



# **Genotoxicity Evaluation of Titanium Dioxide Nanoparticles In Vivo and In Vitro: A Meta-Analysis**

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**Abstract:** Background: Recent studies have raised concerns about genotoxic effects associated with titanium dioxide nanoparticles (TiO<sub>2</sub> NPs), which are commonly used. This meta-analysis aims to investigate the potential genotoxicity of TiO<sub>2</sub> NPs and explore influencing factors. Methods: This study systematically searched Chinese and English literature. The literature underwent quality evaluation, including reliability evaluation using the toxicological data reliability assessment method and relevance evaluation using routine evaluation forms. Meta-analysis and subgroup analyses were performed using R software, with the standardized mean difference (SMD) as the combined effect value. Results: A total of 26 studies met the inclusion criteria and passed the quality assessment. Meta-analysis results indicated that the SMD for each genotoxic endpoint was greater than 0. This finding implies a significant association between TiO<sub>2</sub> NP treatment and DNA damage and chromosome damage both in vivo and in vitro and gene mutation in vitro. Subgroup analysis revealed that short-term exposure to TiO<sub>2</sub> NPs increased DNA damage. Rats and cancer cells exhibited heightened susceptibility to DNA damage triggered by TiO<sub>2</sub> NPs (p < 0.05). Conclusions: TiO<sub>2</sub> NPs could induce genotoxicity, including DNA damage, chromosomal damage, and in vitro gene mutations. The mechanism of DNA damage response plays a key role in the genotoxicity induced by TiO<sub>2</sub> NPs.

Keywords: titanium dioxide; nanoparticles; genotoxicity; hazard evaluation; meta-analysis

## 1. Introduction

Titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) are particles ranging in size from 1 to 100 nm in at least one dimension of three-dimensional space [1]. Compared to coarse TiO<sub>2</sub> particles, TiO<sub>2</sub> NPs exhibit enhanced conductivity, reactivity, photocatalytic activity, and permeability. These outstanding properties have positioned TiO<sub>2</sub> NPs as one of the most extensively used nanomaterials, finding applications in various industries such as cosmetics, toothpaste, and drug carriers [2,3]. They are also widely used as food additives, primarily added to the coatings of dairy and confectionery products [4].

The unique physicochemical properties of nanoparticles bring about both application advantages and safety concerns. One major concern is that the NPs may increase cellular uptake rate and internalization behavior due to their diminutive size and extensive surface area [5]. Once inside a cell, NPs can disrupt normal cellular functions, leading to cell damage [6]. Moreover, nanomaterials have the potential to interact with molecules within



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). organisms, interfering with biochemical reactions and signaling pathways, thereby affecting the entire biological system [7,8]. Additionally, solubility and ionization play important roles in the cellular responses and toxicity induced by NPs at the molecular, cellular, tissue, and systemic levels [9,10]. Therefore, the risk of adverse effects from TiO<sub>2</sub> NPs may be amplified when there are additional routes of exposure and high levels of exposure, such as prolonged dermal contact and inhalation.

Evidence suggests TiO<sub>2</sub> NPs may be genotoxic, including DNA and chromosomal damage. Based on the risk assessment report in 2021, the European Food Safety Authority (EFSA) updated its opinion that food-grade titanium dioxide (E171) was no longer a safe food additive. New research indicated that up to fifty percent of the NPs in E171 could induce DNA strand breakage and chromosome damage [11]. A meta-analysis focusing on the in vitro genotoxicity of TiO<sub>2</sub> NPs revealed significant increases in tail DNA percentage, olive tail moment, and gene mutation rates [12]. Furthermore, Shi et al. [13] conducted a comprehensive review encompassing both in vivo and in vitro studies on TiO<sub>2</sub> NPs, which collectively suggested the potential of these NPs to induce genotoxic effects. Both in vivo and in vitro tests confirmed the genotoxic nature of TiO<sub>2</sub> NPs, with gene mutation and DNA strand breakage serving as sensitive genetic indicators [14]. Notably, the manifestation of genotoxicity depended not only on the particle surface, size, and exposure pathway but also on the duration and concentration of exposure [15].

This study systematically retrieved the latest literature from Chinese and English databases to evaluate the genotoxic effects of  $TiO_2$  NPs in vivo and in vitro; the selection of the eligible literature adhered to predefined inclusion and exclusion criteria. In addition, a comprehensive quality evaluation of the included literature was conducted, including reliability evaluation based on the toxicological data reliability assessment method and relevance evaluation using routine evaluation forms. The meta-analysis was performed separately for different genotoxic endpoints. Subgroup analyses were used to investigate potential influencing factors, such as particle size, experimental subjects, exposure duration, and exposure concentration. The primary objective of this study was to provide an up-to-date and comprehensive reference for assessing  $TiO_2$  NPs' genotoxicity.

# 2. Materials and Methods

#### 2.1. Search Strategy

This study comprehensively scoured relevant articles from databases, including PubMed, Web of Science (WoS), China National Knowledge Infrastructure (CNKI), and EFSA reports. The search was conducted using a set of keywords, which included "TiO<sub>2</sub>", "Titanium dioxide", "TiO<sub>2</sub> NPs", "genotoxicity", "genotoxic", "gene", "DNA", "chromosome", and "mutation". EFSA's 2016 report on evaluating titanium dioxide as a food additive and common terminology found in CNKI's translation assistant influenced the choice of these keywords. All papers in English and Chinese published before 30 June 2022 were considered for inclusion in this study.

#### 2.2. Selection Criteria

The inclusion criteria in the systematic retrieval included (1) experimental research; (2) studies published in either Chinese or English; (3) mammalian cells or mammals as experimental subjects; (4) studies focused on genotoxic effects, such as gene mutation, chromosome aberration, DNA damage, oxidative stress, etc; and (5) the genotoxicity endpoints reported in the study that included the percentage of DNA in tail (T DNA%), tail length (TL), olive tail moment (OTM), mutation frequency (MF), frequency of micronucleus (MN), percentage of chromosomal aberrations (CA), etc.

The exclusion criteria were also established, including (1) non-original research such as case reports, comments, editorials, reviews, letters, or reports; (2) studies on the joint exposure of  $TiO_2$  with other substances or ultraviolet rays; (3) studies performed with  $TiO_2$  nanofibers, nanocomposites, nanotubes, or non-nanoparticles;(4) in vivo studies on non-oral exposure; (5) only epigenetics of genotoxic effects; and (6) no quantitative results or incomplete data.

#### 2.3. Quality Assessment

Based on the reliability definition of Klimisch, the toxicological data reliability assessment method (TRAM) was developed to evaluate the reliability of toxicological data. The evaluation process incorporated the meticulous assessment of the physicochemical properties of the substances, as well as the conformance to established design standards governing toxicity tests. TRAM was an ideal tool for undertaking safety assessment in China, as it considered the soundness and validity of the research methodology employed in the studies under review [16].

The TRAM evaluation team comprised 18 experts from Jiangsu's Center for Disease Control and Prevention (CDC). All members were required to possess professional qualifications in the field of toxicology, including a Master's degree or higher, a minimum of three years of work experience, and intermediate or senior professional titles. Following TRAM training, the experts evaluated studies based on specific evaluation criteria tailored to different types of data. Each criterion was assigned a weighted score, which was then aggregated and converted into a percentage. Studies that received a score below 60% were categorized as having low reliability, those scoring between 60% and 80% were deemed to have medium reliability, and those scoring above 80% were classified as having high reliability. Studies falling into the "low reliability" category were promptly excluded from subsequent analyses to uphold the analytical rigor and integrity of the process.

Routine evaluation forms were used to determine the relevance of toxicological data. Furthermore, 17 experts from the CDC in Beijing were invited to participate in the evaluation process. The outcome of the evaluation was systematically categorized into three distinct classifications, namely "A", "B", or "C", based on the extent of alignment with the research objectives and the applicability for hazard assessment. Notably, data assigned the label "A" signified a robust concurrence with the research objectives, rendering it highly recommended for hazard evaluation. In contrast, data allocated to the designation "B" embodied a moderated degree of correlation and held the potential for inclusion within the assessment framework. Under circumstances where there was a lack of substantial correlation, the corresponding research was assigned the classification of "C" and consequently excised from any further consideration. Furthermore, to indicate the degree of relevance between exposure route, duration, concentration, and risk assessment, a "+" symbol was added to the results.

#### 2.4. Data Extraction

To extract useful information, researchers independently collected and recorded the following contents including (1) basic information, such as the lead author, publication year and country; (2) subject characteristics and interventions, such as species or cells, routes of administration, particle characteristics, treatment time, concentration and sample size (n); and (3) outcome measures, consisting of (a) T DNA%, TL, and OTL in comet assay, (b) MF in gene mutation assay, (c) MN frequency in MN assay, and (d) CA frequency in CA assay. The mean  $\pm$  standard deviation (SD) was used to describe the outcome variables.

#### 2.5. Statistical Analysis

In assessing the combined genotoxic effects of  $TiO_2$  NPs, the standardized mean difference (SMD) and its 95% confidence interval (CI) were employed. An SMD greater than 0 indicated higher genotoxicity in the exposed groups compared to control groups, while an SMD of 0 suggested no difference between the two groups.

Among the included studies, statistical heterogeneity was estimated by *I*-squared ( $I^2$ ) analysis. The significance of heterogeneity was determined by  $I^2 > 50$  or p < 0.05 in the Q-test. In instances where substantial heterogeneity was present among the individual studies, a random-effects model was employed. Conversely, a fixed-effects model was selected for the meta-analysis. Subgroup analysis was performed to identify the potential sources of heterogeneity and to examine the association between treatment variables (e.g., particle size, treatment object, exposure time, and concentration) and the genotoxic effects of TiO<sub>2</sub> NPs. The stability and reliability of the meta-analysis results were assessed through sensitivity analysis.

Considering the limitation of the included literature, a threshold of at least nine studies was established for conducting funnel plots and Egger test analyses to examine the potential for publication bias. All tests were two-tailed, and a significance level of p < 0.05 was adopted. R-4.2.0 software and the meta package were utilized for all statistical analyses.

#### 3. Results

## 3.1. Literature Screening

The process of the literature retrieval and screening is depicted in Figure 1. Of the total retrieved articles, 1876 were obtained from PubMed, 5483 from WoS, and 1311 from CNKI, resulting in a cumulative count of 8670 articles. After excluding 944 duplicate studies, the titles and abstracts of the remaining 7916 records were screened. From this initial filtering, 328 articles were retained for further consideration. Finally, a full-text screening identified 31 studies that met the eligibility criteria for inclusion. Among these, 12 studies were conducted in vivo, while the remaining 19 were conducted in vitro. The focal point of these studies was to discern the genotoxic potential of  $TiO_2$  NPs.



Figure 1. Flow diagram of the literature search and screening.

#### 3.2. Basic Characteristics and Quality Assessment

Information from in vivo genotoxicity research of TiO<sub>2</sub> NPs is summarized in Table 1. The included studies were classified based on outcome indicators as T DNA% (six studies), TL/ $\mu$ m (three studies), OTM/ $\mu$ m (six studies), MN frequency (two studies), and CA frequency (five studies). The results of quality assessments indicated medium to high reliability, with relevance ratings of "B++" and above.

Table 2 provides information on in vitro genotoxicity studies of TiO<sub>2</sub> NPs. An article with a determined reliability assessment of "low" and four articles exhibiting a correlation evaluation result of "C" were excluded from the meta-analysis. Outcome indicators classified the included studies as T DNA% (nine studies), TL/ $\mu$ m (two studies), OTM/ $\mu$ m (six studies), MF (three studies), MN frequency (seven studies), and CA frequency (two studies).

		Test Animals and	TiO <sub>2</sub>	-NP Characteris	stics	Dasa		Exposure		Control	D 11 1 114	
Included Studies	Country	Exposure Methods	Crystal	Size (nm)	Purity (%)	(mg/kg bw)	п	$\mathbf{Mean} \pm \mathbf{SD}$	п	$\mathbf{Mean} \pm \mathbf{SD}$	Evaluation	Correlation Evaluation
					Outcome	s were described	l as T D	NA%				
Shukla R. K. 2014 [17]	India	<i>Male Swiss albino</i> <i>mice</i> (continuous gavage for 14 d)	Anatase	20–50	99.7	10 50 100	5 5 5	$\begin{array}{c} 17.72 \pm 0.72 \\ 18.98 \pm 1.21 \\ 20.28 \pm 1.11 \end{array}$	5 5 5	$\begin{array}{c} 14.29 \pm 0.67 \\ 14.29 \pm 0.67 \\ 14.29 \pm 0.67 \end{array}$	high	A+++
Martins A. D. C., Jr. 2017 [18]	Brazil	Male Wistar rats (continuous gavage for 45 d)	NA	$41.99 \pm 1.63$	NA	0.5	6	$4.64\pm0.82$	6	$3.6\pm0.35$	medium	B+++
Fadda L. M. 2018 [19]	Saudi Arabia	<i>Male Wistar Albino</i> <i>rats</i> (continuous gavage for 21 d)	Anatase	$60 \pm 10$	NA	1000	10	$4.32\pm0.24$	10	$2.26\pm0.31$	medium	B+++
Chakrabarti S. 2019 [20]	India	Female/male Swiss-Albino mice (oral for 90 d)	NA	$58.25 \pm 8.11$	NA	200 500	10 10	$0.07 \pm 0.012$ (liver) $0.085 \pm 0.009$ (kidney) $0.236 \pm 0.066$ (liver) $0.27 \pm 0.075$ (kidney)	10 10	$0.068 \pm 0.007$ (liver) $0.084 \pm 0.004$ (kidney) $0.068 \pm 0.007$ (liver) $0.084 \pm 0.004$ (kidney)	high	A+++
Sallam M. F. 2022 [21]	Egypt	<i>Male SD rats</i> (continuous gavage for 21 d)	NA	$50 \pm 2.4$	NA	50	10	(kidney) 19.25 ± 0.86	10	(kidney) 9.05 ± 0.25	medium	B++
Sallam M. F. 2022 [22]	Egypt	<i>Male SD rats</i> (continuous gavage for 21 d)	NA	28	NA	50	10	$18.74\pm1.77$	10	$9.77 \pm 1.24$	medium	B++
					Outcome	es were describe	d as TL	μm)				
Hassanein K. M. 2016 [23]	Egypt	Adult male SD rats (continuous gavage for 90 d)	NA	21	NA	150	10	$20.39 \pm 1.6$	10	$10.57 \pm 1.3$	medium	A+++
Fadda L. M. 2018 [19]	Saudi Arabia	<i>Male Wistar Albino</i> <i>rats</i> (continuous gavage for 21 d)	NA	$60 \pm 10$	NA	1000	10	$4.27\pm0.10$	10	$1.14\pm0.13$	medium	B+++

**Table 1.** Basic characteristics and quality evaluation of the included studies on in vivo genotoxicity of TiO<sub>2</sub> NPs<sup>1</sup>.

