal size: 5.9 nm, 34.1 nm, 15.5 nm, 1-10 nm. DLS analysis: 460 nm, 400 nm, 420 nm, 600 nm	<i>In vitro</i> Chinese hamster lung fibroblast (V79) cells	One concentration level: 100 mg/l. Exposure duration: 24h	The % Tail DNA and the OTM were increased by twofold in cells treated with 100 mg/l of non-coated nano-TiO ₂ after 24h. Cell viability was more than 40% after exposure to 100 mg/L of nano-TiO ₂ after 24h.	+	[160]
TiO ₂	Nominal size: 25 nm. TEM analysis: 15–30 nm; agglomeration size: 285 ± 52	<i>In vitro</i> primary human nasal epithelial cells	Four concentration levels: 10, 25, 50 and 100 µg/ml. Exposure duration: 24h	No genotoxic effect could be shown for any of the tested concentrations of TiO ₂ -NPs.	-	[161]

	nm					
TiO ₂	Nominal size < 25 nm. TEM analysis: 285 ± 52 nm	<i>In vitro</i> human peripheral blood lymphocytes	Four concentration levels: 20, 50, 100, and 200 µg/ml. Exposure duration: 24h	No evidence for genotoxicity could be shown for any of the tested concentrations of TiO ₂ -NPs.	-	[162]
TiO ₂	TEM analysis: 14 ± 4 nm and 25 ± 6 nm. DLS analysis was carried out (see reference)	<i>In vitro</i> Syrian hamster embryo (SHE) cells	Three concentration levels: 10, 25 and 50 µg/cm ² . Cells were treated for 24h	At the highest particle concentration (50 µg/cm ²), all TiO ₂ particles except rutile NPs caused increased DNA damage after 24 h of exposure.	+	[123]
TiO ₂	Nominal size: 100 nm. AFM analysis: 90–110 nm	<i>In vitro Allium cepa, Nicotiana tabacum</i> root or leaf nuclei and human lymphocyte cells	(Plants) five concentration levels: 2, 4, 6, 8 and 10 mM. Exposure duration: 3, 6 and 24 h. (Lymphocytes) eight concentration levels: 0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75, 2 mM. Exposure duration: 3 h	A uniform pattern of dose response was observed in <i>A. cepa</i> at all treatment schedules, no particular time dependent effect was noticed. Similarly TiO ₂ NPs treated (24h) <i>N. tabacum</i> leaf nuclei showed an initial increase in extent of DNA damage followed by a gradual decrease up to the highest dose. The value was statistically significant at 2 mM. The percentage of tail DNA (% tail DNA) in lymphocytes treated with different concentrations of NPs revealed a distinct	+	[163]

				pattern of genotoxicity. These NPs showed signs of significant DNA damage only at lower concentration (0.25 mM) followed by gradual decrease		
TiO ₂	Nominal size: ~100 nm. TEM analysis: 50.93 ± 7.08 nm. DLS analysis: average hydrodynamic diameter 6180.73 nm	<i>In vitro</i> human lymphocyte cells	Three concentration levels: 25, 50 and 100 µg/ml. Exposure duration: 3 h	DNA fragmentation induced by TiO ₂ NPs in human lymphocytes was statistically significant at a treatment dose of 25 mg/ml. Treatment doses of 50 and 100 mg/ml induced DNA damage, but was not significant compared with the control set	+	[164]
TiO ₂	Nominal size: 21 nm and 50 nm. DLS analysis: 21 ± 0.8 nm for nano-TiO ₂ (21 nm) and 50 ± 0.5 nm for nano-TiO ₂ (50 nm)	<i>In vitro Allium cepa</i> root meristem cells	Three concentration levels: 10,100 and 1000 µg/ml. Exposure duration: 1h	The results obtained from root meristem cells of <i>A. cepa</i> demonstrated that only the highest concentration (1000 µg/mL) of TiO ₂ NPs (21 nm) was statistically significant in comparison to the control, while all concentrations of TiO ₂ NPs (50 nm) were significant for % DNA tail. On the other hand, TiO ₂ NPs tested did show a dose-dependent	+	[165]

				increment for tail moment.		
TiO ₂	Nominal average diameter 21 nm. TEM analysis 24 ± 4.6 nm (fresh); aged nTiO ₂ formed aggregates of particles with average diameters ranging from 27.60 ± 6.9 to 108.40 ± 5.2 nm	<i>In vivo</i> marine mussels (<i>Mytilus galloprovincialis</i>) haemocytes	One dose level: 10 mg/l. Exposure duration: 4 days	All treatments showed significantly higher DNA damage than controls. Interestingly all TiO ₂ treatments resulted in approximately 40% tail DNA and there were no significant differences between the treatments.	+	[166]
TiO ₂	Nominal size: 21 nm and <25 nm. DLS analysis: mean hydrodynamic diameter 160.5 nm and 420.7 nm	<i>In vitro</i> human gastric epithelial cancer (AGS)	One concentration level: 150 µg/ml. Exposure duration: 3h	In the comet assay, there was a 1.88-fold significant increase in %Tail DNA when the cells were treated with TiO ₂ NPs.	+	[167]
TiO ₂	Nominal size: 1–3 nm. DLS analysis: 99.20 ± 6.2 nm (water) 337e5 ± 190e5	<i>In vitro</i> <i>S. cerevisiae</i> cells	One concentration level: 31.25 mg/l. No information about time exposure	A significant amount of DNA damage was detected in NP-exposed cells when compared with controls.	+	[121]

	nm (medium)					
TiO ₂	TEM analysis: 12 ±3 nm. BET analysis: 17 nm	<i>In vitro</i> NRK-52E rat kidney proximal cells	Four concentration levels: 50, 75, 100, 200 µg/ml. Exposure duration: 24h	DNA damage induced by TiO ₂ -CEA increased with exposure concentration and was statistically significant for exposure concentrations equal or higher than 100 µg/ml.	+	[168]
TiO ₂	TiO ₂ at 10 nm (Hombikat UV100) and 20 nm (Millenium PC500) in diameter. No further investigations are reported in the paper. (Anatase form)	<i>In vitro</i> human bronchial epithelial cells, BEAS-2B (ATCC CRL-9609)	Cells were treated with 10 µg/mL of TiO ₂ for 24 h	The results showed that apparent DNA damage was detected in treatment with 10 µg/mL anatase 10 nm particles	+	[169]
TiO ₂	Nominal size: 25 nm. X-ray diffraction analysis (XPD) analysis specific surface area: 50 m ² /g, mean powder size approximately 30 nm. (A mixture of	<i>In vitro</i> human peripheral blood lymphocytes	20, 50, or 100 µg/mL for 6, 12, or 24 h	Positive in alkaline comet assay. Reduced effect was found when cells were pretreated with N-acetylcysteine. The dose- and time-dependent effect on DNA fragmentation was found. Lymphocytes exposed had a significantly greater OTM than those not exposed (<i>P</i> < 0.05).	+	[170]

	anatase (70–85%) and rutile (30–15%)					
TiO ₂	Nanopowder nominal size: 63 nm. TEM analysis: 20-100 nm. DLS analysis: 300 nm.	<i>In vitro</i> A549 cells	1 µg/cm ² (2 µg/mL), 20 µg/cm ² (40 µg/mL), and 40 µg/cm ² (80 µg/mL) for 4 h. FPG sensitive sites were measured at 20 µg/cm ² , and 40 µg/cm ² after 4h.	A dose-response trend in induced DNA damage could be seen in cells treated with TiO ₂ . Positive in alkaline comet assay. In FPG particles caused a slight increase, although this was not statistically significant (19% tail, <i>p</i> < 0.001).	+	[114]
TiO ₂	Nominal average size: 5 nm. (anatase form)	Fish skin cells	1, 10, and 100 µg/mL for 2 or 24 h in cell medium with serum	A modified comet assay using bacterial lesion-specific repair endonucleases (Endo-III, FPG) was employed to specifically target oxidative DNA damage. Negative with endonuclease III. For the comet assay, doses of 1, 10 and 100 g/mL in the absence of UVA caused elevated levels of FPG-sensitive sites, indicating the oxidation of purine DNA bases (i.e. guanine) by TiO ₂ . UVA irradiation of TiO ₂ -treated cells caused further increases in DNA	+	[171]

				damage.		
TiO ₂	<p>Mixture of anatase (25%) and rutile (75%).</p> <p>TEM cross sectional diameter Mean size: 24.4 ± 0.5 nm, minimum = 11.8 nm, maximum = 38.5 nm).</p> <p>Though ENPs (sonicated in H₂O) aggregated while preparing for TEM studies, the NPs could still be characterised as less than <100 nm in diameter.</p>	<p><i>In vivo</i> rainbow trout gonad (RTG-2) cells (fibroblastic cell line)</p>	<p>5 and 50 µg/mL in MEM or PBS for 4 or 24 h with or without 30 min exposure to UV light</p>	<p>Positive in alkaline and FPG comet assay in combination with UV.</p>	+	[172]
TiO ₂	<p>Nominal size powder: 63 nm.</p> <p>TEM analysis: 20-100 nm. DLS analysis: 300 nm.</p>	<p><i>In vitro</i> A549 cells</p>	<p>40 to 80 µg/mL for 4h</p>	<p>In alkaline comet assay significantly higher levels of DNA damage were found compared to control (24% tail, $p < 0.001$).</p> <p>In FPG-modified comet assay, no significant levels of oxidative DNA damage at 80 µg/mL was found compared to control</p>	+	[31]

				were observed. None of the particles caused a significant increase in oxidative DNA damage when cells were exposed to 40 µg/mL.		
TiO ₂	HPPS size: by volume 6.57 nm (100%); by intensity: 8.2 nm (80.4%) and 196.52 nm (19.4%)	<i>In vitro</i> WIL2-NS human B-cell lympho- blastoid cells	WIL2-NS cells were incubated for 6, 24 and 48 h with 0, 26, 65 and 130 µg/mL. Results of comet assay were reported for 65 µg/mL for 24 h.	There was a 3-fold significant (P < 0.05) increase in %Tail DNA when the cells were treated with UF-TiO ₂ at a dose of 65g/mL for 24 h exposure (16±3% tail).	+	[173]
TiO ₂	TEM dry size distribution: 10x30 nm (They were heavily aggregated not only in dry powder but also in solutions (the aggregated sizes was approximately 130–170 nm); (anastase form)	<i>In vitro</i> TK6 human lymphoblastoid cells	50, 100, 150, 200 µg/mL for 24 h	The standard comet assay and endonucleases enzyme-modified comet assay were performed. None of the TiO ₂ -NPs treatments increased DNA damage in either of the assays.	-	[174]
TiO ₂	TEM primary particle size: 21 nm. BET specific surface area: 50 ± 15 m ² /g. DLS agglomerates range: 21-1446	<i>In vivo</i> mice peripheral blood was collected by submandibular vein puncture	60, 120, 300, and 600 µg/mL concentrations in drinking water for 5 days	Tail moment significantly increased after TiO ₂ NPs treatment. The average tail moment was 0.0102 ± 0.001 before treatment and 0.0137 ± 0.0011 after TiO ₂ NPs treatment.	+	[89]

