## Appendix N- In vitro genotoxicity studies from OECD dossier (OECD, 2016)

The evaluation of the studies has been performed according the approach set in Appendix D

Test system/Test object	Exposure conditions (concentration/ duration /metabolic activation	Information on the characteristic s of the test substance	Scoring for nanoscale considerations (dispersion and/or confirmation of internal exposure), assigned according to Appendix E	Result	Reliability/ Comments	Relevance of the result	Reference authors_year
Micronucleus test Peripheral blood lymphocytes (PBL)	20, 50, 100 µg/ml TiO2NPs Treatment 24h after lymphocytes stimulation 20h later CytoB, 72 h harvesting.	TiO <sub>2</sub> NPs (P25), anatase/rutile, 15-24 nm	NSC: 2 Protocol for dispersion including sonication in the media, but no results presented.	<b>Positive</b> concentration- related increase Cytotoxicity: concentration- and time-dependent decrease in cell viability	Reliability: 2 no positive control No concurrent measurement of cell proliferation (CBPI or RI)	Limited	Kang et al. (2008)
Comet assay in human peripheral blood mononuclear cells (PBMC) isolated by buoyancy density centrifugation (Ficoll-paque)	0, 50, or 100 µg /mL for 0, 6, 12, and 24 hr To determine the protective effects of antioxidants, lymphocytes were pre-treated with 1 mM N- acetylcysteine (NAC; for 1 hr prior to TiO2 NPs treatment Measure of Olive tail moment (OTM)	TiO <sub>2</sub> NPs (P25), anatase/rutile, 15-24 nm	NSC: 2 Protocol for dispersion including sonication in the media, but no results presented.	Positive Statistically significant and concentration- related increase of OTM. NAC significantly decreased TiO <sub>2</sub> NPs- induced DNA breakage. Cytotoxicity: concentration- and time-dependent decrease in cell	Reliability: 3 No positive control, only OTM is reported. Methodology insufficiently described. Not clear how many samples (hence cells) per concentration were analysed. No data on stability of the	Low	Kang et al. (2008)

Cell viability: NPs viability trypan blue ROS increase, the ROS: DCFDA probe level is reduced by fluorescence. NAC treatment Shi et al. (2010) Neutral and 0, 0.01, 0.1 and TiO<sub>2</sub>NPs (P25), NSC:3 Negative Reliability: 3 Low no specific indications on 1.0 µg/mL anatase/rutile, alkaline comet dispersion or need for 15-24 nm TiO<sub>2</sub> NPs did not No positive assay 24 hr exposure. All increase statistically considerina control was L-02 cell line experiments agglomeration and no significantly used. were performed in indications of Only tail (human fetus OTM level in triplicate and hepatic cells) ultrasonication alkaline or neutral moment is repeated three versions. reported. 8-OHdG No effect on cell No data on times Analysis (-S9) viability by both stability of the Measure of OTM ATP level or (HPLC/EC) NPs in culture Negative control: apoptosis medium. DMSO No proof of assessment. Positive control: the NPs TiO2NPs increased internalisation. none cellular 8-OHdG levels at 1  $\mu$ g/ml. Method for Viability: determination of double strand intracellular ATP breaks level ROS generation: measurement TiO<sub>2</sub> NPs induced (neutral comet Apoptosis: flow significant increase assay in ROS level at 1 procedure cytometry µg/ml. All described) is **ROS** generation not adequately concentrations of TiO<sub>2</sub> NPs increased reported. and lipid peroxidation: flow MDA levels cytometry significantly.



					Concentrations in tables are in µg/L, while in the text µg/mL.		
Micronucleus test L-02 cell line (human fetus hepatic cells)	0, 0.01, 0.1 and 1.0 μg/L 24 hr exposure. All experiments were performed in triplicate and repeated three times (-S9)	TiO2NPs (P25), anatase/rutile, 15-24 nm	NSC: 3 no specific indications on dispersion or need for considering agglomeration and no indications of ultrasonication	Negative	Reliability: 3 No positive control was used. Low concentration range tested ("trace TiO <sub>2</sub> NPs " according to the study authors). Concentrations in tables are in µg/L, while in the text µg/mL	Low	Shi et al. (2010)
Comet assay NRK-52E rat kidney proximal cells (CRL-1571)	cells exposed for 24h (Comet) or 48 h (cytotoxicity) to TiO <sub>2</sub> NPs (from CEA) from 20 to 200 µg/mL Cytotoxicity: MTT and LDH assay ROS generation: spectrofluorimetry with 2',7'- dichlorodihydro-	TiO2NPs, anatase, 12 nm (TEM)	NSC: 1 specific dispersion protocol, different levels of agglomeration observed, but cell internalisation confirmed (Fig 3 reports onlyTiO <sub>2</sub> NPs (12nm), but text indicates confirmation for all NPs)	POSITIVE Comet: Tail moment is reported, statistically significant and concentration- dependent increase. Cytotoxicity: low, dependent on NPs size (smallest NPs are more cytotoxic)	Reliability: 2 No results on positive and negative controls reported. Only tail moment is reported.	Limited	Barillet et al. (2010)

