In vitro/In vivo	Type of assay	Reference authors_year	Evaluation by EFSA or SCCS	Test system/Test object	Exposure conditions (concentration/duration/metabolic activation)	TiO2 material tested	Test substance	Information on the characteristics of the test substance	Presence of nanofraction in fine TiO2 proved/possible	Cellular uptake	Results	Route of exposure	Genotoxicity result	Reliability/comments	Relevance [for EFSA data of only high or limited relevance taken]	Crystalline Phase	Shape of Particle	Particle size, nominal particle size given by manufacturer (size range/ distribution) [nm]	Particle size measured by the authors of the publication (size range/ distribution) [nm]	Aspect ratio value (SD)	Crystal structure, ratio of anatase/rutile	Specific Surface area [m2/g] by BET method	Hydrodynamic diameter [nm] by DLS in distilled water	Hydrodynamic diameter [nm] by DLS in saline, culture media, etc.	Zeta-potential [mV] at pH 7 in distilled water	Zeta-potential [mV] in saline, culture media	Polydispersity Index (PdI) in distilled water	Polydispersity Index (PdI) in saline, culture medium	Chemical composition of particles	Purity of particles [%]	Surface chemistry Coating	Impurities of concern	Other
in vitro	Micronucleus test in vitro	Bhattacharya et al., 2008	SCCS	V79 Chinese hamster lung fibroblasts	**nanoparticles:** TiO2 NPs, anatase **type of assay:** micronucleus test **guideline:** **treatment time**: 24, 48, 72 h **concentrations**: 1, 5, 10, 15, 25 ug/cm2 **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : RPMI-1640 supplemented with 10% fetal bovine serum, 2% L-glutamine, and 1% penicllin/streptomycin **cytotoxicity assessment:** Trypan Blue Exclusion Test after 1–100 µg/cm2 for different time periods (24, 48, or 72 h). **positive control**: **methodology:** cells were seeded in a density of 2 × 104 cells/cm2 on each well of Quadriperm dishes (Greiner, Germany) and cultured up to a maximum of 80% confluence. Then the cells were incubated for 24, 48, or 72 h with various concentrations from 1 to 25 µg/cm2. After exposure, cells were fixed in cold methanol (−20°C) for at least 30 min. Nuclei were stained with bisbenzimide (5 µg/ml) for 1 min. The slides were mounted for fluorescence microscopy and examined for the presence of MN. **statistics:** at least 2000 cells were evaluated for the presence of MN in each case. The experiments were repeated three times. The chi-square test was used for statistical analysis of the MN results. The criterion for significance was set at p<.05. **Additional measurements**: Hydroxyl radical formation by Electron Spin Resonance (ESR) with spin trapping in the presence of H2O2. Thiobarbituric Acid-Reactive Substances (TBARS) (1, 2, 5, or 10 µg/cm2) for 24, 48, or 72 h.	NANOMATERIAL	TiO2 NPs, anatase, from Degussa (Essen, Germany)	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : NO **result:** inconclusive the particles did not show any genotoxicity in the applied test system at non-cytotoxic concentrations. **result for positive control:** **cytotoxicity:** 70% viability after exposure to 15 ug/cm2 **ROS:** natural anatase only produced 15,000 ESR units, compared to untreated (3,000 ESR units) and negative control (CaSO4, 8,000 ESR units) **GSH:** **internalisation:**		Inconclusive	3 No dispersion protocol, no stability data, no positive control used, procedure of scoring of micronuclei not clear, no historical control data	Low no internalisation proof, insufficient methodology description	anatase	spheroid	30–50				20-120											
in vitro	Micronucleus test in vitro	Bhattacharya et al., 2008	SCCS	V79 Chinese hamster lung fibroblasts	**nanoparticles:** TiO2 NPs, anatase coated with V2O5 **type of assay:** micronucleus test **guideline:** **treatment time**: 24, 48, 72 h **concentrations**: 1, 5, 10, 15, 25 ug/cm2 **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : RPMI-1640 supplemented with 10% fetal bovine serum, 2% L-glutamine, and 1% penicllin/streptomycin **cytotoxicity assessment:** Trypan Blue Exclusion Test after 1–100 µg/cm2 for different time periods (24, 48, or 72 h). **positive control**: **methodology:** cells were seeded in a density of 2 × 104 cells/cm2 on each well of Quadriperm dishes (Greiner, Germany) and cultured up to a maximum of 80% confluence. Then the cells were incubated for 24, 48, or 72 h with various concentrations from 1 to 25 µg/cm2. After exposure, cells were fixed in cold methanol (−20°C) for at least 30 min. Nuclei were stained with bisbenzimide (5 µg/ml) for 1 min. The slides were mounted for fluorescence microscopy and examined for the presence of MN. **statistics:** at least 2000 cells were evaluated for the presence of MN in each case. The experiments were repeated three times. The chi-square test was used for statistical analysis of the MN results. The criterion for significance was set at p<.05. **Additional measurements**: Hydroxyl radical formation by Electron Spin Resonance (ESR) with spin trapping in the presence of H2O2. Thiobarbituric Acid-Reactive Substances (TBARS) (1, 2, 5, or 10 µg/cm2) for 24, 48, or 72 h.	NANOMATERIAL	TiO2 NPs, anatase coated with V2O5, from the Institute of Environmental Process Engineering and Plant Design of the University Duisburg–Essen. The flame synthesis method (Nakazawa et al., 2005) was applied for production of V2O5-treated TiO2 particles. The high temperature of the flame leads to sintered dense nanoparticles with a thin coating of V2O5 without micropores exhibiting high surface areas.	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : YES **result:** positive V2O5-treated anatase induced significant genotoxic effects. The number of induced MN was 3-fold increased after exposure to V2O5-anatase (2 µg/cm2) after 24 and 48 h of exposure. **result for positive control:** **cytotoxicity:** no cytotox at 2 ug/cm2; 60%, 70%, 80% cell death at 25 µg/cm2 after 24 h, 48 h, and 72 h of exposure **ROS:** V2O5-anatase induced 30,000 ESR units, while natural anatase only produced 15,000 ESR units, compared to untreated (3,000 ESR units) and negative control (CaSO4, 8,000 ESR units) **GSH:** **internalisation:**		Positive	3 No dispersion protocol, no stability data, no positive control used, procedure of scoring of micronuclei not clear, no historical control data	Low no internalisation proof, insufficient description of methodology	anatase/V2O5	spheroid	30–50 No formation of distinct vanadia particles was visible using high-resolution transmission electron microscopy (HRTEM)				20-120									V2O5		
in vitro	Micronucleus test in vitro	Jaeger et al., 2012	SCCS	HaCaT Human keratinocytes	**nanoparticles:** P25, anatase/rutile **type of assay:** micronucleus test +UVA **guideline:** **treatment time**: 24 and 48 h plus UVA (4, 8, 12, 48, 96 J/cm2) **concentrations**: 0.5, 1, 5, 10 and 50 ug/mL equates to 0.17, 0.33, 1.66, 3.32 and 16.6 ug/cm2 **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : **cytotoxicity assessment:** **positive control**: **methodology:** cells grown on coverslips in medium containing 2 ug/mL cytochalasin B and were treated with different doses of TiO2-NPs with UVA (4, 8, 12, 48, 96 J/cm2). After 24 h and 48 h post-exposure cover slips were washed, fixed, air-dried, stained with Hoechst 33258 and evaluated for the presence of micronuclei. **statistics:** frequency of micronuclei was assayed in at least three biological replicates of 3 × 500 binucleated cells per data point by using a fluorescence microscope **Additional measurements**: apoptosis and ROS by flow cytometry	NANOMATERIAL	P25, TiO2 NPs, anatase/rutile, from Degussa, Germany	**Suspension stability**: **Cell internalisation**: TEM **Sonication**: sterilization by heating to 120 °C for 2 h, suspended in sterilized phosphate-buffered saline (1× PBS) to a 12.5 mM stock solution (1 mg/ml) and kept at 4 °C until used. Before application to the cells, the particle suspension was treated with an ultrasonicator for 3 min at cycle count 20% and 70% of maximum power.		TEM	**any statistical increase at any concentration** : YES, +UVA - significant at 10 ug/ml after 24 h and 1, 5, 10, 50 ug/mL after 48 h **result:** positive; 2.2-fold of control 48 h after exposure to 5 ug/mL TiO2-NPs **result for positive control:** **cytotoxicity:** BN rate was significantly decreased about 10% at 48 h post-exposure to TiO2-NPs (0.5, 1, 5, 10, 50 ug/ml) **ROS:** increase after 4 h at 5 and 50 ug/mL **GSH:** **internalisation:** TiO2-NPs formed aggregates found to agglomerate inside and outside of HaCaT cells		Positive	3 exposure was with UVA, no data on conditions without UVA	Low	anatase/rutile		21 primary crystallite size ~30 nm			anatase (70%)/rutile (30%)	52.7 ± 3.6				-11.6 ± 1.2 (DMEM, pH 7.5)							
in vitro	Micronucleus test in vitro	Jaeger et al., 2012	SCCS	HaCaT Human keratinocytes	**nanoparticles:** TiO2 pigmentary **type of assay:** micronucleus test +UVA **guideline:** **treatment time**: 24 and 48 h plus UVA (4, 8, 12, 48, 96 J/cm2) **concentrations**: 0.5, 1, 5, 10 and 50 ug/mL equates to 0.17, 0.33, 1.66, 3.32 and 16.6 ug/cm2 **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : **cytotoxicity assessment:** **positive control**: **methodology:** cells grown on coverslips in medium containing 2 ug/mL cytochalasin B and were treated with different doses of TiO2-NPs with UVA (4, 8, 12, 48, 96 J/cm2). After 24 h and 48 h post-exposure cover slips were washed, fixed, air-dried, stained with Hoechst 33258 and evaluated for the presence of micronuclei. **statistics:** frequency of micronuclei was assayed in at least three biological replicates of 3 × 500 binucleated cells per data point by using a fluorescence microscope	PIGMENTARY GRADE other than "E171-equivalent or E171-similar"	TiO2 pigmentary, anatase, from Sigma–Aldrich, Germany			not reported	**any statistical increase at any concentration** : YES +UVA - significant at 1, 5 ug/ml after 24 h and 0.5, 5, 10 ug/mL after 48 h **result:** positive **result for positive control:** **cytotoxicity:** BN rate was significantly decreased about 10% at 48 h post-exposure to TiO2-MPs (0.5, 1, 10 ug/ml). **ROS:** increase after 4 h at 5 and 50 ug/mL **GSH:** **internalisation:**		Positive	3 exposure was with UVA, no data on conditions without UVA	Low	anatase		200															