T 1 1. 1		Test Animals and	TiO <sub>2</sub> -	-NP Characteris	stics	Dece		Exposure		Control	D.11.1.111	Gamelation
Studies	Country	Exposure Methods	Crystal	Size (nm)	Purity (%)	(mg/kg bw)	n	$\mathbf{Mean} \pm \mathbf{SD}$	п	$\mathbf{Mean} \pm \mathbf{SD}$	Evaluation	Evaluation
Chakrabarti S. 2019 [20]	India	Female/male Swiss-Albino mice (oral for 90 d)	NA	$58.25 \pm 8.11$	NA	200 500	10 10	$\begin{array}{c} 0.579 \pm 0.041 \\ (\text{liver}) \\ 0.655 \pm 0.009 \\ (\text{kidney}) \\ 2.213 \pm 0.059 \\ (\text{liver}) \\ 1.858 \pm 0.041 \\ (\text{kidney}) \end{array}$	10 10	$\begin{array}{c} 0.575 \pm 0.028 \\ (\text{liver}) \\ 0.651 \pm 0.007 \\ (\text{kidney}) \\ 0.575 \pm 0.028 \\ (\text{liver}) \\ 0.651 \pm 0.007 \\ (\text{kidney}) \end{array}$	high	A+++
					Outcomes	were described	as OTM	Λ (μm)		-		
Shukla R. K. 2014 [17]	India	Male Swiss albino mice (continuous	Anatase	20–50	99.7	10 50	5 5 F	$2.71 \pm 0.25 \\ 2.98 \pm 0.22 \\ 2.76 \pm 0.22$	5 5	$1.93 \pm 0.14$ $1.93 \pm 0.14$ $1.02 \pm 0.14$	high	A+++
Mohamed H. R.	Egypt	Male Swiss Webster mice (continuous	Anatase/ Rutile	$46.23 \pm 3.45$	99.5	5 50 500	5 5 5	$3.76 \pm 0.23$ $3.01 \pm 0.36$ $3.43 \pm 0.71$ $5.78 \pm 2.02$	5 5 5 5	$1.93 \pm 0.14$ $1.86 \pm 0.26$ $1.86 \pm 0.26$ $1.86 \pm 0.26$	medium	B+++
2013 [24]		gavage for 5 d)				500	8	$5.78 \pm 2.02$ $1.43 \pm 0.15$ (liver) $2.06 \pm 0.28$	8	$1.86 \pm 0.26$ $0.84 \pm 0.30$ (liver) $0.61 \pm 0.24$		
Shi Z. 2015 [25]	China	Female/male wild-type ICR mice, Nrf2(-/-) ICR mice (continuous gavage for 7 d)	Anatase	10–25	99.7	1000	8	(kidney) 3.29 ± 0.21 (liver) 4.33 ± 0.36 (kidney)	8	(kidney) $0.84 \pm 0.30$ (liver) $0.61 \pm 0.24$ (kidney)	high	A+++
		1017 (4)				2000	8	$8.59 \pm 2.67$ (liver) $8.07 \pm 2.91$	8	$0.84 \pm 0.30$ (liver) $0.61 \pm 0.24$		
Chakraharti		Female/male				200	10	(kidney) $0.546 \pm 0.041$ (liver) $0.554 \pm 0.01$ (kidney)	10	(kidney) $0.523 \pm 0.025$ (liver) $0.549 \pm 0.007$ (kidney)		
Chakrabarti S. 2019 [20]	India	Female/male Swiss-Albino mice (oral for 90 d)	ice NA l)	58.25 ± 8.11	NA	500	10	(kidney) $0.835 \pm 0.074$ (liver) $0.758 \pm 0.026$ (kidney)	10	$\begin{array}{c} \text{(Rulley)} \\ 0.523 \pm 0.025 \\ \text{(liver)} \\ 0.549 \pm 0.007 \\ \text{(kidney)} \end{array}$	high	A+++

**TiO<sub>2</sub>-NP** Characteristics Control Exposure **Test Animals and** Dose Reliability Included Correlation Country Size Purity (mg/kg bw) **Exposure Methods** Studies Evaluation Evaluation n Crystal Mean  $\pm$  SD n  $Mean \pm SD$ (%) (nm)Sallam M. F. Male SD rats (continuous Egypt NA  $50 \pm 2.4$ NA 50 10  $2.74 \pm 0.17$ 10  $1.08 \pm 0.04$ medium B++2022 [21] gavage for 21 d) Sallam M. F. Male SD rats (continuous Egypt NA 28 NA 50 10  $3.57 \pm 0.14$ 10  $1.12\pm0.02$ medium B++ 2022 [22] gavage for 21 d) Outcomes were described as MN frequency (MN/1000 PCEs) 5 10 5  $1.50\pm0.51$  $1.20\pm0.20$ Shukla R. K. Male Swiss albino mice 5 50  $2.25 \pm 0.49$ 5  $1.20 \pm 0.20$ 20 - 5099.7 high India Anatase A+++ 2014 [17] (continuous gavage for 14 d) 5 100  $3.0\pm0.68$ 5  $1.20 \pm 0.20$ Female/male Swiss-Albino mice 10 10 Chakrabarti 200  $5.83 \pm 0.75$  $0.16\pm0.40$ India NA  $58.25\pm8.11$ high NA A+++ S. 2019 [20] (oral for 90 d) 500 10  $7.16 \pm 0.75$ 10  $0.16 \pm 0.40$ Outcomes were described as CA frequency 50  $13.30 \pm 0.98$ 15 15  $4.72 \pm 0.24$ Ali S. A. Male Swiss albino mice Egypt 250 15  $15.80\pm0.34$ 15  $4.72\pm0.24$ NA 21 NA medium A+++ (continuous oral for 5 d) 2019 [26] 500 15  $31.70 \pm 0.67$ 15  $4.72\pm0.24$ 50 15  $12.00 \pm 0.66$ 15  $4.72 \pm 0.24$ Ali S. A. Male Swiss albino mice Egypt 250 15  $15.00 \pm 0.69$ 15  $4.72\pm0.24$ NA 80 NA medium A+++ 2019 [26] (continuous oral for 5 d) 500  $24.00 \pm 1.67$  $4.72\pm0.24$ 15 15 5 0.2  $0.05\pm0.04$ 5  $0.01\pm0.01$ Manivannan Male Swiss albino mice 5 5 0.4  $0.14\pm0.04$  $0.01 \pm 0.01$ high  $25.074 \pm 3.593$ NA B+++ India Rutile J. 2019 [27] (continuous gavage for 28 d) 0.8 5  $0.19 \pm 0.03$ 5  $0.01\pm0.01$ Chakrabarti Female/male Swiss-Albino mice 200 10  $0.83 \pm 0.23$ 10  $0.76 \pm 0.29$ India NA  $58.25\pm8.11$ high NA A+++ S. 2019 [20] (oral for 90 d) 500 10  $1.9\pm0.20$ 10  $0.76 \pm 0.29$ Salman A. *Male Balb/c mice* (continuous Germany NA 28.9 NA 25 6  $13.2 \pm 0.35$ 6  $1.6 \pm 0.2$ high A+++ S. 2021 [28] gavage for 21 d)

<sup>1</sup> NA: not applicable; *n*: sample size; SD: standard deviation; T DNA%: the percentage of DNA in tail; TL: tail length; OTM: olive tail moment; MF: mutation frequency; MN/1000 PCEs: no. of micronucleus/1000 polychromatic erythrocytes; CA frequency: percentage of cells exhibiting chromosomal aberrations.

Included Country Test Cells and Exposure Concentration Concentration	Evalua-	
Since $(nm)$ (%) ( $\mu g/n(L)$ $n$ Mean $\pm$ SD $n$ Mean $\pm$ SD	tion	Evalua- tion
Outcomes were described as T DNA%		
$0.008$ 3 $9.72 \pm 0.78$ 3 $9.36 \pm 0.69$		
$0.08$ 3 $9.76 \pm 0.40$ 3 $9.36 \pm 0.69$		
Shukla R. K. Human epidermal cells line 2014 Isola India A127 I C Anatase 50 99.7 $0.8$ 3 $11.79 \pm 0.94$ 3 $9.36 \pm 0.69$	high	В
2011 [29] A431, exposed for 6 h 8 3 $2.35 \pm 0.43$ 3 $9.36 \pm 0.69$	0	
$80 \qquad 3 \qquad 12.89 \pm 0.47 \qquad 3 \qquad 9.36 \pm 0.69$		
$25 \qquad 25 \qquad 9.94 \pm 6.72 \qquad 25 \qquad 5.53 \pm 3.70$		
Hong L. Human lung adenocarcinoma $50$ 25 $14.26 \pm 13.67$ 25 $5.53 \pm 3.70$		
2011 [30] China cells, exposed for 6 h NA 5-10 >99.9 100 25 $12.37 \pm 5.16$ 25 $5.53 \pm 3.70$	medium	A+++
200		
$1 \qquad 3 \qquad 8.61 \pm 0.67 \qquad 3 \qquad 7.75 \pm 0.36$		
$HepG2$ human hepatocellular $10$ $3$ $9.13 \pm 0.54$ $3$ $7.75 \pm 0.36$		
Shukia K. K. India carcinoma cells, exposed for Anatase $30-70$ 99.7 20 3 $10.53 \pm 0.49$ 3 $7.75 \pm 0.36$	high	В
$\begin{array}{c} 2013 \ [31] \\ 6 \ h \\ \end{array} \qquad \qquad$	0	
$80 \qquad 3 \qquad 13.55 \pm 0.43 \qquad 3 \qquad 7.75 \pm 0.36$		
$12.863 \pm 11.00(6 \text{ h})$ $11.836 \pm 6.073(6 \text{ h})$		
$5$ $7.557 \pm 6.846(24 \text{ h})$ $3$ $6.000 \pm 6.866(24 \text{ h})$		
20 $11.470 \pm 8.074(6 \text{ h})$ $11.836 \pm 6.073(6 \text{ h})$		
20 3 $9.007 \pm 10.417(24 \text{ h})$ 3 $6.000 \pm 6.866(24 \text{ h})$		
$100$ $2$ $12.094 \pm 7.677(6 \text{ h})$ $11.836 \pm 6.073(6 \text{ h})$		
Chen Z. China V/9 cells, exposed for 6 h, Anatase $75 \pm 15$ 99.90 $100$ $3$ $9.005 \pm 7.177(24 h)$ $3$ $6.000 \pm 6.866(24 h)$	high	A+++
2014 [14] $16.5 \pm 1.9(4 \text{ h})$ $12.1 \pm 1.8(4 \text{ h})$	0	
20 2 $14.0 \pm 3.7(24 \text{ h})$ 2 $13.7 \pm 2.3(24 \text{ h})$		
$20.3 \pm 5.3(48 \text{ h})$ $20.3 \pm 6.6(48 \text{ h})$		
$18.6 \pm 3.3(4 \text{ h})$ $12.1 \pm 1.8(4 \text{ h})$		
50 2 $16.3 \pm 5.7(24 \text{ h})$ 2 $13.7 \pm 2.3(24 \text{ h})$		
$20.9 \pm 1.7(48 \text{ h})$ $20.3 \pm 6.6(48 \text{ h})$		
Frenzilli G. Human fibroblast (HuDE), $12.1 \pm 1.8(4 \text{ h})$ $12.1 \pm 1.8(4 \text{ h})$	1.	D
2014 [32] $100   2   17.3 \pm 2.9(24 \text{ h})   2   13.7 \pm 2.3(24 \text{ h})$	medium	B+
$48 \text{ h} 21.0 \pm 4.1(48 \text{ h}) 20.3 \pm 6.6(48 \text{ h})$		
$25.0 \pm 2.6(4 \text{ h})$ $12.1 \pm 1.8(4 \text{ h})$		
150 2 $16.4 \pm 8.6(24 \text{ h})$ 2 $13.7 \pm 2.3(24 \text{ h})$		
$20.8 \pm 6.7(48 \text{ h}) \qquad \qquad 20.3 \pm 6.6(48 \text{ h})$		

**Table 2.** Basic characteristics and quality evaluation of the included studies on in vitro genotoxicity of TiO<sub>2</sub> NPs<sup>1</sup>.

**TiO<sub>2</sub>-NP Characteristics** Control Exposure Reliability Correlation Included **Test Cells and Exposure** Concentration Country Evalua-Evalua-Size Purity  $(\mu g/mL)$ Studies Methods Crystal n Mean  $\pm$  SD n Mean  $\pm$  SD (%) tion tion (nm)  $34.6 \pm 10.5(4 \text{ h})$  $22.6 \pm 6.5(4 \text{ h})$ 20 2  $38.4 \pm 2.5(24 \text{ h})$ 2  $17.6 \pm 2.1(24 \text{ h})$  $32.7 \pm 14.8(48 \text{ h})$  $13.5 \pm 5.2(48 \text{ h})$  $31.1 \pm 8.0(4 \text{ h})$  $22.6 \pm 6.5(4 \text{ h})$ 50 2  $25.6 \pm 5.1(24 \text{ h})$ 2  $17.6 \pm 2.1(24 \text{ h})$ Bottlenose dolphin fibroblast Frenzilli G.  $27.3 \pm 9.3(48 \text{ h})$  $13.5 \pm 5.2(48 \text{ h})$ Italy 99.7 B+ (*BDF*), exposed for 4 h, 24 h Anatase 20 - 50medium 2014 [32]  $34.8 \pm 7.2(4 \text{ h})$  $22.6 \pm 6.5(4 \text{ h})$ and 48 h 100 2  $21.9 \pm 1.9(24 \text{ h})$ 2  $17.6 \pm 2.1(24 \text{ h})$  $25.2 \pm 2.4(48 \text{ h})$  $13.5 \pm 5.2(48 \text{ h})$  $21.2 \pm 9.6(4 \text{ h})$  $22.6 \pm 6.5(4 \text{ h})$ 150 2  $25.0 \pm 0.1(24 \text{ h})$ 2  $17.6 \pm 2.1(24 \text{ h})$  $25.9 \pm 7.6(48 \text{ h})$  $13.5 \pm 5.2(48 \text{ h})$  $24.4 \pm 3.1(4 \text{ h})$  $17.2 \pm 4.2(4 \text{ h})$ 20 2  $21.4 \pm 14.9(24 \text{ h})$ 2  $14.5 \pm 2.7(24 \text{ h})$  $18.3 \pm 5.1(48 \text{ h})$  $22.1 \pm 5.3(48 \text{ h})$  $21.8 \pm 4.3(4 \text{ h})$  $17.2 \pm 4.2(4 \text{ h})$ 2 50  $26.0 \pm 9.1(24 \text{ h})$ 2  $14.5 \pm 2.7(24 \text{ h})$ Mouse fibroblast (3 T3), Frenzilli G.  $28.3 \pm 10.1(48 \text{ h})$  $22.1 \pm 5.3(48 \text{ h})$ Italy 20-50 99.7 B+ exposed for 4 h, 24 h and Anatase medium 2014 [32]  $13.8 \pm 2.7(4 \text{ h})$  $17.2 \pm 4.2(4 \text{ h})$ 48 h 100 2  $14.5 \pm 4.8(24 \text{ h})$ 2  $14.5 \pm 2.7(24 \text{ h})$  $21.0 \pm 3.9(48 \text{ h})$  $22.1 \pm 5.3(48 \text{ h})$  $18.8 \pm 2.0(4 \text{ h})$  $17.2 \pm 4.2(4 \text{ h})$ 2 150  $15.9 \pm 1.8(24 \text{ h})$ 2  $14.5 \pm 2.7(24 \text{ h})$  $26.3 \pm 4.9(48 \text{ h})$  $22.1 \pm 5.3(48 \text{ h})$  $10.6 \pm 4.5(4 \text{ h})$  $8.3 \pm 2.3(4 \text{ h})$ 20 2  $14.6 \pm 5.9(24 \text{ h})$ 2  $11.8 \pm 3.2(24 \text{ h})$  $14.7 \pm 3.2(48 \text{ h})$  $10.0 \pm 2.1(48 \text{ h})$  $12.3 \pm 4.4(4 \text{ h})$  $8.3 \pm 2.3(4 \text{ h})$ Human leukocytes (HL), Frenzilli G. 50 2  $11.2 \pm 2.5(24 \text{ h})$ 2  $11.8 \pm 3.2(24 \text{ h})$ Italv exposed for 4 h, 24 h and 48 Anatase 20 - 5099.7 medium B+  $14.4 \pm 7.8(48 \text{ h})$ 2014 [32]  $10.0 \pm 2.1(48 \text{ h})$ h  $13.2 \pm 4.8(4 \text{ h})$  $8.3 \pm 2.3(4 \text{ h})$ 100 2  $13.1 \pm 2.5(24 \text{ h})$ 2  $11.8 \pm 3.2(24 \text{ h})$  $12.6 \pm 5.1(48 \text{ h})$  $10.0 \pm 2.1(48 \text{ h})$ 