	nm and mean size: 160 ± 5 nm. (A mixture of 75% anatase and 25% rutile TiO_2 , purity was at least 99.5% TiO_2)					
TiO_2	TEM mean size: 33.2 ± 16.7 nm. DLS analysis: (anatase form)	<i>In vivo</i> CBAB6F1 mice, brain, liver and bone marrow	40, 200 mg/kg body weight, daily oral for seven days	Increased DNA strand breaks in bone marrow cells was found. The % tail DNA in the comet tail significantly increased after treatment, from 3.66 in the control group to 7.99 ± 1.21 and 6.8 ± 1.13 in the treated groups ($p < 0.05$). No statistically significant changes have been found in the cells of liver and brain.	+	[87]
TiO_2	Nominal mean diameter: 28nm. DLS size of particles and agglomerates in cell culture medium analysis: 280 nm.	<i>In vitro</i> human lung epithelial A549 cells	0, 5, and 15 $\mu\text{g/mL}$ for 12 h	Alkaline comet assay, no change has been found comparing to the control.	-	[175]
TiO_2	TEM size: 50 nm. DLS mean hydrodynamic	<i>In vitro</i> human epidermal cells (A431)	Concentrations ranging from 80 to 0.008 $\mu\text{g/mL}$ (0.008, 0.08, 0.8, 8, 80	A statistically significant ($p < 0.05$) induction in the DNA damage was	+	[176]

	size (in water): 124.9 nm.		µg/mL)	observed by the FPG-modified comet assay in cells exposed to 0.8 µg/mL NPs (2.20 ± 0.26 vs. control 1.24 ± 0.04) and higher concentrations for 6 h.		
TiO ₂	TEM size: 30-70 nm. DLS mean hydrodynamic size (in water and medium respectively): 124.9-192.5 nm	<i>In vitro</i> human liver cells (HepG2)	1, 10, 20, 40 and 80 µg/mL after 6h	The FPG-modified comet assay revealed a significant ($p < 0.05$) concentration-dependent increase in oxidative DNA damage in response to TiO ₂ NP exposure as analysed using qualitative and quantitative parameters of the comet assay viz. OTM and % Tail DNA respectively. FPG elicited a significantly greater response at all the concentrations of TiO ₂ NPs	+	[177]
TiO ₂	Two samples of TiO ₂ : A1 and A2. XRD crystal form analysis: respectively anatase and mixture anatase:rutile 2:8 ratio. SEM diameter:	<i>In vitro</i> Erythrocytes	4.8 µg/mL for 1h	To investigate the presence of DNA damage due to oxidation of pyrimidine and purine bases, Endo III and FPG enzymes were used, respectively. A statistically significant increase in the % tail DNA was observed in the	+	[178]

	spherical 10-20 nm, spherical 20-150 nm			presence of A1 and A2 TiO ₂ NPs when the slides were incubated with both enzymes. TiO ₂ NPs (4.8 µg/mL) induce DNA damage and the entity of the damage is independent from the type (A1 or A2) of TiO ₂ NPs used.		
TiO ₂	TEM average particle size: 15 nm. DLS average hydrodynamic radius: 820 nm. BET surface area: 190-290 m ² /g	<i>In vitro</i> and <i>in vivo</i> Tetrahymena thermophila cells	Two different concentrations (1 - 0.1 µg/mL and 2 - 100 µg/mL). Three different exposure scenarios (acellular, <i>in vitro</i> , <i>in vivo</i>) were applied and two different protocols of comet assay (alkaline lysis and neutral lysis)	After alkaline lysis indicated significant damage of DNA in <i>T. thermophila</i> in both <i>in vivo</i> and <i>in vitro</i> treatments. This was independent of the concentration of particles. Statistical analysis of comet assays by neutral lysis showed that in cells treated, the average DNA tail length does not significantly differ from that in control cells	+	[179]
TiO ₂	XRD average size: 30.6 nm. DLS diameter in medium and water respectively: aggregates and	<i>In vitro</i> human amnion epithelial (WISH) cells	0.625, 1.25, 2.5, 5.0, 10, 20 µg/kg for 6 h	A significant induction ($p < 0.05$) in DNA damage (% DNA tail: 23.94 ± 0.66) at a concentration of 20 µg/mL in neutral comet assay was observed	+	[180]

	particles of 13 and 152 nm and in water aggregates of 380 nm. (Crystalline polyhedral rutile synthesized for this study)					
TiO ₂	Listed by the manufacturer: Primary particle size: 27.5 nm. Specific surface area: 49 m ² /g.. DLS analysis of the NPs in different media is also reported. (Crystal form 86% anastase and 14% rutile)	<i>In vitro</i> human lung cells (BEAS-2B) in three different dispersion media (KF, KB, and DM)	Different concentrations from 0 to 100 µg/mL for 24 h	There was a concentration-dependent increase in DNA damage after TiO ₂ NP exposure in all three treatment media that was genotoxic but statistically significant. All concentrations in all treatment media induced DNA damage that was significantly greater than the concurrent negative control except for two points: 10 µg/mL in KF and 50 µg/mL in DM	+	[181]
TiO ₂	Listed by the manufacturer: Primary particle size: 27.5 nm. Specific surface area: 49 m ² /g. DLS analysis of the NPs in different media is also reported.	<i>In vitro</i> human liver cells (HepG2)	Different concentrations from 0 to 100 µg/mL for 24h	DNA damage increased significantly with increasing concentrations of nano-TiO ₂ in both treatment media, indicating a genotoxic effect. The responses at the two highest concentrations were significantly greater than	+	[182]

	(Crystal form 86% anatase and 14% rutile.)			the control; however, the type of medium used did not influence the level of DNA damage		
TiO ₂	Listed by the manufacturer: Field-emission-gun scanning electron microscopy (FEG-SEM) particle size within the agglomerates and aggregates TiO ₂ -An and TiO ₂ -Ru: <25 and <100 nm. XRD analysis of crystal: TiO ₂ -An anatase and TiO ₂ -Ru rutile form. BET specific surface area: 129.3 and 116.7 m ² /g	<i>In vitro</i> human hepatic carcinoma cells (HepG2)	Four concentration levels: 0, 1, 10, 100, 250 µg/mL for 2, 4 and 24 h	TiO ₂ -An, but not TiO ₂ -Ru, caused a persistent increase in DNA strand breaks (comet assay) and oxidized purines (FPG-comet). In HepG2 cells exposed to TiO ₂ -An NPs we detected slight, however statistically significant (P < 0.05) greater amount of DNA strand breaks than in the control	+	[183]
TiO ₂	Nominal size: <25 nm. BET specific surface area: 129.3 m ² /g. XRD analysis: 18 nm. FEG-SEM: 1	HepG2 cells	0, 1, 10, 100, 250 µg/mL for 2, 4 and 24 h	UV pre-irradiated TiO ₂ -B induced significant (p < 0.05) increases in FPG-sensitive sites after 2 h and 4 h exposure at all of the concentrations tested.	+	[184]

TiO ₂	Nanopowder nominal size: <25 nm. DLS mean hydrodynamic diameter: 92±3.6 at 0h in the dispersion of 12.5 µg/mL.	Allium cepa Root Tip	When the roots reached 2 to 3 cm in length they were treated with different concentrations (12.5, 25, 50, 100 µg/mL) of TiO ₂ NP suspensions for 4 h	The lowest reported exposure concentration of TiO ₂ NPs to exert a significant damage to DNA was 20 µg/mL. Olive Tail Moments of about 2.3460.74 and 8.662.81% was observed at the test concentrations of 12.5 µg/mL and 100 µg/mL respectively indicating damaged DNA structure	+	[185]
TiO ₂	XRD analysis of different samples: 9, 10 and 10 nm. (NM101, NRCWE001, NRCWE002, NRCWE003 that are in anatase form, respectively with no coating, no coating, positive charged and negative charged). TEM analysis respectively: 4-8/50-100, 80-400, 80-400 and 80-400 nm) A sample of	<i>In vitro</i> HK-2 cells	0.8, 40, 20, 60, and 40 µg/cm ² after a 4h treatment	Significant tail increase in FPG-modified comet for one of the samples of TiO ₂ , others small but significant	+	[186]

	XRD size: approximately 100 nm (NRCWE004). In this sample five different particle types were identified.					
TiO ₂	Two different types of TiO ₂ were used; Nominal size: 20 and 7 nm. BET surface area: 66.604 and 300.81 m ² /g	<i>In vivo</i> in the freshwater crustacean Daphnia magna and the larva of the aquatic midge Chironomus riparius	The fish were collected 24 h from the control and experimental tanks after exposure to 1 µg/L of NPs	No genotoxic effect on either species as no significant increase in the tail/Olive Tail Moments was observed in these species when exposed	-	[111]
V ₂ O ₃	Nominal size of spherical diameter: approximately 70 nm. TEM average diameter: 25 nm. TEM length: 100 – 1.000 nm. (Needle-like structure)	<i>In vitro</i> human epithelial lung cell line (A549)	Concentrations: 1 and 2 µg/cm ² , time: 24, 36, 48 h via inhalation	Positive	+	[52]
V ₂ O ₅	Nominal size of spherical diameter: 170 – 180 nm.	<i>In vitro</i> human epithelial lung cell line (A549)	Concentrations: 1 and 2 µg/cm ² , time: 24, 36, 48 h via inhalation	Negative	-	[52]

	They are up to several hundred nanometer in length and usually have a diameter of less than 50 nm. (Rod-shaped)					
ZnO	<p>XRD analysis of different samples: 70 to > 100 and 58-93 nm. (NM110 and NM111, with no coating,). TEM size: 20-200/10-450 and 20-200/10-450 nm. BET surface area: 14 and 18 m²/g. Mainly 2 euhedral morphologies: 1) aspect ratio close to 1; 2) ratio 2 to 7.5. Minor amounts of particles with irregular morphologies observed.</p>	<i>In vitro</i> HK-2 cells	0.8, 40, 20, 60, and 40 µg/cm ² after a 4h treatment	No significant tail increase in % tail in FPG-modified comet for one of the samples of ZnO, others small but significant	+	[186]

	M111 as M110 but with different size distributions. 1) particles with aspect ratio close to 1; 2) particles with aspect ratio 2 to 8.					
ZnO	Two NPs of ZnO have been evaluated. EZ-1 (coated) and EZ-2 (uncoated) with the following characterization results respectively: TEM primary size: 30±20, 40±20 nm. XRD powder hydrodynamic diameter in water: 70-150 and 90-160 nm.	<i>In vitro</i> A549, HT29, HaCaT cells	0, 0.1, 1, 10 µg/mL for	The results show a small, but significant increase in DNA damage compared to that of controls at concentrations of 10 µg/mL ZnO for HT29 cells for the polymer coated NPs. For The HaCaT cells, only the polymer coated NPs (EZ-1) show an increase in DNA damage at 1 µg/mL, while the A549 cells are not significantly affected by any of the NPs.	+	[187]
ZnO	Respectively Nominal size and BET surface area: 10 nm, 70 m ² /g	<i>In vitro</i> human colon epithelium cell line (CaCo-2)	Cells were treated 4h with 20 µg/cm ² particles (in MEM without serum)	Significant effects found in FPG-modified comet assay.	+	[133]