fluorescein and on crystalline diacetate acetyl phase (anatase was ester (H<sub>2</sub>DCF-DA) the most cytotoxic). probe ROS generation: Positive control: etoposide ROS increase, but not correlated to Negative control: cytotoxicity untreated cells NPs observed in cytoplasm either in vesicles or isolated, maybe NPs can enter cells via nonspecific adsorptive endocytosis or by direct diffusion NSC: 1 vH2AX NRK-52E cells Barillet et al. 1) TiO<sub>2</sub>NPs, no effect on yH2AX Reliability: 1 Limited anatase, 12 nm specific dispersion yH2AX assay (2010) immunostainin exposed to foci concentrations (TEM) protocol, different levels is not a q of agglomeration from 20 to 200 standardised NRK-52E rat  $\mu g/mL;$ 2) TiO<sub>2</sub>NPs observed, but cell test. The internalisation confirmed (P25), kidney method is proximal cells anatase/rutile, Negative control: (Fig 3 reports only not validated TiO<sub>2</sub>NPs (12nm), but text (CRL-1571) untreated cells 15-24 nm for indicates confirmation for regulatory 3) TiO2, all NP) Positive control: purposes. etoposide anatase, 142 nm (TEM) NSC: 3 Positive: TiO<sub>2</sub> NPs Alkaline comet 10 µg/mL 1) TiO<sub>2</sub>NPs, Reliability: 3 Gurr et al. Low (2005) Exposure for 1h in anatase, 10 nm assay No information on anatase sizes 10 dispersion, however, low nm, 20 nm, and +/- Fpg dark 2) TiO<sub>2</sub>NPs, Not Measure of tail anatase, 20 nm concentration TiO<sub>2</sub> rutile size 200 appropriate 3) TiO2, Human moment nm induced conditions and statically significant bronchial Negative control: anatase, ≥200 time of oxidative DNA epithelial cells, untreated nm, exposure: 4) TiO2, BEAS-2B damages (tail

Cytotoxicity: MTT	anatase, 200	moment).	BEAS-2B cells	
assay (0.001, 0.1,	nm		embedded in	
1 and 10 µg/ml)	5) TiO2, rutile,	Negative: TiO2	gel	
3 days of exposure	200 nm	anatase, sizes 200	on slides were	
		nm and >200 nm	treated with	
Measurement of		did not induce	TiO2 for 1h in	
hydrogen		oxidative DNA	dark.	
peroxide:		damages.	It is not clear	
fluorogenic		-	to what extent	
probe, Amplex red		(A preliminary	the NPs	
		study was	migrated	
Measurement of		performed, cells	through the	
lipid peroxidation:		treated with	gels.	
MDA		TiO2 particles >200	One	
		nm and with TiO2	concentration	
		NPs anatase (10	tested for	
		nm) 0, 5, and 10	rutile. For	
		µg/mL for 1 h.	anatase 2	
		DNA damage was	concentrations	
		detected in	positive but no	
		treatment with 10	concentration	
		µg/mL	response.	
		TiO <sub>2</sub> NPs anatase	No	
		(10 nm). No	demonstration	
		damage was	of uptake.	
		detected with 5	No positive	
		µg/ml anatase	control was	
		TiO <sub>2</sub> NPs (10 nm)	used. Only tail	
		or with TiO <sub>2</sub>	moment is	
		particles >200 nm).	reported in the	
		Treatment with	comet assay.	
		anatase-rutile		
		mixture (10 µl		
		each) induced		
		higher level of		
		oxidative DNA		
		damage than		

		treatment with		
		either anatase or		
		rutilo		
		particles alone.		
		Cytotoxicity:		
		treatment with		
		10 ug/ml anatase-		
		cized TiO <sub>2</sub> ND <sub>2</sub> (20		
		Sized TIO2 INFS (20		
		nm) for 3		
		days caused cell		
		growth inhibition,		
		$IC_{50} = 6.5 \text{ µa/ml}$		
		Hydrogon porovido		
		levels: statistically		
		significant increase		
		of cellular levels by		
		TiO <sub>2</sub> NPs anatase		
		sized		
		(10  and  20  nm)		
		and TiO rutile		
		sized (200 nm) and		
		not by anatase-		
		sized (200 and		
		>200 nm) TiO2.		
		-		
		Lipid peroxidation:		
		(10  and  20  nm)		
		increased the		
		cellular MDA level,		
		while TiO <sub>2</sub> anatase		
		(200 nm and >200		
		nm), and TiO <sub>2</sub> rutile		
		(200  nm) did not		

Micronucleus test10 ug/ml of TiO2 for 24 hi presence of CytoB1) TiO2,NPs, anatase, 10 mm anatase, 20 mm 3) TiO2, anatase, 200 nm, 4) TiO2, anatase, 200 nm, 4) TiO2, rutile, 200 nmNSC: 3 No information on dispersion, however, low concentrationPositive: anatase-sized (C) 200 nm) and TiO2 200 nm)Reliability: 3 matase-sized (C) 200 nm) and TiO2 200 nm) anatase, 200 nm, 4) TiO2, rutile, 200 nmNSC: 3 no, dispersion, however, low concentrationPositive: anatase-sized (200 nm) and rutile-sized (200 nm) TiO2LowCurr et al. (2005)Alkaline comet gasayBEAS 2B, A549, Caco-2: 0, 50, 100 16 HBE: 0, 2, 8, pulmonary (200 concentration1) TiO2 (NM- 100), anatase, 201 TiO3, NPs (NM- 102), anatase, 105), manatase, 201 TiO3, NPs (NM- 102), anatase, 201 TiO3, NPs (NM- 102), anatase, 21 TiO3, NPs (NM- 102), anatase, 16 HBE: 0, 2, 8, alveolar A549) intestinal (Caco-2; exposure: 3h and persive: concent for 30 TiO3, NPs (NM- 105), anatases/rutile, 1150, NPs (NM- 105), anatase/rutile, 21 TiO3, NPs (NM- 105), anatase/rutile, 1150, NNSC: 1 NSC: 1 <b< th=""><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></b<>								
Test presence of CytoBInformation of presence of CytoBantatase, 10 nm antase, 20 nm 3) TiO2, antase, 2200 nm, 4) TiO2, antase, 2200 nm, 5) TiO2, rutile, 200 nmNo intormation on dispersion, however, low concentrationantase-sized (TiO, NPs 10 nm and TiO, 200 nm) Negative: anatase-sized (200 nm) and artase-sized (200 nm) and tratement with 100/mi TiO: NPs and TiO: particles (N- No sand TiO: particles (N- No sand TiO: particles (NM- 100) antase, 30, 1102, antase, 31 TiO: NPs (NM- 102, antase, 21-22 nm 30 TiO:NPs (NM- 105), antase/rutile, 15-24 nmNSC: 1 NASC: 1 NANOGENOTOX Project 201 tiop: particles (NHEX: 0, 15, 33) antase/rutile, 15-24 nmNSC: 1 NANOGENOTOX Project 201 tiop: particles (NHEX: 0, 15, 33) antase/rutile, 15-24 nmNSC: 1 NANOGENOTOX Project 201 tiop: particles No r24-h treatment. No r24-h treatment	Micronucleus	10 µg/ml of TiO2	1) TiO <sub>2</sub> NPs,	NSC: 3	Positive:	Reliability: 3	Low	Gurr et al.