in vitro	Micronucleus test in vitro	Llewellyn et al., 2022	SCCS	Primary Human multicellular microtissue liver model (PHH)	**nanoparticle:** NM-105, TiO2 NPs, anatase **type of assay:** micronucleus test 3D hepato models **guideline: the OECD Test No. 487** **treatment time**: 24 h **concentrations**: 25, 50 ug/ml **FBS serum:** 10% **biological medium for NPs dispersion** : InSightTM human liver maintenance medium (CS-07-305B-01, DMEM for liver spheroids+10% FBS **cytotoxicity assessment:** CBPI, Adenylate kinase **positive control**: B[a]P, 4 concentrations **methodology:** To assess cytotoxicity and genotoxicity following acute (24 h) exposure to B[a]P, the CBMN Assay was undertaken in conjunction with the Cytokinesis-Block Proliferation Index (CBPI) post-acute exposure. Liver spheroids were then trypsinisedand a proportion of the cells taken to make slides for 20% Giemsa staining and manual cytotoxicity assessment. The remaining cells in suspension were then fixed for semi-automated micronucleus scoring. A minimum of 1000 binucleated cells were counted per exposure dose per biological replicate (n = 3). **statistics:** minimum 1000 binucleated cells were counted per exposure dose per biological replicate (n = 3). Prism 8, GraphPad Software, Inc. (USA). Shapiro-Wilk test was used to calculate normality for each data set, followed by a two-way ANOVA, with Tukey's multiple comparisons test **Additional measurements**: IL-8	NANOMATERIAL	NM-105, TiO2 NPs, anatase/rutile, from the Joint Research Centre (JRC, Ispra, Italy)	NM stock solution prepared (2.56 mg/mL) and dispersed for 16 min in 0.05% BSA with the probe sonication (Jensen et al., 2014). Working stocks of NMs were made fresh for each experiment.		not reported	Data on TiO2 micronucleus not reported - aim comprison and transferability of the methods to other labs.		No data reported	3	Low	anatase/rutile																	
in vitro	Chromosomal aberration test in vitro	Theogaraj et al., 2007	SCCS	CHO-WBL Chinese hamster ovary epithelial cells	**nanoparticles:** anatase 80%/ rutile 20% **type of assay:** chromosomal aberration -/+UV irradiation **guideline:** **treatment time**: 3h + 17h **concentrations**: 512, 640, 800 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : particles were suspended in absolute ethanol, and probably diluted in culture medium: McCoy's 5A medium including 10% (v/v) foetal calf serum (FCS) and 100 ug/mL gentamycin **cytotoxicity assessment:** **positive control**: 8-methoxypsoralen (8-MOP) (1 ug/mL) and 4-nitroquinoline-1-oxide (NQO) (0.125 ug/mL) **methodology:** 25-cm2 tissue–culture flasks were seeded with approximately 2.5×105 cells per flask in 4.95 ml culture medium. After 15 min incubation in the dark (to allow internalisation), the cultures were exposed to UV to 750 mJ/cm3. After irradiation, cultures were incubated in the dark until 3 h, washed and incubated for a further 17 h. On the basis of cell-count reductions in the absence of UV, a top concentration that induced close to approximately 50% toxicity was chosen. **statistics:** 100 cells per replicate (i.e. 200 metaphases from each treatment condition) were analyzed (where appropriate) for structural chromosome aberrations. **Additional measurements**:	NANOMATERIAL	TiO2 NPs, anatase/rutile, from Degussa AG, Hanau, Germany	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : NO **result:** negative -/+UV irradiation **result for positive control:** NQO 10x increase; 8-MOP 35x increase **cytotoxicity:** <42% **ROS:** **GSH:** **internalisation:**		Negative	2 excessively high concentrations used, no dispersion control, no proof on internalisation, relatively short time of exposure	Low	anatase/rutile		21 (TEM)			anatase (80%)/rutile (20%)										Trimethoxy caprylylsilane		
in vitro	Chromosomal aberration test in vitro	Theogaraj et al., 2007	SCCS	CHO-WBL Chinese hamster ovary epithelial cells	**nanoparticles:** anatase 80%/ rutile 20% **type of assay:** chromosomal aberration -/+UV irradiation **guideline:** **treatment time**: 3h + 17h **concentrations**: 392, 560, 800 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : particles were suspended in physiological saline, and probably diluted in culture medium: McCoy's 5A medium including 10% (v/v) foetal calf serum (FCS) and 100 ug/mL gentamycin **cytotoxicity assessment:** **positive control**: 8-methoxypsoralen (8-MOP) (1 ug/mL) and 4-nitroquinoline-1-oxide (NQO) (0.125 ug/mL) **methodology:** 25-cm2 tissue–culture flasks were seeded with approximately 2.5×105 cells per flask in 4.95 ml culture medium. After 15 min incubation in the dark (to allow internalisation), the cultures were exposed to UV to 750 mJ/cm3. After irradiation, cultures were incubated in the dark until 3 h, washed and incubated for a further 17 h. On the basis of cell-count reductions in the absence of UV, a top concentration that induced close to approximately 50% toxicity was chosen. **statistics:** 100 cells per replicate (i.e. 200 metaphases from each treatment condition) were analyzed (where appropriate) for structural chromosome aberrations. **Additional measurements**:	NANOMATERIAL	TiO2 NPs, anatase/rutile, from Degussa AG, Hanau, Germany	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : NO **result:** negative -/+UV irradiation **result for positive control:** NQO 2.5x increase; 8-MOP 20x increase **cytotoxicity:** >65% -UV; >52%+UV **ROS:** **GSH:** **internalisation:**		Negative	2 excessively high concentrations used, unacceptable level of cytotoxicity; no dispersion control, no proof on internalisation, relatively short time of exposure	Low	anatase/rutile		21 (TEM)			anatase (80%)/rutile (20%)										Doped di-iron trioxide (2±1%)		
in vitro	Chromosomal aberration test in vitro	Theogaraj et al., 2007	SCCS	CHO-WBL Chinese hamster ovary epithelial cells	**nanoparticles:** anatase 80%/ rutile 20% **type of assay:** chromosomal aberration -/+UV irradiation **guideline:** **treatment time**: 3h + 17h **concentrations**: 209.7, 640, 800 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : particles were suspended in physiological saline, and probably diluted in culture medium: McCoy's 5A medium including 10% (v/v) foetal calf serum (FCS) and 100 ug/mL gentamycin **cytotoxicity assessment:** **positive control**: 8-methoxypsoralen (8-MOP) (1 ug/mL) and 4-nitroquinoline-1-oxide (NQO) (0.125 ug/mL) **methodology:** 25-cm2 tissue–culture flasks were seeded with approximately 2.5×105 cells per flask in 4.95 ml culture medium. After 15 min incubation in the dark (to allow internalisation), the cultures were exposed to UV to 750 mJ/cm3. After irradiation, cultures were incubated in the dark until 3 h, washed and incubated for a further 17 h. On the basis of cell-count reductions in the absence of UV, a top concentration that induced close to approximately 50% toxicity was chosen. **statistics:** 100 cells per replicate (i.e. 200 metaphases from each treatment condition) were analyzed (where appropriate) for structural chromosome aberrations. **Additional measurements**:	NANOMATERIAL	TiO2 NPs, anatase/rutile, from Degussa AG, Hanau, Germany	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : NO **result:** negative -/+UV irradiation **result for positive control:** NQO 28x increase; 8-MOP 94x increase **cytotoxicity:** >47% -UV; >35%+UV **ROS:** **GSH:** **internalisation:**		Negative	3 excessively high concentrations used, unacceptable level of cytotoxicity; no dispersion control, no proof on internalisation, relatively short time of exposure	Low	anatase/rutile		21 (TEM)			anatase (80%)/rutile (20%)												
in vitro	Chromosomal aberration test in vitro	Theogaraj et al., 2007	SCCS	CHO-WBL Chinese hamster ovary epithelial cells	**nanoparticles:** rutile 100% **type of assay:** chromosomal aberration -/+UV irradiation **guideline:** **treatment time**: 3h + 17h **concentrations**: 1470, 2100, 3000 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : particles were suspended in DMSO, and probably diluted in culture medium: McCoy's 5A medium including 10% (v/v) foetal calf serum (FCS) and 100 ug/mL gentamycin **cytotoxicity assessment:** **positive control**: 8-methoxypsoralen (8-MOP) (1 ug/mL) and 4-nitroquinoline-1-oxide (NQO) (0.125 ug/mL) **methodology:** 25-cm2 tissue–culture flasks were seeded with approximately 2.5×105 cells per flask in 4.95 ml culture medium. After 15 min incubation in the dark (to allow internalisation), the cultures were exposed to UV to 750 mJ/cm3. After irradiation, cultures were incubated in the dark until 3 h, washed and incubated for a further 17 h. On the basis of cell-count reductions in the absence of UV, a top concentration that induced close to approximately 50% toxicity was chosen. **statistics:** 100 cells per replicate (i.e. 200 metaphases from each treatment condition) were analyzed (where appropriate) for structural chromosome aberrations. **Additional measurements**:	NANOMATERIAL	TiO2 NPs, rutile, by various companies, members of the Physical Sunscreen Manufacturers Association, Brussels, Belgium	**Suspension stability**: reported solubility problems above 3000 ug/mL **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : NO **result:** negative -/+UV irradiation **result for positive control:** NQO 110x increase; 8-MOP 30x increase **cytotoxicity:** <57% -UV; <20%+UV **ROS:** **GSH:** **internalisation:**		Negative	2 excessively high concentrations used; no dispersion control, no proof on internalisation, relatively short time of exposure	Low	rutile		14 (XRD)			rutile (100%)										Alumina (8–11%), simethicone (1–3%)		