	(nanoactive aggregates) and 20 nm, 50 m ² /g (nanoscale particles).					
ZnO	TEM mean size 30 nm. DLS: 165 nm.	<i>In vitro</i> human epidermal cell line (A431)	0.001, 0.008, 0.08, 0.8, 5 µg/mL for 6h	A significant induction (p < 0.05) in DNA damage was observed in cells exposed to ZnO NPs for 6 h at 5 and 0.8 µg/mL concentrations compared to control.	+	[188]
ZnO	TEM analysis: 30, 50 and 70 nm. DLS size in water: 272 nm.	<i>In vivo</i> liver and kidney cells of mice after oral exposure	50 and 300 mg/kg of NPs for 14 days	In both alkaline and FPG-modified comet assay, at the highest concentration DNA % tail was significantly increased (16.15±1.56)	+	[189]
ZnO	TEM analysis: 30 nm. DLS hydrodynamic size in water: 165 nm.	<i>In vitro</i> A431 cells	Cells were exposed to 0.001, 0.008, 0.08, 0.8, 5 µg/mL for 6 h	A significant induction (p < 0.05) in DNA damage was observed in cells exposed to ZnO NPs for 6 h at 5 and 0.8 µg/mL concentrations compared to control respectively % DNA tail were 10.6 ± 0.76 and 13 ± 1.5.	+	[188]
ZnO	Respectively Nominal size and BET: 50 and 70 nm. TEM average size: 30 nm. DLS mean hydrodynamic	<i>In vivo</i> mice liver and kidney cells	50 and 300 mg/kg mice oral exposure for 14 consecutive days	A statistically significant (p < 0.05) qualitative and quantitative increase in the oxidative DNA damage was observed in the liver of mice exposed to the	+/-	[189]

	diameter (water): 272 nm.			higher dose (300 mg/kg) in the presence of FPG. However, no significant DNA damage was observed in the mice administered with the lower dose (50 mg/kg). There was no significant difference in the comet parameters in the kidney cells of control and ZnO NPs exposed mice.		
ZnO	TEM analysis: 20.2 ± 6.4 nm	<i>In vitro</i> primary mouse embryo fibroblasts (PMEF)	5 and 10 µg/mL particles in DMEM for 24 h	There were significant increases in tail length, percentage of DNA in tail, tail moment and Olive Tail Moment after PMEF cells were treated with at both examined concentrations (18.8% tail DNA, $p < 0.01$) in alkaline comet assay.	+	[55]
ZnO	Nanopowder nominal size: 71 nm. TEM analysis: 20-200 nm. DLS analysis: 320 nm.	<i>In vitro</i> A549 cells	1 µg/cm ² (2 µg/mL), 20 µg/cm ² (40 µg/mL), and 40 µg/cm ² (80 µg/mL) for 4 h. FPG sensitive sites were measured at 20 µg/cm ² , and 40 µg/cm ² after 4h.	DNA damage was induced when cells were exposed for 4 h to 40 µg/cm ² (12% tail, $p < 0.05$). ZnO showed statistically significant ($p < 0.05$) increased levels of oxidative DNA lesions compared to those of the control in the highest dose.	+	[114]
ZnO	DLS analysis	<i>In vitro</i> human	20-30-40 µg/mL for 3h	These increases were	+	[190]

	hydrodynamic diameter: 243.7 nm (in water), 273.4 (medium). Nominal size (BET analysis): 100 nm. Surface area: 15-25 mg(Provided by the supplier)	neuroblastoma SHSY5Y cell line	and 6h	statistically significant in all the conditions tested in the comet assay, except for the highest concentration after the 6 h exposure.		
ZnO	Nominal size: 20 and 70 nm. SEM analysis: 35 ± 5 , 28 ± 8 , 70 ± 19 , and 72 ± 11 nm. DLS hydrodynamic size ranges from 200 to 400 nm, 180 to 300 nm, 300 to 900 nm, and 200to 500 nm [20 nm (+) charge, 20 nm (-) charge, 70 nm (+) charge, and 70 nm (-) charge NPs, respectively	<i>In vivo</i> male Crl: CD (SD) rats liver and stomach single cells	Three dose levels: 500, 1000, and 2000 mg/kg body weight. Test substance was administered three times by gavage at 0, 24, and 45h	Tail intensity of liver and stomach single cells treated with ZnO NPs with 20 nm (+) and (-) charge had no significant increase in comparison with solvent control group. The results of 70 nm (+) and (-) charged ZnO NPs also revealed no significant increase in tail intensity.	-	[191]
ZnO	DLS particle size: 45-150 nm. TEM: Spherical, triangolare and	<i>In vitro</i> human lymphocyte cells	0, 125, 500, and 1000 µg/mL for 3h	Significant ($p < 0.05$) increase in DNA fragmentation at 1000 µg/mL which is much	-	[192]

	hexagonal structures size 45-150 nm and average diameter: 75 ± 5 nm.			higher than predicted concentrations. These NPs are safe up to 500 µg/mL.		
ZnO	Nominal size: 100 nm (NM 110). TEM analysis: 20-250/50-350 nm. DLS analysis (MEM): 306 nm	<i>In vitro</i> human hepatoblastoma C3A cells	Three concentration levels: 0.62, 1.25 and 2.5 µg/cm ² . Exposure duration: 4h	A small but significant increase in percentage tail DNA following exposure was observed	+	[156]
ZnO	Nominal size: 10-20 nm. No further characterization reported in the paper	<i>In vivo</i> earthworm <i>Eisenia fetida</i> (Savigny, 1826)	Four dose levels: 0.1, 0.5, 1.0, 5.0 g/kg dry soil. Exposure duration: 7 days	Earthworms exhibited DNA damage when exposed to ZnO at 1 and 5 g/kg. At 5 g/kg the degrees of DNA damage were significant when compared to controls.	+	[159]
ZnO	Nominal size < 100 nm. TEM analysis: rod shaped 86 ± 41 nm X 42 ± 21 nm; mean diameter was 353 nm	<i>In vitro</i> primary human nasal mucosa cells	Five concentration levels: 0.01, 0.1, 5, 10 and 50 µg/ml. Exposure duration: 24h	A ZnO-NP concentration-dependent increase in the Olive Tail Moment (OTM) as an indicator for genotoxic effects could be seen. The enhanced DNA migration was significant at 10 µg/ml and 50 µg/ml.	+	[193]
ZnO	Nominal size: 50–80 nm size and average	<i>In vitro Allium cepa</i> root	Three concentration levels: 10,100 and 1000 µg/ml. Exposure	The results obtained in the comet assay show that both tested ZnO NPs	+	[165]

	<p>particle size \leq 35 nm. DLS analysis: 50 ± 0.3 nm for ZnO 50 nm NPs and 35 ± 1.1 nm for ZnO NPs (\leq 35 nm)</p>	meristem cells	duration: 1h	are genotoxic in the root meristem cells of <i>A. cepa</i> in terms of both the percentage of DNA in tail and tail moment.		
ZnO	<p>Nominal size: 50–80 nm size and average particle size \leq35 nm. DLS analysis: 50.75 ± 0.0 nm for ZnO 50–80 nm) NPs and 36.42 ± 0.1 nm for ZnO (\leq35 nm) NPs</p>	<p><i>In vitro</i> human embryonic kidney (HEK293) and mouse embryonic fibroblast (NIH/3T3) cells</p>	<p>Three concentration levels: 10,100 and 1000 μg/ml. Exposure duration: 1h</p>	<p>The results show that both tested ZnO NPs are genotoxic in the two cell lines used. The significance of the genotoxicity results were independently of using the percent-age of DNA in tail or the tail moment. The induced genotoxicity followed a direct dose–response effect with positive induction at 100 and 1000 μg/mL concentrations</p>	+	[194]
ZnO	<p>TEM analysis: 17 nm. DLS analysis: average hydrodynamic size 263.0 nm (in medium)</p>	<p><i>In vitro</i> human skin melanoma (A375) cells</p>	<p>Three concentration levels: 5, 10 and 20 μg/ml. Cells were treated for 24 and 48h</p>	<p>Cells exposed to different concentrations of ZnO NPs showed significantly more DNA damage than did the control cells.</p>	+	[195]

ZnO	Nominal average particle size: 50 nm. TEM analysis: 22 nm. DLS analysis: average hydrodynamic size 264.8 nm (in water)	<i>In vivo</i> freshwater snail <i>Lymnaea luteola</i> digestive gland cells	Three dose levels: 10, 21 and 32 µg/ml. Isolation of digestive gland cells was done at intervals of 24 and 96h	The cells exposed to different concentrations of ZnO NPs exhibited significantly higher DNA damage in cells than those of the control groups.	+	[196]
ZnO	Nominal size: <100 nm. DLS analysis: 612 ± 10.9 nm (water) 5294 ± 3184 nm (medium)	<i>In vitro</i> <i>S. cerevisiae</i> cells	One concentration level: 31.25 mg/l. No information about time exposure	A significant amount of DNA damage was detected in NP-exposed cells when compared with controls.	+	[121]
ZnO	SEM analysis: 40-70 nm.	<i>In vitro</i> lymphocytes and sperm cells	Approximately 4–93 µg/mL in PBS for 30 min in the dark, preirradiated and simultaneous irradiation with UV	The ZnO particles are capable of inducing genotoxic effects on human sperm and lymphocytes and that the effect of ZnO is enhanced by UV both in case of lymphocytes and sperm, but effects are only statistically significantly different from responses in the dark at the highest	+	[155]

				does after pre-irradiation and simultaneous irradiation.		
ZrO ₂	Nominal size: 6 nm. TEM analysis: average hydrodynamic diameter 6 ± 0.8nm.	<i>In vitro</i> human embryonic kidney (HEK293) cells and human peripheral blood lymphocytes	Concentrations of 1, 10, or 100 µg/mL	Results demonstrated that at all concentration NP did not induce any marked genotoxicity.	-	[103]

Table 2. Current review of genotoxicity studies (Micronucleus test) on metal oxide NPs (+ positive; - negative; +/- equivocal; bw = body weight). As explained in section 3, each row in this table summarizes all genotoxicity data found for this test for all nanomaterials with a given core chemical composition reported in a given publication.

Nanomaterial core chemical composition	Characteristics (Size)	Cell type and assay	Exposure	Results	Summary	Ref.
Al ₂ O ₃	Two aluminium oxide NMs were studied. Nominal diameter: 30 nm and 40 nm. TEM analysis: 39.85±31.33 nm and 47.33±36.13 nm	<i>In vivo</i> inbred female albino Wistar rats bone marrow cells	Three dose levels (oral administration): 500, 1000 and 2000 mg/kg body weight. The study was performed at 30 and 48 h of sampling times	Significantly increased frequency of MN was observed with 1000 and 2000 mg/kg body weight dose levels of Aluminum oxide 30 nm and Aluminum oxide 40 nm over control at 30 h. Likewise, at 48 h sampling time a significant increase in frequency of MN was evident at 1000 and 2000 mg/kg body weight dose levels of Aluminum oxide 30 nm and Aluminum oxide 40 nm	+	[197]

				compared to control.		
Al ₂ O ₃	Two aluminium oxide NMs were studied. Nominal diameters: 30 nm and 40 nm. TEM analysis: 39.85 ± 31.33 nm and 47.33 ± 36.13 nm. DLS analysis: average diameters 212.0 and 226.1 nm in water.	<i>In vivo</i> female Wistar rat peripheral blood cells	Three dose levels (via gavage): 500, 1000 and 2000 mg/kg body weight. Whole blood was collected at 48 and 72 h	Data indicated statistically significant effects on micronuclei frequency after treatment with both NMs compared to the control group at 1000 and 2000 mg/kg dose levels after 48 and 72 h.	+	[81]
CeO ₂	Nominal size < 25 nm. TEM analysis: 25 ± 1.512 nm. DLS analysis: hydrodynamic diameter 269.7 ± 27.398 nm	<i>In vitro</i> human neuroblastoma (IMR32) cells	Five concentration levels: 10, 20, 50, 100, and 200 mg/ml. Exposure duration: 24 h	At concentration levels of 100 and 200 mg/ml the frequency of micronucleus in binucleated cells was increased significantly.	+	[106]
CeO ₂	Nominal size: 7 nm. DLS analysis: hydrodynamic diameter 15 nm	<i>In vitro</i> human dermal fibroblasts	Four concentration levels: 0.06, 0.6, 6 and 60 mg/l. Exposure duration: 48 h	Binucleated micronucleated fibroblast frequencies were significantly increased in a dose-dependence manner from the lowest tested concentrations (0.06 mg/l)	+	[107]
CuO	Four different CuO nanoparticles were studied, with the following size measurements and shapes determined by	<i>In vitro</i> human murine macrophages RAW 264.7 cells and peripheral blood	Three concentration levels: 0.1, 1 and 10 µg/ml. Exposure duration: 48 h	In all tested NMs, macrophages showed a higher number of micronucleated cells than lymphocytes, except for the NM (1). Spheres and spindles showed a dose-dependent increase in the	+	[115]