Included Country			TiO <sub>2</sub> -N	NP Characte	ristics	Concentration		Exposure		Control	Reliability	Correlation
Studies	Country	lest Cells and Exposure Methods	Crystal	Size (nm)	Purity (%)	(μg/mL)	n	$\mathbf{Mean} \pm \mathbf{SD}$	n	$\mathbf{Mean} \pm \mathbf{SD}$	Evalua- tion	Evalua- tion
						20	2	$33.8 \pm 15.1(4 \text{ h})$ $44.5 \pm 22.6(24 \text{ h})$	2	$25.5 \pm 10.6(4 \text{ h})$ $35.2 \pm 19.5(24 \text{ h})$		
						20	-	$29.5 \pm 9.7(48 \text{ h})$	-	$36.1 \pm 14.3(48 \text{ h})$		
								$27.8 \pm 7.8(4 \text{ h})$		$25.5 \pm 10.6(4 \text{ h})$		
Frenzilli G.	T. 1	Bottlenose dolphin leukocytes				50	2	$50.4 \pm 19.4(24 \text{ h})$	2	$35.2 \pm 19.5(24 \text{ h})$		-
2014 [32]	Italy	(BDL), exposed for4 h, 24 h	Anatase	20–50	99.7			$44.9 \pm 18.8(48 \text{ h})$		$36.1 \pm 14.3(48 \text{ h})$	medium	B+
		and 48 h						$35.3 \pm 15.9(4 \text{ h})$		$25.5 \pm 10.6(4 \text{ h})$		
						100	2	$47.5 \pm 16.2(24 \text{ h})$	2	$35.2 \pm 19.5(24 \text{ h})$		
								$43.9 \pm 12.1(48 \text{ h})$		$36.1 \pm 14.3(48 \text{ h})$		
						10	4	$14.11\pm0.21$	4	$11.31 \pm 0.67$		
				21	$\geq 99.5$	100	4	$15.11\pm0.22$	4	$11.31\pm0.67$		
Demir E.	Crain	Human embryonic kidney cells	D (1			1000	4	$32.21\pm0.77$	4	$11.31\pm0.67$	la i a la	
2015 [33]	2015 [33]	(HEK293), cultured for 1 h	Rutile			10	4	$12.89\pm0.75$	4	$11.31\pm0.67$	nign	А
			50	$\geq 98$	100	4	$13.88\pm0.65$	4	$11.31\pm0.67$			
					1000	4	$30.29\pm0.67$	4	$11.31\pm0.67$			
						10	4	$14.10\pm0.27$	4	$12.31\pm0.17$		
				21	$\geq 99.5$	100	4	$15.41\pm0.29$	4	$12.31\pm0.17$		
Demir E.	Spain	Mouse embryonic kidney cells	Derth			1000	4	$35.91\pm0.57$	4	$12.31\pm0.17$	high	
2015 [33]	Spam	(NIH/3 T3), cultured for 1 h	Rutile			10	4	$12.10\pm0.78$	4	$12.31\pm0.17$	nign	А
				50	$\geq 98$	100	4	$13.59\pm0.73$	4	$12.31\pm0.17$		
						1000	4	$31.77\pm0.60$	4	$12.31\pm0.17$		
						25	3	$5.14\pm0.12$	3	$4.48\pm0.11$		
Kansara K.	T 1.	Human lung cancer cell line	Derth	4 0	00 7	50	3	$6.06\pm0.15$	3	$4.48\pm0.11$	1.	р
2015 [34]	India	( <i>A549</i> ), exposed for 6 h	Kutile	4-8	99.7	75	3	$8.25\pm0.24$	3	$4.48\pm0.11$	mealum	Б
						100	3	$9.49\pm0.25$	3	$4.48\pm0.11$		
						10	4	$1.14\pm0.23$	4	$0.52\pm0.12$		
Andreoli C.	Italu	Peripheral blood monocytes,	A	20 (0	. 00 F	50	4	$1.62\pm0.47$	4	$0.52\pm0.12$	1.	٨
2018 [35]	Italy	exposed for 24 h	Anatase	20-60	>99.5	100	4	$2.01\pm0.66$	4	$0.52\pm0.12$	mealum	А
						200	4	$1.54\pm0.52$	4	$0.52\pm0.12$		
						10	4	$1.19\pm0.19$	4	$0.44\pm0.05$		
Andreoli C.	Italy	Peripheral blood monocytes, exposed for 24 h	Rutile	30 × 100	>99.5	50	4	$2.33\pm0.68$	4	$0.44\pm0.05$	madiur	٨
2018 [35]	itary					100	4	$2.62\pm0.54$	4	$0.44\pm0.05$	mealum	А
						200	4	$3.48 \pm 1.59$	4	$0.44\pm0.05$		

**TiO<sub>2</sub>-NP Characteristics** Control Exposure Reliability Correlation Concentration Included **Test Cells and Exposure** Country Evalua-Evalua-Size Purity  $(\mu g/mL)$ Studies Methods Crystal n  $Mean \pm SD$ n Mean  $\pm$  SD (%) tion tion (nm)10 4  $1.30 \pm 0.04$ 4  $0.34 \pm 0.01$ Peripheral blood monocytes, 50  $2.51\pm0.96$  $0.34 \pm 0.01$ Andreoli C. 4 4 Anatase/ Italy 45-262 >99.5 medium А 2018 [35] exposed for 24 h 100 4  $4.44 \pm 0.18$ 4  $0.34 \pm 0.01$ Rutile  $4.45\pm0.09$ 200 4 4  $0.34 \pm 0.01$ *Lymphocytes from patients* 10 40  $17.7 \pm 5.4$ 40  $15.4 \pm 5.3$ Osman I. F. UK with respiratory diseases, 40-70 30 40  $19.0 \pm 5.5$ 40  $15.4 \pm 5.3$ Anatase 99.7 high В 2018 [36] exposed for 72 h 50 40  $23.3\pm6.5$ 40  $15.4 \pm 5.3$ 10 12  $12.4 \pm 6.1$ 12  $10.2 \pm 4.7$ Osman I. F. Lymphocytes from healthy UK 30 40-70 99.7 12  $13.8\pm5.5$ 12  $10.2 \pm 4.7$ high В Anatase *people*, exposed for 72 h 2018 [36] 50 12  $15.3 \pm 6.3$ 12  $10.2 \pm 4.7$ Outcomes were described as TL (µm)  $65.23 \pm 26.86$ 25 25 25  $37.50 \pm 15.35$ Hong L. Human lung adenocarcinoma 50 25  $78.19 \pm 37.43$ 25  $37.50 \pm 15.35$ NA 5 - 10>99.9 China medium A+++ 2011 [30] cells, exposed for 6 h 100 25  $69.54 \pm 20.61$ 25  $37.50 \pm 15.35$ 200 25  $66.18 \pm 17.87$ 25  $37.50 \pm 15.35$ 3 20  $51.60\pm0.64$ 3  $52.70\pm0.55$ 40 3  $53.49 \pm 0.68$ 3  $52.70\pm0.55$ Ünal F. Human lymphocytes, exposed Turkev 3  $54.29\pm0.70$ NA <100 NA 60 3  $52.70\pm0.55$ medium A+++ 2021 [37] for 30 min 80 3  $54.38 \pm 0.63$ 3  $52.70\pm0.55$ 100 3  $57.59 \pm 1.02$ 3  $52.70\pm0.55$ Outcomes were described as OTM (µm) 0.01 9  $0.91 \pm 0.75$ 9  $0.79 \pm 0.74$ Human fetal liver L-02 cells, Shi Y. Anatase/ 9 9 China 30-50 NA 0.1  $1.28 \pm 0.96$  $0.79 \pm 0.74$ high С 2010 [38] exposed for 24 h Rutile 1 9  $1.30 \pm 1.01$ 9  $0.79\pm0.74$ 0.001 3  $0.67 \pm 0.09$ 3  $0.65 \pm 0.06$ 0.01 3  $0.68 \pm 0.10$ 3  $0.65\pm0.06$ Du H. Human fetal liver L-02 cells, 3 NA 25 - 50>99.5 0.1  $0.71\pm0.08$ 3  $0.65\pm0.06$ С China median 2012 [39] exposed for 24 h 1 3  $0.73 \pm 0.09$ 3  $0.65 \pm 0.06$ 10 3  $0.76\pm0.09$ 3  $0.65\pm0.06$ 3 0.008  $1.27 \pm 0.05$ 3  $1.20 \pm 0.01$ 3 0.08  $1.30 \pm 0.03$ 3  $1.20\pm0.01$ Shukla R. K. Human epidermal cell line 50 0.8 3  $1.43 \pm 0.09$ 3  $1.20 \pm 0.01$ high В 99.7 India Anatase 2011 [29] A431, exposed for 6 h 8 3  $1.20\pm0.01$  $1.79 \pm 0.08$ 3 80 3  $1.91 \pm 0.04$ 3  $1.20\pm0.01$ 

Included Country		TiO <sub>2</sub> -N	IP Characte	eristics	Concentration		Exposure		Control	Reliability	Correlation	
Studies	Country	Methods	Crystal	Size (nm)	Purity (%)	Concentration (μg/mL)	n	$\textbf{Mean} \pm \textbf{SD}$	п	$\mathbf{Mean} \pm \mathbf{SD}$	Evalua- tion	Evalua- tion
						25	25	$12.08\pm8.45$	25	$4.27\pm2.76$		
Hong L.		Human lung adenocarcinoma	NT A	F 10		50	25	$12.43\pm10.79$	25	$4.27\pm2.76$	1.	<b>A</b>
2011 [30]	China	<i>cells,</i> exposed for 6 h	NA	5-10	>99.9	100	25	$12.48\pm2.71$	25	$4.27\pm2.76$	medium	A+++
						200	25	$8.46 \pm 4.73$	25	$4.27\pm2.76$		
						1	3	$1.13\pm0,06$	3	$0.94\pm0.06$		
						10	3	$1.20\pm0.05$	3	$0.94\pm0.06$		
Shukla R. K.	T 1.	HepG2 human hepatocellular	A	20 70	00 7	20	3	$1.40\pm0.02$	3	$0.94\pm0.06$	hiah	р
2013 [31]	India	hepatoma cells, exposed for	Anatase	30-70	99.7	40	3	$1.55\pm0.07$	3	$0.94\pm0.06$	nign	В
		6 h				80	3	$1.76\pm0.09$	3	$0.94\pm0.06$		
						F	2	$5.857 \pm 6.198(6 \text{ h})$	2	$4.698 \pm 3.375(6 \text{ h})$		
						5	3	$3.113 \pm 4.285(24 \text{ h})$	3	$2.576 \pm 3.928(24 \text{ h})$		
Chen Z.	<u></u>	V79 cells, exposed for 6 h,	A		00.00	20	2	$5.086 \pm 4.700(6 \text{ h})$	2	$4.698 \pm 3.375(6 \text{ h})$	hiah	<b>A</b>
2014 [14] China	24 h	Anatase	$75 \pm 15$	15 99.90	20	3	$4.174 \pm 7.453(24 \text{ h})$	3	$2.576 \pm 3.928(24 \text{ h})$	nign	A+++	
						100	2	$4.999 \pm 4.594$ (6 h)	2	$4.698 \pm 3.375(6 \text{ h})$		
						100	3	$3.870 \pm 4.116(24 \text{ h})$	3	$2.576 \pm 3.928(24 \text{ h})$		
Ryu A. R.	<b>I</b> Z	Peripheral blood lymphocytes of	NTA	NT A	NTA	60	6	$23.08\pm0.52$	6	$8.79 \pm 2.18$	1	р
2016 [40]	Korea	rats, exposed for 30 min	NA	NA	NA	80	6	$25.66\pm6.11$	6	$8.79 \pm 2.18$	low	В
Osmora I.E.		Lymphocytes from patients				10	40	$4.3\pm1.6$	40	$37\pm1.5$		
Osman I. F.	UK	with respiratory diseases,	Anatase	40-70	99.7	30	40	$5.0\pm2.0$	40	$37\pm1.5$	high	В
2010 [50]		exposed for 72 h				50	40	$6.2\pm2.2$	40	$37\pm1.5$	-	
		-				10	12	$2.3\pm1.0$	12	$1.8\pm0.7$		
Osman I. F.		Lymphocytes from healthy		10 70	00 7	30	12	$2.7\pm1.0$	12	$1.8\pm0.7$	hiah	р
2018 [36]	UK	people, exposed for 72 h	Anatase	40-70	99.7	50	12	$3.2\pm1.2$	12	$1.8\pm0.7$	nign	В
						20	3	$1.01\pm0.11$	3	$1.03\pm0.09$		
						40	3	$1.59\pm0.29$	3	$1.03\pm0.09$		
Ünal F.	Truelcore	Human lymphocytes, exposed	NT A	<100	NT A	60	3	$1.73\pm0.36$	3	$1.03\pm0.09$	1.	<b>A</b>
2021 [37]	Turkey	for 30 min	NA		NA	80	3	$1.49\pm0.25$	3	$1.03\pm0.09$	medium	A+++
	2021 [07]	for 30 min				100	3	$1.90\pm0.41$	3	$1.03\pm0.09$		