in vitro	Chromosomal aberration test in vitro	Theogaraj et al., 2007	SCCS	CHO-WBL Chinese hamster ovary epithelial cells	**nanoparticles:** anatase 100% **type of assay:** chromosomal aberration -/+UV irradiation **guideline:** **treatment time**: 3h + 17h **concentrations**: 1715, 3500, 5000 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : particles were suspended in DMSO, and probably diluted in culture medium: McCoy's 5A medium including 10% (v/v) foetal calf serum (FCS) and 100 ug/mL gentamycin **cytotoxicity assessment:** **positive control**: 8-methoxypsoralen (8-MOP) (1 ug/mL) and 4-nitroquinoline-1-oxide (NQO) (0.125 ug/mL) **methodology:** 25-cm2 tissue–culture flasks were seeded with approximately 2.5×105 cells per flask in 4.95 ml culture medium. After 15 min incubation in the dark (to allow internalisation), the cultures were exposed to UV to 750 mJ/cm3. After irradiation, cultures were incubated in the dark until 3 h, washed and incubated for a further 17 h. On the basis of cell-count reductions in the absence of UV, a top concentration that induced close to approximately 50% toxicity was chosen. **statistics:** 100 cells per replicate (i.e. 200 metaphases from each treatment condition) were analyzed (where appropriate) for structural chromosome aberrations. **Additional measurements**:	NANOMATERIAL	TiO2 NPs, anatase, by various companies, members of the Physical Sunscreen Manufacturers Association, Brussels, Belgium	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : NO **result:** negative -/+UV irradiation **result for positive control:** NQO 110x increase; 8-MOP 30x increase **cytotoxicity:** <28% -UV; <32%+UV **ROS:** **GSH:** **internalisation:**		Negative	2 excessively high concentrations used; no dispersion control, no proof on internalisation, relatively short time of exposure	Low	anatase		60 (X-ray disc centrifugation, XDC) aggregate rather than particle size			anatase											Alumina (37%), silica (12–18%)		
in vitro	Chromosomal aberration test in vitro	Theogaraj et al., 2007	SCCS	CHO-WBL Chinese hamster ovary epithelial cells	**nanoparticles:** rutile 100% **type of assay:** chromosomal aberration -/+UV irradiation **guideline:** **treatment time**: 3h + 17h **concentrations**: 2450, 3000, 5000 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : particles were suspended in DMSO, and probably diluted in culture medium: McCoy's 5A medium including 10% (v/v) foetal calf serum (FCS) and 100 ug/mL gentamycin **cytotoxicity assessment:** **positive control**: 8-methoxypsoralen (8-MOP) (1 ug/mL) and 4-nitroquinoline-1-oxide (NQO) (0.125 ug/mL) **methodology:** 25-cm2 tissue–culture flasks were seeded with approximately 2.5×105 cells per flask in 4.95 ml culture medium. After 15 min incubation in the dark (to allow internalisation), the cultures were exposed to UV to 750 mJ/cm3. After irradiation, cultures were incubated in the dark until 3 h, washed and incubated for a further 17 h. On the basis of cell-count reductions in the absence of UV, a top concentration that induced close to approximately 50% toxicity was chosen. **statistics:** 100 cells per replicate (i.e. 200 metaphases from each treatment condition) were analyzed (where appropriate) for structural chromosome aberrations. **Additional measurements**:	NANOMATERIAL	TiO2 NPs, rutile by various companies, members of the Physical Sunscreen Manufacturers Association, Brussels, Belgium	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : NO **result:** negative -/+UV irradiation **result for positive control:** NQO 110x increase; 8-MOP 30x increase **cytotoxicity:** <38% -UV; <1%+UV **ROS:** **GSH:** **internalisation:**		Negative	2 excessively high concentrations used; no dispersion control, no proof on internalisation, relatively short time of exposure	Low	rutile		20 (XRD)			rutile (100%)											Alumina (5–6.5%), dimethicone (1–4%)		
in vitro	Chromosomal aberration test in vitro	Theogaraj et al., 2007	SCCS	CHO-WBL Chinese hamster ovary epithelial cells	**nanoparticles:** rutile 100% **type of assay:** chromosomal aberration -/+UV irradiation **guideline:** **treatment time**: 3h + 17h **concentrations**: 348.1, 823.9, 1950 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : particles were suspended in DMSO, and probably diluted in culture medium: McCoy's 5A medium including 10% (v/v) foetal calf serum (FCS) and 100 ug/mL gentamycin **cytotoxicity assessment:** **positive control**: 8-methoxypsoralen (8-MOP) (1 ug/mL) and 4-nitroquinoline-1-oxide (NQO) (0.125 ug/mL) **methodology:** 25-cm2 tissue–culture flasks were seeded with approximately 2.5×105 cells per flask in 4.95 ml culture medium. After 15 min incubation in the dark (to allow internalisation), the cultures were exposed to UV to 750 mJ/cm3. After irradiation, cultures were incubated in the dark until 3 h, washed and incubated for a further 17 h. On the basis of cell-count reductions in the absence of UV, a top concentration that induced close to approximately 50% toxicity was chosen. **statistics:** 100 cells per replicate (i.e. 200 metaphases from each treatment condition) were analyzed (where appropriate) for structural chromosome aberrations. **Additional measurements**:	NANOMATERIAL	TiO2 NPs, rutile by various companies, members of the Physical Sunscreen Manufacturers Association, Brussels, Belgium	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : NO **result:** negative -/+UV irradiation **result for positive control:** NQO 20x increase; 8-MOP 19x increase **cytotoxicity:** <58% -UV; <55%+UV **ROS:** **GSH:** **internalisation:**		Negative	2 excessively high concentrations used; no dispersion control, no proof on internalisation, relatively short time of exposure	Low	rutile		15 (TEM)			rutile (100%)											Alumina (3–8%), stearic acid (5–11%)		
in vitro	Chromosomal aberration test in vitro	Theogaraj et al., 2007	SCCS	CHO-WBL Chinese hamster ovary epithelial cells	**nanoparticles:** rutile 100% **type of assay:** chromosomal aberration -/+UV irradiation **guideline:** **treatment time**: 3h + 17h **concentrations**: 1582, 3750, 5000 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : particles were suspended in DMSO, and probably diluted in culture medium: McCoy's 5A medium including 10% (v/v) foetal calf serum (FCS) and 100 ug/mL gentamycin **cytotoxicity assessment:** **positive control**: 8-methoxypsoralen (8-MOP) (1 ug/mL) and 4-nitroquinoline-1-oxide (NQO) (0.125 ug/mL) **methodology:** 25-cm2 tissue–culture flasks were seeded with approximately 2.5×105 cells per flask in 4.95 ml culture medium. After 15 min incubation in the dark (to allow internalisation), the cultures were exposed to UV to 750 mJ/cm3. After irradiation, cultures were incubated in the dark until 3 h, washed and incubated for a further 17 h. On the basis of cell-count reductions in the absence of UV, a top concentration that induced close to approximately 50% toxicity was chosen. **statistics:** 100 cells per replicate (i.e. 200 metaphases from each treatment condition) were analyzed (where appropriate) for structural chromosome aberrations. **Additional measurements**:	NANOMATERIAL	TiO2 NPs, rutile by various companies, members of the Physical Sunscreen Manufacturers Association, Brussels, Belgium	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : NO **result:** negative -/+UV irradiation **result for positive control:** NQO 47x increase; 8-MOP 75x increase **cytotoxicity:** <54% -UV; <51%+UV **ROS:** **GSH:** **internalisation:**		Negative	2 excessively high concentrations used; no dispersion control, no proof on internalisation, relatively short time of exposure	Low	rutile		20-22 (XRD)			rutile (100%)											Alumina (10.5–12.5%), silica (3.5–5%)		
