HESI-ILSI
Webinar

Genotoxicity Testing of Nanomaterials

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Content

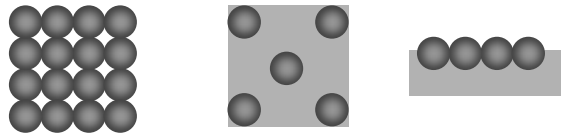
- **General Thoughts on Nanomaterials in the Body**

- **Test and Testmethod Overview**
- **Role of the Particle Size, the Testsubstance and the Testmethod**
- **Recomendations**
- **Conclusion**

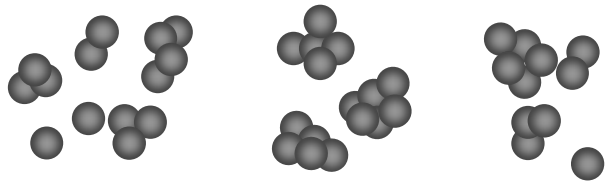
- ***Example*:**
  Inhalation study with *ex vivo* Comet assay in the lung

Nanomaterials in the Body

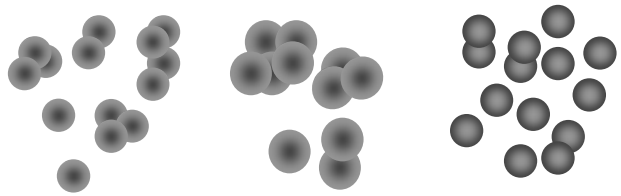
Nanomaterial
Powder
Embedded on Surface or in Matrix



Dispersion
Aerosol
Suspension

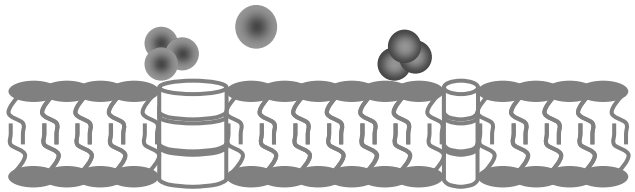


Uptake in the body
deposition in the lung,
alveolar, intestinal, dermal penetration



Modification in the body
surface coating changes
agglomeration, deagglomeration

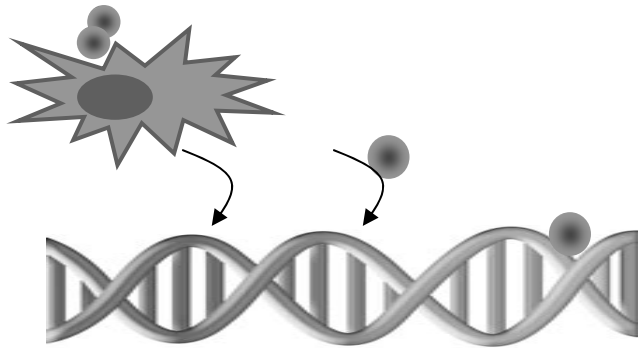
Distribution in the body
penetration of biological barriers
tissue distribution, intracellular distribution



Primary Effect
Inflammation
catalysing formation of reactive compounds
direct interaction with DNA or spindle

Genotoxic Effect
gene mutations
chromosomal aberrations
and malsegregation

Mutation
DNA oxidation, DNA-base adducts,
attachment to DNA



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OECD Criteria Characterization of Nanomaterials for Toxicological Testing

Physical-Chemical Properties and Material Characterization

- Agglomeration/aggregation
- Water solubility
- Crystalline phase
- Dustiness
- Crystallite size
- Representative TEM picture(s)
- Particle size distribution
- Specific surface area
- Zeta potential (surface charge)
- Surface chemistry (where appropriate)
- Photocatalytic activity
- Pour density
- Porosity