	TEM: (1) 10-100 nm (unspecified shape); (2) 7±1 nm (spheres); (3) 7±1×40±10 nm (rods); (4) 1200±250×270±50×30±10 nm (spindles)	lymphocytes (PBL).		micronuclei frequency in macrophages.		
CuO	Two different CuO NMs were studied. Nominal average size between 30 and 40 nm. SEM analysis: diameter between 70 and 100 nm. TEM analysis: protein-NPs complex had a total average size of 356 ± 70 nm	<i>In vitro</i> mouse neuroblastoma (Neuro-2A) cells	Four concentration levels: 12.5, 25, 50 and 100. Exposure duration: 24 h	Treated cells showed a significant increase in the frequency of micronuclei at the lowest concentration level	+	[198]
CuO	Nominal size between 27.2 and 95.3 nm	<i>In vivo</i> female ICR mice peripheral blood cells	Two dose levels (intraperitoneal injection): 1 mg/mouse and 3 mg/mouse. Exposure time: 24, 48 and 72 h	A significant differences was observed between control and 3 mg doses treated cells	+	[86]
Fe ₂ O ₃	Nominal size < 50 nm. TEM analysis: mean size 29.75 ± 1.87 nm. DLS analysis: hydrodynamic diameter 363 nm (water)	<i>In vivo</i> albino Wistar female rat peripheral blood and bone marrow cells	Three dose levels (oral administration): 500, 1000 and 2000 mg/kg body weight. Peripheral blood cells exposure duration: 48 and 72 h. Bone marrow cells exposure duration: 24 and 48 h	The frequencies of micronuclei were statistically insignificant at all doses in both cell lines and at every exposure duration	-	[126]
Fe ₂ O ₃	Nominal size between 60 and 100 nm	<i>In vivo</i> female ICR mice peripheral	Two dose levels: 1 mg/mouse and 3 mg/mouse. Exposure	A significant increase in micronucleated reticulocytes cells was observed	+	[86]

		blood cells	time: 72 h			
Fe ₃ O ₄	Nominal size between 20 nm and 60 nm. Photon correlation spectroscopy (PCS) analysis: mean diameter 311 nm	<i>In vitro</i> human lung adenocarcinoma type-II alveolar epithelial cells A549	Four concentration levels: 1, 10, 50 and 100 µg/cm ² . Exposure duration: 24 h	A significantly enhanced MN induction was already observed at 10 µg/cm ² , reaching a maximum at 100 µg/cm ²	+	[131]
Fe ₃ O ₄	TEM analysis: primary diameter of 26.1 ± 5.2 nm	<i>In vivo</i> kunming mice bone marrow cells	Four dose levels: 5, 2.5, 1.25, and 0.625 g/kg. The 30 h injection method was used, ie, a 24 h interval between two injections with a 6-hour wait after the second injection	No significant difference was found between the test animals and the negative controls	-	[199]
Fe ₃ O ₄	Nominal size: 80 nm	<i>In vivo</i> female ICR mice peripheral blood cells	Two dose levels: 1 mg/mouse and 3 mg/mouse. Exposure time: 72 h	Significant increases in micronucleated reticulocytes cells was observed	+	[86]
MnO ₂	MnO ₂ nanopowder nominal size of <30 nm. TEM analysis: mean size distribution 45 ± 17 nm. DLS size in the Milli Q water suspension was 334.4 nm	<i>In vivo</i> bone marrow cells extracted from the femurs of female albino Wistar rats	Three dose levels: 100, 500 and 1000 mg/kg body weight. The study was performed at 24 and 48 h after treatment	The data revealed statistically significant enhancement in the MN frequency in the groups treated with 1000 mg/kg body weight of MnO ₂ -45 nm at 24 and 48 h of sampling times	+	[128]
SiO ₂ (amorphous)	Nominal size: 15 nm and 55 nm. DLS analysis: z-average particle diameter (pH 7.5) 31.6 nm and 105.1 nm	<i>In vitro</i> human peripheral blood lymphocytes (HPBLs); <i>In vivo</i> Wistar rat	(<i>In vitro</i>) Four concentration levels: 31.6, 100, 316, 1000 µg/mL. Exposure duration 24h. (<i>In vivo</i>) Single concentration	For both the 15 nm and 55 nm silica NPs, no increase in the % MN was observed with any of these particles at any dose tested in this <i>in vitro</i> system in HPBLs. (<i>In vivo</i>) Injection of silica NPs resulted in a	+/-	[82]

		peripheral blood cells	for 15 nm SiO ₂ : 50 mg/kg; two concentration levels for 55 nm SiO ₂ : 25 mg/kg and 125 mg/kg. Rats injected i.v. at 48h, 24h, and 4h prior to tissue collection	dose-dependent increase in DNA damage in liver and lung tissue and in white blood cells		
SiO ₂ (amorphous)	DLS analysis: hydrodynamic diameter 12 nm in DMEM and 75 nm in DMEM + 10%FBS	<i>In vitro</i> A549 human lung carcinoma cells	Three concentration levels: 1.5, 2.5, 5 µg/mL. Cells were treated for 40h	No induction of MN was observed compared to the untreated control both in 10% serum and in 0% serum	-	[200]
SiO ₂ (amorphous)	DLS hydrodynamic diameter: ranging from 12 nm to 174 nm without foetal calf serum (FCS) and from 52 nm to 258 nm in FCS	<i>In vitro</i> A549 human lung carcinoma cells	Concentrations range between 0 µg/mL and 1056 µg/mL. Cells were treated for 40h with different doses of the SNPs either in presence or absence of 10% FCS	A statistically significant increase in MN frequencies was observed after treatment with L-40 in the absence of serum as well as with L-40 and S-174 in the presence of serum and after treatment with L-40, S-59 and S-139 in the absence of serum. No dose dependency was observed	+	[201]
SiO ₂ (amorphous)	Nominal size: ranging from 5 nm to 80 nm. DLS analysis: mean particle diameter between 17.42 ± 0.16 and 185.1 ± 7.51 in ultrapure water; between 16.08 ± 0.81 and 332.6 ± 11.42 in serum-free cell culture medium	<i>In vitro</i> Balb/3T3 mouse fibroblasts	Cells were treated at the fixed concentration of 100 µg/mL for 24h	SiO ₂ NPs did not trigger the formation of micronuclei, suggesting that neither the size diameter nor the particles' synthesis procedure induces genotoxicity	-	[202]
SiO ₂ (quartz)	Nominal size: diameter <5 µm.	<i>In vitro</i> WIL2-NS (ATCC, CRL	Two concentration levels: 60, and 120	The results show that the frequency of	+	[138]

	High-performance particle sizer (HPPS) analysis after filtration: Z-Average size 12.2 nm	8155) human lymphoblastoid B-cell	µg/mL. Cyt-B was added and the cultures were incubated for 26h	MNed BNCs increased significantly with the increase of particle dose, from 5 MN per 1000 BNed cells at untreated control to 12 at 120 µg/mL of particles		
TiO ₂	TEM analysis: 12.1 ± 3.2 nm. Agglomerates in the treatment solution was found to be around 130 nm and around 170 nm in cell culture medium	<i>In vivo</i> male B6C3F1 mice blood cells	Three dose level: 0.5, 5.0 and 50 mg/kg for three consecutive days	No difference in %MN-RET frequencies between TiO ₂ -NP treated and control animals was observed	-	[203]
TiO ₂	X-ray diffraction (XRD) analysis: diameter ranging from 7 nm to 10 nm. DLS analysis: hydrodynamic diameter ranging from 139 nm to 211 nm in DMEM and from 109 nm to 233 nm in DMEM + 10%FBS	<i>In vitro</i> A549 human lung carcinoma cells	Four concentration levels: 50, 75, 125, 250 µg/mL. Cells were treated for 40h	Results are not available as the MN were obscured by NM agglomerates over the cells and thus could not be scored	+/-	[200]
TiO ₂	Nominal size: 25 nm. DLS analysis: mean particle size 300 nm	<i>In vitro</i> human lymphocytes and hamster lung fibroblasts V79 cells	Four concentration levels: 20, 60, 100 and 250 µg/mL. Cells were exposed without metabolic activation system for 24h	Weak mutagenic effect on human lymphocytes at 60–250 µg/mL	+	[146]
TiO ₂	Nominal size: 40 ± 5 nm. SEM analysis: average size distribution 42.30 ± 4.60 nm	<i>In vivo</i> ICR mice bone marrow cells	Four dose levels: 140, 300, 645, and 1387 mg/kg body weight. Blood samples were collected 14 days after treatment	Micronucleus test result 14 days after a single intravenous injection of different doses of TiO ₂ NPs shows no significant increase in micronucleus cell number	-	[204]
TiO ₂	Nominal size < 25 nm.	<i>In vivo</i> P.	The fish were exposed	Micronuclei were not detected, but	-	[148]

	DLS hydrodynamic size (Z-average): 1611 ± 21 nm after 24h	mesopotamicus (pacu caranha) erythrocytes cells	(with visible light or ultraviolet and visible light) to the following concentrations of nano-TiO ₂ during a 96 h period: 0 (control), 1, 10, and 100 mg/l.	the extent of morphological alterations in the erythrocyte nuclei revealed an influence of the type of illumination, since the alterations were more prevalent in groups exposed to UV light		
TiO ₂	Nominal size: 21 nm. DLS hydrodynamic diameter: 129.50 ± 2.6 nm	<i>In vivo</i> Adult male Wistar rats bone marrow cells	Single dose of 5 mg/kg body weight. Animals were sacrificed at 24h, 1 week and 4 weeks after the injections	A significantly elevated frequency of MN was observed for TiO ₂ NPs after 24h. The frequencies of micronuclei were statistically insignificant after 1 and 4 weeks.	+	[147]
TiO ₂	Nominal average size: 75 ± 15 nm. ZetaSizer Nano ZS90 hydrodynamic diameter: 473.6 nm and 486.8 nm size when suspended in H ₂ O and FBS-free DMEM	<i>In vivo</i> Sprague-Dawley male rat bone marrow cells	Three dose levels: 10, 50 and 200 mg/kg body weight every day for 30 days	These results showed that TiO ₂ NPs could induce DNA double strand breaks in bone marrow cells after oral administration, but no significant chromosomes or mitotic apparatus damage and toxicity were found in bone marrow cells.	+	[149]
TiO ₂	UF-TiO ₂ particle size ≤ 20 nm. No further investigations are reported in the paper	<i>In vitro</i> Syrian hamster embryo (SHE) cells.	Cells were treated with different concentrations: 0.5, 1.0, 5, and 10 µg/cm ² , for different periods: 12, 24, 48, 66, and 72 h	UF-TiO ₂ induced MN, which significantly increased at concentrations between 0.5 and 5.0 µg/cm ²	+	[205]
TiO ₂	TiO ₂ anatase at 10 nm (Hombikat UV100) and 20 nm (Millenium PC500) in diameter. No further investigations are reported in the paper	<i>In vitro</i> human bronchial epithelial cells, BEAS-2B (ATCC CRL-9609)	Cells were treated with 10 g/mL of TiO ₂ for 24 h	The results indicated that treatment with anatase-sized (10 and 200 nm) TiO ₂ increased micronuclei	+	[169]
TiO ₂	No characterization of the NM is reported in the	<i>In vitro</i> Chinese hamster ovary-	Cells were treated with various	Results show that TiO ₂ significantly induced MN in CHO-K1 cells using	+	[206]