in vitro	Comet in vitro	Kang et al., 2011	SCCS	Peripheral blood mononuclear cells human, isolated	**nanoparticles:** TiO2 Aeoroxide P25 **type of assay:** alkaline comet assay -/+UVA irradiation **guideline:** **treatment time**: 24h **concentrations**: 1 and 5 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : **cytotoxicity assessment:** Trypan blue exclusion test -/+UVA (isolated lymphocytes: 1, 5 ug/mL for 24 h) **positive control**: **methodology:** lymphocytes isolated by buoyancy density centrifugation at 400 × g using Ficoll-Paque. The isolated lymphocytes cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 1% phytohemagglutinin. At 24 hours after the start of culture, cells were treated with TiO2 in the absence or presence of UVA for 0–48 hours. Komet 5.5 image analysis system **statistics:** olive tail moments of 60 randomly selected cell images from each sample were analyzed **Additional measurements**: on isolated lymphocytes: comet assay, ROS (DCFDA by flow cytometry), apoptosis (PI staining by flow cytometry), MMP (3,3'dihexyloxacarbocyanine by flow cytometry)	NANOMATERIAL	P25, TiO2 NPs, Aeroxide, from Degussa (Hanau, Germany)	**Suspension stability**: **Cell internalisation**: **Sonication**: Before inoculation into the in vitro cell systems, the particles were suspended in sterile phosphate-buffered saline (PBS) and then dispersed for 30 minutes by sonication to prevent aggregation.		not reported	**any statistical increase at any concentration** : YES -/+UVA **result:** positive +UVA with concentration-related increase; Pretreatment with NAC significantly decreased the TiO2+UVA–induced DNA damage **result for positive control:** **cytotoxicity:** 70% and 60% viability after 5 ug/mL +UVA after 24 and 48h, resp.; increase in sub-G1 after 5 ug/mL +UVA **ROS:** Nano-TiO2 (but not normal-TiO2) increased ROS production, and this was dramatically enhanced by UVA (160 and 340%, -/+UVA, respectively). **GSH:** **internalisation:**		Positive	3 no positive control, no proof of internalisation; only OTM is reported; methodology insufficiently described; not clear how many samples (hence cells) per concentration were analysed; no data on stability of the NPs	Low	anatase/rutile		20			anatase (70–85%)/rutile (30–15%)	48,08												
in vitro	Comet in vitro	Kang et al., 2011	SCCS	Peripheral blood mononuclear cells human, isolated	**nanoparticles:** TiO2, pigmentary, rutile **type of assay:** alkaline comet assay -/+UVA irradiation **guideline:** **treatment time**: 24h **concentrations**: 1 and 5 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : **cytotoxicity assessment:** Trypan blue exclusion test -/+UVA (isolated lymphocytes: 1, 5 ug/mL for 24 h) **positive control**: **methodology:** lymphocytes isolated by buoyancy density centrifugation at 400 × g using Ficoll-Paque. The isolated lymphocytes cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 1% phytohemagglutinin. At 24 hours after the start of culture, cells were treated with TiO2 in the absence or presence of UVA for 0–48 hours. Komet 5.5 image analysis system **statistics:** olive tail moments of 60 randomly selected cell images from each sample were analyzed **Additional measurements**: on isolated lymphocytes: comet assay, ROS (DCFDA by flow cytometry), apoptosis (PI staining by flow cytometry), MMP (3,3'dihexyloxacarbocyanine by flow cytometry)	PIGMENTARY GRADE other than "E171-equivalent or E171-similar"	TiO2 pigmentary, rutile, from Sigma-Aldrich, St. Louis, Missouri, USA	**Suspension stability**: **Cell internalisation**: **Sonication**: Before inoculation into the in vitro cell systems, the particles were suspended in sterile phosphate-buffered saline (PBS) and then dispersed for 30 minutes by sonication to prevent aggregation.	possible	not reported	**any statistical increase at any concentration** : YES +UVA **result:** positive +UVA; pretreatment with NAC significantly decreased the TiO2+UVA–induced DNA damage; negative -UVA **result for positive control:** **cytotoxicity:** no cytotoxicity after 5 ug/mL -/+UVA after 24 and 48h; no increase in sub-G1 after 5 ug/mL +UVA **ROS:** no increase -/+UVA **GSH:** **internalisation:**		Positive	3 no positive control, no proof of internalisation; only OTM is reported; methodology insufficiently described; not clear how many samples (hence cells) per concentration were analysed; no data on stability of the particles	Low	rutile		1 µm (primary particle size)			rutile ~99%													
in vitro	Comet in vitro	Pan et al., 2011	SCCS	HEp-2 Human larynx epidermoid carcinoma, contaminated with HeLa cells	**nanoparticles:** P25 **type of assay:** alkaline comet assay **guideline:** **treatment time**: 2h **concentrations**: 7.8 and 62.5 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : RPMI 1640 medium with 10% FBS, 100 U/ml penicillin, and 100 ug/ml streptomycin **cytotoxicity assessment:** MTT ((7.8–500 ug/mL) for 24h **positive control**: **methodology:** SYBR Green staining, no information on scoring method **statistics:** For each data point a minimum of 30 random cells were visually analyzed. **Additional measurements**: GSH by spectrophotometry	NANOMATERIAL	P25, TiO2 NPs, anatase/rutile, from Degussa Shanghai	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : YES, <2 fold increase in %DNA in tail **result:** equivocal; slight increase in %DNA in tail, however no data on historical control values makes it impossible to evaluate **result for positive control:** **cytotoxicity:** MTT IC50: 180 ug/mL **ROS:** **GSH:** no increase after 4 h **internalisation:**		Equivocal	3 no positive control, no proof of internalisation; methodology insufficiently described; only two concentratons used, not clear how many samples (hence cells) per concentration were analysed; no data on stability of the particles	Low	anatase/rutile					anatase (80%)/rutile (20%)													
in vitro	Comet in vitro	Petkovic et al., 2011a	SCCS	HepG2 Human hepatocellular carcinoma cells	**nanoparticles:** TiO2 rutile **type of assay:** alkaline comet assay -/+Fpg **guideline:** **treatment time**: 2, 4, 24h **concentrations**: 1, 10, 100, 250 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : **cytotoxicity assessment:** MTT assay, cells incubated for 4, 24 and 48 h **positive control**: 0.5mM t-BOOH as positive control for 2 and 4 h treatments and 50 mM BaP for 24 h treatments **methodology:** 12-well microtiter plates, percent of tail DNA, images of 50 randomly selected nuclei per experimental point analyzed with image analysis software Comet Assay IV (Perceptive Instruments, UK). **statistics:** Three independent experiments were performed for each of the treatment conditions. **Additional measurements**: DCFH-DA assay by spectrofluorimetry	NANOMATERIAL	TiO2 NPs, rutile, from Sigma-Aldrich	**Suspension stability**: Particle size distribution of TiO2 NPs in the treatment medium was measured by laser scattering, using a particle size distribution analyzer Horiba LA-920 (Japan). The particles were dispersed in EMEM medium at 30 ug/ml and sonicated using the same conditions as for preparation of particle suspensions for the cell treatments. **Cell internalisation**: **Sonication**: Powdered TiO2 NPs were suspended in PBS at a concentration 1 mg/ml and sonicated for 30 min in an ultrasonic bath (Sonorex, Bandelin electronic, Germany) at a frequency of 60 kHz, voltage of 220 V and an electric current of 0.5 A to ensure uniform suspension. This stock solution was subsequently diluted in the complete cell growth medium to yield concentrations ranging from 1–250 ug/ml. These samples were then sonicated for 30 min to produce a stable, less-agglomerated nanocrystalline suspension before exposure of cells in culture.		Not reported	**any statistical increase at any concentration** : equivocal -/+FpG (high SD values); equivocal -/+ENDOIII (high SD values); single slight increases without concentration dependence **result:** equivocal **result for positive control:** 2-3x increase **cytotoxicity:** no changes up to 48 h **ROS:** significant increase only at 250 ug/mL **GSH:** **internalisation:**		Equivocal	3 high SD values for all experiments, especially for positive controls; in many cases single significant measurements with slight increase and without concentration dependent increase; no proof of internalisation	Low	rutile	elongated	10x40	<100		rutile	116,7													XRD: tetragonal crystallographic system; P42/mnm space group (according to JCPDS: 34–0180); characteristic reflections (110) at 27.3; (101) at 36.0; (211) at 54.1, 2q. UV-Vis spectroscopy: absorption peaks at lmax = 305 nm and lmax = 205 nm
in vitro	Comet in vitro	Botelho et al., 2014	SCCS	AGS Human gastric cancer cells	**nanoparticles:** TiO2 NPs (Sigma) **type of assay**: alkaline comet assay **guideline**: **treatment time**: 3h **concentrations**: 150 ug/mL **FBS serum**: **S9 mix:** **biological medium for NPs dispersion** : RPMI supplemented with 2% BSA in phosphate-buffered saline (PBS) cytotoxicity assessment: Trypan blue exclusion assay, MTS assay methodology: Comet Assay IV software (Perceptive Instruments); percentage of tail DNA was the DNA damage parameter **statistics:** One hundred randomly selected cells (50 per replicate) were examined for each dose Additional measurements: GSH by spectrophotometry, apoptosis by TUNEL staining	NANOMATERIAL	TiO2 NPs, nanopowder, anatase, from Sigma-Aldrich (Cat #637254)	**Suspension stability**: DLS in MilliQ water **Cell internalisation**: **Sonication**: RPMI supplemented with 2% BSA in phosphate-buffered saline (PBS) and probe sonicated at 30 W for 5 min (1.5 min pulse on and 1 min pulse off for two times and a final pulse of 2 min).		not reported	**any statistical increase at any concentration** : NO **result:** negative **result for positive control:** 1.88x increase in %tail DNA **cytotoxicity:** increased proliferation **ROS:** **GSH:** increased concentration of GSSG after 150 ug/mL **internalisation:**		Negative	3 only 1 quite high concentration tested, no positive control, no proof of internalisation; rather unexpected reported increased proliferation of cells in spite of increased of GSSG and DNA damage level	Low	anatase					anatase		420,7		-9,96									