Human bronchial epithelial cells, BEAS-28BEAS 28, A549, (Caco-2; 0, 50, 100) 16 HBE; abveolar A549)1) TiO; (NM- 10, 20, anatase, 200 nmdispersion, however, low concentration(HO, NPS 10 nm and TiO; 200 nm) Negative: anatase-szed (>200 nm) and trutle-sized (200 nm) TiO2 namameterials.Ihe experimental protocol, with co-exposure of cells to TiO2 anatase-sized (200 nm) TiO2 namameterials.Alkaline comet assayBEAS 28, A549, (Caco-2; 0, 50, 100) 16 HBE; 0, 2, 8, 32, 128 and 5121) TiO; (NM- 100, anatase, 200 nmNSC: 1 NSC: 1 NSC: 1Positive TiO; project, 2013 (Cause observable cells or 16 HBE; og 10, 21, 22 nmHigh NANOGENOTOX Project, 2013, anatase, 21, 70, NPS (NM- 100), anatase, 21, 70, NPS (NM- 105), anatase/rutile, 16 HBE; adveolar A549 (Caco-2; tormainity undifferentiate dispersion resonance project, 2013, anatase, project, 2013, anatase, prostive; trutile, 15-24 nmNSC: 1 NANOGENOTOX Project, 2013, anatase, prostive; trutile, 15-24 nmNSC: 1 NANOGENOTOX Project, 2013, anatase, prostive; trutile, 15-24 nmNSC: 1 Nanogenotox Project, tios nm trutile, 16 HBE; opsitive; trutile, 16 HBE; prostive; 31 and 10;High NANOGENOTOX Project, 2013, anatase, tore 4-h treatment. Horizand MM-105; NM- treatment.High NANOGENOTOX Project, 2013, Course- tore 4-h treatment.Intestinal (Caco-2; provided to EFSA No. 7,8 and 10;100, Nas only anatase/rutile, 15-24 nmNas tore 4-h treatment.National (AM-105) tore 4-h treatment.High tore 4-h treatment.Intestinal (	test	for 24 h in	anatase, 10 nm	No information on	anatase-sized			(2005)
Human bronchial epithelial cells, BEAS-28CytoBanatase, 20 m anatase, 2200 nm, 4) TiO2, anatase, 200 nm, mConcentrationand Irb2 20 mm) Negative: anatase-sized (>200 nm) and rutile-sizedexperimental co-exposure of cells to TiO2 and cytoB, is inadequate for nanomaterials.BEAS-28BEAS-28, 258, 2549, (200 nm)1) TiO2, rutile, 200 nm1) TiO2, rutile, 200 nmCell cycle progression (% of binucleated cells): Treatment with 10µg/m1 TiO2, PAPs and TiO2, particles for 24 h did not case observable cell cycle delay.A single concentration was tested.Alkaline comet pulmonary (bronchial epithelial for 16 HBE: og 30-skin: 0, 82, and 226 µg/m1 16 HaBE; 16 HBE; 16 HBE; <br< td=""><td></td><td>presence of</td><td>2) <math>I_1O_2NPs</math>,</td><td>dispersion, however, low</td><td><math>(IIO_2 NPs 10 nm)</math></td><td>The</td><td></td><td></td></br<>		presence of	2) $I_1O_2NPs$ ,	dispersion, however, low	$(IIO_2 NPs 10 nm)$	The		
Human bronchial epithelial cells, BEAS-28Santase, 2200 nm, 4) TiO2, nantases, 2200 nmNegative: anatase, 2200 nmProtocol, with calls to TiO2 and cytoB, is inadequate for nanomaterials.Alkaline comet assayBEAS 2B, A549, and 256 µg/ml1) TiO2, (NM- 100, anatase, 200 nmNSC: 1 No positive tortolNSC: 1 No positive tortol and 256 µg/mlNSC: 1 100, nantase, 201 TiO2, natase, 201 TiO3, natase, 21 TiO3, NSC NINSC: 1 Nanogenotox Project tispersion protocolPositive TiO2, particles tortol was used.NANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)NANOGENOTOX TiO2, particles (NM- 100, natase, 21 TiO2NPS (IM- anatase, 21-22 nm tispersion protocolPositive TiO2, particles (NM- 100, anatase, 21 TiO2NPS (IM- 105, natase, 21-22 nm tispersion protocolReliability: 1 HighHigh NANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)Pulmonary (fornochial µg/ml (forcochial µg/ml (locaco-2; 24A daid 246 µg/ml (locaco-2; 24A daid 246 µg/ml (locaco-2; 24A daid 246 µg/ml (locaco-2; 24A daid 246 µg/ml (locaco-2; 24A daid 246 µg/ml (locaco-2; 24A 24ANANOS (IM- 105, anatase, 21-22 nm anatase/turble, anatase/ 21-22 nm anatase/turble, anatase/ 21-22 nm anatase/turble, anatase, 21-22 nm anatase/turble, anatase, 21-22 nm anatase/turble, anatase/ 21-22 nm 21-22 nm 21-22 nm 21-22 nmNegative: and 50 µg/ml anatase/turble, anatase/ <td></td> <td>CytoB</td> <td>anatase, 20 nm</td> <td>concentration</td> <td>and <math>IIO_2 200 \text{ nm}</math>)</td> <td>experimental</td> <td></td> <td></td>		CytoB	anatase, 20 nm	concentration	and $IIO_2 200 \text{ nm}$ )	experimental		