	paper	K1 (CHO-K1) cells	concentrations of TiO ₂ (0 to 20 µM) for 18h	cytokinesis block technique. Furthermore, the frequency of MN was slightly enhanced by TiO ₂ in the conventional MN assay system, i.e., without cytokinesis block		
TiO ₂	Ultrafine TiO ₂ (UF1 - uncoated anatase) average crystal sizes: 20 nm. No further investigations are reported in the paper	<i>In vitro</i> rat liver epithelial cell (RLE)	Three concentrations: 5, 10 and 20 µg/cm ² . All the cultures were treated with cytochalasin B and incubated for 20 h. A duplicate series of experiments was carried out by irradiating the TiO ₂ exposed cells with near-UV light	No observed increase of the number of micronucleated cells. Exposure of the cells to UV light gave a slight but not statistically significant effect, Interestingly, TiO ₂ appeared to have a slight decreasing effect on the frequency of micronuclei at the lowest treatment concentrations both in the presence and in the absence of UV irradiation.	-	[207]
TiO ₂	Particle size distribution measured by high-performance particle sizer (HPPS): by volume 6.57 nm (100%); by intensity: 8.2 nm (80.4%) and 196.52 nm (19.4%)	<i>In vitro</i> WIL2-NS human lymphoblastoid cells	Cells were treated with 26, 65 and 130 µg/mL of UF-TiO ₂ . Cyt-B was added and the cultures were incubated for 26h	Exposure to UF-TiO ₂ resulted in significant increases in MNBNCs compared to untreated control.	+	[173]
TiO ₂	Nominal size: 19.7–101.0 nm. No further investigations are reported in the paper	<i>In vivo</i> female ICR mice peripheral blood cells	Two dose levels: 1 mg/mouse and 3 mg/mouse. Exposure time: 72h	Significant increases in micronucleated reticulocytes (MNRETs) observed	+	[86]
TiO ₂	TEM analysis: cross sectional diameter 24.4 ± 0.5 nm	<i>In vitro</i> RTG-2 gonadal tissue fish cell line from rainbow trout	Two concentration levels: 5 and 50 µg/mL. 48h time exposure before adding the	No significant difference in micronuclei induction over the control. Decreases in frequencies of MN were observed with the ENP treatments, which in	-	[172]

			cyto-B for 48 h	addition, had little effect on cell division or cytotoxicity		
V ₂ O ₃	Nominal size of spherical diameter: approximately 70 nm. TEM average diameter: 25 nm. TEM length: 100 – 1.000 nm. (Needle-like structure)	<i>In vitro</i> human epithelial lung cell line (A549)	Two exposure levels: 1 and 2 µg/cm ² . 24 hours time of exposure via inhalation	No induction of micronuclei was observed in the micronucleus test but morphological changes in cell nuclei.	-	[52]
V ₂ O ₅	Nominal size of spherical diameter: 170 –180 nm. They are up to several hundred nanometer in length and usually have a diameter of less than 50 nm. (Rod-shaped)	<i>In vitro</i> human epithelial lung cell line (A549)	Two exposure levels: 1 and 2 µg/cm ² . 24 hours time of exposure via inhalation	No induction of micronuclei was observed in the micronucleus test but morphological changes in cell nuclei.	-	[52]
WO ₃	No characterization of the NM is reported in the paper	<i>In vivo</i> bone marrow cells of male Sprague-Dawley rats	Three dose levels: 25, 50 and 100 mg/kg body weight. Animals received daily intraperitoneal injections of WO ₃ for 30 days.	No statistically significant difference was found between 25 mg WO ₃ applied and control group. On the contrary, the higher doses of WO ₃ (50 and 100 mg) caused increases of MN rates	+	[83]
ZnO	Nominal size: 20 and 70 nm. SEM analysis: 35 ± 5, 28 ± 8, 70 ± 19, and 72 ± 11 nm. DLS hydrodynamic size ranges from 200 to 400 nm, 180 to 300 nm, 300 to 900 nm, and 200 to 500 nm [20 nm (+) charge, 20 nm (-) charge,	<i>In vivo</i> Out-bred mice of strain ICR, 6–7 bone marrow cells	Three dose levels: 500, 1000, and 2000 mg/kg body weight. The test substance was given twice with a 24h interval	The frequencies of MNPCE (micronucleated polychromatic erythrocyte) were not represented statistical significance and dose-dependent response at any dose on four kinds of ZnO NPs	-	[191]

	70 nm (+) charge, and 70 nm (–) charge NPs, respectively					
ZnO	X-ray diffraction (XRD) analysis: diameter ranging from 71 to >100. DLS analysis: hydrodynamic diameter 250 (±100) in DMEM and 258 (±93) in DMEM + 10%FBS	<i>In vitro</i> A549 human lung carcinoma cells	Three concentration levels: 10, 25, 50 µg/mL. Cells were treated for 40h	No significant increase in MN was shown for ZnO NM, except at the highest dose tested (50 µg/mL) in the presence of 10% serum, where high toxicity was observed	-	[200]
ZnO	Nominal size < 100 nm. DLS analysis: effective diameter 120 ± 2.6 nm	<i>In vitro</i> A. cepa root cells	Four concentration levels: 25, 50, 75 and 100 µg/mL. Exposure duration: 4h	Dose dependent increase of MN was observed.	+	[208]
ZnO	TEM analysis: particle size 29 ± 10 nm	<i>In vitro</i> WIL2-NS human lymphoblastoid cells	Cells were cultured with 10 mg/l NPs for 24h	The assessment of DNA damage indicated significant increases in the frequency of MNi in cells exposed to OA-coated and PMAA-coated NPs, respectively, whereas there was no significant elevation of MNi found in cells exposed to uncoated or medium-coated NPs	+/-	[209]

Table 3. Current review of genotoxicity studies (Ames test) on metal oxide NMs. As discussed in section 3.3, in the Ames test one or more strains of *Salmonella* (*S. typhimurium*) and/or *E. coli* are used e.g. the *S. typhimurium* strains (TA) TA97a, TA98, TA100, TA102, TA1535 and TA1537 or the *E. coli* strain WP2u-vrA⁺ referred to in Table 3. It can also be used with or without metabolic activation i.e. typically with or without “S9- mix”. Typically, a single “positive” result in any one of these combinations results in the outcome of the Ames test being “positive” [97]. As explained in section 3, each row in this table summarizes all genotoxicity data found for this test for all nanomaterials with a given core chemical composition reported in a given publication.

Nano material core chemical composition	size (nm)	Ames outcome	TA97a	TA97a +S9	TA98	TA98 +S9	TA100	TA100 +S9	TA102	TA102 +S9	TA153 5	TA153 5 +S9	TA153 7	TA153 7 +S9	E. coli WP2u -vrA ⁻	E. coli WP2u -vrA ⁻ +S9	publications
Al ₂ O ₃	<50	-	-	-			-	-							-	-	[210]
Al ₂ O ₃	30-40	-	-	-	-	-	-	-	-	-	-	-					[81, 197]
Co ₃ O ₄	<50	-	-	-			-	-							-	-	[210]
CuO	<50	+	-	+			+	+							+	colony inhibition	[210]
Fe ₃ O ₄	8.0 ±2 (10-30ppm)	-			-	-	-	-	-	-							[132]
Fe ₃ O ₄	8.0 ±2 (70ppm)	+			-	-	-	+	-	-							[132]
TiO ₂	50	+			+	+	-	-			-	-	+	+	+	+	[211]
TiO ₂	<100	+	-				-								+	+	[210]
TiO ₂	10	-			-		-		-		-		-				[174]
TiO ₂	<100	+			+	+	-	+									[213]
ZnO	5.4	-			-	-	-	-					-	-	-	-	[214]
ZnO	30	+			-	+	-	-			-	-	-	+	-	+	[211]
ZnO	<100	+	-				-								+	+	[210]
ZnO	100	-			-		-						-		-		[215]

ZnO	20 and 70	-			-	-	-	-			-	-	-	-	-	-	[191]
WO ₃	<100	+			+		-				-				-		[216]
In ₂ O ₃	<100	+			+		-				-				-		[216]
Dy ₂ O ₃	<100	+			+		+				+				+		[216]

4. Survey of available genotoxicity data for metal oxide NMs in the literature

In order to estimate an overall situation with genotoxicity tests for NPs, we have performed a literature search in the Scopus online database, searching articles published from January 1997 until July 2014 using the keywords: “genotoxicity” and “nanomaterial” or “nanoparticle”. The results of literature search were last updated in July 2014. The search identified more than 600 publications which contained the keywords mentioned above; the distribution of years of publication is shown in Figure 2. From these publications, 165 reporting experimental data relating only to metal oxide/silica NMs’ genotoxicity were selected. The data presented in these publications are summarized in Tables 1-3.

Furthermore, the selected publications were analyzed, identifying the particular genotoxicity test used, with Figure 3 showing the trend in the number of publications per year for each test type. Interestingly, the comet assay appears to be the most popular genotoxicity test for NMs at the current time. Furthermore, the number of publications reporting NM studies using the comet assay increases year on year, while the number of publications reporting use of the, currently second most popular, micronucleus test appears to be declining. Among the other two tests considered, the Ames and chromosome aberration tests, the use of the chromosome aberration test would appear to be declining whilst the Ames test is becoming increasingly popular for NMs, in spite of warnings regarding its suitability for NMs [37, 101].

From the 165 articles with genotoxicity data (Tables 1-3), 137 genotoxicity studies describe the use of the comet assay, 38 the micronucleus assay, 20 the Ames test and 6 the chromosome aberrations test (some papers include two or three tests), (Figure 4).

Based on Table 4 we can see that TiO_2 , SiO_2 and ZnO NMs are the most assessed NMs, out of the group of metal oxides/silica considered in this review, in genotoxicity studies (Table 4). The other types of NMs evaluated in these studies, out of the group of metal oxides/silica considered in this review, are, in descending order of number of publications presenting genotoxicity studies of NMs, as follows: Fe_3O_4 , Fe_2O_3 , CuO , Al_2O_3 , CeO_2 , Co_3O_4 , NiO , MnO_2 , WO_3 , V_2O_5 , Dy_2O_3 , In_2O_3 , ZrO_2 , MgO , V_2O_3 , Bi_2O_3 NMs.