in vitro	Comet in vitro	Boyadzhiev et al., 2022	SCCS	FE1 Immortalized cells from the Muta™Mouse transgenic rodent model. The cells retain the characteristics of type I and type II pulmonary alveolar epithelial cells.	**nanoparticles:** TiO2 pigmentary, nanowires (Sigma Aldrich Cat# 774510) **type of assay:** alkaline comet assay -/+Fpg **guideline:** **treatment time:** 2 and 4 h **concentrations:** 0.65, 12.5, 25, 50, 100 ug/mL **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion:** F12 HAM (1:1), DMEM/F12 (1:1) (1X), supplemented with 2% FBS **positive control: 1h 100 uM H2O2** **cytotoxicity**: Trypan Blue exclusion **methodology:** CometChip high throughput, 50 comet scored, 2-8 replicates; paarameter measured - % DNA in Tail; modelling for concentration response PROAST version 67.0	PIGMENTARY GRADE other than "E171-equivalent or E171-similar"	TiO2 pigmentary, no data on crystalline form; additional PC characteristics in doi:10.3390/nano10091812	**Suspension stability**: **Cell internalisation**: **Sonication**: NPs suspended in ultrapure dH2O at 5 mg/mL, sonicated, BSA added to a final concentration of 2 mg/mL. The suspension then vortexed and incubated for 10 min. Sonicated stock suspensions were used to produce desired serial dilutions in a cell culture medium. Each exposure dilution was inverted 20 times to mix and the dilutions were used within 15 min of preparation.	possible	not reported	**Negative** **negative control:** 5-6 % DNA in Tail **positive control:** 60-80% DNA in Tail **no cytotoxicity**		Negative	2 no data on crystalline form, detailed characterisation, robust tests with many TiO2, statistical analysis and modelling performed, no proof of internalisation	Low no data on crystalline form	NO INFORMATION ON CRYSTALLINE FORM - the study not analysed	rods	100 x 10 000	106.5 (SD 147.5) crystallite size 25 +/- 22.9 nm (XRD) and equivalent diameter, deq, mean Feret =790 (std Error 40, SD 377) minFeter= 65 (std Error 3, SD 32) and aspect ratio = 13, according to doi:10.3390/nano10091812			24 according to doi:10.3390/nano10091812												
in vitro	Comet in vitro	Boyadzhiev et al., 2022	SCCS	FE1 Immortalized cells from the Muta™Mouse transgenic rodent model. The cells retain the characteristics of type I and type II pulmonary alveolar epithelial cells.	**nanoparticles:** TiO2 pigmentary (US Research Nanomaterials Inc. US1017M) **type of assay:** alkaline comet assay -/+Fpg **guideline:** **treatment time:** 2 and 4 h **concentrations:** 0.65, 12.5, 25, 50, 100 ug/mL **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion:** F12 HAM (1:1), DMEM/F12 (1:1) (1X), supplemented with 2% FBS **positive control: 1h 100 uM H2O2** **cytotoxicity**: Trypan Blue exclusion **methodology:** CometChip high throughput, 50 comet scored, 2-8 replicates; paarameter measured - % DNA in Tail; modelling for concentration response PROAST version 67.0	PIGMENTARY GRADE other than "E171-equivalent or E171-similar"	TiO2 pigmentary, 1500 nm anatase, 1500 nm rutile	**Suspension stability**: **Cell internalisation**: **Sonication**: NPs suspended in ultrapure dH2O at 5 mg/mL, sonicated, BSA added to a final concentration of 2 mg/mL. The suspension then vortexed and incubated for 10 min. Sonicated stock suspensions were used to produce desired serial dilutions in a cell culture medium. Each exposure dilution was inverted 20 times to mix and the dilutions were used within 15 min of preparation.	possible	not reported	**Negative** **negative control:** 5-6 % DNA in Tail **positive control:** 60-80% DNA in Tail **no cytotoxicity**		Negative	2 no data on crystalline form, detailed characterisation, robust tests with many TiO2, statistical analysis and modelling performed, no proof of internalisation	Low no data on crystalline form	NO INFORMATION ON CRYSTALLINE FORM - the study not analysed		1500 (anatase), 1500 (rutile)	1500 (anatase), 1500 (rutile)		anatase/rutile	5-8												
in vitro	Comet in vitro	Freire et al., 2021	SCCS	A549 Human lung cancer cells	**Nanoparticles**: TiO2 NPs **Type of assay**: alkaline comet assay **Exposure:** 24h **Cytotoxicity:** MTT **Positive control**: bleomycin **Concentrations**: 5, 50, 100 and 500 ug/mL. **Statistical analysis:** parameter tail moment; STATGRAPHICS, analysis by ANOVA, followed by the Fisher' Least Significant Difference (LSD) **Additional measurements**: ROS and GSH/GSSG ratio	NANOMATERIAL	TiO2 NPs, anatase/brookite, prepared by the sol–gel method using titanium isopropoxide as precursor and isopropanol as the solvent. HNO3 was used as a catalyst.	**Suspension stability:** DLS **Cell internalisation:** detailed uptake by TEM; TiO2 Nps inhibited the caveolae-mediated endocytosis or macropinocytosis **Sonication:** TiO2 NPs in PBS at 10 mg/mL, sonicated for 60 min, and stored at 4 ◦C the dark. The stock suspension was diluted with DMEM.		TEM	**any statistical increase at any concentration:** YES 100 and 500 ug/mL have borderline cytotoxicity (over 20-30%) **result:** equivocal **result for positive control:** **cytotoxicity:** 20-30% cytotoxicity ROS negative		Equivocal	2 Parameter tail moment - extent of damage difficult to assess, no positive control was used	Low	anatase/brookite	spherical		12.9 ± 2.8		anatase/brookite													

in vitro	Comet in vitro	Hamzeh et al., 2013	SCCS	V79 Chinese hamster lung fibroblasts	**nanoparticles:** Vive Nano Titania rutile polymer coated **type of assay:** alkaline comet assay **guideline:** **treatment time**: 24h **concentrations**: 100 ug/ml **FBS serum:** 10% **S9 mix:** **biological medium for NPs dispersion** : DMEM without phenol red, and supplemented with 10% FBS, 1% sodium pyruvate, 2% L-glutamine, and 1% pen-strep antibiotics. **cytotoxicity assessment:** MTT: 1, 10, and 100 ug/mL for 24 and 48h (Benzalkonium chloride as positive control) **positive control**: (H2O2) at 100 uM for 10 min **methodology:** KOMET 6.0 image analysis software (Andor, Gamble Technologies Ltd, ON, Canada). The olive tail moment and the percentage of tail DNA were assessed. **statistics:** at least 50 randomly selected images were analyzed from each 2 samples; 3 separate and independent experiments. **Additional measurements**: ROS DCFDA 24 h (visualisation by fluorescence microscope); apoptosis 24 h (annexin V/propidium iodide) by flow cytometry	NANOMATERIAL	TiO2 NPs, Vive Nano Titania (–) (rutile), from Vive Nano Inc. (Toronto, ON)	**Suspension stability**: DLS in culture medium **Cell internalisation**: **Sonication**: nanoparticles were suspended at 200 mg/L in a serum-containing culture media and were sonicated (Sonics & Materials Inc., Newtown, CT) for 60 s at 30% amplitude and 20 kHz f. The dispersions were cooled during sonication with an ice/water bath in order to prevent heating of the dispersion. The suspension was immediately added to cultured cells seeded in multi-well plates.		not reported	**any statistical increase at any concentration** : NO **result:** negative **result for positive control:** 3x increase in %tail DNA **cytotoxicity:** no change **ROS:** **GSH:** **internalisation:**		Negative	2 only one concentration used; no demonstration of uptake	Limited	rutile		1-10			rutile 22% (polymer 78%)			600 SEM of each value (not reported) is less than 1% of its mean		-19		0,5	polymer (Allosperse-A; 78% (w/w)) and TiO2 NPs (22%)		Polymer coated (Allosperse-A; 78% (w/w)) and TiO2 NPs (22%)		
in vitro	Other genotoxicity endpoints in vitro - gene expression	El-Said et al., 2014	SCCS	HepG2 Human hepatocellular carcinoma cells	**nanoparticles:** TiO2 NPs **type of assay:** DNA damage signaling gene expression changes **guideline:** **treatment time**: 48h **concentrations**: 10 ug/ml **FBS serum:** **biological medium for NPs dispersion** : **cytotoxicity assessment:** **positive control**: **methodology:** PCR array and RT-PCR of genes in the human DNA damage signaling pathways **statistics:**	NANOMATERIAL	TiO2 NPs, anatase/rutile, P25 (Aeroxide), from Sigma-Aldrich, St Louis, MO, USA)	**Suspension stability:** DLS **Cell internalisation:** **Sonication:** TiO2 NPs was dispersed in distilled water and autoclaved at 120°C for 20 min. The suspension was cooled to room temperature and then sonicated for 10 min at 200 kHz using a high-frequency ultrasonic sonicator (MidSonic 600, Kaijo Corp., Tokyo, Japan).		not reported	Genes for: apurinic/apyrimidinic exonuclease 1 (APEX1), ataxia telangiectasia mutated (ATM), growth arrest and DNA-damage-inducible, alpha (GADD45A), inositol hexaphosphate kinase 3 (IP6K3), methyl-CpG binding domain protein 4 (MBD4), and structural maintenance of chromosomes 1A (SMC1A) were increased by >1.5 fold, with the remainder of the genes in the PCR array exhibiting <1.5 fold changes.		Positive	1 The stydy does not address a routine genotoxicity testing. The results can be treated as supportive only.	Low	anatase/rutile			216 ± 70 The size of the aggregated TiO2 NPs remained stable for several weeks under the indicated storage conditions.		anatase/rutile, P25												