			TiO <sub>2</sub> -N	P Characte	ristics	Concentration		Exposure		Control	Reliability	Correlation
Included Studies	Country	Test Cells and Exposure Methods	Crystal	Size (nm)	Purity (%)	Concentration (μg/mL)	n	$\mathbf{Mean} \pm \mathbf{SD}$	п	$\mathbf{Mean} \pm \mathbf{SD}$	Evalua- tion	Evalua- tion
				0	utcomes w	ere described as N	1F					
Xu A. 2009 [41]	US	Primary embryonic fibroblasts of transgenic mice, incubated in medium for 24 h	Anatase	5	99.7	0.1	3	$12.52\pm4.11$	3	5.69 ± 1.87	medium	В
Chen Z. 2014 [14]	China	V79 cells, exposed for 24 h	Anatase	$75\pm15$	99.9	100	3	$22.7\pm3.0$	3	$8.7\pm1.2$	high	A+++
Jain A. K. 2017 [42]	India	<i>Chinese hamster lung</i> <i>fibroblasts (V-79),</i> exposed for 6 h	Anatase	12–25	99.7	100	3	23.0 ± 2.6	3	$7.7\pm2.1$	medium	A++
				Outcomes v	vere descri	bed as MN freque	ncy (Bi	MN)				
Shi Y.	China	Human fetal liver L-02 cells,	Anatase/	30–50	NA	0.01 0.1	9 9	$0.91 \pm 0.75 \\ 1.28 \pm 0.96$	9 9	$\begin{array}{c} 0.79 \pm 0.74 \\ 0.79 \pm 0.74 \end{array}$	high	С
2010 [38]		exposed for 24 h	Rutile			1 20	9 3	$1.30 \pm 1.01 \\ 15.00 \pm 1.00$	9 3	$0.79 \pm 0.74 \\ 9.33 \pm 1.52$	Ū	
Kang S. J. 2008 [43]	South Korea	exposed for 20 h	Anatase/ Rutile	25	NA	50 100	3 3	$\begin{array}{c} 18.33 \pm 2.08 \\ 23.67 \pm 0.58 \end{array}$	3 3	$9.33 \pm 1.52 \\ 9.33 \pm 1.52$	median	С
Reis É.deM	Brazil	V79 cells, exposed for 3 h	Anatase	3.4	99.7	30 60	3 3	$\begin{array}{c} 6.67 \pm 1.15 \\ 12.00 \pm 1.00 \end{array}$	3 3	$\begin{array}{c} 7.00 \pm 1.00 \\ 7.00 \pm 1.00 \end{array}$	high	С
2016 [44] Rois É doM						120 30	3 3	$\begin{array}{c} 14.67 \pm 2.06 \\ 11.33 \pm 2.31 \end{array}$	3 3	$\begin{array}{c} 7.00 \pm 1.00 \\ 7.00 \pm 1.00 \end{array}$		
2016 [44]	Brazil	<i>V79 cells,</i> exposed for 3 h	Anatase	6.2	99.7	60 120	3 3	$\begin{array}{c} 8.33 \pm 1.15 \\ 10.00 \pm 2.00 \end{array}$	3 3	$\begin{array}{c} 7.00 \pm 1.00 \\ 7.00 \pm 1.00 \end{array}$	high	С
Reis É.deM 2016 [44]	Brazil	V79 cells, exposed for 3 h	Anatase	78	99.7	30 60 120	3 3 3	$5.33 \pm 1.53$ $7.67 \pm 1.15$ $12.33 \pm 2.52$	3 3 3	$7.00 \pm 1.00$ $7.00 \pm 1.00$ $7.00 \pm 1.00$	high	С
						0.008	3 3	$11.67 \pm 1.20$ $12.67 \pm 0.88$	3 3	$9.33 \pm 1.00$ $9.33 \pm 1.00$ $9.33 \pm 1.00$		
Shukla R. K. 2011 [29]	India	<i>Human epidermal cell line</i> A431, exposed for 6 h	Anatase	50	99.7	0.8 8	3 3	$\begin{array}{c} 14.67 \pm 1.20 \\ 15.67 \pm 0.88 \end{array}$	3 3	$9.33 \pm 1.00 \\ 9.33 \pm 1.00$	high	В
		** * ***				80	3	$16.00\pm0.58$	3	$9.33 \pm 1.00$		
Srivastava R. K. 2013 [45]	India	Human lung cancer cell line (A549), exposed for 24 h	Anatase	<25	NA	10 50	3 3	$\begin{array}{c} 12.66 \pm 0.33 \\ 17.33 \pm 0.33 \end{array}$	3 3	$5.33 \pm 0.33 \\ 5.33 \pm 0.33$	medium	В

Included Country		TiO <sub>2</sub> -N	P Characte	ristics	C		Exposure	Control		Reliability	Correlation	
Included Studies	Country	Test Cells and Exposure Methods	Crystal	Size (nm)	Purity (%)	μg/mL)	n	$\mathbf{Mean} \pm \mathbf{SD}$	n	$\mathbf{Mean} \pm \mathbf{SD}$	Evalua- tion	Evalua- tion
						1	3	$8.00 \pm 1.15$	3	$7.00\pm0.58$		
						10	3	$11.00\pm1.53$	3	$7.00\pm0.58$		
Shukla K.	T 1.	HepG2 human hepatocellular	A	20 70	00 7	20	3	$15.00\pm0.58$	3	$7.00\pm0.58$	hich	р
N. 2012 [21]	India	carcinoma cells, exposed for 6 h	Anatase	30-70	99.7	40	3	$12.33\pm0.33$	3	$7.00\pm0.58$	nign	В
2013 [51]						80	3	$10.67\pm0.88$	3	$7.00\pm0.58$		
						25	3	$7.33 \pm 1.20$	3	$6.00\pm2.80$		
Kansara K.	India	Human lung cancer cell line (A549),	Apatasa	1 9	00.7	50	3	$9.66 \pm 2.84$	3	$6.00\pm2.80$	madium	P
2015 [34]	mala	exposed for 6 h	Anatase	4-0	99.7	75	3	$12.33\pm2.96$	3	$6.00\pm2.80$	mealum	D
						100	3	$14.66\pm2.33$	3	$6.00\pm2.80$		
Andreoli C.	Italy	Peripheral blood monocytes, exposed for 24 h	Anataca	20 60	>00.5	50	2	$9.0\pm1.41$	2	$8.5\pm0.71$	modium	٨
2018 [35]	itary	for 24 h	Allalase	20-00	299.0	100	2	$10.0\pm4.24$	2	$8.5\pm0.71$	meanum	A
Androoli C		Devintened blood way age too averaged				50	2	$9.0\pm2.83$	2	$7.5\pm3.54$		
2018 [35]	Italy	for 24 h	Rutile	$30 \times 100$	>99.5	100	2	$7.0\pm2.83$	2	$7.5\pm3.54$	medium	А
2010 [00]		101 24 11				200	2	$8.0\pm1.41$	2	$7.5\pm3.54$		
Androoli C		Devintened blood way age too averaged	Amataca			50	2	$9.5\pm0.71$	2	$9.5\pm0.71$		
2018 [35]	Italy	for 24 h	Anatase/	45-262	>99.5	100	2	$8.0\pm4.24$	2	$9.5\pm0.71$	medium	А
2010 [55]		10r 24 ft	Kuthe			200	2	$5.5\pm2.12$	2	$9.5\pm0.71$		
Osman I. F.	UK	Lymphocytes from patients with	Apatasa	40.70	00.7	5	40	$8.29 \pm 1.55$	40	$8.54 \pm 1.40$	high	P
2018 [36]	UK	respiratory diseases, exposed for 72 h	Anatase	40-70	99.7	10	40	$11.03 \pm 1.70$	40	$8.54 \pm 1.40$	Ingit	D
						5	12	$4.47\pm2.39$	12	$1.87 \pm 1.63$		
Osman I. F.	UV	Lymphocytes from healthy people,	Amataca	40.70	00.7	10	12	$7.21 \pm 1.69$	12	$1.87 \pm 1.63$	high	P
2018 [36]	UK	exposed for 72 h	Anatase	40-70	99.7	20	3	$0.30\pm0.099$	3	$0.13\pm0.066$	Ingit	D
						40	3	$0.30\pm0.099$	3	$0.13\pm0.066$		
Ünal E						60	3	$0.30\pm0.099$	3	$0.13\pm0.066$		
Unai F.	Turkey	Human lymphocytes, exposed for 48 h	NA	<100	NA	80	3	$0.17\pm0.075$	3	$0.13\pm0.066$	medium	A+++
2021 [37]						100	3	$0.13\pm0.066$	3	$0.13\pm0.066$		

**TiO<sub>2</sub>-NP Characteristics** Control Exposure Reliability Correlation **Test Cells and Exposure** Concentration Included Country Evalua-Evalua-Size Purity  $(\mu g/mL)$ Studies Methods Crystal n Mean  $\pm$  SD n Mean  $\pm$  SD (%) tion tion (nm) Outcomes were described as CA frequency  $1.25 \pm 1.26(24 \text{ h})$  $0.75 \pm 0.96(24 \text{ h})$ 6.25 2  $0.50 \pm 0.58(48 \text{ h})$ 2  $0.00 \pm 0.00(48 \text{ h})$  $0.25 \pm 0.50(72 \text{ h})$  $0.50 \pm 1.00(72 \text{ h})$  $0.50 \pm 0.58(24 \text{ h})$  $0.75 \pm 0.96(24 \text{ h})$ 12.5 2  $0.50 \pm 0.58(48 \text{ h})$ 2  $0.00 \pm 0.00(48 \text{ h})$  $1.25 \pm 0.96(72 \text{ h})$  $0.50 \pm 1.00(72 \text{ h})$  $0.00 \pm 0.00(24 \text{ h})$  $0.75 \pm 0.96(24 \text{ h})$ 25 2  $0.25 \pm 0.50(48 \text{ h})$ 2  $0.00 \pm 0.00(48 \text{ h})$  $0.25 \pm 0.50(72 \text{ h})$  $0.50 \pm 1.00(72 \text{ h})$  $0.50 \pm 0.58(24 \text{ h})$  $0.75 \pm 0.96(24 \text{ h})$ Catalán J. Human lymphocytes, exposed 50 2  $0.25 \pm 0.50(48 \text{ h})$ 2  $0.00 \pm 0.00(48 \text{ h})$ 99.7 high Finland Anatase <25 A++ 2011 [46] for 24 h, 48 h and 72 h  $0.50 \pm 1.00(72 \text{ h})$  $0.50 \pm 1.00(72 \text{ h})$  $0.00 \pm 0.00(24 \text{ h})$  $0.75 \pm 0.96(24 \text{ h})$ 100 2  $1.00 \pm 0.82(48 \text{ h})$ 2  $0.00 \pm 0.00(48 \text{ h})$  $0.75 \pm 0.96(72 \text{ h})$  $0.50 \pm 1.00(72 \text{ h})$  $0.25 \pm 0.50(24 \text{ h})$  $0.75 \pm 0.96(24 \text{ h})$ 2  $1.25 \pm 0.50(48 \text{ h})$ 2 150  $0.00 \pm 0.00(48 \text{ h})$  $0.50 \pm 0.58(72 \text{ h})$  $0.50 \pm 1.00(72 \text{ h})$  $1.00 \pm 1.15(24 \text{ h})$  $0.75 \pm 0.96(24 \text{ h})$ 300 2  $1.00 \pm 0.82(48 \text{ h})$ 2  $0.00 \pm 0.00(48 \text{ h})$  $0.50 \pm 0.58(72 \text{ h})$  $0.50 \pm 1.00(72 \text{ h})$  $6.00 \pm 1.37(24 \text{ h})$  $1.33 \pm 0.66(24 \text{ h})$ 3 3 20  $5.33 \pm 1.30(48 \text{ h})$  $1.33 \pm 0.66(48 \text{ h})$  $6.67 \pm 1.44(24 \text{ h})$  $1.33 \pm 0.66(24 \text{ h})$ 3 3 40  $3.00 \pm 0.98(48 \text{ h})$  $1.33 \pm 0.66(48 \text{ h})$  $4.33 \pm 1.17(24 \text{ h})$  $1.33 \pm 0.66(24 \text{ h})$ 3 60 3 Ünal F. Human lymphocytes, exposed Turkev <100  $3.33 \pm 1.03(48 \text{ h})$  $1.33 \pm 0.66(48 \text{ h})$ NA NA medium A+++ 2021 [37] for 24 h, 48 h  $5.00 \pm 1.26(24 \text{ h})$  $1.33 \pm 0.66(24 \text{ h})$ 80 3 3  $3.33 \pm 1.03(48 \text{ h})$  $1.33 \pm 0.66(48 \text{ h})$  $6.00 \pm 1.37(24 \text{ h})$  $1.33 \pm 0.66(24 \text{ h})$ 3 100 3  $4.00 \pm 1.13(48 \text{ h})$  $1.33 \pm 0.66(48 \text{ h})$ 

<sup>1</sup> NA: not applicable; *n*: sample size; SD: standard deviation; T DNA%: the percentage of DNA in tail; TL: tail length; OTM: olive tail moment; MF: mutation frequency; BiMN: no. of micronucleus/1000 binucleated cells; CA frequency: percentage of cells exhibiting chromosomal aberrations.

## 3.3. Meta-Analysis for In Vivo Genotoxicity of TiO<sub>2</sub> NPs

## 3.3.1. Heterogeneity Test and Meta-Analysis

The results of the I<sup>2</sup> analysis for different genotoxic endpoints showed significant heterogeneity (p < 0.01, I<sup>2</sup>  $\geq$  50%). Consequently, the random-effects model was employed to estimate the combined effects.

Meta-analysis of in vivo genotoxicity of TiO<sub>2</sub> NPs summarized the SMDs of five categories of genotoxicity endpoints (as shown in Figure 2). The forest plots illustrated significant increases in T DNA% (Z = 4.02, p < 0.0001), TL (Z = 2.38, p = 0.0174), and OTM (Z = 5.44, p < 0.0001). The SMDs and 95%CIs were 4.19 (2.15–6.24), 16.73 (2.94–30.51), and 5.62 (3.59–7.64), respectively, indicating that treatment with TiO<sub>2</sub> NPs could cause DNA damage. Similarly, MN frequency (Z = 2.59, p = 0.0097) and CA frequency (Z = 3.58, p = 0.0003) in the exposed group also significantly increased, with SMDs and 95%CIs of 5.07 (1.23–8.91) and 15.81 (7.16–24.45). This evidence suggested that TiO<sub>2</sub> NPs may induce chromosome damage.