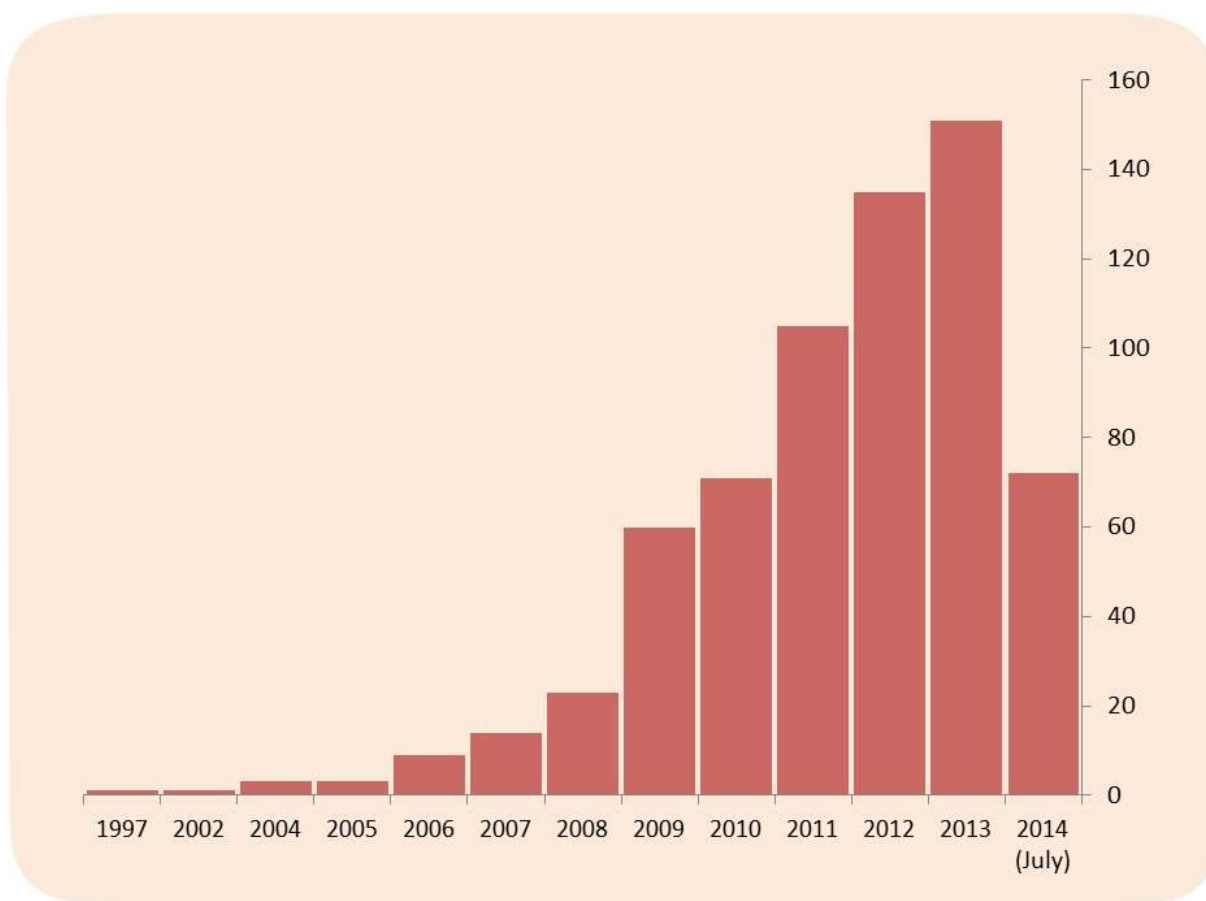


Figure 2. Literature results in terms of number of papers per year on genotoxicity of NMs performed in the Scopus online database from year 1997 using “nanoparticle” or “nanomaterial” and “genotoxicity” as key words.

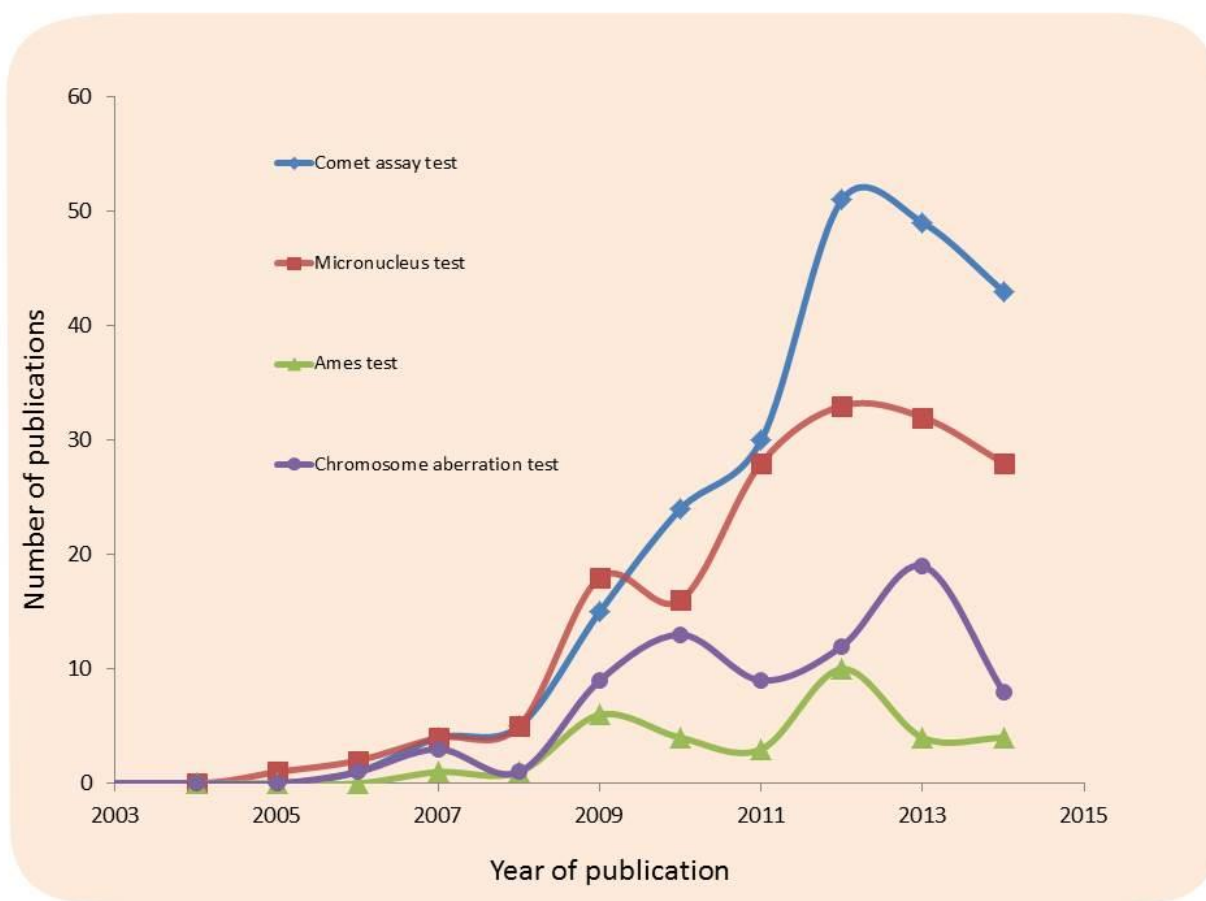


Figure 3. Number of publications per each genotoxicity test in a range from 1997 to 2014 (July).

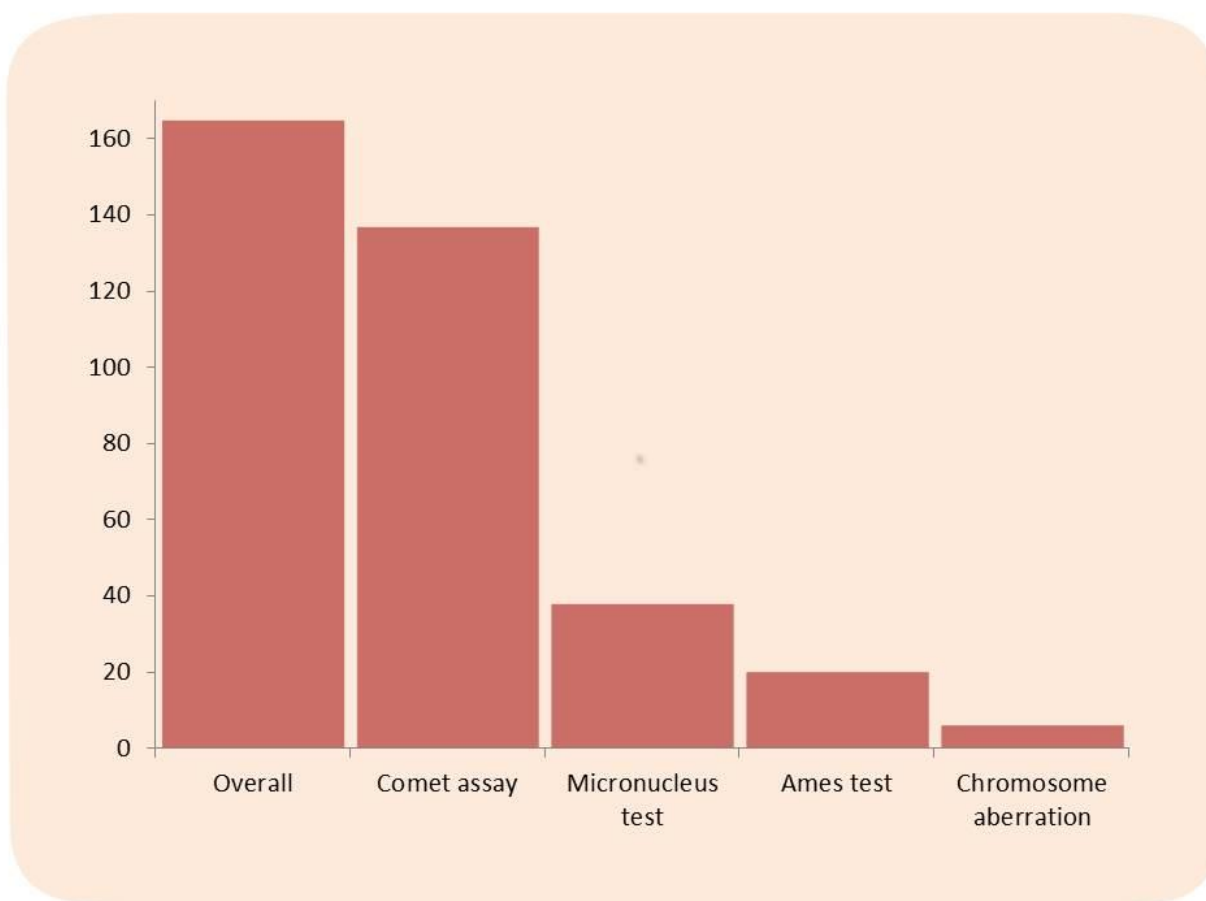


Figure 4. Ranking of genotoxicity tests by the number of publications reporting their use for evaluating - the tests that are more frequently used for evaluation of MeOx and silica NMs genotoxicity. N.B. This figure reflects the information on total number of publications, given in Table 4.

Table 4. Ranking of different kinds of metal oxide/silica NMs by: a) Number of publications reporting genotoxicity evaluations by different tests, b) Datum point for each assay performed for each type of nano metal oxide/silica.

Nano metal oxides	Number of publications ^a	Comet ^b	Micronucleus ^b	Ames ^b	Chromosome aberration ^b
Total	165	137	39	20	6
TiO ₂	63	53	14	5	2
ZnO	25	21	4	5	1
SiO ₂	18	15	5		
CuO	14	11	3	2	
Fe ₃ O ₄	10	9	3	2	

Fe ₂ O ₃	9	8	2		
CeO ₂	7	7	2		
Al ₂ O ₃	5	3	2	2	1
Co ₃ O ₄	3	2		1	
MnO ₂	2	2	1		1
V ₂ O ₅	1	1	1		
Bi ₂ O ₃	1	1			
Dy ₂ O ₃	1			1	
In ₂ O ₃	1			1	
MgO	1	1			
NiO	1	1			
V ₂ O ₃	1	1	1		
WO ₃	1		1	1	1
ZrO ₂	1	1			

5. Mechanisms of metal oxide/silica NM-induced genotoxicity

The knowledge of the various possible mechanisms of NMs' toxicity and genotoxicity, in particular, is critically important in order to assess the level of hazard posed by NMs towards the environment and living organisms. Inorganic materials can interfere with the delicate balance of cellular homeostasis and hereby alter intracellular signaling pathways, resulting in cascade of possible effects. As for all NMs, the detailed mechanisms of genotoxicity for metal oxide/silica NMs are still not well understood, and as was discussed in [26, 60] it is often not clear if an effect on DNA is "nano-specific". By "nano-specific effect", we mean that the mechanism of toxic action is specific to particles with initial dimensions within the size range 1-100 nm as opposed to also being associated with particles of different size but with the same chemical composition. In general, particle induced genotoxicity may be classified as either "primary genotoxicity" or "secondary genotoxicity", where "secondary genotoxicity" refers to the induction of genotoxicity via reactive oxygen species (ROS) generated during particle-elicited inflammation and "primary genotoxicity" refers to genotoxicity induced in the absence of inflammation [217]. There are studies that suggest primary genotoxicity could be the result of direct interaction of NMs with DNA, as well as studies that confirm indirect damage from NM-induced reactive oxygen species (ROS) formation, or by toxic ions released from soluble or even from low soluble NMs [57, 107, 116, 139, 172, 189, 218-220]. At the same time, secondary genotoxicity may result from DNA attack by ROS generated via activated phagocytes (neutrophils, macrophages) during NM-elicited inflammation [60, 221]. A more detailed discussion regarding possible metal oxide/silica NM genotoxicity mechanisms of action is given below.