bronchial epithelial cells, BEAS-2B Alkaline comet pulmonary	Human		3) 1102,		Negative:	protocol, with		
epithelial cells, BEAS-2Bnm, 4) TiO2, anatase, 200 nmnm, 4) TiO2, anatase, 200 nmNm, 4) TiO2, anatase, 200 nmCells to 1iO2 inadequate for nanomaterials.Alkaline comet assayBEAS 2B, A549, and 256 µg/ml1) TiO2 (NM- 100, anatase, 200 nmNSC: 1 Nanogenotox Project dispersion protocolPositive treatment with 10µg/ml TiO2, NPs and TiO2, particles for 24 h did not case observable cell cycle delay.Ne positive concentration was tested.NANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10) and 256 µg/mlNITiO2, (NM- 100), anatase, 20 TiO2, NPs (NM- 100), anatase, 21 TiO2, NPs (NM- 101, anatase, 21 TiO2, NPs (NM- 102), anatase, 21 TiO2, NPs (NM- 102), anatase, 21 TiO2, NPs (NM- 102), anatase, 21 TiO2, NPs (NM- 102), anatase, 21 TiO2, NPs (NM- 105), and 65 µg/mlNITIO2, NM- 105, anatase, 21 TiO2, NPs (NM- 105), anatase, 21 TiO2, NPs (NM- 105), anatase, 21 TiO2, NPs (NM- 105), and 452 Pad and 452 Pad 105, anatase, 21 TiO2, NPs (NM- 105, anatase, 21 TiO2, NPs (NM- 105, NM- 105, NM- 105, NM- 106 HBE; 30 Tio2, NPs (NM- 107, NPs (NM-102) and AM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.High HighNANOGENOTOX NM- NM- EFSA No. 7,8 and 10)Intestinal (Caco-2, µg/cm2Exposure: 3h and 22/h 24/h undifferentiate d cells used)NHE Stive control for StiveNHE Stive control for StiveNHE Stive Stive An and 250 Stive An and 250 StiveNHE Stive Stive StiveNHE Stive Stive <td>bronchial</td> <td></td> <td>anatase, ≥200</td> <td></td> <td>anatase-sized</td> <td>co-exposure of</td> <td></td> <td></td>	bronchial		anatase, ≥200		anatase-sized	co-exposure of		
BEAS-2B4) TiO2, anatase, 200 nmanatase, 200 nmA single concentration was tested.anatase, 200 nanomaterials.Alkaline comet assayBEAS 2B, A549, 16 HBE: 0, 2, 8, pulmonary (bronchial B HEK: 0, 15, 33 nitestinal (Caco-2, primarily undifferentiate primarily 24h1) TiO2 (NM- 100, anatase, 2) TiO2NPS (NM- 100, anatase, 100, anatase,	epithelial cells,		nm,		(>200 nm) and	cells to 1102		
Alkaline comet assayBEAS 2B, A549, and 256 µg/ml intestinal (Cao-2, primarily undifferentiate for and 246 µg/m21) TiO2 (NM- 100, anatase, 30 TiO2 (NM- 100, anatase, 16 HBE: 0, 2, 8, 16 HBE: 0, 2, 8, 170 2 methodNSC: 1 NSC: 1 Nanogenotox Project HO/0 was only tested in 16 HBE cells and was positive; 16 HBE: 0, 2, 8, 16 HBE: 0, 2, 8, 100, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2,	BEAS-2B		4) TiO2,		rutile-sized	and cytoB, is		
Image: Normal systemImage: Normal systemNmm (S)TiO2, rutile, 200 nmCell cycle progression (% of binucleated cells): Treatment with 10µg/ml TiO2 NPs and TiO2 particles for 24 h did not cause observable cell cycle delay.A single concentration was tested.A single concentration was tested.Alkaline comet assayBEAS 2B, A549, 100, anatase, 50-150 nm 16 HBE: 0, 2, 8, 100, anatase, pulmonary (bronchial ef HBE: 0, 2, 8, 16 HBE: 0, 2, 8, and 65 µg/ml 16 HBE: 0, 2, 8, 102), anatase, 102), anatase, 102), anatase, 102), anatase, 102), anatase, 102), anatase, 102), anatase, 102, anatase, 102), anatase, 102, anatase, 103, anatase, 104 and 246 105, 104 and 246 105, 105, 104 and 246 105, 105, 105, 104 and 246 105, 105, 105, 104 and 246 105, 105,			anatase, 200		(200 nm) TiO2	inadequate for		
Cell cycle progression (% of binucleated cells): Treatment with 10µg/ml TiO2, PPs and TiO2 particles for 24 h did not cause observable cell cycle delay.A single concentration was tested.Alkaline comet assayBEAS 2B, A549, Caco-2: 0, 50, 100 and 256 µg/ml 16 HBE: 0, 2, 8, 32, L28 and 512 (bronchial epitheliai1) TiO2 (NM- 100), anatase, 50-150 nm 2) TiO2NP's (NM- 2) TiO2NP's (NM- 2) TiO2NP's (NM- 16 HBE: alwelar A549)NSC: 1 No positive calco-2: 0, 50, 100 100), anatase, 50-150 nm 2) TiO2NP's (NM- 2) TiO2NP's (NM- 2) TiO2NP's (NM- 2) TiO2NP's (NM- 100), anatase, 2) TiO2NP's (NM- 100), anatase, 2) TiO2NP's (NM- 102), anatase, 102, anatase, <td></td> <td></td> <td>nm</td> <td></td> <td></td> <td>nanomaterials.</td> <td></td> <td></td>			nm			nanomaterials.		