		Ex	posure			Control	Standardised Mean			
Study	Total	Mean	SD	Total	Mean	SD	Difference	SMD	95%-CI	Weight
[ Shukla R. K. 2013 ]	5	17.72	0.7200	5	14.29	0.6700		4.45	[1.70; 7.21]	8.8%
[Shukla R. K. 2013]	5	18.98	1.2100	5	14.29	0.6700		4.33	[1.63; 7.02]	8.8%
[Shukla R. K. 2013]	5	20.28	1.1100	5	14.29	0.6700		5.90	[2.41; 9.39]	8.0%
[Martins A. D. C., Jr. 2017]	6	4.64	0.8200	6	3.60	0.3500		1.52	[0.17; 2.87]	10.0%
[Fadda L. M. 2018]	10	4.32	0.2400	10	2.26	0.3100		7.12	[4.52; 9.71]	8.9%
[Chakrabarti S. 2019]	10	0.07	0.0120	10	0.07	0.0070	in 1	0.19	[-0.68; 1.07]	10.2%
[Chakrabarti S. 2019]	10	0.08	0.0090	10	0.08	0.0040		0.14	[-0.74; 1.02]	10.2%
[Chakrabarti S. 2019]	10	0.24	0.0660	10	0.07	0.0070	-	3.43	[1.96; 4.90]	9.9%
[Chakrabarti S. 2019]	10	0.27	0.0750	10	0.08	0.0040		3.35	[1.91; 4.80]	9.9%
[Sallam M. F. 2022]	10	19.25	0.8600	10	9.05	0.2500		- 15.42	[10.05; 20.80]	6.1%
[ Sallam M. F. 2022 ]	10	18.74	1.7700	10	9.77	1.2400		5.62	[ 3.50; 7.74]	9.4%
Random effects model	91			91			\$	4.19	[2.15; 6.24]	100.0%
Heterogeneity: $I^2 = 90\%$ , $\tau^2 = 1$	0.4713	B, p < 0.	01							
						-	20 -10 0 10	20		

## (a) Outcomes were described as T DNA%

		Ex	posure			Control	S	tanda	rdised	Mea	n			
Study	Total	Mean	SD	Total	Mean	SD		Di	ferenc	e		SMD	95%-CI	Weight
[Hassanein K. M. 2016]	10	20.39	1.6000	10	10.57	1.3000			-+-			6.45	[4.07; 8.84]	17.6%
[Fadda L. M. 2018]	10	4.27	0.1000	10	1.14	0.1300					-	25.85	[16.92; 34.77]	16.4%
[Chakrabarti S. 2019]	10	0.58	0.0410	10	0.57	0.0280						0.11	[-0.77; 0.99]	17.6%
[Chakrabarti S. 2019]	10	0.66	0.0090	10	0.65	0.0070			+			0.48	[-0.42; 1.37]	17.6%
[Chakrabarti S. 2019]	10	2.21	0.0590	10	0.57	0.0280				-	•	33.97	[22.26; 45.68]	15.7%
[Chakrabarti S. 2019]	10	1.86	0.0410	10	0.65	0.0070				-		39.30	[25.77; 52.84]	15.1%
Random effects model Heterogeneity: $I^2 = 96\% \tau^2$	<b>60</b> = 280	2237 n	< 0.01	60					<		_	16.73	[ 2.94; 30.51]	100.0%
notorogonoty: / cont, r	200.		0.01				-40	-20	0	20	40			

(**b**) Outcomes were described as TL

Figure 2. Cont.

Study	Total	Ex Mean	posure SD	Total	Mean	Control SD	Standardised Mean Difference	SMD	95%-CI	Weight
[ Shukla R. K. 2013 ]	5	2.71	0.2500	5	1.93	0.1400		3.48	[ 1.19; 5.76]	5.9%
[Shukla R. K. 2013]	5	2.98	0.2200	5	1.93	0.1400		5.14	[2.04; 8.24]	5.5%
[Shukla R. K. 2013]	5	3.76	0.2300	5	1.93	0.1400	<u> </u>	8.68	[ 3.72; 13.63]	4.6%
[Mohamed H. R. 2015]	5	3.01	0.3600	5	1.86	0.2600	-	3.31	[1.10; 5.51]	5.9%
[Mohamed H. R. 2015]	5	3.43	0.7100	5	1.86	0.2600		2.65	[0.73; 4.57]	6.0%
[Mohamed H. R. 2015]	5	5.78	2.0200	5	1.86	0.2600		2.46	[0.62; 4.30]	6.0%
[Shi Z. 2015]	8	1.43	0.1500	8	0.84	0.3000	-	2.35	[1.00; 3.70]	6.2%
[ Shi Z. 2015 ]	8	2.06	0.2800	8	0.61	0.2400		5.26	[2.96; 7.56]	5.9%
[ Shi Z. 2015 ]	8	3.29	0.2100	8	0.84	0.3000		8.94	[ 5.27; 12.62]	5.3%
[ Shi Z. 2015 ]	8	4.33	0.3600	8	0.61	0.2400		11.49	[ 6.84; 16.15]	4.8%
[ Shi Z. 2015 ]	8	8.59	2.6700	8	0.84	0.3000		3.86	[2.04; 5.67]	6.1%
[ Shi Z. 2015 ]	8	8.07	2.9100	8	0.61	0.2400		3.42	[1.75; 5.08]	6.1%
[Chakrabarti S. 2019]	10	0.55	0.0410	10	0.52	0.0250		0.65	[-0.26; 1.55]	6.3%
[Chakrabarti S. 2019]	10	0.55	0.0100	10	0.55	0.0070		0.55	[-0.34; 1.45]	6.3%
[Chakrabarti S. 2019]	10	0.83	0.0740	10	0.52	0.0250	100	5.41	[3.35; 7.47]	6.0%
[Chakrabarti S. 2019]	10	0.76	0.0260	10	0.55	0.0070		10.51	[ 6.79; 14.23]	5.2%
[ Sallam M. F. 2022 ]	10	2.74	0.1700	10	1.08	0.0400		12.87	[ 8.36; 17.38]	4.8%
[ Sallam M. F. 2022 ]	10	3.57	0.1400	10	1.12	0.0200		- 23.46	[15.35; 31.57]	3.1%
Random effects model Heterogeneity: $l^2 = 89\%$ , $\tau$	<b>138</b> <sup>2</sup> = 16.7	7611, p	< 0.01	138			-20 -20 -10 0 10 20	5.62	[ 3.59; 7.64]	100.0%

## (c) Outcomes were described as OTM

		Ex	posure			Control	Standardised Mean			
Study	Total	Mean	SD	Total	Mean	SD	Difference	SMD	95%-CI	Weight
[ Shukla R. K. 2013 ]	5	1.50	0.5100	5	1.20	0.2000	÷	0.70	[-0.60; 2.00]	21.5%
[ Shukla R. K. 2013 ]	5	2.25	0.4900	5	1.20	0.2000		2.53	[0.66; 4.40]	20.9%
[ Shukla R. K. 2013 ]	5	3.00	0.6800	5	1.20	0.2000		3.24	[1.06; 5.42]	20.6%
[Chakrabarti S. 2019]	10	5.83	0.7500	10	0.16	0.4000		9.03	[5.81; 12.26]	19.1%
[Chakrabarti S. 2019]	10	7.16	0.7500	10	0.16	0.4000		- 11.15	[7.22; 15.09]	17.9%
Random effects model Heterogeneity: $I^2 = 90\%$ , $\tau^2$	35 2 = 17.3	3961, p	< 0.01	35				<b>5.07</b>	[1.23; 8.91]	100.0%
						_	15 -10 -5 0 5 10 1	5		

# (d) Outcomes were described as MN frequency

		Ex	posure			Control	Standard	ised Mean			
Study	Total	Mean	SD	Total	Mean	SD	Diffe	rence	SMD	95%-CI	Weight
[Ali S A 2019]	15	13 30	0.9800	15	4 72	0 2400			11 70	[ 8 46: 14 95]	87%
[ Ali S. A. 2019 ]	15	15.80	0.3400	15	4.72	0.2400			36.63	[26.70: 46.57]	7.9%
[ Ali S. A. 2019 ]	15	31.70	0.6700	15	4.72	0.2400		-	- 52.16	[38.04; 66.29]	7.2%
[ Ali S. A. 2019 ]	15	12.00	0.6600	15	4.72	0.2400			14.26	[10.34; 18.19]	8.7%
[ Ali S. A. 2019 ]	15	15.00	0.6900	15	4.72	0.2400			19.36	[14.08; 24.65]	8.6%
[ Ali S. A. 2019 ]	15	24.00	1.6700	15	4.72	0.2400		12	15.72	[11.41; 20.04]	8.7%
[Manivannan J. 2019]	5	0.05	0.0400	5	0.01	0.0100			1.24	[-0.18; 2.65]	8.8%
[Manivannan J. 2019]	5	0.14	0.0400	5	0.01	0.0100		-	4.03	[1.48; 6.57]	8.8%
[Manivannan J. 2019]	5	0.19	0.0300	5	0.01	0.0100			7.27	[ 3.06; 11.47]	8.7%
[Chakrabarti S. 2019]	10	0.83	0.2300	10	0.76	0.2900	1		0.26	[-0.62; 1.14]	8.8%
[Chakrabarti S. 2019]	10	1.90	0.2000	10	0.76	0.2900		-	4.38	[2.64; 6.13]	8.8%
[Salman A. S. 2021]	6	13.20	0.3500	6	1.60	0.2000			37.55	[19.42; 55.68]	6.4%
Random effects model Heterogeneity: $l^2 = 96\%$ , $\tau$	<b>131</b> <sup>2</sup> = 219	.8812, 4	0 < 0.01	131					15.81	[ 7.16; 24.45]	100.0%
<b>°</b> ,							-60 - 40 - 20	0 20 40 6	0		

(e) Outcomes were described as CA frequency

**Figure 2.** Meta-analysis for in vivo genotoxicity of TiO<sub>2</sub> NPs. (**a**–**e**) Show the forest plots for genotoxicity endpoints of T DNA%, TL, OTM, MN frequency, and CA frequency, respectively. 'Total' is the sample size; 'SD' is the standard deviation; 'SMD' is the standardized mean difference; '95%CI' is the 95% confidence interval; ' $I^{2'}$ ' is Higgins's inconsistency statistic; ' $\tau^{2'}$ ' is the estimate of between-study variance. Significance is at p < 0.05.

#### 3.3.2. Subgroup Analysis

Given the limited available literature on the in vivo genotoxicity of TiO<sub>2</sub> NPs, our subgroup analysis focused on T DNA% and OTM data. Figure 3 depicts that the observed heterogeneity in the results may be attributed to the exposure time (p < 0.01) and the species used in experiments (p = 0.01). Specifically, the TiO<sub>2</sub> NPs-treated group exhibited significantly higher T DNA% in short-term exposures ( $\leq 21$  days) (SMD = 6.56, 95%CI: 4.12–9.00) compared to long-term exposures (>21 days) (SMD = 1.64, 95%CI: 0.22–3.06). Additionally, OTM was significantly higher in rats (SMD = 17.61, 95%CI: 7.29–27.93) than in mice (SMD = 4.39, 95%CI: 2.93–5.84). However, no statistically significant results were observed when considering particle size and treatment dose for T DNA% or OTM. These findings suggested that short-term exposure could potentially contribute to in vivo DNA damage caused by TiO<sub>2</sub> NPs. Furthermore, rats seem more sensitive to the genotoxic impacts of TiO<sub>2</sub> NP-induced DNA damage than mice.

Variable	n		SMD(95%CI)	<i>p</i> value
Size (nm)		7		
≤30	25	⊢∎⊣	5.08(3.76-6.40)	0.56
>30	66	j⊢_∎i	4.00(0.56-7.43)	
Species				
Mice	55	F-■-1	2.77(1.14, 4.41)	0.41
Rats	36	↓ <b></b>	7.00(1.67, 12.33)	
Time (d)				
≤21	45	<b>⊢</b> ∎1	6.56(4.12, 9.00)	< 0.01
>21	46	, ⊨∎⊣	1.64(0.22, 3.06)	
Dose (mg/kg bw)				
≤80	36	·	5.81(1.72, 9.89)	0.25
>80	55	·-∎	3.09(0.85, 5.34)	
			12 1 2 2	
		-10 0 10 20		
Variable	n		SMD(95%CI)	<i>p</i> value
Variable Size (nm)	n		SMD(95%CI)	p value
Variable Size (nm) ≤30	<b>n</b> 73		SMD(95%CI) 6.70(3.78-9.62)	<i>p</i> value 0.28
Variable Size (nm) ≤30 >30	n 73 65		SMD(95%CI) 6.70(3.78-9.62) 4.44(1.54-7.34)	<i>p</i> value 0.28
Variable Size (nm) ≤30 >30 Species	n 73 65		<b>SMD(95%CI)</b> 6.70(3.78-9.62) 4.44(1.54-7.34)	<i>p</i> value 0.28
Variable Size (nm) ≤30 >30 Species Mice	n 73 65 118		SMD(95%CI) 6.70(3.78-9.62) 4.44(1.54-7.34) 4.39(2.93-5.84)	<i>p</i> value 0.28 0.01
Variable Size (nm) ≤30 >30 Species Mice Rats	n 73 65 118 20		SMD(95%CI) 6.70(3.78-9.62) 4.44(1.54-7.34) 4.39(2.93-5.84) 17.61(7.29-27.93)	<i>p</i> value 0.28 0.01
Variable Size (nm) ≤30 >30 Species Mice Rats Time (d)	n 73 65 118 20		<b>SMD(95%CI)</b> 6.70(3.78-9.62) 4.44(1.54-7.34) 4.39(2.93-5.84) 17.61(7.29-27.93)	<i>p</i> value 0.28 0.01
Variable     Size (nm)     ≤30     >30     Species     Mice     Rats     Time (d)     ≤21	n 73 65 118 20 98		SMD(95%CI) 6.70(3.78-9.62) 4.44(1.54-7.34) 4.39(2.93-5.84) 17.61(7.29-27.93) 6.13(3.80-8.45)	<i>p</i> value 0.28 0.01 0.41
Variable     Size (nm)     ≤30     >30     Species     Mice     Rats     Time (d)     ≤21     >21	n 73 65 118 20 98 40		SMD(95%CI) 6.70(3.78-9.62) 4.44(1.54-7.34) 4.39(2.93-5.84) 17.61(7.29-27.93) 6.13(3.80-8.45) 4.03(-0.41-8.47)	<i>p</i> value 0.28 0.01 0.41
Variable     Size (nm)     ≤30     >30     Species     Mice     Rats     Time (d)     ≤21     >21     Dose (mg/kg bw)	n 73 65 118 20 98 40		SMD(95%CI) 6.70(3.78-9.62) 4.44(1.54-7.34) 4.39(2.93-5.84) 17.61(7.29-27.93) 6.13(3.80-8.45) 4.03(-0.41-8.47)	<i>p</i> value 0.28 0.01 0.41
Variable     Size (nm)     ≤30     >30     Species     Mice     Rats     Time (d)     ≤21     >21     Dose (mg/kg bw)     ≤80	n 73 65 118 20 98 40 40		SMD(95%CI) 6.70(3.78-9.62) 4.44(1.54-7.34) 4.39(2.93-5.84) 17.61(7.29-27.93) 6.13(3.80-8.45) 4.03(-0.41-8.47) 7.80(1.97-13.63)	<i>p</i> value 0.28 0.01 0.41 0.34

**Figure 3.** Subgroup analyses of TiO<sub>2</sub> NPs genotoxicity on in vivo T DNA% (**a**) and OTM (**b**). 'n' is the sample size; 'SMD' is the standardized mean difference; '95%CI' is the 95% confidence interval; and '*p* value' represents the heterogeneity between subgroups. Significant heterogeneity between subgroups is at *p* < 0.05.

#### 3.3.3. Sensitivity Analysis and Publication Bias

The presence of heterogeneity among in vivo studies focusing on various genotoxic endpoints was noted. This analysis did not reveal any significant differences in the study outcomes, as indicated by the SMD and its 95%CI. Considering the relatively limited number of included studies for each genotoxicity endpoint, no publication bias test was performed.

#### 3.4. Meta-Analysis for In Vitro Genotoxicity of TiO<sub>2</sub> NPs

### 3.4.1. Heterogeneity Test and Meta-Analysis

Due to the observed heterogeneity among in vitro studies with outcome indicators of T DNA%, OTM, and MN frequency (p < 0.01), the random-effects model was utilized to analyze the combined effects. Conversely, for the outcome indicators of TL, MF, and CA frequency, which showed no significant heterogeneity, the fixed-effects model was considered appropriate.