in vivo	Micronucleus test in vivo	Shakeel et al., 2016	SCCS	Blood cells (not explicitly stated if these were erythrocytes)	**nanoparticles:** TiO2 anatase 36.15 nm **type of assay:** micronucleus assay in blood cells **guideline**: **treatment time**: 28 days **concentrations**: . Group 1 - control, group 2 - 1ml of saline, 3, 4 and 5 were injected subcutaneously with 50 or 100 or 150 mg of TiO2 (<5 µm) bulk salt (BS) per kilogramme body weight of the rats, respectively. While groups 6, 7 and 8 received subcutaneous injections of 50 or 100 or 150 mg of TiO2 NPs, respectively, per kg body weight of rats days for 28 days **toxicity assessment:** physiological alterations, weight loss, mortality, haematological alterations, liver function tests, assay and histological alterations **positive control:** **methodology:** subcutaneous injection, MN methodology not clear (described in paper by Jorge et al. which is on completely dofferent topic), analysis and calculation described. **statistics:** Tukey's test **additional measurements**: heamatological parameters, liver function tests, ALT, AST, alkaline phosphatase, SOD, GST, glutathione, histology	NANOMATERIAL	TiO2 NPs, anatase, generated from titanium tetraisopropoxide (Ti(C3H7O)4, 97%, (Sigma–Aldrich, St. Louis, MO) The dried gel was sintered at 400 °C for 4 h to get the desired TiO2 NPs	**Suspension stability**: **Cell internalisation**: TiO2 content was measured in liver by ICP-MS, data expresed in ng/g fresh tissue weight **Sonication**: Yes TiO2 NPs were heated at 125 °C for 15 min. Then, the powder was dispersed into an aqueous solution buffered with 0.15 % (w/w) sodium chloride solution. Solutions containing TiO2 particles were treated by ultrasound for 15–20 min and mechanically vibrated for 2–3 min to sufficiently disperse the particles.		not reported TiO2 content measured in liver	According to authors: TiO2 and TiO2 NPs caused dose-dependent genotoxicity in the blood cells of the treated rat as revealed by micronuclei test. The highest frequency of micronuclei was observed in rats treated with NPs at the dose of 150 mg/kg bw which was significantly different (P < 0.001) from all other experimental groups after 28 days of exposure. (P < 0.001). For TiO2 NPs clear weight loss during 28 days.	subcutaneous	Positive	3 no stability data, no proof of internalisation (although content of Ti in liver was measured so systemic exposure was proved), MN methodology not clear, subcutaneous injection; no data on historical controls; no information on the proportion of immature erythrocytes among total erythrocytes and no information on micronucleated immature erythrocytes, given separately for each animal; the number or Ret scored is not described.	Low	anatase		36.15 (30–80)	36.15 (30–80)		anatase												
in vivo	Comet in vivo	Dekanski et al., 2018	SCCS	Peripheral blood mononuclear cells mice	**nanoparticles:** TiO2 NPs not modified, TiO2 NPs surface modified with caffeic acid (ICT complex) **type of assay:** alkaline comet assay **guideline: OECD TG420** (acute oral toxicity) **, TG489** (comet assay in vivo) **treatment time and conditions** : female NMRI/HAN mice; oral acute exposue 2 and 24h (the total dose was divided over four treatments; 20.0 mL/kg of experimental animal); sampling day 0, 1, 2, 4, 7 and 14; comet assay exposure after 2 and 24h exposure **concentrations**: limit dose 1000 or 2000 mg/kg, N=5, **treatment medium for NPs dispersion** : NPs in water with HCl (0.01 M), pH 2 **toxicity assessment:** clinical oservation for abnormal behavioral signs, lethargy, dizziness, restlessness, neurological signs, respiratory distress or mortality conducted until the end of the study. The liver function examined by alanine aminotransferase (ALT) and aspartate aminotransferase (AST); the kidneys function was evaluated with serum levels of urea and creatinine **negative control:** vehicle HCl (0.01M) in water; **positive control**: not reported **methodology:** at the day 14 animal sacrificed; blood collected by cardial puncture, and liver, spleen and kidneys and gastric and intestinal mucosa evaluated for gross pathological changes. Samples for histopathology; comet assay - whole blood from vein tail collected 2 and 24h after last administration; standard alkaline version of comet assay, 100 nuclei, 2 replicates, visual scoring, 5 classes **statistics:** mean +/- SD. Anova with Turkey's post-hoc test **Additional measurements**: ALT, AST, urea, creatinine	NANOMATERIAL	TiO2 NPs, anatase unmodified and TiO2 NPs, anatase surface modified with Cafeic Acid (CA); more details in Bajic et al., 2017 (doi:10.1016/j.colsurfb.2017.04.032)	**Suspension stability:** no information **Cell internalisation:** **Sonication:** no information - probably no sonication		not reported	Negative for comet assay acute toxicity results: sleepiness and lethargy after highest dose of TiO2, 2 animals died histopathology: extensive histological changes in liver	oral	Inconclusive According to authors negative, but no proof of internalisation	2 both acute toxicity and comet assay according to guideline. No proof of internalisation. Very limited information on nanoparticles stability and unusual way of presenting data for the comet assay - only cells with damage	Low	anatase (from Bajic et al., 2017)	octahedral or square piramid (when NPs are below 20 nm) (from Bajic et al., 2017)	45A (from Bajic et al., 2017)	No information		anatase										TiO2 bare and surface modified with cafeinic acid		
in vitro	Comet in vitro	Pedrino et al., 2022	SCCS	LA-9 Mouse fibroblast cells	**nanoparticles:** TiO2 NP surface-functionalized with sodium carboxylic ligands **type of assay:** alkaline comet assay, visually evaluated according to the tail size and classified in classes **guideline:** **treatment time**: 24 h **concentrations**: 100, 10, and 1 µg/mL **FBS serum:** 10% **S9 mix:** not used **biological medium for NPs dispersion** : DMEM without phenol and supplemented with 10% fetal bovine serum (FBS); mixing and stirring **cytotoxicity assessment:** MTT, LDH **positive control**: 70 µM methyl methanesulfonate (MMS) solution **methodology:** DNA damage was visually evaluated according to the tail size and classified in class 0: without visual damage; 1: small damage; 2: average damage; 3: large damage (Kobayashi et al. 1995). Score DNA damage was calculated for all treatment groups based on Equation **statistics:** one-away ANOVA (Bonferroni post-hoc correction) **Additional measurements**: Intracellular ROS measurement; Apoptosis induction	NANOMATERIAL	TiO2 NPs, anatase, surface-functionalized with sodium carboxylic ligands	**Suspension stability**: **Cell internalisation**: not verified **Sonication**: not used		not reported	**any statistical increase at any concentration** : Yes, at 100, 10, and 1µg/mL **result:** genotoxic effects in fibroblasts exposed to 100, 10, and 1 µg/mL compared with unexposed cells The damage was dose-dependent on this period and achieved the highest score at 100 µg/mL compared with other treatments. **result for positive control:** positive (no statistics) **cytotoxicity:** negative **ROS: s**ignificant increase in ROS generation in fibroblasts exposed at 100 and 1000 µg/mL **GSH:** **internalisation: not reported** **pro-inflammatory response: not reported**		Positive	3 no stability data, methodology for comet scoring subjective	Low	anatase	tetragonal		12.3 (10 µg/mL) 13.9 (100 µg/mL) 14.3 (1000 µg/mL)		anatase			23.77 ± 0.50 (10 µg/mL) 29.81 ± 1.044 (100 µg/mL) 87.26 ± 6.3 (1000 µg/mL)		-10.1 ± 4.9 (10 µg/mL) -21.7 ± 5. 2 (100 µg/mL) -22.9 ± 0.75 (1000 µg/mL)		0.47 ± 0.01 (10 µg/mL) 0.43 ± 0.03 (100 µg/mL) 0.28 ± 0.01 (1000 µg/mL)			surface-functionalized with sodium carboxylic ligands		
in vivo	Comet in vivo	Kämpfer et al., 2021	SCCS	Colonocytes Mice	**nanoparticles:** TiO2, P25 **type of assay:** Alkaline Comet Assay **guideline:** **treatment time**: Mice orally exposed to control or ENM-enriched feed pellets (0.2% and 1% TiO2) for 28 days **biological medium for NPs dispersion** : water **cytotoxicity assessment:** **positive control**: **methodology:** DNA damage was quantified in freshly isolated murine colonocytes **statistics:** **Additional measurements**:	NANOMATERIAL	P25, TiO2 NPs, anatase/rutile	**Suspension stability**: The TiO2 sedimentation was estimated to be of an overall lower rate, reaching ≈30% in DMEM and 70% in Eagle's MEM after 24 h in 24-well format. **Cell internalisation**: **Sonication**: ENMs were suspended in sterile H2O at 4 mg mL −1 and sonicated using a cup horn sonifier (Sonifier 450, Branson Ultrasonics, USA) at a duty cycle of 0.2 s, output of 240 W for 10 min		not reported	**any statistical increase at any concentration** : no **result:** No Intestinal DNA Damage **result for positive control:** **cytotoxicity:** negative **ROS:** **GSH:** **internalisation:** **pro-inflammatory response:** No enhanced Proinflammatory Marker Expression in Murine Intestine	diet	Negative	3 no reference to guideline, effective dose ingested not measured, no proof of target organ exposure, no positive control reported	Low	anatase/rutile		21 nm	21		anatase/rutile, P25		43.49 ± 19.26 (5 mg/mL, HBSS)		-3.23 ± 1.19 (5 mg/mL, HBSS)								
in vitro	Other genotoxicity endpoints in vitro - DNA methylation	Malakootian et al., 202	SCCS	Peripheral blood mononuclear cells human, isolated	**nanoparticles:** TiO2, anatase phase **type of assay:** methylation, epigenetic alterations **guideline:** **treatment time**: **concentrations**: 25, 50 and 100 and 200 µg/ml. **FBS serum:** **S9 mix:** **biological medium for NPs dispersion** : **cytotoxicity assessment:** MTT **positive control**: **methodology:** **statistics:** Analysis of variance (ANOVA) test and post-hoc test of TukeyHSD **Additional measurements**:	NANOMATERIAL	TiO2 NPs, anatase, 39–74 nm	**Suspension stability**: **Cell internalisation**: **Sonication**:		not reported	**any statistical increase at any concentration** : yes **result:** cause DNA hypomethylation in PBMCs in a dose-response manner **result for positive control:** **cytotoxicity:** 100 µg/ml did not alter cell viability by >20%. **ROS:** **GSH:** **internalisation:** **pro-inflammatory response:**		Positive	2 no uptake demonstrated	Low not clear association with health effects	anatase		100	39–74		anatase	14	43,82					1,62					melting point (350◦C) and density (4.26 g/ml at 25◦C).