5.1. Direct primary genotoxicity

As soon as particles enter the nucleus, they have the potential to interact directly with DNA molecules. During these interactions, the metal oxide/silica NMs might bind and influence DNA replication or disturb other DNA processes, for example, transcription to RNA [222].

Several studies support the hypothesis of direct primary genotoxicity of NMs indicating binding with DNA [223-236], although many of them are computational studies [228-230, 232-235]. Palchoudhury et al [224], using gel electrophoresis, studied platinum-attached iron oxide NMs' interaction with DNA and showed that DNA has strong interaction with iron oxide NMs' attached to platinum. Tang et al [225] investigated the interaction of cadmium quantum dots with DNA and, using circular dichroism spectroscopy, indicated that the Cd-MAA complex might interact with DNA fragments. Rice et al [226] studied the interactions of TiO₂-NMs with DNA and, using adsorption studies, showed that terminal phosphate groups influence binding of DNA to TiO₂. In another study, Wahab et al [227] investigated ZnO-NMs by various spectroscopy methods and observed the interaction of zinc oxide NMs with DNA by UV-vis and atomic force microscopy (AFM) spectroscopy. The dissociation of double-stranded DNA (dsDNA) by small metal NMs (5nm Au) was observed through a series of DNA melting transition measurements by Yang et al [236], which confirms the strong non-specific interaction between DNA and metal NMs. In addition to the experimental studies discussed, a number of computational studies support the scarce experimental evidence that metal oxide NMs can interact with DNA bases and DNA fragments [228-230, 232-235]. For example, Shewale et al [234], applying first-principles calculations, identified possible interactions of ZnO clusters with DNA nucleobases. Fahrenkopf et al [228], using density functional theory (DFT) calculations, showed the interaction between hafnium dioxide NMs and DNA, suggesting that the interactions were predominantly mediated by the terminal phosphate in an oriented manner. Jin et al [229] used DFT calculations to indicate strong interactions between Al₁₂X (X=Al, C, N and P) NMs and DNA base pairs, suggesting that Al-based NMs might affect structural stability of DNA and cause structural damage. In another computational study, Paillusson et al [232] investigated interactions between model NMs and DNA. They investigated the influence on the effective interaction of the following conditions: the shape of the NP, the magnitude of the nanoparticle charge and its distribution, the value of the pH of the solution, the magnitude of Van der Waals interactions, depending on the nature of the constitutive material of the NM (metal vs. dielectric), and showed that, for positively charged concave NPs, the effective interaction is repulsive at short distances i.e. the interaction energy shows a minimum at a finite distance from the DNA.

5.2. Indirect primary genotoxicity

The indirect mechanisms of nanoparticle primary genotoxicity were recently reviewed systematically [60]. Here we discuss only those mechanisms applicable to metal oxide NPs. In fact, to cause damage, metal oxide NPs do not need to be in direct contact with DNA. Some possible indirect genotoxicity mechanisms for metal oxide NPs include: interaction with nuclear proteins (involved in replication, transcription, and repair), disturbance of cell cycle checkpoint functions, ROS arising from the NP surface, release of toxic metal ions from the NP surface, ROS produced by cell components, and inhibition of antioxidant defense [54, 110, 237]. Several experimental studies have shown that indirect DNA damage might be caused by oxidative stress initiated by ROS species generated by metal oxide NPs [92, 109, 114, 116, 238-246].

5.3. Secondary genotoxicity

Metal oxide/silica NMs interactions may cause secondary genotoxicity via the following pathway: NMs trigger ROS production by inflammatory cells (neutrophils and macrophages), i.e. in this case ROS are not generated by the NM itself or by ions leaching from the NM surface, but by inflammatory cells via an inflammation signaling pathway. Several publications confirmed the genotoxicity of metal oxide NMs being associated with inflammation processes [59, 87, 89, 186, 247-252].

6. Brief overview of experimental data identified and comparative analysis of genotoxicity for metal oxide/silica NMs

This section provides a brief overview of the experimental data identified generalizing the data represented in Tables 1-3 and discussing the main findings.

6.1. Toxicity of NMs compared to their micrometer-sized and bulk counterparts

Karlsson et al [31] compared the ability of nano-sized (<100 nm) and micrometer-sized (<5 μm) particles of some metal oxides (Fe_2O_3 , Fe_3O_4 , TiO_2 and CuO) to cause cell death, mitochondrial damage, DNA damage and oxidative DNA lesions after exposure to the human cell line A549. This publication reported that NPs of CuO were much more toxic compared to CuO micrometer-sized particles. One key mechanism may be the ability of CuO NPs to damage the mitochondria. In contrast, micrometer-sized particles of TiO_2 caused more DNA damage compared to the NPs, although this may be explained by differences in their crystal structures. The iron oxides showed low toxicity and no clear difference between the different particle sizes.

Singh et al [126] studied Fe_2O_3 (< 50 nm) - and Fe_2O_3 -bulk (< 5 μm) particles in female Wistar rats. The genotoxicity was evaluated at 6, 24, 48 and 72 hours by the comet assay in leucocytes, at 48 and 72 hours by the micronucleus test in peripheral blood cells, at 18 and 24 hours by the chromosomal aberration assay in bone marrow cells and at 24 and 48 hours by the micronucleus test in bone marrow cells. The tail DNA (comet), frequencies of micronuclei (micronucleus test) and chromosome aberrations were statistically insignificant ($p > 0.05$) at all doses. These results suggested that 30nm and bulk Fe_2O_3 were not genotoxic at the doses tested.

In a similar study, Singh et al [128] assessed MnO_2 nano- (45 nm) and micrometer-sized particles (< 5 μm). Nano- MnO_2 elicited genotoxicity in rats as determined using the micronucleus test, comet and chromosomal aberration assays at 1000 mg/kg but bulk particles did not. A significant ($p < 0.05$) increase in the percentage tail DNA was observed in the peripheral blood leukocytes (PBLs) of rats exposed to MnO_2 -45 nm at the highest dose of 1000 mg/kg body weight at 24 and 48 h sampling times; however, no significant DNA damage was observed at 6 and 72 h. In rats treated orally with 100, 500 and 1000 mg/kg body weight of MnO_2 -bulk particles at 6, 24, 48, and 72 h, no significant DNA damage was observed. Moreover, there was a clear size dependent biodistribution as well as toxicity. These findings support the view that NMs may have both higher toxicity and distribution rates compared to their bulk counterparts.

Midander et al [113] assessed the toxic aspects of nano-sized (50 nm) and micrometer-sized (<10 μm) particles of copper(II) oxide in contact with cultivated lung cells. The nano-sized particles caused a higher degree of DNA damage (single-strand breaks) and caused a significantly higher percentage of cell death than micrometer-sized particles. Since these authors also observed higher release of copper for the nano-sized particles, under similar conditions to the toxicity assays, their results suggest that both the observed genotoxicity and cytotoxicity were caused by the release of copper from the particles.

In another comparative study by Guichard et al [253], commercially available nanosized (<90 nm) and micro-sized (<0.75 μm) anatase TiO_2 , rutile TiO_2 , Fe_3O_4 , and Fe_2O_3 particles were compared in Syrian hamster embryo (SHE) cells. Similar levels of DNA damage were observed in the comet assay after 24 h of exposure to anatase NPs and microparticles. Rutile microparticles were found to induce more DNA damage than the nanosized particles. However, no significant increase in DNA damage was detected from nanosized and micro-sized iron oxides. None of the samples tested showed significant induction of micronuclei formation after 24 h of exposure.

Balasubramanyam et al. stated that [81], Al_2O_3 -bulk particles (50–200 μm) did not induce statistically significant changes over control values when assessed via the comet assay. The nanosized Al_2O_3 however, produced a genotoxic effect in the comet assay.

The studies highlighted above suggest that (at least in terms of their genotoxic effects) NPs do not always have higher toxicity than micrometer-sized particles or their bulk counterparts of the same chemical composition. However, the higher toxicity of some NPs compared to their micrometer-sized counterparts arguably justifies caution when moving from the micrometer to nanometer scale.

One important issue needs to be appreciated. Although the number of studies on the genotoxicity of metal oxide/silica NMs is increasing, many results are inconsistent and need to be confirmed by additional experiments. In previous sections we have discussed the results that may be conflicting, but overall, show some trends in metal oxides NMs' genotoxicity. We assume that experimental data for NMs of the same core chemical composition may vary to some extent because of the following reasons, along with other possible variations: 1) various average sizes of NMs used; 2) various size distributions; 3) various purity of NMs; 4) various surface areas of NMs with the same average size; 5) different coatings; 6) differences in crystal structures of the same types of NMs; 7) different sizes of aggregates in different media; 8) differences in assays; 9) different concentrations of NMs in assay tests; 10) variation in concentration of analytes in assays. The situation of conflicting reports (experimental data) and inherent problems with nanotoxicology studies was discussed in the following publications [254-259]. For example, in a recent research article [255], the authors evaluated publications related to engineered NMs' safety assessments where evaluation was spurred by conflicting reports demonstrating different degrees of toxicity with the same NMs. They found that that ca. 95% of papers from 2010 using biochemical techniques to assess nanotoxicity did not account for potential interference of NMs (i.e. interference of NMs properties with analytical techniques), and this number had not substantially improved in 2012. Based on these findings, they provided recommendations for authors of future nanotoxicology studies [255].

6.2. Discussion of results from the experimental studies for each type of metal oxide/silica NM

The 165 publications obtained from the literature search refer to nano oxides of different metals (cerium oxide, copper oxide, iron oxides, titanium oxide, nickel oxide, manganese oxide, magnesium oxide, cobalt oxide, bismuth oxide, and zirconium oxide) as well as silica (silicon dioxide). The data obtained using the comet and micronucleus assay are summarized in Table 1 and Table 2 respectively. The experimental protocols clearly differ in many respects. The main differences between the different studies are the heterogeneity of cell types and methods used. In addition, the nano-sized metal oxides/silica were of varying sizes etc. Nonetheless, in spite of this inconsistency, it is possible to draw some general findings in some cases. The Ames

test results are reported in Table 3. The number of studies concerning this test on NMs is low. Furthermore, a few studies consider Ames test not appropriate for NMs [52].

Overall genotoxicity of each nano metal oxide/silica considering the data gathered in this review:

Comet assay results were as following for each compound (Table 1):

Al₂O₃

Results from the comet assay are reported in three publications [81, 103, 104]. In two *in vitro* studies [103, 104] this nano metal oxide gave different outcomes; it increased the DNA damage significantly comparing to control in mouse lymphoma (L5178Y) cells and human bronchial epithelial (BEAS-2B) cells and had no significant effect on human embryonic kidney (HEK293) cells and peripheral blood lymphocytes. The third publication reported an increase in DNA damage in an *in vivo* test [81].