The meta-analysis of in vitro genotoxicity of TiO<sub>2</sub> NPs revealed significant findings across six categories of outcome indicators (as shown in Figure 4). The results from the forest plots indicate that the experimental group exposed to TiO<sub>2</sub> NPs has significantly higher levels of T DNA% (Z = 10.12, p < 0.0001), TL (Z = 9.42, p < 0.0001), and OTM (Z = 7.09, p < 0.0001) than controls. The SMDs and 95%CIs were 0.84 (0.68–1.01), 1.46 (1.16–1.77), and 1.12 (0.79–1.45), respectively. These findings suggested that TiO<sub>2</sub> NP treatment could cause DNA damage. There was a significant increase in MF (Z = 2.83, p = 0.0046) with a result of 2.70 (0.83–4.56), indicating the potential of TiO<sub>2</sub> NPs to induce gene mutations. Moreover, significant increases were observed in MN frequency (Z = 5.68, p < 0.0001) and CA frequency (Z = 2.90, p = 0.0037). The SMDs and 95%CIs were 1.11 (0.65–1.56) and 0.72 (0.23–1.20), respectively, suggesting chromosomal damage effects.

#### 3.4.2. Subgroup Analysis

In the subgroup analysis conducted on in vitro studies, a specific focus was placed on the examination of T DNA% and OTM data. As illustrated in Figure 5, the potential origins of heterogeneity were identified as the exposure time and the type of experimental cells (p = 0.02). For the TiO<sub>2</sub> NPs-treated group, the results of subgroup analysis revealed that OTM was significantly higher during short-term exposure ( $\leq 12$  h) (SMD = 1.55, 95%CI: 0.99–2.12) compared to long-term exposure (>12 h) (SMD = 0.78, 95%CI: 0.46–1.10). Furthermore, the OTM value for cancer cells (SMD = 1.98, 95%CI: 1.08–2.88) was significantly higher than that of normal cells (SMD = 0.83, 95%CI: 0.54–1.11). Nevertheless, neither particle size nor exposure concentration exhibited statistically significant differences in relation to T DNA% and OTM. In summation, brief periods of exposure to TiO<sub>2</sub> NPs may potentially result in DNA damage in vitro. Additionally, cancer cells were discerned to manifest a heightened sensitivity to in vitro DNA damage elicited by TiO<sub>2</sub> NPs.

#### 3.4.3. Sensitivity Analysis and Publication Bias

The sensitivity analysis of the data from the in vitro assay indicated that no single study significantly impacted the overall results. Furthermore, the merged effect values remained consistent, suggesting that the original results of forest plots were statistically reliable and robust.

A publication bias test was performed specifically for the studies with a genotoxicity endpoint of T DNA%. The funnel plot in Figure 6a revealed an uneven distribution of points representing the effect values for each study. A significant proportion of these points were positioned to the right of the combined effect value and lay outside the associated confidence interval. In addition, the *p*-value of the Egger test was found to be less than 0.05. These findings collectively suggested the presence of publication bias, which possibly affected the accuracy of meta-analysis. To eliminate publication bias, an additional 15 studies were needed, as indicated by hollow origin in Figure 6b.

Study	Exposure Total Mean SD	Control Total Mean SD	Standardised Mean Difference	SMD	95%-CI Weight
[Shukla R. K. 2011]	3 9.72 0.7800	3 9.36 0.6900	t	0.39	[-1.25; 2.03] 0.9%
[ Shukla R. K. 2011 ] [ Shukla R. K. 2011 ]	3 9.76 0.4000 3 11.79 0.9400	3 9.36 0.6900 3 9.36 0.6900	ĥ	0.57	[-1.11; 2.25] 0.9% [-0.32; 5.02] 0.4%
[Shukla R. K. 2011]	3 2.35 0.4300	3 9.36 0.6900		-9.73	[-18.71; -0.75] 0.0%
[Hong L. 2011]	25 9.94 6.7200	25 5.53 3.7000	<u>k</u>	0.80	[ 0.22; 1.38] 5.8%
[ Hong L. 2011 ] [ Hong L. 2011 ]	25 14.26 13.6700 25 12.37 5.1600	25 5.53 3.7000 25 5.53 3.7000		0.86	[ 0.28; 1.44] 5.8% [ 0.87; 2.13] 5.1%
[Hong L. 2011]	25 9.47 4.9700	25 5.53 3.7000	ė.	0.89	[0.30; 1.47] 5.8%
[ Shukla R. K. 2013 ]	3 9.13 0.5400	3 7.75 0.3600	•	2.40	[-0.30; 5.10] 0.4%
[ Shukla R. K. 2013 ] [ Shukla R. K. 2013 ]	3 10.53 0.4900 3 11.61 0.3800	3 7.75 0.3600	+	5.16 8.32	[ 0.21; 10.11] 0.1% [ 0.60; 16.04] 0.0%
[ Shukla R. K. 2013 ] [ Chen Z. 2014 ]	3 13.55 0.4300 3 12.86 11.0000	3 7.75 0.3600 3 11.84 6.0730	ļ.	11.67	[ 0.95; 22.39] 0.0% [ -1.51: 1.69] 1.0%
[Chen Z. 2014]	3 11.47 8.0740	3 11.84 6.0730		-0.04	[-1.64; 1.56] 1.0%
[ Chen Z. 2014 ]	3 7.56 6.8460	3 6.00 6.0730		0.03	[-1.42; 1.80] 1.0%
[ Chen Z. 2014 ] [ Chen Z. 2014 ]	3 9.01 10.4170 3 9.01 7.1770	3 6.00 6.0730 3 6.00 6.0730	Î	0.28	[-1.34; 1.90] 1.0% [-1.27; 1.99] 1.0%
[Frenzilli G. 2014]	2 16.50 1.9000	2 12.10 1.8000	t	1.34	[-1.94; 4.62] 0.2%
[ Frenzilli G. 2014 ]	2 23.40 4.7000	2 12.10 1.8000	÷	1.79	[-2.23; 5.81] 0.2%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 25.00 2.6000 2 14.00 3.7000	2 12.10 1.8000 2 13.70 2.3000	+	3.25 0.05	[-3.42; 9.93] 0.1% [-1.91; 2.02] 0.7%
[Frenzilli G. 2014]	2 16.30 5.7000	2 13.70 2.3000	t	0.34	[-1.73; 2.41] 0.6% [-1.70; 3.26] 0.4%
[Frenzilli G. 2014]	2 16.40 8.6000	2 13.70 2.3000	-	0.24	[-1.77; 2.26] 0.6%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 20.30 5.3000 2 20.90 1.7000	2 20.30 6.6000 2 20.30 6.6000	1	0.00	[-1.96; 1.96] 0.7% [-1.89; 2.04] 0.7%
[Frenzilli G. 2014] [Frenzilli G. 2014]	2 21.00 4.1000	2 20.30 6.6000	ţ	0.07	[-1.89; 2.04] 0.7% [-1.92; 2.00] 0.7%
[Frenzilli G. 2014]	2 34.60 10.5000	2 22.40 6.5000	ł	0.79	[-1.71; 3.28] 0.4%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 31.10 8.0000 2 34.80 7.2000	2 22.40 6.5000 2 22.40 6.5000	ţ	1.02	[-1.69; 3.04] 0.5% [-1.78; 3.82] 0.3%
[Frenzilli G. 2014] [Frenzilli G. 2014]	2 21.20 9.6000	2 22.40 6.5000	1	-0.08	[-2.05; 1.88] 0.7% [-5.07: 15.24] 0.0%
[Frenzilli G. 2014]	2 25.60 5.1000	2 17.60 2.1000	ł	1.16	[-1.84; 4.16] 0.3%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 25.00 0.1000	2 17.60 2.1000	Ť.	2.81	[-1.87; 4.29] 0.3% [-3.03; 8.65] 0.1%
[Frenzilli G. 2014] [Frenzilli G. 2014]	2 32.70 14.8000	2 13.50 5.2000	ţ	0.98	[-1.76; 3.72] 0.3% [-1.79: 3.85] 0.3%
[Frenzilli G. 2014]	2 25.20 2.4000	2 13.50 5.2000	÷	1.63	[-2.12; 5.38] 0.2%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 24.40 3.1000	2 17.20 4.2000	Ţ	1.10	[-1.80; 3.95] 0.3% [-1.81; 4.01] 0.3%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 21.80 4.3000 2 13.80 2.7000	2 17.20 4.2000	ļ	0.61	[-1.69; 2.91] 0.5% [-2.77: 1.69] 0.5%
[Frenzilli G. 2014]	2 18.80 2.0000	2 17.20 4.2000	ł	0.27	
[ Frenzilli G. 2014 ]	2 26.00 9.1000	2 14.50 2.7000	ł	0.30	[-1.76; 3.69] 0.4%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 14.50 4.8000 2 15.90 1.8000	2 14.50 2.7000 2 14.50 2.7000	ţ	0.00 0.34	[-1.96; 1.96] 0.7% [-1.73; 2.42] 0.6%
[Frenzilli G. 2014] [Frenzilli G. 2014]	2 18.30 5.1000	2 22.10 5.3000	t	-0.41	[-2.53; 1.71] 0.6% [-1.70; 2.57] 0.6%
[Frenzilli G. 2014]	2 21.00 3.9000	2 22.10 5.3000		-0.13	[-2.11; 1.84] 0.7%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 10.60 4.5000	2 8.30 2.3000	Į	0.46	[-1.72; 2.45] 0.6%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 12.30 4.4000 2 13.20 4.8000	2 8.30 2.3000 2 8.30 2.3000	ţ	0.64	[-1.69; 2.97] 0.5% [-1.70; 3.17] 0.4%
[Frenzilli G. 2014]	2 14.60 5.9000	2 11.80 3.2000	İ	0.33	[-1.73; 2.40] 0.6%
[Frenzilli G. 2014]	2 13.10 2.5000	2 11.80 3.2000	ł	0.26	[-1.77; 2.28] 0.6%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 14.40 7.8000	2 10.00 2.1000	Ţ	0.98	[-1.70; 2.57] 0.3%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 12.60 5.1000 2 33.80 15.1000	2 10.00 2.1000 2 25.50 10.6000	ţ	0.38	[-1.72; 2.47] 0.6% [-1.72; 2.44] 0.6%
[Frenzilli G. 2014]	2 27.80 7.8000	2 25.50 10.6000	t	0.14	[-1.84; 2.12] 0.7%
[ Frenzilli G. 2014 ]	2 44.50 22.6000	2 35.20 19.5000	ł	0.25	[-1.77; 2.27] 0.6%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 50.40 19.4000 2 47.50 16.2000	2 35.20 19.5000	Ī	0.44	[-1.70; 2.58] 0.6%
[ Frenzilli G. 2014 ] [ Frenzilli G. 2014 ]	2 29.50 9.7000 2 44.90 18.8000	2 36.10 14.3000 2 36.10 14.3000	ţ	-0.30 0.30	[-2.35; 1.74] 0.6% [-1.75; 2.34] 0.6%
[Frenzilli G. 2014]	2 43.90 12.1000	2 36.10 14.3000	1	0.33	[-1.73; 2.40] 0.6%
[ Demir E. 2015 ]	4 15.11 0.2200	4 11.31 0.6700	+	6.62	[ 1.98; 11.26] 0.1%
[ Demir E. 2015 ] [ Demir E. 2015 ]	4 12.89 0.7500	4 11.31 0.6700		1.93	[ 0.04; 3.82] 0.7%
[ Demir E. 2015 ] [ Demir E. 2015 ]	4 13.88 0.6500 4 30.29 0.6700	4 11.31 0.6700 4 11.31 0.6700	*	3.38 24.61	[ 0.73; 6.03] 0.4% [ 8.09; 41.12] 0.0%
[ Demir E. 2015 ]	4 14.10 0.2700	4 12.31 0.1700	+	6.89	[ 2.08; 11.70] 0.1% [ 3.63; 19.03] 0.0%
[ Demir E. 2015 ]	4 35.91 0.5700	4 12.31 0.1700		48.74	[16.11; 81.36] 0.0%
[ Demir E. 2015 ] [ Demir E. 2015 ]	4 13.59 0.7300	4 12.31 0.1700	ŀ	2.10	[ 0.13; 4.07] 0.7%
[ Demir E. 2015 ] [ Kansara K. 2015 ]	4 31.77 0.6000 3 5.14 0.1200	4 12.31 0.1700 3 4.48 0.1100	+	38.33 4.57	[12.66; 64.01] 0.0% [0.12; 9.03] 0.1%
[Kansara K. 2015]	3 6.06 0.1500	3 4.48 0.1100	+	9.58	[0.74; 18.43] 0.0%
[Kansara K. 2015]	3 9.49 0.2500	3 4.48 0.1100		20.70	[ 1.84; 39.56] 0.0%
[ Andreoli C. 2018 ] [ Andreoli C. 2018 ]	4 1.14 0.2300 4 1.62 0.4700	4 0.52 0.1200 4 0.52 0.1200	*	2.94 2.79	[ 0.53; 5.34] 0.5% [ 0.46; 5.11] 0.5%
[ Andreoli C. 2018 ] [ Andreoli C. 2018 ]	4 2.01 0.6600 4 1.54 0.5200	4 0.52 0.1200 4 0.52 0.1200	*	2.73	[ 0.44; 5.02] 0.5% [ 0.25; 4.44] 0.6%
[Andreoli C. 2018]	4 1.19 0.1900	4 0.44 0.0500	+	4.69	[ 1.26; 8.12] 0.2% [ 0.74: 6.07] 0.4%
[ Andreoli C. 2018 ]	4 2.62 0.5400	4 0.44 0.0500	+	4.94	[ 1.36; 8.52] 0.2%
[ Andreoli C. 2018 ] [ Andreoli C. 2018 ]	4 3.48 1.5900 4 1.30 0.0400	4 0.44 0.0500 4 0.34 0.0100	·	2.35 28.60	[ 0.25; 4.44] 0.6% [ 9.42; 47.78] 0.0%
[Andreoli C. 2018] [Andreoli C. 2018]	4 2.51 0.9600	4 0.34 0.0100	*	2.78	[ 0.46; 5.09] 0.5% [ 9.20; 46.67] 0.0%
[Andreoli C. 2018]	4 4.45 0.0900	4 0.34 0.0100	<u> </u>	- 55.75	[18.44; 93.07] 0.0%
[ Osman I. F. 2018 ]	40 17.70 5.4000	40 15.40 5.3000		0.43	[ 0.21; 1.11] 8.2%
[ Osman I. F. 2018 ] [ Osman I. F. 2018 ]	40 23.30 6.5000 12 12.40 6.1000	40 15.40 5.3000 12 10.20 4.7000	4	1.32 0.39	[ 0.83; 1.80] 7.4% [ -0.42; 1.20] 3.4%
[ Osman I. F. 2018 ] [ Osman I. F. 2018 ]	12 13.80 5.5000 12 15.30 6.3000	12 10.20 4.7000 12 10.20 4.7000		0.68 0.89	[-0.15; 1.51] 3.3% [ 0.04; 1.73] 3.2%
Random effects model	520	520		0.84	0.68; 1.011 100.0%
Heterogeneity: $I^2 = 44\%$ , $\tau^2$	<sup>2</sup> = 0.0318, <i>p</i> < 0.01	1.05 <sup>10</sup> (15 <sup>2</sup> 0)	-50 0 50		

(a) Outcomes were described as T DNA%

Figure 4. Cont.