Alkaline comet assayBEAS 2B, A549, Caco-2: 0, 50, 100 and 256 µg/ml 			5) TiO2, rutile,		Cell cycle			
Alkaline comet assayBEAS 2B, A549, Caco-2: 0, 50, 100 and 256 µg/ml1) TiO2 (NM- 100), anatase, 50-150 nmNSC: 1 Nanogenotox Project dispersion protocolPositive Treatment with nd 102, particles for 24 h did not used.Reliability: 1 No positive control was used.HighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 102 NPS (NM- 100), anatase, 101, anatase, 21, 120, anatase, 21-22 nmPositive to 150, nm 21-102, natase, 21-22 nmReliability: 1 Nanogenotox Project dispersion protocolHighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)pulmonary epithelial BEAS 2B and 16 HBE; alveolar A549101, anatase, 21-22 nm 105, anatase/rutile, 15-24 nmNSC: 1 Nanogenotox Project 102, anatase, 21-22 nm 105, anatase/rutile, 15-24 nmNANOGENOTOX Project 2013 (Documentation provided to EFSA No. 7,8 and 10)NANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)16 HBE; alveolar A549 undifferentate d cells used)NEK: 0, 15, 33 164 and 246 µg/cm2NEX: 1 15-24 nmNEX: 1 Nangenotox Project 15-24 nmNEX: 1 Nangenotox Project 21-22 nm 105, NM- 102 and NM-105 Were tested in all cell lines and were positive, with the 3- h or 24-h treatment.NEX: 1 No NM- 102 and NM-105 Were meative inNEX: 1 NANOGENOTOX NM- NONEX: 1 NANOGENOTOX Project, 2013 NO NO NM- NO16 HBE; alveolar A549 bo 2 or methylicNEX: 1 NANOGENOTOX NANOGENOTOX NO NANOGENOTOX NO NO NO N			200 nm		progression (% of	A single		
Alkaline comet assayBEAS 2B, A549, and TiO2 particles for 24 h did not cause observable cell cycle delay.No positive control was used.NANOGENOTOX Project, 2013Alkaline comet assayBEAS 2B, A549, and TiO2 particles for 24 h did not cause observable and TiO2 particles (NM- 100) anatase, 100 anatase, 20 TiO2 NPs (NM- 20 TiO2 NPs (NM- 20 TiO2 NPs (NM- 102) anatase, 20 TiO2 NPs (NM- 20 TiO2 NPs (NM- 					binucleated cells):	concentration		
Alkaline comet assayBEAS 2B, A549, Caco-2: 0, 50, 100 and 256 µg/ml1) TiO2 (NM- 100), anatase, 20 TiO2 NPS (NM- 100), anatase, 20 TiO2 NPS (NM- 102), anatase, 21 TiO2 NPS (NM-<					Treatment with	was tested.		