Bi₂O₃

One publication [105] reports that DNA damage in the root cells of *Allium cepa* for different concentrations of the NM (25, 50, 75, 100 ppm) exhibit statistically significant differences compared to the control.

CeO₂

Reports are provided in seven publications of the comet assay being employed to assess NMs of CeO₂. The type of cells and duration of exposure varied. Each study was performed on a different cell type and exposure criteria. This might explain the contrasting results: five publications [92, 106, 107, 109, 111] reported a positive comet assay outcome and two [108, 110] did not report significant genotoxic effects. However, another possible explanation for the contrasting results could be differences in size or surface functionalization etc.

Co₃O₄

Two publications have reported genotoxic effects observed in comet assay for NMs of this cobalt oxide [109, 112].

CuO

In ten publications (on human lung epithelial cells (A549) cells and human murine macrophages cells (RAW)) the CuO NMs ranging in size from 10-100nm, induced DNA strand breakages as assessed by the alkaline comet assay [31, 113-118, 120-122]. A publication reported no

genotoxicity observed following exposure to the metal oxide in aquatic organism (*Macoma balthica*) [119].

Fe₂O₃

Reports in seven publications stated that Fe₂O₃ of different dimensions and preparations elicited no significant genotoxic effect as determined with the alkaline comet assay [31, 114, 123, 124, 126, 127]. However, in one publication [31], these kinds of particles were found to be genotoxic according to the comet assay.

Fe₃O₄

Six publications conducted on A549 and BEAS-2B cells and human lymphocytes reported that these kinds of nano particles induced DNA breakages as detected by the comet assay [109, 129-132, 260]. Three publications reported no genotoxic effect of which one study conducted on peripheral blood lymphocytes HEK293 and HPL cells showed no significant genotoxicity at all concentrations after 1 h incubation with both types of cells [123] and no DNA damage was observed at the tested concentration levels on *in vitro* human lung type II epithelial (A549) cells in two studies [31, 114].

MnO₂

Two publications report the *in vivo* genotoxic effects of the MnO₂ nano metal oxides: A statistically significant ($p < 0.05$) increase in the percentage of tail DNA was observed in the PBLs of rats exposed to MnO₂-45 nm at the highest dose of 1000 mg/kg body weight at 24 and 48 h sampling times; however, no significant DNA damage was observed at 6 and 72 h [128]. Singh et al [134] reported a statistically significant ($P < 0.01$) increase in the DNA damage (percentage of tail DNA) with the highest and medium doses. No significant increase was found with the lowest dose.

NiO

One publication has reported genotoxic effects observed in comet assay for NPs of NiO [109].

MgO

Particle-induced DNA strand breakage and oxidative DNA damage in Caco-2 cells was detected using the FPG variant of the comet assay. DNA strand breakage and oxidative DNA damage was determined in Caco-2 cells by the FPG-modified comet assay following 4 h treatment at 20 mg/cm². After treatment, all samples were processed in the comet assay. MgO produced no significant change compared to the control [133].

SiO₂

Fifteen publications were obtained for silica, with different crystalline structures (amorphous and quartz forms) as well as differences in surface functionalization. These studies showed the genotoxic [55, 82, 140, 142, 144] or non- genotoxic behavior of this NM [109, 111, 133, 135-144].

TiO₂

Fifty three publications were retrieved for titanium dioxide NMs [31, 85, 87, 89, 103, 111, 114, 121, 123, 125, 133, 146-168, 170-176, 178-180, 182-186, 249, 261-263]. These studies included data on different forms of TiO₂, such as anatase, rutile and the mixture of both forms. Genotoxic effects, as determined with the alkaline comet assay, were observed in forty of these publications - whilst the other thirteen detected no significant genotoxic effect for this kind of NMs.

V₂O₃ and V₂O₅

One study has reported genotoxic effects observed in comet assay for NPs of V₂O₃ and no genotoxic effects of V₂O₅ [52].

ZnO

Twenty one comet publications were found for these nano metal oxides [55, 114, 121, 133, 152, 155, 156, 159, 165, 186-196]. Genotoxic effects were observed in CaCo-2 cells [133], lymphocytes and sperm cells [155], A549 [114, 152], human epidermal cell line [189], primary mouse embryo fibroblasts [55], HK2 cells [186]. Among all the studies, two reported non-genotoxic results from the comet assay, where the size of agglomerates was about 200nm [191, 192]. The large size (over 100nm) of NPs studied is one possible reason of non-genotoxic results and therefore it is questionable if these results are truly relevant for most NPs.

ZrO₂

In a single publication performed on nano metal oxides of ZrO₂, no significant induction in DNA damage by the comet assay was observed with or without the Endo III and FPG enzymes at all concentrations.

Micronucleous test results for each compound (Table 2):

Al₂O₃

Two publications reported positive results in the *in vivo* micronucleous assays on the same type of Al₂O₃ NMs (30-40 nm Nominal size). One publication reported results of the test performed after oral administration in bone marrow cells [197]. The other publication reported results on peripheral blood cells after gavage administration [81].

CeO₂

In two publications [106, 107] genotoxic effects of these NMs were observed for the micronucleous assay on *in vitro* human cells (dermal fibroblasts and neuroblastoma).

CuO

In two publications *in vitro* micronucleous test results reported genotoxic effects of these NMs [115, 198]. Song et al [86] reported an *in vivo* micronucleous test genotoxic outcome.

Fe₂O₃

Two publications reported results of *in vivo* micronucleous assay for this compound; whereas one paper reported statistically insignificant results [126], the other publication reported genotoxic effects for these NMs [86]. The studies were conducted on albino Wistar female rat peripheral blood and bone marrow cells and female ICR mice peripheral blood cells.

Fe₃O₄

Three publications reported results of the micronucleous assay, a study conducted *in vivo* on female ICR mice peripheral blood cells identified significant increases in micronucleated reticulocytes cells [131]. Chen et al. found no significant difference between the test animals and the negative controls in the *In vivo* kunming mice bone marrow cells [199]. Song et al. observed a significantly enhanced MN induction on human lung adenocarcinoma type-II alveolar epithelial cells A549 (*in vitro*) at 10 µg/cm² [86].

MnO₂

In a study performed by Singh et al [128] the micronucleous assay conducted on bone marrow cells extracted from the femurs of female albino Wistar rats (*in vivo*) the data revealed statistically significant enhancement in the MN frequency in the groups treated with 1000 mg/kg body weight of MnO₂-45 nm at 24 and 48 h of sampling times.

SiO₂

Micronucleous assay was reported in five publications on different crystalline structures. These studies reported genotoxic [201, 202] and non genotoxic [138] [200] effects of these NMs. One publication showed equivocal results [82].

TiO₂

Micronucleous assay was reported in fourteen publications. Among these, in five non-genotoxic effects were observed [148, 172, 203, 204, 207] and genotoxic effects in the remaining studies [86, 137, 146, 147, 169, 199, 200, 205, 206].

For a detailed report on genotoxicity of these nanoparticles see reviews [264, 265].

V₂O₃ and V₂O₅

No induction of the micronuclei has been observed for NPs of V₂O₃ and V₂O₅ [52].

ZnO

Four publications reported micronucleous test results for these NMs: An *in vivo* study, described in [191], on mice bone marrow cells reported non-genotoxic micronucleous test results. In three publications [16, 200, 209] *in vitro* studies on different cells (A549 human lung carcinoma cells 199, A549 human lung carcinoma cells, and A. cepa root cells) showed negative, positive and equivocal results. In one of these *in vitro* studies [209] coated and uncoated nano ZnO were tested. The uncoated ZnO NMs were not genotoxic in the micronuclei assay. The coated ZnO was, however, genotoxic.

WO₃

One publication [83] reported micronucleous test conducted on the nanoparticles of WO₃. No statistically significant difference was found between 25 mg WO₃ applied and control group. On the contrary, the higher doses of WO₃ (50 and 100 mg) caused increases of MN rates. No characterization of the NMs was reported in the paper.

Ames test results; an overall view (Table 3):

Mutagenicity assessed by the Ames test is reported in Table 3. Al₂O₃, Co₃O₄, Fe₃O₄ tested on various strains of *S. typhimurium* and *E. coli*, with and without metabolic activation gave negative results in Ames test. Fe₃O₄ was reported in one publication to be positive in the Ames test. Nano TiO₂ in four publications gathered in this review, showed at least one positive result in Ames test. Woodruff et al [174] reported negative mutagenicity of the nano TiO₂. Hasegawa et al [216] reported positive Ames results for nano WO₃, In₂O₃, and Dy₂O₃. In two publications ZnO was found to have positive Ames test data results and to be negative in three other publications.

7. Conclusions

The number of published genotoxicity studies on metal oxide/silica NMs is still limited, although this endpoint has recently received more attention for NMs and the number of related publications has increased. However, more, well designed, genotoxicity studies are required, with a particular need for more *in vivo* experiments. We can expect an increasing number of genotoxicity studies of NMs, with our literature analysis showing an increasing number of genotoxicity publications every year. In particular, we can see that the number of papers reporting comet assay studies for NMs is increasing year on year, although this is not true for the micronucleus test which was the second most popular test amongst those considered in this review. Among the other two tests considered in this review, the Ames and chromosome aberration tests, the former is much more popular for NMs analysis and its use is increasing, in spite of concerns raised about the validity of the Ames test for NMs, whilst the use of the latter would seem to be decreasing in popularity for nanomaterials. Although the number of studies of the genotoxicity of metal oxide/silica NMs is increasing, many results, for the same core chemical composition, are inconsistent: these may need to be confirmed by additional experiments or they may reflect genuine differences due to differences in particles sizes, functionalization etc. In this review, we have discussed the results that may be conflicting. We assume that experimental data for genotoxicity, for NMs with the same core chemical composition, may vary to some extent because of the following reasons: 1) various sizes of NPs used; 2) various size distribution; 3) various purity of NMs; 4) various surface areas for NMs with the same average size; 5) different coatings; 6) differences in crystal structures of the same types of NMs; 7) different sizes of aggregates in solution/media; 8) differences in assays; 9) different concentrations of NMs in assay tests; 10) variation in concentration of analytes in assays etc. The experimental data in the public domain are still quite scarce and exhibit considerable heterogeneity. Ideally, all experimental studies would need to be performed using the same protocol to be able to properly compare these data. As a result of these issues, the genotoxicity data for NMs are quite difficult to compare and make robust conclusions.

As can be seen from the above discussion, different kinds of metal-oxide/silica NMs exhibited varying degrees of genotoxicity. In the majority of the literature references analysed, the NMs considered caused DNA strand breaks or oxidative DNA lesions. Our analysis of these data shows that NMs based on ZnO, NiO, CuO, V₂O₃, Al₂O₃, TiO₂ exhibited at least one positive genotoxic response in most of the references analyzed, while Fe₂O₃ and SiO₂ based NMs mainly showed non-genotoxic responses in these references. Nonetheless, caution is advised regarding these generalizations as considerable inconsistency in the experimental protocols was observed as well as variation in the characteristics of the studied NMs, of any given chemical composition, such as particle size, functionalization etc. In addition to considering the outcome of the tests (i.e. “positive” or “negative” study calls), it should be noted that metal oxide/silica

NMs may induce genotoxicity via various mechanisms. For example, metal oxide NMs may induce genotoxicity via primary or secondary ROS generation pathways. There is a great need for careful scrutiny of the genotoxicity of metal oxide/silica NMs at the molecular level.

This review should help to improve genotoxicity testing of metal oxide/silica NMs, as well as help in understanding of mechanisms and, crucially, provides a valuable summary of genotoxicity data for these NMs reported in the literature up until July 2014.

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