		E	xposure			Control	Standardised Mean			
Study	Total	Mean	SD	Total	Mean	SD	Difference	SMD	95%-CI	Weight
[Hong L. 2011]	25	65.23	26.8600	25	37.50	15.3500		1.25	[ 0.64; 1.86]	24.9%
[Hong L. 2011]	25	78.19	37.4300	25	37.50	15.3500	-	1.40	[0.78; 2.02]	23.8%
[Hong L. 2011]	25	69.54	20.6100	25	37.50	15.3500		1.74	[ 1.08; 2.39]	21.4%
[Hong L. 2011]	25	66.18	17.8700	25	37.50	15.3500		1.69	[ 1.04; 2.35]	21.7%
[ ÜNal F. 2011 ]	3	51.60	0.6400	3	52.70	0.5500		-1.47	[-3.56; 0.61]	2.1%
[ ÜNal F. 2011 ]	3	53.49	0.6800	3	52.70	0.5500		1.02	[-0.83; 2.87]	2.7%
[ ÜNal F. 2011 ]	3	54.29	0.7000	3	52.70	0.5500		2.02	[-0.42; 4.45]	1.6%
[ ÜNal F. 2011 ]	3	54.38	0.6300	3	52.70	0.5500	+	2.27	[-0.34; 4.87]	1.4%
[ÜNal F. 2011]	3	57.59	1.0200	3	52.70	0.5500		- 4.76	[ 0.15; 9.37]	0.4%
<b>Common effect model</b> Heterogeneity: $J^2 = 33\%$	115 $\tau^2 < 0.0$	001. p	= 0.15	115			· · · · · · · · · · · · · · · · · · ·	1.46	[ 1.16; 1.77]	100.0%
							-5 0 5			

# (**b**) Outcomes were described as TL

		E	xposure		Control	Standardised Mean			
Study	Total	Mean	SD	Total	Mean SD	Difference	SMD	95%-CI	Weight
[ Shukla R. K. 2011 ]	3	1.27	0.0500	3	1.20 0.0100	+	1.55	[-0.58: 3.68]	1.9%
[ Shukla R. K. 2011 ]	3	1.30	0.0300	3	1.20 0.0100		3.57	[-0.05: 7.18]	0.8%
[Shukla R. K. 2011]	3	1.43	0.0900	3	1.20 0.0100	-	2.87	[-0.19; 5.92]	1.0%
[Shukla R. K. 2011]	3	1.79	0.0800	3	1.20 0.0100	÷	8.26	[ 0.59; 15.92]	0.2%
[Shukla R. K. 2011]	3	1.91	0.0400	3	1.20 0.0100	· ·	- 19.43	[ 1.72; 37.15]	0.0%
[Hong L. 2011]	25	12.08	8.4500	25	4.27 2.7600		1.22	[0.62; 1.83]	7.0%
[Hong L. 2011]	25	12.43	10.7900	25	4.27 2.7600		1.02	[0.43; 1.61]	7.1%
[Hong L. 2011]	25	12.48	2.7100	25	4.27 2.7600	13	2.95	[2.14; 3.77]	5.9%
[Hong L. 2011]	25	8.46	4.7300	25	4.27 2.7600		1.07	[0.47; 1.66]	7.1%
[Shukla R. K. 2013]	3	1.13	0.0600	3	0.94 0.0600	-	2.53	[-0.27; 5.32]	1.2%
[Shukla R. K. 2013]	3	1.20	0.0500	3	0.94 0.0600		3.76	[-0.01; 7.52]	0.7%
[Shukla R. K. 2013]	3	1.40	0.0200	3	0.94 0.0600	÷	8.21	[ 0.59; 15.83]	0.2%
[Shukla R. K. 2013]	3	1.55	0.0700	3	0.94 0.0600	<u> </u>	7.47	[ 0.50; 14.43]	0.2%
[Shukla R. K. 2013]	3	1.76	0.0900	3	0.94 0.0600	÷	8.55	[ 0.62; 16.48]	0.2%
[Chen Z. 2014]	3	5.86	6.1980	3	4.70 3.3750	÷	0.19	[-1.42; 1.79]	2.9%
[Chen Z. 2014]	3	5.09	4.7000	3	4.70 3.3750	÷	0.08	[-1.53; 1.68]	2.9%
[ Chen Z. 2014 ]	3	5.00	4.5940	3	4.70 3.3750	÷	0.06	[-1.54; 1.66]	2.9%
[ Chen Z. 2014 ]	3	3.11	4.2850	3	2.58 3.9280	+	0.10	[-1.50; 1.71]	2.9%
[ Chen Z. 2014 ]	3	4.17	7.4530	3	2.58 3.9280	÷	0.21	[-1.40; 1.83]	2.9%
[Chen Z. 2014]	3	3.87	4.1160	3	2.58 3.9280	÷	0.26	[-1.36; 1.87]	2.9%
[Osman I. F. 2018]	40	4.30	1.6000	40	3.70 1.5000	li ili	0.38	[-0.06; 0.83]	7.9%
[Osman I. F. 2018]	40	5.00	2.0000	40	3.70 1.5000	101	0.73	[0.28; 1.18]	7.9%
[Osman I. F. 2018]	40	6.20	2.2000	40	3.70 1.5000		1.31	[0.83; 1.80]	7.7%
[Osman I. F. 2018]	12	2.30	1.0000	12	1.80 0.7000	i i i i i i i i i i i i i i i i i i i	0.56	[-0.26; 1.38]	5.9%
[Osman I. F. 2018]	12	2.70	1.0000	12	1.80 0.7000	(III)	1.01	[0.15; 1.86]	5.7%
[Osman I. F. 2018]	12	3.20	1.2000	12	1.80 0.7000	(3)	1.38	[0.47; 2.28]	5.4%
[ ÜNal F. 2021 ]	3	1.01	0.1100	3	1.03 0.0900	+	-0.16	[-1.77; 1.45]	2.9%
[ ÜNal F. 2021 ]	3	1.59	0.2900	3	1.03 0.0900	-	2.08	[-0.40; 4.56]	1.5%
[ÜNal F. 2021]	3	1.73	0.3600	3	1.03 0.0900	*	2.13	[-0.38; 4.64]	1.4%
[ ÜNal F. 2021 ]	3	1.49	0.2500	3	1.03 0.0900	<del>]</del>	1.95	[-0.44; 4.34]	1.6%
[ ÜNal F. 2021 ]	3	1.90	0.4100	3	1.03 0.0900	*	2.34	[-0.32; 5.00]	1.3%
Random effects model	319			319			1.12	[ 0.79; 1.45]	100.0%
Heterogeneity: $I^2 = 58\%$ , $\tau$	$^2 = 0.30$	048, p <	0.01					•	
						-30-20-10 0 10 20 30			

# (c) Outcomes were described as OTM

		Ex	posure			Control	Standardised Mean			
Study	Total	Mean	SD	Total	Mean	SD	Difference	SMD	95%-CI	Weight
[Xu A. 2009]	3	12.52	4.1100	3	5.69	1.8700	+	1.71	[-0.52; 3.93]	70.2%
[ Chen Z. 2014 ]	3	22.70	3.0000	3	8.70	1.2000		- 4.89	[0.17; 9.61]	15.6%
[ Jain A. K. 2017 ]	3	23.00	2.6000	3	7.70	2.1000		- 5.17	[ 0.21; 10.12]	14.2%
Common effect model	9			9			~	2.70	[ 0.83; 4.56]	100.0%
Heterogeneity: $I^2 = 21\%$ ,	$t^2 = 1.7$	206, p =	= 0.28			-1(		10	•	

# (d) Outcomes were described as MF

Figure 4. Cont.

		Ex	posure			Control	Standar	dised Mean			
Study	Total	Mean	SD	Total	Mean	SD	Diff	erence	SMD	95%-CI	Weight
[Shukla R. K. 2011]	3	11.67	1.2000	1	9.33	1.0000		÷	1.20	[-1.74: 4.15]	1.9%
[ Shukla R. K. 2011 ]	3	12.67	0.8800	3	9.33	1.0000		*	2.83	[-0.20: 5.86]	1.8%
[Shukla R. K. 2011]	3	14.67	1.2000	3	9.33	1.0000		-	3.86	[0.01; 7.71]	1.2%
[Shukla R. K. 2011]	3	15.67	0.8800	3	9.33	1.0000			5.37	[0.24; 10.50]	0.7%
[Shukla R. K. 2011]	3	16.00	0.5800	3	9.33	1.0000		<b>⊢</b> ⊷	6.51	[ 0.39; 12.63]	0.5%
[Srivastava R. K. 2013]	3	12.66	0.3300	3	5.33	0.3300		<u>⊢</u>	17.72	[ 1.55; 33.89]	0.1%
[Srivastava R. K. 2013]	3	17.33	0.3300	3	5.33	0.3300			- 29.01	[2.62; 55.41]	0.0%
[Shukla R. K. 2013]	3	8.00	1.1500	3	7.00	0.5800		<u>6</u>	0.88	[-0.91; 2.66]	3.9%
[Shukla R. K. 2013]	3	11.00	1.5300	3	7.00	0.5800		*	2.76	[-0.21; 5.73]	1.9%
[Shukla R. K. 2013]	3	15.00	0.5800	3	7.00	0.5800		<b>⊢</b>	11.01	[0.89; 21.12]	0.2%
[Shukla R. K. 2013]	3	12.33	0.3300	3	7.00	0.5800		<b>⊢</b> ⊷	9.01	[0.67; 17.35]	0.3%
[Shukla R. K. 2013]	3	10.67	0.8800	3	7.00	0.5800		-	3.93	[0.02; 7.84]	1.2%
[Kansara K. 2015]	3	7.33	1.2000	3	6.00	2.8000		<b></b>	0.49	[-1.17; 2.15]	4.2%
[Kansara K. 2015]	3	9.66	2.8400	3	6.00	2.8000		<b></b>	1.04	[-0.82; 2.89]	3.7%
[Kansara K. 2015]	3	12.33	2.9600	3	6.00	2.8000		÷	1.75	[-0.50; 4.01]	2.9%
[Kansara K. 2015]	3	14.66	2.3300	3	6.00	2.8000		*	2.68	[-0.23; 5.60]	1.9%
[Andreoli C. 2018]	2	9.00	1.4100	2	8.50	0.7100		÷	0.25	[-1.77; 2.27]	3.3%
[Andreoli C. 2018]	2	10.00	4.2400	2	8.50	0.7100		÷	0.28	[-1.76; 2.31]	3.3%
[Andreoli C. 2018]	2	9.00	2.8300	2	7.50	3.5400		<b></b>	0.26	[-1.76; 2.29]	3.3%
[Andreoli C. 2018]	2	7.00	2.8300	2	7.50	3.5400		<b></b>	-0.09	[-2.06; 1.88]	3.5%
[Andreoli C. 2018]	2	8.00	1.4100	2	7.50	3.5400		÷.	0.10	[-1.87; 2.08]	3.4%
[Andreoli C. 2018]	2	9.50	0.7100	2	9.50	0.7100		<b></b>	0.00	[-1.96; 1.96]	3.5%
[Andreoli C. 2018]	2	8.00	4.2400	2	9.50	0.7100		<b></b>	-0.28	[-2.31; 1.76]	3.3%
[Andreoli C. 2018]	2	5.50	2.1200	2	9.50	0.7100		そ	-1.43	[-4.84; 1.99]	1.5%
[Osman I. F. 2018]	40	8.29	1.5500	40	8.54	1.4000		li li	-0.17	[-0.61; 0.27]	9.0%
[Osman I. F. 2018]	40	11.03	1.7000	40	8.54	1.4000			1.58	[1.08; 2.09]	8.8%
[Osman I. F. 2018]	12	4.47	2.3900	12	1.87	1.6300		i i i i i i i i i i i i i i i i i i i	1.23	[0.34; 2.11]	7.2%
[Osman I. F. 2018]	12	7.21	1.6900	12	1.87	1.6300			3.11	[ 1.86; 4.35]	5.6%
[ ÜNal F. 2021 ]	3	0.30	0.0990	3	0.13	0.0660		÷	1.61	[-0.56; 3.78]	3.0%
[ ÜNal F. 2021 ]	3	0.30	0.0990	3	0.13	0.0660		ř	1.61	[-0.56; 3.78]	3.0%
[ ÜNal F. 2021 ]	3	0.30	0.0990	3	0.13	0.0660		÷	1.61	[-0.56; 3.78]	3.0%
[ ÜNal F. 2021 ]	3	0.17	0.0750	3	0.13	0.0660		- p	0.45	[-1.20; 2.10]	4.3%
[UNal F. 2021]	3	0.13	0.0660	3	0.13	0.0660		Î	0.00	[-1.60; 1.60]	4.4%
Random effects model	183			181				•	1.11	[ 0.65; 1.56]	100.0%
Heterogeneity: $I^{*} = 62\%$ , $\tau$	= 0.54	424, p <	0.01				-10 -20	0 20 40			
							-40 -20	0 20 40			

# (e) Outcomes were described as MN frequency

		Ex	posure		Control	Standardised Mean			
Study	Total	Mean	SD	Total	Mean SD	Difference	SMD	95%-CI	Weight
Cotolán I 2011 1	2	1 05	1 0000	0	0.75 0.0000	def.	0.05	1 4 77. 0 071	E 70/
[Catalan J. 2011]	2	1.25	1.2600	2	0.75 0.9600		0.25	[-1.11; 2.21]	5.1%
[Catalan J. 2011]	2	0.25	0.5000	2	0.50 1.0000		-0.18	[-2.17; 1.01]	5.9%
[Catalan J. 2011]	2	0.50	0.5800	2	0.75 0.9600		-0.18	[-2.17; 1.81]	5.9%
[Catalan J. 2011]	2	1.25	0.9600	2	0.50 1.0000		0.43	[-1.70; 2.57]	5.1%
[ Catalán J. 2011 ]	2	0.25	0.5000	2	0.50 1.0000		-0.18	[-2.17; 1.81]	5.9%
[ Catalán J. 2011 ]	2	0.50	0.5800	2	0.75 0.9600		-0.18	[-2.17; 1.81]	5.9%
[ Catalán J. 2011 ]	2	0.50	1.0000	2	0.50 1.0000		0.00	[-1.96; 1.96]	6.1%
[Catalán J. 2011]	2	0.75	0.9600	2	0.50 1.0000		0.14	[-1.84; 2.12]	6.0%
[ Catalán J. 2011 ]	2	0.25	0.5000	2	0.75 0.9600		-0.37	[-2.46; 1.72]	5.3%
[Catalán J. 2011]	2	0.50	0.5800	2	0.50 1.0000		0.00	[-1.96; 1.96]	6.1%
[ Catalán J. 2011 ]	2	1.00	1.1500	2	0.75 0.9600		0.13	[-1.84; 2.11]	6.0%
[ Catalán J. 2011 ]	2	0.50	0.5800	2	0.50 1.0000		0.00	[-1.96; 1.96]	6.1%
[ÜNal F. 2021]	3	6.00	1.3700	3	1.33 0.6600		3.47	[-0.06; 7.00]	1.9%
[ ÜNal F. 2021 ]	3	6.67	1.4400	3	1.33 0.6600		- 3.80	[-0.00; 7.61]	1.6%
[ ÜNal F. 2021 ]	3	4.33	1.1700	3	1.33 0.6600	+ <u>i</u>	2.52	[-0.27: 5.31]	3.0%
[ ÜNal F. 2021 ]	3	5.00	1,2600	3	1.33 0.6600	↓ <del>↓</del>	2.91	[-0.18: 6.00]	2.4%
ÜNal F. 2021 1	3	6.00	1.3700	3	1.33 0.6600		3.47	[-0.06: 7.00]	1.9%
[ ÜNal F. 2021 ]	3	5.33	1,3000	3	1.33 0.6600		3.10	[-0.14: 6.33]	2.2%
[ ÜNal F. 2021 ]	3	3.00	0.9800	3	1.33 0.6600		1.59	[-0.56: 3.75]	5.0%
[ ÜNal F. 2021 ]	3	3.33	1.0300	3	1.33 0.6600		1.84	[-0.47: 4.16]	4.3%
[ÜNal E 2021]	3	3 33	1 0300	3	1.33 0.6600		1.84	[-0.47:4.16]	4.3%
[ ÜNal E 2021 ]	3	4 00	1 1300	3	1.33 0.6600	1 100	2.30	[-0.33:4.93]	3.4%
	5	1.00	1.1500	0	1.00 0.0000		2.00	[ 0.00, 4.00]	0.470
Common effect model	54			54		-	0.72	[ 0.23: 1.20]	100.0%
Heterogeneity: $l^2 = 10\%$	2<00	001 n =	0 33	54			5.112		
riotorogeneity. 7 = 10%,	0.0	oor, p -	0.00			-6 -1 -2 0 2 1 6			
						-0 -4 -2 0 2 4 0			