in vitro	Other genotoxicity endpoints in vitro - 53BP1	Marucco et al., 2020	SCCS	HCT-116 Human colorectal carcinoma cells	**nanoparticles:** NM-101 (JRCNM01001a), TiO2 NPs, additional characterisation of one food-grade TiO2 (10-350 nm) and other nanometric TiO2 (70 nm), not used for toxicity study **type of assay:** 53BP1 **guideline:** **treatment time**: 24h **concentrations**: 10, 20, 50 ug/mL **FBS serum:** 0 **S9 mix: no** **biological medium for NPs dispersion** : water **cytotoxicity assessment:** WST-1 Assay **positive control**: 100 uM etoposide for 53BP1; H2O2 for ROS **methodology:** simulated human digestive system (SHDS); genotoxicity assessed using 53BP1 (p53 binding protein 1; DSB-responsive protein promoting repair of DSB by nonhomologous end-joining while preventing homologous recombination); immuno-labelling and foci count **statistics:** Experiments on cells were reproduced three times independently (n = 3), with 5 technical replicates per independent experiment. Results from these 5 technical replicates were averaged. Kruskall–Wallis one-way ANOVA followed by pairwise comparison using Mann-Whitney test **Additional measurements**: oxidative stress	NANOMATERIAL	NM-101, TiO2 NPs, anatase, from the Joint Research Centre (JRC, Ispra, Italy)	Dispersion verified by DLS		not reported	**any statistical increase at any concentration** : No **result:** Negative for both pristine and digested No significant impact of simulated digestion on TiO2-NM101 cytotoxicity or genotoxicity was observed. **result for positive control:** positive - (CoO)(NiO) NPs **cytotoxicity:** negative (WST-1 10-100 ug/mL for 24 h) **ROS:** slight (<30%), time dependent increases at 10, 20 and 50 ug/mL, both pristine and digested **GSH:** **internalisation:** **pro-inflammatory response:**		Negative	2 no uptake demonstrated	Low genotoxicity assay of limited value since it should be used complimentary to other assays such as yH2AX	anatase		5-6			anatase (98.1%)	316.07 ± 15.80	Aggregates/agglomerates 100-1000 nm	A dramatic increase of the hydrodynamic size was observed in simulated gastric fluid. Irreversible aggregation occured.						98,10		Al, Na, P, S	
in vivo	Micronucleus test in vivo	Akagi et al., 2023	SCCS	Hepatocytes isolated	**nanoparticles:** TiO2 anatase 6 nm **type of assay:** micronucleus assay in isolated hepatocytes from formalin-fixed liver tissue **guideline**: subacute 28 days toxicity OECD TG 407 **treatment time**: 28 days **doses**: 10, 100 and 1000 mg/kg bw/day **toxicity assessment:** hematology, biochemistry, histology **positive control:** **methodology:** Exposure: suspension of TiO2 NPs was administered once daily by oral gavage MN: ten 3 mm-cubes of diced and fixed liver tissue were incubated in 50% potassium hydroxide at RT for 16 h and washed with water. The cubes were homogenized using a grinder, filtered through a cell strainer with a pore size of 100 µm, and resuspended in water. The suspension was centrifuged at 50×g for 5 min and washed three times with 10% neutral-buffered formalin. The suspension was stained with SYBR Gold, dropped onto glass slides, and covered with a glass coverslip. Analysis by fluorescence microscopy. **statistics:** at least 2000 hepatocytes were analyzed for each animal **additional measurements**: hematological parameters, ALT, AST, alkaline phosphatase, gamma-glutamyl transferase, histology; γ-H2AX in liver and bone marrow; nuclear/cytoplasmic translocation of β-catenin and Ki-67 in colonocytes	NANOMATERIAL	TiO2 NPs, anatase, 6 nm, AMT-100 from Tayca, Osaka, Japan	**Suspension stability**: 0.2% Na2HPO4 was used as a dispersant, as previously described [35], and its administration volume was set at 10 mL/kg bw to intend to prevent aggregation. Particle size in suspension determined by dynamic light scattering using a Zeta-potential and Particle size Analyzer ELSZ-2 (Otsuka Electronics, Hirakata, Osaka, Japan). The stability of the particle size in the suspension was confirmed at 3 h after preparation. TiO2 suspension was freshly prepared daily, mixed well for each dose, and administered within 2 h of preparation. **Cell internalisation**: Titanium content analysis in tissues by ICP-MS **Sonication**:		Titanium concentrations in the liver, kidneys, and spleen of all animals were measured using inductively coupled plasma mass spectrometry (ICP-MS) at Japan Food Research Laboratories (Tokyo, Japan).	**any statistical increase at any concentration** : no increase in micronucleated hepatocytes was observed in either the male or female 1000 mg/kg group **result:** inconclusive (distribution of TiO2 to liver was at trace levels) **result for positive control:** **organ toxicity:** no biologically meaningful changes in hematology, liver, or systemic toxic parameters. **Authors: "histopathological examination and quantification of Ti in the liver suggested that the liver was not exposed to orally administered TiO2 NPs in this study."** **presence in tissues:** by ICP-MS: 1. in comparison with the control groups, a significant increase in Ti concentration in the liver was observed in the 1000 mg/kg bw/day group of females in the 28-day study 2. no changes of Ti content in kidney, and spleen **histopathology**: the examination revealed yellowish-brown materials in the tissues of the nasal cavity, trachea, lungs (bronchus-associated lymphoid tissue),	oral, stomach tube	inconclusive	1	Low Distribution of TiO2 NPs to organs after p.o. administration not conclusively demonstrated	anatase		3.5–7, modal diameter of 5 nm accounting for over 75% frequency (by TEM)	6		anatase	280	206.3 ± 44.2 a small fraction with particles < 100 nm in diameter (4.9 ± 11.1%) was occasionally observed							93,00			
in vivo	Other genotoxicity endpoints in vivo - H2AX	Akagi et al., 2023	SCCS	Hepatocytes isolated, bone marrow cells, nasal cavity, BALT, trachea, Peyer's patches, cervical and mediastinal lymph nodes tissues	**nanoparticles:** TiO2 anatase 6 nm **type of assay:** γ-H2AX staining in formalin-fixed tissues **guideline**: subacute 28 days, subchronic 90 days toxicity OECD TG 407 and 408 **treatment time**: 28 and 90 days **doses**: 28 days: 10, 100 and 1000 mg/kg bw/day; 90 days: 100, 300 and 1000 mg/kg/bw/day **toxicity assessment:** hematology, biochemistry, histology **positive control:** **methodology:** Exposure: suspension of TiO2 NPs was administered once daily by oral gavage H2AX: for number of γ-H2AX positive hepatocytes per liver area, the area of the liver parenchymal region in the left lateral lobe and medial lobe sections was measured, excluding major vacuoles, bile ducts, blood vessels, and stromal regions. The number of γ-H2AX positive hepatocytes within that area was counted under a microscope. Besides the liver and bone marrow (28 days), γ-H2AX staining was done in in the nasal cavity, BALT, trachea, Peyer's patches, and cervical and mediastinal lymph nodes in the 90-day study. **statistics:** **additional measurements**: hematological parameters, ALT, AST, alkaline phosphatase, gamma-glutamyl transferase, histology; MN in liver; nuclear/cytoplasmic translocation of β-catenin and Ki-67 in colonocytes	NANOMATERIAL	TiO2 NPs, anatase, 6 nm, AMT-100 from Tayca, Osaka, Japan	**Suspension stability**: 0.2% Na2HPO4 was used as a dispersant, as previously described [35], and its administration volume was set at 10 mL/kg bw to intend to prevent aggregation. Particle size in suspension determined by dynamic light scattering using a Zeta-potential and Particle size Analyzer ELSZ-2 (Otsuka Electronics, Hirakata, Osaka, Japan). The stability of the particle size in the suspension was confirmed at 3 h after preparation. TiO2 suspension was freshly prepared daily, mixed well for each dose, and administered within 2 h of preparation. **Cell internalisation**: Titanium content analysis in tissues by ICP-MS **Sonication**:		Titanium concentrations in the liver, kidneys, and spleen of all animals were measured using inductively coupled plasma mass spectrometry (ICP-MS) at Japan Food Research Laboratories (Tokyo, Japan).	**any statistical increase at any concentration** : no induction of H2AX was observed in either the male or female 1000 mg/kg group **result:** inconclusive (distribution of TiO2 to liver was at trace levels) **result for positive control:** **organ toxicity:** no biologically meaningful changes in hematology, liver, or systemic toxic parameters. **Authors: "histopathological examination and quantification of Ti in the liver suggested that the liver was not exposed to orally administered TiO2 NPs in this study."** **presence in tissues:** by ICP-MS: 1. in comparison with the control groups, a significant increase in Ti concentration in the liver was observed in the 1000 mg/kg bw/day group of females in the 28-day study 2. no changes of Ti content in kidney, and spleen **histopathology**: the examination revealed yellowish-brown materials in the tissues of the nasal cavity, trachea, lungs (bronchus-associated lymphoid tissue),	oral, stomach tube	inconclusive	1	Low Distribution of TiO2 NPs to organs after p.o. administration not conclusively demonstrated	anatase		3.5–7, modal diameter of 5 nm accounting for over 75% frequency (by TEM)	6		anatase	280	206.3 ± 44.2 a small fraction with particles < 100 nm in diameter (4.9 ± 11.1%) was occasionally observed							93,00			