Alkaline comet assayBEAS 2B, A549, Caco-2: 0, 50, 100 and 256 µg/ml 16 HBE: 0, 2, 8, pulmonary gibre thelial1) TiO2 (NM- 100), anatase, 50-150 nm 2) TiO2NPS (NM- 102), anatase, 2) TiO2NPS (NM- 105), and 65 µg/ml 16 HBE: 0, 2, 8, and 65 µg/ml 16 HBE; 3D-skni: 0, 82, alveolar A549)1) TiO2 (NM- 102, natase, 2) TiO2NPS (NM- 105), anatase/rutile, 105, and 105, and 105, and 105, and 105, and 105, and 106, lawNSC: 1 Nanogenotox Project dispersion protocolPositive TiO2 particles (NM- TiO2 particles (NM- 100) was only tested in 16 HBE cells and was positive; TiO2 NPS (NM-102) and NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.HighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)Intestinal (Caco-2, µg/cm2Exposure: 3h and 24h undifferentiate d cells used)15-24 nmNanogenotox Project and NM-105 were positive, with the 3- h or 24-h treatment.Reliability: 1 HighHighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)					10µg/ml TiO <sub>2</sub> NPs			
Alkaline comet assayBEAS 2B, A549, Caco-2: 0, 50, 100 and 256 µg/ml 16 HBE: 0, 2, 8, 232, 128 and 512 µg/ml1) TiO2 (NM- 100), anatase, 50-150 nm 20 TiO2NPS (NM- 102), anatase, 21-22 nmNSC: 1 Nanogenotox Project dispersion protocolPositive TiO2 particles (NM- 100) was only tested in 16 HBE cells and was positive; TiO2 NPS (NM-102) and 05 µg/ml and 256 µg/ml 102), anatase, 21-22 nm 3) TiO2NPS (NM- 105), anatase/rutile, alveolar A549)NANOGENOTOX Project, 2013 (Documentation provided to and 25 µg/ml 102), anatase, 21-22 nm 3) TiO2NPS (NM- 105), anatase/rutile, 15-24 nmNSC: 1 NANOGENOTOX Project, 2013 (Documentation provided to and NM-105) were and NM-105) were and NM-105 were positive, with the 3- h or 24-h treatment.Manogenotox Project tios (NM- 102) anatase, 21-22 nm anatase/rutile, 15-24 nmNANOGENOTOX Project, 2013 (Documentation provided to and NM-105) were and NM-105 were positive, with the 3- h or 24-h treatment.HighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)Intestinal (Caco-2, primarily undifferentiate d cells used)10 and NM-105 tho or methylIs an an and to 2 and NM-105 were <b>negative</b> inBoth TiO2 NPS, NM- 102 and NM-105 were <b>negative</b> inHigh the documentation the documentation					and TiO <sub>2</sub> particles	No positive		
Alkaline comet assayBEAS 2B, A549, Caco-2: 0, 50, 100 and 256 µg/ml 16 HBE: 0, 2, 8, 21, 128 and 512 (bronchial epithelial1) TiO2 (NM- 100), anatase, 50-150 nm 2) TiO2NPS (NM- 102), anatase, 21-22 nmNSC: 1 Nanogenotox Project dispersion protocolPositive TiO2 particles (NM- 100) was only tested in 16 HBE cells and was positive; TiO2 NPS (NM-102 and 10), anatase, tested in 16 HBE cells and was positive; TiO2 NPS (NM-102 and 10), anatase, 21-22 nmNSC: 1 Nanogenotox Project dispersion protocolPositive TiO2 particles (NM- 100) was only tested in 16 HBE cells and was positive; TiO2 NPS (NM-102 and NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.HighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)Intestinal (Caco-2, (Caco-2, Uells used)NHEK: 0, 15, 33 40, 07 methyl3) TiO2NPS (NM- 105, N15-24 nmNanogenotox Project dispersion protocolNH-102 and NM-105 h or 24-h treatment.NANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)					for 24 h did not	control was		
Alkaline comet assayBEAS 2B, A549, Caco-2: 0, 50, 100 and 256 µg/ml 16 HBE: 0, 2, 8, 2) TiO2NPS (NM- 100), anatase, 22, 128 and 512 (bronchial epithelial BEAS 2B and 16 HBE; alveolar A549)1) TiO2 (NM- 100), anatase, 2) TiO2NPS (NM- 102), anatase, 21-22 nm 21-22 nm 21-22 nm 21-22 nm 21-22 nm 21-22 nm 21-22 nm 105), and 256 µg/ml 105), and 55 µg/ml 164 and 246 µg/cm²1) TiO2 (NM- 102), anatase, 21-22 nm 105), anatase/rutile, alveolar A549)NBC: 1 NBEAS 2B and 164 and 246 µg/cm²NSC: 1 NAnogenotox Project dispersion protocolReliability: 1 TiO2 particles (NM- 100) was only tested in 16 HBE cells and was positive; TiO2 NPs (NM-102 and NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.HighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)intestinal (Caco-2, primarily undifferentiate d cells used)10 TiO2 NPs (NM- HEAS 2B and 164 and 246 µg/cm²15-24 nmNanogenotox Project dispersion protocolTiO2 NPs (NM-102 and NM-105 Nor Hor 24-h treatment.HighHighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)intestinal (Caco-2, positive control for SBs: Hor 2 or methylNANOGENOTOX Project, 2013 (Documentation Documentation provided to SBs: Hor 2 or methylNBC Project, 2013 NM- 102 and NM-105 were <b>negative</b> inHighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)					cause observable	used.		
Alkaline comet assayBEAS 2B, A549, Caco-2: 0, 50, 100 and 256 µg/ml 16 HBE: 0, 2, 8, 32, 128 and 512 µg/ml1) TiO2 (NM- 100), anatase, 50-150 nm 2) TiO2NPS (NM- 102), anatase, 21-22 nm epithelial 16 HBE; 0, 15, 33 and 65 µg/mlNSC: 1 Nanogenotox Project dispersion protocolReliability: 1 TiO2 particles (NM- 100) was only tested in 16 HBE cells and was positive; TiO2 NPS (NM-102 and MM-105) were tested in all cell lines and were pg/cm2HighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)Intestinal (Caco-2, primarily undifferentiate d cells used)NEK: 0, 15, 33 H2O, or methyd3) TiO2NPS (NM- 105, anatase/rutile, 15-24 nmNANOGENOTOX Project, 2013 (Documentation provided to tested in all cell lines and were positive, with the 3- h or 24-h treatment.HighNANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)					cell cycle delay.			