# (f) Outcomes were described as CA frequency

**Figure 4.** Meta-analysis for in vitro genotoxicity of TiO<sub>2</sub> NPs. (**a**–**f**) Show the forest plots for genotoxicity endpoints of T DNA%, TL, OTM, MF, MN frequency, and CA frequency, respectively. 'SD' is the standard deviation; 'SMD' is the standardized mean difference; '95%CI' is the 95% confidence interval; ' $I^{2'}$ ' is Higgins's inconsistency statistic; and ' $\tau^{2'}$ ' is the estimate of between-study variance. Significance is at p < 0.05.

136 384 247 273 235 285 357 163		0.82(0.57-1.06) 0.86(0.63-1.09) 0.99(0.70-1.27) 0.74(0.52-0.96) 0.98(0.75-1.21) 0.74(0.53-0.96) 0.78(0.59-0.97) 0.84(0.68-1.32)	0.80 0.18 0.14 0.23
136 384 247 273 235 285 357 163		0.82(0.57-1.06) 0.86(0.63-1.09) 0.99(0.70-1.27) 0.74(0.52-0.96) 0.98(0.75-1.21) 0.74(0.53-0.96) 0.78(0.59-0.97) 0.84(0.68-1.32)	0.80 0.18 0.14 0.23
384 247 273 235 285 357 163		0.86(0.63-1.09) 0.99(0.70-1.27) 0.74(0.52-0.96) 0.98(0.75-1.21) 0.74(0.53-0.96) 0.78(0.59-0.97) 0.84(0.68-1.32)	0.18 0.14 0.23
247 273 235 285 357 163		0.99(0.70-1.27) 0.74(0.52-0.96) 0.98(0.75-1.21) 0.74(0.53-0.96) 0.78(0.59-0.97) 0.84(0.68-1.32)	0.18 0.14 0.23
247 273 235 285 357 163		0.99(0.70-1.27) 0.74(0.52-0.96) 0.98(0.75-1.21) 0.74(0.53-0.96) 0.78(0.59-0.97) 0.84(0.68-1.32)	0.18 0.14 0.23
273 235 285 357 163		0.74(0.52-0.96) 0.98(0.75-1.21) 0.74(0.53-0.96) 0.78(0.59-0.97) 0.84(0.68-1.32)	0.14
235 285 357 163		0.98(0.75-1.21) 0.74(0.53-0.96) 0.78(0.59-0.97) 0.84(0.68-1.32)	0.14
235 285 357 163		0.98(0.75-1.21) 0.74(0.53-0.96) 0.78(0.59-0.97) 0.84(0.68-1.32)	0.14
285 357 163		0.74(0.53-0.96) 0.78(0.59-0.97) 0.84(0.68-1.32)	0.23
357 163		0.78(0.59-0.97) 0.84(0.68-1.32)	0.23
357 163		0.78(0.59-0.97) 0.84(0.68-1.32)	0.23
163		0.84(0.68-1.32)	
	0.0 0.5 1.0 1.5		
	0.0 0.5 1.0 1.5		
n		SMD(95%CI)	<i>n</i> valu
			P
100	<b>⊢</b>	1.53(0.67-2.40)	0.17
219	<b>⊢</b> ∎(	0.89(0.61-1.18)	
115	·•	1.98(1.08-2.88)	0.02
204	<b>⊢</b> ∎1	0.83(0.54-1.11)	
		1999 - 1990 - 1999 - 19	
154	<b>⊢</b>	1.55(0.99-2.12)	0.02
165	<b>-</b> i	0.78(0.46-1.10)	
260	<b>⊢</b> ∎1	0.95(0.70-1.21)	0.52
59	· · · · · · · · · · · · · · · · · · ·	1.35(0.18-2.51)	
	100 219 204 154 165 260 59	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	100 $1.53(0.67-2.40)$ 219 $1.53(0.67-2.40)$ 115 $0.89(0.61-1.18)$ 115 $1.98(1.08-2.88)$ 204 $1.55(0.99-2.12)$ 165 $0.78(0.46-1.10)$ 260 $0.95(0.70-1.21)$ 1.59 $1.35(0.18-2.51)$

**Figure 5.** Subgroup analyses of TiO<sub>2</sub>-NPs genotoxicity on in vitro T DNA% (**a**) and OTM (**b**). 'n' is the sample size; 'SMD' is the standardized mean difference; '95%CI' is the 95% confidence interval; and '*p* value' represents heterogeneity between subgroups. Significant heterogeneity between subgroups is at p < 0.05.



**Figure 6.** Egger funnel diagram of in vitro T DNA% before (**a**) and after (**b**) publication bias correction by the trim and fill method. The middle line shows the overall estimated standard mean difference. Black dots represent the original studies included, and white dots indicate studies that need supplementation.

#### 4. Discussion

In this paper, a comprehensive analysis of 12 in vivo and 14 in vitro studies was conducted to assess the genotoxic effects of  $TiO_2$  NPs. These studies were selected based on meeting the reliability and relevance assessment criteria. The meta-analysis results showed that the SMD for each genotoxic endpoint was greater than 0, suggesting that  $TiO_2$  NPs significantly induced DNA damage and chromosome damage both in vivo and in vitro. Furthermore, there was a significant association between  $TiO_2$  NP treatment and gene mutation in vitro. These findings confirmed the potential risks of genotoxicity associated with human exposure to  $TiO_2$  NPs. Evidently, the duration of exposure and experimental subjects emerged as significant variables influencing DNA damage in the  $TiO_2$  NPs-treated group. Short-term exposure to  $TiO_2$  NPs displayed a higher likelihood of inducing DNA damage. The in vivo comet assay revealed that rats exhibited greater sensitivity to DNA damage induced by  $TiO_2$  NPs than mice. Furthermore, the in vitro comet assay demonstrated that cancer cells exhibited heightened susceptibility to DNA damage induced by  $TiO_2$  NPs than normal cells. However, it was essential to be cautious about the potential influence of publication bias on the accuracy of the meta-analysis results.

Currently, three mechanisms have been proposed for the genotoxicity of  $TiO_2$  NPs. The first mechanism involves direct interaction with DNA. The second one refers to an indirect mechanism in which  $TiO_2$  NPs interact with other molecules and affect the genetic material. Finally, reactive oxygen species (ROS) are generated due to the catalytic potential of the particles [46]. However, the available evidence questions the direct effect of  $TiO_2$  NPs on DNA and favors the role of the latter two mechanisms. According to the French Agency for Food, Environmental, and Occupational Health and Safety, there was no evidence of direct interaction between  $TiO_2$  NPs and DNA or the mitotic apparatus. However, they suggested that direct effects on molecules interacting with genetic material could not be completely excluded [47]. A comprehensive weight of evidence assessment suggested that observed genotoxic effects of  $TiO_2$  (nano and other forms) were secondary to physiological stress rather than direct DNA damage [48]. Nanoparticle-induced oxidative stress was viewed as a signal transducer for further physiological effects, including genotoxicity and cytotoxicity [49,50]. EFSA concluded that the relative contribution of different molecular mechanisms triggered by  $TiO_2$  NPs remained unknown [11].

Extensive research has demonstrated that  $TiO_2$  NP exposure is associated with increased occurrence of DNA damage. This propensity for DNA damage appears to be particularly pronounced following short-term exposure to  $TiO_2$  NPs. This conclusion is substantiated by the collective findings of all in vivo comet assays and the majority of in vitro comet assays encompassed within this meta-analysis. This aligned with the findings of Ling et al. [12], who also observed severe DNA damage following brief exposure to  $TiO_2$  NPs. This phenomenon can likely be attributed to the insufficient time for effective DNA repair due to the constricted exposure window. Additionally, comet assay studies showed a correlation between longer exposure periods and reduced DNA damage [51,52]. This implied that  $TiO_2$  NPs possibly cause early and reversible DNA damage, but cells adapt to the  $TiO_2$  NPs environment and initiate repair mechanisms during prolonged exposures. The potential impact of genotoxicity includes influencing cellular responses like DNA repair, cell cycle arrest, and apoptosis. Inadequate DNA repair before or during damaged DNA replication could potentially trigger mutagenic and oncogenic events [53].

In comet assay, rats and cancer cells subjected to TiO<sub>2</sub> NP exposure exhibited a pronounced susceptibility to DNA damage, as evidenced by their significantly higher OTM than mice and normal cells. This observed discrepancy most likely depended on the inherent capacity of DNA damage response (DDR). Cancer cells showed a broad spectrum of mutations and abnormal gene expressions within the domain of DNA repair responses, which set in motion a state of genome instability [54,55]. The frequent compromise of certain DDR pathways in cancer cells facilitated the accumulation of genomic instability. As a result, the loss of functional DDR pathways rendered cancer cells more prone to DNA damage and additional defects within the DDR network [56]. Conversely, the meticulously controlled replication observed in normal cells acted as a buffer against the onset of a hyperactivated DDR [57]. This observation was validated by evidence that the incidence of DNA lesions within cancer cell lines was elevated compared to primary cells cultivated under controlled laboratory conditions [58]. Close attention must be paid to the risks of cancer treatments based on  $TiO_2$  NP drug delivery systems [59]. Studies have shown that exposure to  $TiO_2$  NPs of high concentrations or small size is usually associated with higher genotoxicity. A literature review concluded that genotoxicity exhibited an increasing trend with decreasing particle size and increasing concentrations of  $TiO_2$  NPs [13]. Moreover, Dubey et al. [60] observed a dose-dependent escalation in DNA damage, lipid peroxidation, and protein carbonylation as concentrations of exposed nanoparticles increased. In this study, no difference in DNA damage induced by  $TiO_2$  NPs was observed under varying particle sizes and exposure concentrations. More high-quality literature is needed to be included in the comprehensive analysis.

The impact of  $TiO_2$  NPs on gene mutation and chromosome aberrations has been extensively studied. Jain et al. [43] reported a linear correlation between the mutation rates and the exposure levels of  $TiO_2$  NPs. Moreover, the mutagenic potential of  $TiO_2$ NPs in V-79 cells was evaluated via mammalian HGPRT gene forward mutation assay, showing a 2.98-fold increase in 6TG<sup>R</sup> HGPRT mutant frequency [42]. The presence of heightened levels of ROS could interact with cellular components, including DNA bases or the deoxyribosyl backbone of DNA, resulting in the formation of damaged bases or strand breaks. Certain oxidative DNA lesions, which might not be fully repaired, could act as precursors to mutagenesis. This phenomenon is particularly relevant to mismatch repair or incomplete repair mechanisms, which can give rise to specific mutational events [42,61]. The study employing transmission electron microscopy yielded evidence suggesting that the internalization of  $TiO_2$  NPs by cells is observable within cytoplasmic vesicles and close to and inside the nucleus. Notably, larger agglomerates of  $TiO_2$  NPs were believed to possess the capacity to disrupt or damage chromosomal structures, potentially leading to chromosome aberrations [62]. This meta-analysis incorporated the most recent studies of in vivo and in vitro genotoxicity and underwent rigorous quality assessments to enable quantitative analysis. However, there were still some limitations. The available data were primarily limited as only the Chinese and English literature was included in the screening process. However, the high reliability and relevance of the included literature increased confidence in the results. Secondly, it is suggested that future studies pay closer attention to the substance characterization of  $TiO_2$  NPs, such as shape, size, and charge. The association between these important characteristics and genotoxicity is worth discussing in depth. Finally, more high-quality genotoxicity studies on  $TiO_2$  NPs are needed to help minimize the impact of publication bias.

Moving forward, there are several key aspects that researchers should focus on in future studies concerning TiO<sub>2</sub> NPs and genotoxicity. Long-term animal studies would be valuable to explore the underlying molecular mechanisms of genotoxicity induced by TiO<sub>2</sub> NPs further. Researchers should also investigate the catabolism of TiO<sub>2</sub> NPs once they enter the human body. Study results will provide valuable insights into the internal exposure dose of nanoparticles within target organs or cells. Furthermore, establishing a cut-off value for TiO<sub>2</sub> particle size in relation to genotoxicity is an important area of research. Establishing stringent regulations and guidelines for the judicious application of TiO<sub>2</sub> NPs is essential to mitigate their potential genotoxic effects, thus ensuring effective protection of public health.

#### 5. Conclusions

This meta-analysis has provided evidence that  $TiO_2$  NPs could induce genotoxicity, including DNA damage and chromosomal damage both in vivo and in vitro, as well as in vitro gene mutations. Short-term exposure to  $TiO_2$  NPs would lead to increased DNA damage. Rats were more sensitive to  $TiO_2$  NPs-induced DNA damage in vivo than mice, and cancer cells exhibited heightened susceptibility to in vitro DNA damage induced by

 $TiO_2$  NPs than normal cells. The interaction between  $TiO_2$  NPs and DNA, along with the activation of ROS, influenced the DNA repair response and induced genotoxicity. Therefore, it is necessary to raise public awareness about the potential risks associated with using  $TiO_2$  NPs, particularly in products intended for consumption as food and drugs.

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#### Abbreviations

Nanoparticles Titanium Dioxide Nanoparticles
European Food Safety Authority
Web of Science
China National Knowledge Infrastructure
the percentage of DNA in tail
tail length
olive tail moment
mutation frequency
micronucleus
chromosomal aberrations
toxicological data reliability assessment method
Center for Disease Control and Prevention
standardized mean difference
confidence interval
reactive oxygen species
DNA damage response

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