in vitro	Micronucleus test in vitro	Vignard et al., 2023	SCCS	TR146 squamous cell carcinoma from human buccal cells	**nanoparticles:** E171, anatase **type of assay:** micronucleus assay in vitro **guideline:** no **treatment time:** 2 h + 22 h **concentrations:** 5, 50 or 100 ug/mL **FBS serum:** 0 **S9 mix:** not used **biological medium for NPs dispersion:** water **cytotoxicity assessment:** Cells exposed to 25 uM of etoposide as a positive control, or 5, 50 or 100 ug/ml of food-grade TiO2 (E171) and TiO2 test materials for 2 h. Cells were then washed with PBS and incubated in fresh culture medium for 72 h. Alamar blue assay. TEER measured immediately or within 48 h. **positive control:** etoposide 50uM **methodology:** No cytokinesis block; immunofluorescence staining (not described); micronucleated cells were scored in at least 100 cells per sample; triplicate experiments **statistics:** one-way or two-way analysis of variance (ANOVA) followed by the appropriate post hoc test **Additional measurements:** E171 particle translocation in vivo through the pig buccal mucosa and in vitro on human buccal TR146 cells; antibodies directed against gammaH2AX and 53BP1 (DNA damage biomarkers); oxidative stress	E171-equivalent	E171, anatase, from French commercial supplier	**Suspension stability**: in ultrapure water (pH 7.75) and in TR146 cell culture medium (Ham's F12, pH 7.54) **Cell internalisation**: TEM; npSCOPE analyses combining SE, STIM and SIMS imaging; confocal microscopy **Sonication**: sonicated in ultrapure water (1 mg/ml) placed in an ice bath for 1 min at 40% amplitude (VCX 750-230 V, Sonics Materials) to obtain a stable dispersion of TiO2 particles for 15 days at 4oC.	possible (TEM image)	Confirmed by TEM; npSCOPE analyses combining SE, STIM and SIMS imaging; confocal microscopy	**any statistical increase at any concentration** : no **result**: negative **result for positive control**: 2.8 fold increase **cytotoxicity**: In proliferating cells, the tested TiO2 samples induced a significant cytotoxic activity at all tested concentrations compared to nontreated cells, except with 5 ug/mL for E171, with a dose-response tendency. In contrast, after cell differentiation, no viability drop was observed in any tested condition. **TEER**: Regardless of the time point tested during the 48 h after treatment, no alterations were detected at any dose, suggesting that epithelial barrier permeability and monolayer integrity were not affected. **Internalisation**: Electron dense (TiO2) particles were isolated or recovered as small aggregates and then larger agglomerates of submicron-sized particles mixed with NPs into the cytoplasm. Conformed by npSCOPE analyses.		Negative	3 low number of cells scored	Low	anatase		55% of the NPs by number were 20 to 440 nm for a mean size of 105 ± 45 nm					297.9 ± 5.1	318.7 ± 14.9	- 32.1 ± 0.79	- 8.97 ± 0.54	0,21	0,4					

in vitro	Micronucleus test in vitro	Vignard et al., 2023	GCS	TR146 squamous cell carcinoma from human buccal cells	**nanoparticles:** TiO2 NPs, anatase, 21 nm, from Sigma–Aldrich, Saint-Quentin-Fallavier, France **type of assay:** micronucleus assay in vitro **soluble:** no **treatment time:** 2 h + 22 h **concentrations:** 5, 50 or 100 ug/mL **FBS serum:** 0 **S9 mix:** not used **biological medium for NPs dispersion:** water **cytotoxicity assessment:** Cells exposed to 25 uM of etoposide as a positive control, or 5, 50 or 100 ug/ml of food-grade TiO2 (E171) and TiO2 test materials for 2 h. Cells were then washed with PBS and incubated in fresh culture medium for 72 h. Alamar blue assay. TEER measured immediately or within 48 h. **positive control:** etoposide 50uM **methodology:** No cytokinesis block; immunofluorescence staining (not described); micronucleated cells were scored in at least 100 cells per sample; triplicate experiments **statistics:** one-way or two-way analysis of variance (ANOVA) followed by the appropriate post hoc test **Additional measurements:** E171 particle translocation in vivo through the pig buccal mucosa and in vitro on human buccal TR146 cells; antibodies directed against gamma#2AX and 53BP1 (DNA damage biomarkers); oxidative stress	NANOMATERIAL	TiO2 NPs, anatase, 21 nm, from Sigma–Aldrich, Saint-Quentin-Fallavier, France			Confirmed by TEM; np|SCOPE analyses combining SE, STEM and SIMS imaging; confocal microscopy			**Suspension stability**: in ultrapure water (pH 7.75) and in TR146 cell culture medium (Ham's F12, pH 7.54) **Cell internalisation** : TEM; np|SCOPE analyses combining SE, STEM and SIMS imaging; confocal microscopy **Sonication**: sonicated in ultrapure water (1 mg/mL) placed in an ice bath for 1 min at 40% amplitude (VCX 750-230 V, Sonics Materials) to obtain a stable dispersion of TiO2 particles for 15 days at 4oC.			**any statistical increase at any concentration** : no **result**: negative **result for positive control** : 2.8 fold increase **cytotoxicity**: In proliferating cells, the tested TiO2 samples induced a significant cytotoxic activity at all tested concentrations compared to nontreated cells, with a dose-response tendency. In contrast, after cell differentiation, no viability drop was observed in any tested condition. **TEER**: Regardless of the time point tested during the 48 h after treatment, no alterations were detected at any dose, suggesting that epithelial barrier permeability and monolayer integrity were not affected. **internalisation**: Electron dense (TiO2) particles were isolated or recovered as small aggregates and then larger agglomerates of submicron-sized particles mixed with NPs into the cytoplasm. Confirmed by np|SCOPE analyses.		Negative	0 low number of cells scored	Low		amateur								173.9 ± 1.7	226.4 ± 3.1	11.1 ± 2.44	8.99 ± 1.96	0,16	0,39						
in vitro	Micronucleus test in vitro	Vignard et al., 2023	GCS	TR146 squamous cell carcinoma from human buccal cells	**nanoparticles:** NM-102, TiO2 NPs, anatase, referenced to JRCNM10200a **type of assay:** micronucleus assay in vitro **soluble:** no **treatment time:** 2 h + 22 h **concentrations:** 5, 50 or 100 ug/mL **FBS serum:** 0 **S9 mix:** not used **biological medium for NPs dispersion:** water **cytotoxicity assessment:** Cells exposed to 25 uM of etoposide as a positive control, or 5, 50 or 100 ug/ml of food-grade TiO2 (E171) and TiO2 test materials for 2 h. Cells were then washed with PBS and incubated in fresh culture medium for 72 h. Alamar blue assay. TEER measured immediately or within 48 h. **positive control:** etoposide 50uM **methodology:** No cytokinesis block; immunofluorescence staining (not described); micronucleated cells were scored in at least 100 cells per sample; triplicate experiments **statistics:** one-way or two-way analysis of variance (ANOVA) followed by the appropriate post hoc test **Additional measurements:** E171 particle translocation in vivo through the pig buccal mucosa and in vitro on human buccal TR146 cells; antibodies directed against gamma#2AX and 53BP1 (DNA damage biomarkers); oxidative stress	NANOMATERIAL	NM-102, TiO2 NPs, anatase, referenced to JRCNM10200a			Confirmed by TEM; np|SCOPE analyses combining SE, STEM and SIMS imaging; confocal microscopy			**Suspension stability**: in ultrapure water (pH 7.75) and in TR146 cell culture medium (Ham's F12, pH 7.54) **Cell internalisation** : TEM; np|SCOPE analyses combining SE, STEM and SIMS imaging; confocal microscopy **Sonication**: sonicated in ultrapure water (1 mg/mL) placed in an ice bath for 1 min at 40% amplitude (VCX 750-230 V, Sonics Materials) to obtain a stable dispersion of TiO2 particles for 15 days at 4oC.			**any statistical increase at any concentration** : no **result**: negative **result for positive control** : 2.8 fold increase **cytotoxicity**: In proliferating cells, the tested TiO2 samples induced a significant cytotoxic activity at all tested concentrations compared to nontreated cells, with a dose-response tendency. In contrast, after cell differentiation, no viability drop was observed in any tested condition. **TEER**: Regardless of the time point tested during the 48 h after treatment, no alterations were detected at any dose, suggesting that epithelial barrier permeability and monolayer integrity were not affected. **internalisation**: Electron dense (TiO2) particles were isolated or recovered as small aggregates and then larger agglomerates of submicron-sized particles mixed with NPs into the cytoplasm. Conformed by np|SCOPE analyses.		Negative	0 low number of cells scored	Low		amateur								601.6 ± 0.4	337.0 ± 20.1	34.3 ± 0.66	8.19 ± 0.59	0,17	0,35						