Ansame ContectDEAS 2D, N3-13, rolog (NM - 100), anatase, and 256 µg/ml 16 HBE: 0, 2, 8, 2) TiO2NPS (NM- 100), anatase, 2) TiO2NPS (NM- 102), anatase, 2) TiO2NPS (NM- 102), anatase, 2) TiO2NPS (NM- 102), anatase, 21-22 nm BEAS 2B and 16 HBE; and 65 µg/mlNanogenotox Project dispersion protocolPrositive restrict (SNM- 100) was only tested in 16 HBE cells and was positive; TiO2 NPS (NM-102 and NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)intestinal (Caco-2, tentestinal (Caco-2, d cells used)NHCK: 0, 15, 33 SR: Ho2 or methyl3) TiO2NPS (NM- 105), anatase/rutile, 15-24 nmTiO2 NPS (NM-102 and NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.Project, 2013 (Documentation provided to EFSA No. 7,8 and 10)	Alkaline comet	ΒΕΔ <u>ς</u> 2Β. Δ540	1) $TiO_2$ (NM-	NSC: 1	Positiva	Reliability: 1	High	
Late 2: 0, 50, 100100, initiality, 100, initialit		$C_{2}C_{2}C_{2}C_{2}C_{2}C_{2}C_{2}C_{2}$	1002 (NM	Nanogenotov Project	TiO <sub>2</sub> particles (NM-	Itenability. 1	riigii	Project 2013
and 250 µg/m30-150 mmdispersion protocol100 was only16 HBE: 0, 2, 8, 32, 128 and 5122) TiO2NPs (NM- 102), anatase, up/ml21-22 nmtested in 16 HBE cells and was positive;EFSA No. 7,8 and 10)epithelial BEAS 2B and and 65 µg/mlNHEK: 0, 15, 33 105), anatase/rutile, 16 HBE; alveolar A549)3) TiO2NPs (NM- 105), anatase/rutile, 15-24 nmTiO2 NPs (NM-102 and NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.md 10)intestinal (Caco-2, primarily undifferentiate d cells used)Exposure: 3h and SBS: HoO2 or methylBoth TiO2 NPs, NM- 102 and NM-105	assay	and 256 µg/ml	$50_{-150}$ nm	dispersion protocol	1002 particles (NM-			(Documentation
pulmonary (bronchial epithelial32, 128 and 512 µg/ml102), anatase, 21-22 nmcells and was positive; and 05 µg/mlEFSA No. 7,8 and 10)epithelial BEAS 2B and 16 HBE; alveolar A549)NHEK: 0, 15, 33 16 and 246 µg/cm23) TiO <sub>2</sub> NPs (NM- 105), anatase/rutile, 15-24 nmTiO <sub>2</sub> NPs (NM-102 and NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.and 10)intestinal (Caco-2, primarily undifferentiateExposure: 3h and Positive control for SBs: HO2 or methylBoth TiO <sub>2</sub> NPs, NM- 102 and NM-105 were <b>negative</b> in		16 HBE: 0 2 8	2 TiO <sub>2</sub> ND <sub>5</sub> (NM <sub>-</sub>		tested in 16 HBE			provided to
pullinolitaryJ22, 120 and J12ID27, initialse, 21-22 nmpositive; positive;positive; and 10)epithelialNHEK: 0, 15, 333) TiO2NPs (NM-BEAS 2B and 16 HBE; alveolar A549)and 65 µg/ml105), anatase/rutile, anatase/rutile, 15-24 nmTiO2 NPs (NM-102 and NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.and 10)intestinal (Caco-2, primarily undifferentiate d cells used)Exposure: 3h and SBs: H2O2 or methyl15-24 nm	nulmonary	32 128 and 512	102 no2nes (nn=		cells and was			FESA No. 7.8
(c) of childμg/mi21 22 minepithelialNHEK: 0, 15, 333) TiO <sub>2</sub> NPs (NM-BEAS 2B andand 65 µg/ml105),16 HBE;3D-skin: 0, 82,anatase/rutile,alveolar A549)164 and 24615-24 nmµg/cm <sup>2</sup> 15-24 nmlines and wereµg/cm <sup>2</sup> h or 24-h(Caco-2,Exposure: 3h andprimarily24hundifferentiatePositive control ford cells used)SBs:H2O2 or methylH2O2 or methyl	(bronchial	120 and 512	$21_{-}22$ nm		nositive			and 10)
BEAS 2B and 16 HBE; alveolar A549)and 65 µg/ml 105, anatase/rutile, 15-24 nm105), anatase/rutile, 15-24 nmand NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.intestinal (Caco-2, primarily undifferentiate d cells used)Exposure: 3h and SBs: H2O2 or methylSo Tro2 NPS (NM-102 and NM-105) were tested in all cell lines and were positive, with the 3- h or 24-h treatment.	onitholial	NHEK: 0 15 33	3) $TiO_{2}ND_{2}$ (NM_		$TiO_2 NDc (NM_102)$			
DLAS 2D andand 00 pg/m100),16 HBE;3D-skin: 0, 82,anatase/rutile,alveolar A549)164 and 24615-24 nmintestinalintestinal15-24 nm(Caco-2,Exposure: 3h andprimarily24hundifferentiatePositive control ford cells used)SBs:HxO2 or methylHxO2 or methyl	BEAS 2B and	and 65 µg/ml	105)		and NM-105) were			
alveolar A549)   164 and 246   15-24 nm   lines and were     intestinal   intestinal   intestinal   h or 24-h     (Caco-2,   Exposure: 3h and   treatment.     primarily   24h   Both TiO2 NPs, NM-     undifferentiate   Positive control for   Both TiO2 NPs, NM-     d cells used)   SBs:   102 and NM-105     were   negative in	16 HBE	3D-skin: 0.82	anatase/rutile		tested in all cell			
intestinal (Caco-2, Exposure: 3h and primarily 24h undifferentiate Positive control for d cells used) SBs: H2O2 or methyl	alveolar 4549)	164 and 246	15-24 nm		lines and were			
intestinal (Caco-2, Exposure: 3h and primarily 24h undifferentiate Positive control for d cells used) SBs: H2O2 or methyl		$\mu a/cm^2$	15 21 1111		nositive with the 3-			
(Caco-2,   Exposure: 3h and   treatment.     primarily   24h   both TiO2 NPs, NM-     undifferentiate   Positive control for   Both TiO2 NPs, NM-     d cells used)   SBs:   102 and NM-105     HaO2 or methyl   were <b>negative</b> in	intestinal	M3/ 5/11			h or 24-h			
primarily 24h   undifferentiate Positive control for   d cells used) SBs:   H2O2 or methyl	(Caco-2.	Exposure: 3h and			treatment.			
undifferentiate Positive control for   d cells used) SBs:   H2O2 or methyl Both TiO2 NPs, NM-   102 and NM-105   were <b>negative</b> in	primarily	24h						
d cells used) SBs: H <sub>2</sub> O <sub>2</sub> or methyl	undifferentiate	Positive control for			Both TiO <sub>2</sub> NPs, NM-			
H <sub>2</sub> O <sub>2</sub> or methyl	d cells used)	SBs:			102 and NM-105			
	, , , , , , , , , , , , , , , , , , , ,	H <sub>2</sub> O <sub>2</sub> or methyl			were <b>negative</b> in			

3-dimensional epidermal methane (NHEK sulphonate (MMS) human reconstructed full keratinocytes) thickness skin No positive control 3-dimensional for Fpg model. human reconstructed full thickness skin model NSC: 1 Micronucleus BEAS 2B, NM-102 1) TiO<sub>2</sub>NPs (NM-Positive Reliability: 1 High NANOGENOTOX 102), anatase, Nanogenotox Project TiO<sub>2</sub> NPs (NM-102 test and NM 105: Project, 2013 dispersion protocol 0, 32, 64, 128 and 21-22 nm and NM-105) (Documentation 256 µg/ml 2) TiO<sub>2</sub>NPs (NM-In NHEK cells provided to pulmonary (bronchial EFSA No. 7 and 105), epithelial 16 HBE, NM-102: anatase/rutile, Equivocal: 8) BEAS 2B and 0, 20, 40 and 60 15-24 nm TiO<sub>2</sub> NPs NM-102 in 16 HBE; µg/ml lymphocytes alveolar A549) 16 HBE, NM-105: Negative 0, 8, 12 and 16 TiO<sub>2</sub> NPs NM-105 in intestinal µg/ml lymphocytes (Caco-2, primarily A549, NM-102: 0, undifferentiate 16, 32, 64 and 128 **Negative** TiO<sub>2</sub> NPs d cells used) µg/ml (NM-102 and NM-A549, NM-105: 0, 105) 16, 32, 64, 128, In: human BEAS 2B, 256 and 512 µg/ml primary lymphocytes 16 HBE Caco-2, NM-102: A549 epidermal 0, 9.5, 28, 85 and Caco-2 cell lines (NHEK 128 µg/ml keratinocytes) Caco-2, NM-105: 0, 28, 85, 128 and 256 µg/ml Lymphocytes, NM-

	102 and NM-105: 0, 15, 45, 125 and 250 μg/ml NHEK, NM-102 and NM-105: 0, 20, 40, 60 and 80 μg/ml Exposure: 1.5-2 cell cycles CytoB added 24h after the start of the treatment for						
	Caco-2 cells and 6h after the start of the treatment for other cells Positive control:						
	mitomycin C						
Mouse lymphoma gene mutation assay L5178Y TK+/- cells	NM-102: two series of concentrations: 1) 0, 32, 64, 128 and 256 µg/ml 2) 0, 312.5, 625, 1250 and 2500 µg/ml NM-105: two series of concentrations: 1) 0, 32, 64, 128 and 256 µg/ml 2) 0, 625, 1250, 2500 and 5000	1) TiO <sub>2</sub> NPs (NM- 102), anatase, 21-22 nm 2) TiO <sub>2</sub> NPs (NM- 105), anatase/rutile, 15-24 nm	NSC: 1 Nanogenotox Project dispersion protocol	<b>Negative</b> for all forms of TiO <sub>2</sub> NPs tested	Reliability: 1 Only minor deficiency in data reporting.	High	NANOGENOTOX Project, 2013 (Documentation provided to EFSA No. 7 and 8)



Positive control: MMS			

CytoB: cytochalasin B; DCFH-DA or DCFDA: 2', 7'-dichlorofluorescein diacetate; Fpg: enzyme formamidopyrimidine glycosylase; LDH: Lactate dehydrogenase; MDA: Malonaldehyde; MMS: methyl methane sulphonate; NAC: N-acetylcysteine; NSC: Nanoscale considerations; RI: replication index; OTM: Olive Tail Moment; ROS: reactive oxygen species; SB: strand breaks; TEM: Transmission electron microscopy.