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Dispersion of Nanomaterials

Compound Bacterial reverse mutation test	CAS No.	Purity	Conc [µg /plate]	Precipi-tation [µg/plate]	Muta-genicity	Best dispersed in
Titanium dioxide (hydrophilic)	13463-67-1	>99.5 %	5000	500	no	Fetal Calf Serum
Zinc oxide, nanopowder	1314-13-2	-	5000	2500	no	Fetal Calf Serum
Titanium(IV) oxide, nanopowder, 99.9%	13463-67-7	99.9%	5000	500	no	Fetal Calf Serum
Titanium(IV) oxide, nanopowder, 99.7%	1317-70-0	99.7%	5000	500	no	Fetal Calf Serum
Iron (II,III) oxide, nanopowder, 98+%	1317-61-9	>98%	5000	2500	no	Fetal Calf Serum
Titanium dioxide	13463-67-7	99.4%	5000	500	no	Fetal Calf Serum
Carbon nanopowder, 99+%	7440-44-0	>99%	2500	50	no	Fetal Calf Serum
Zinc oxide, powder < 7µ	1314-13-2	>99.9 %	5000	500	no	Fetal Calf Serum
Multi-walled Carbon Nanotubes	-	-	2500	50	no	-
Titanium dioxide, modified (T-Lite SF)	13463-67-3	-	2500	20	no	Fetal Calf Serum

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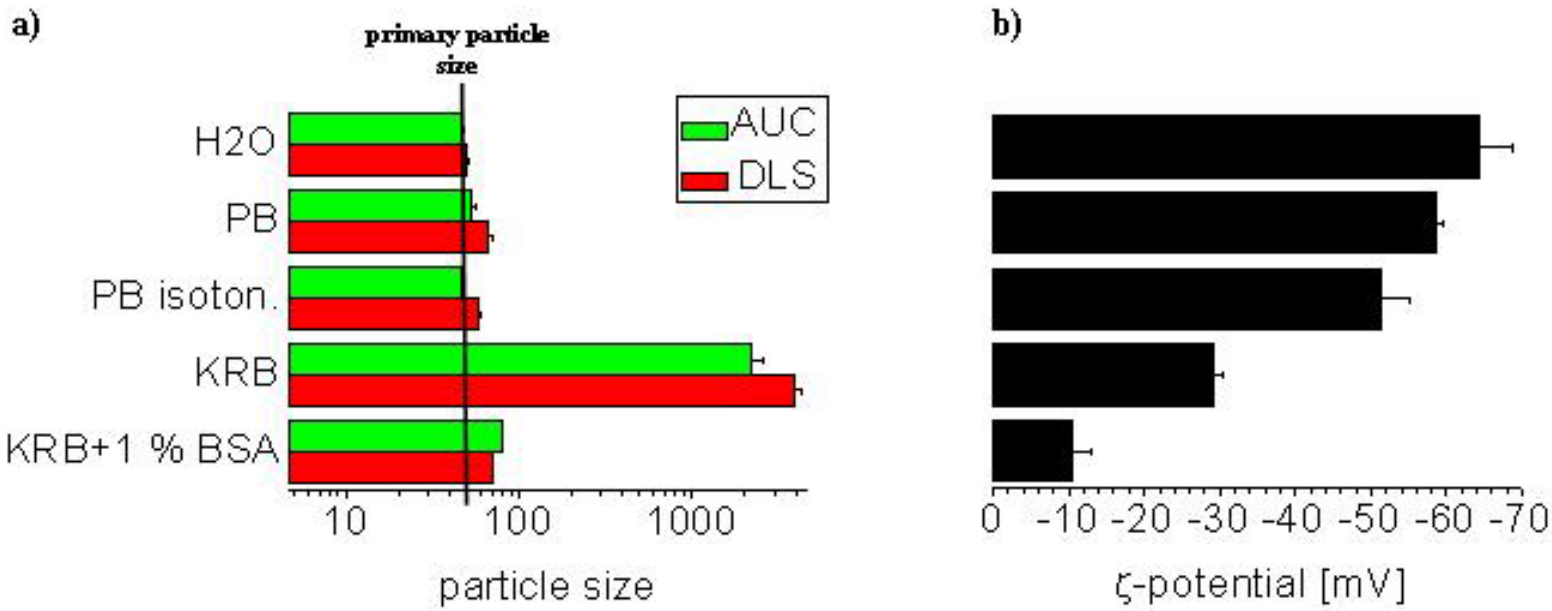
Analytics impossible

Significant amount of fine particles

Predominantly fine particles

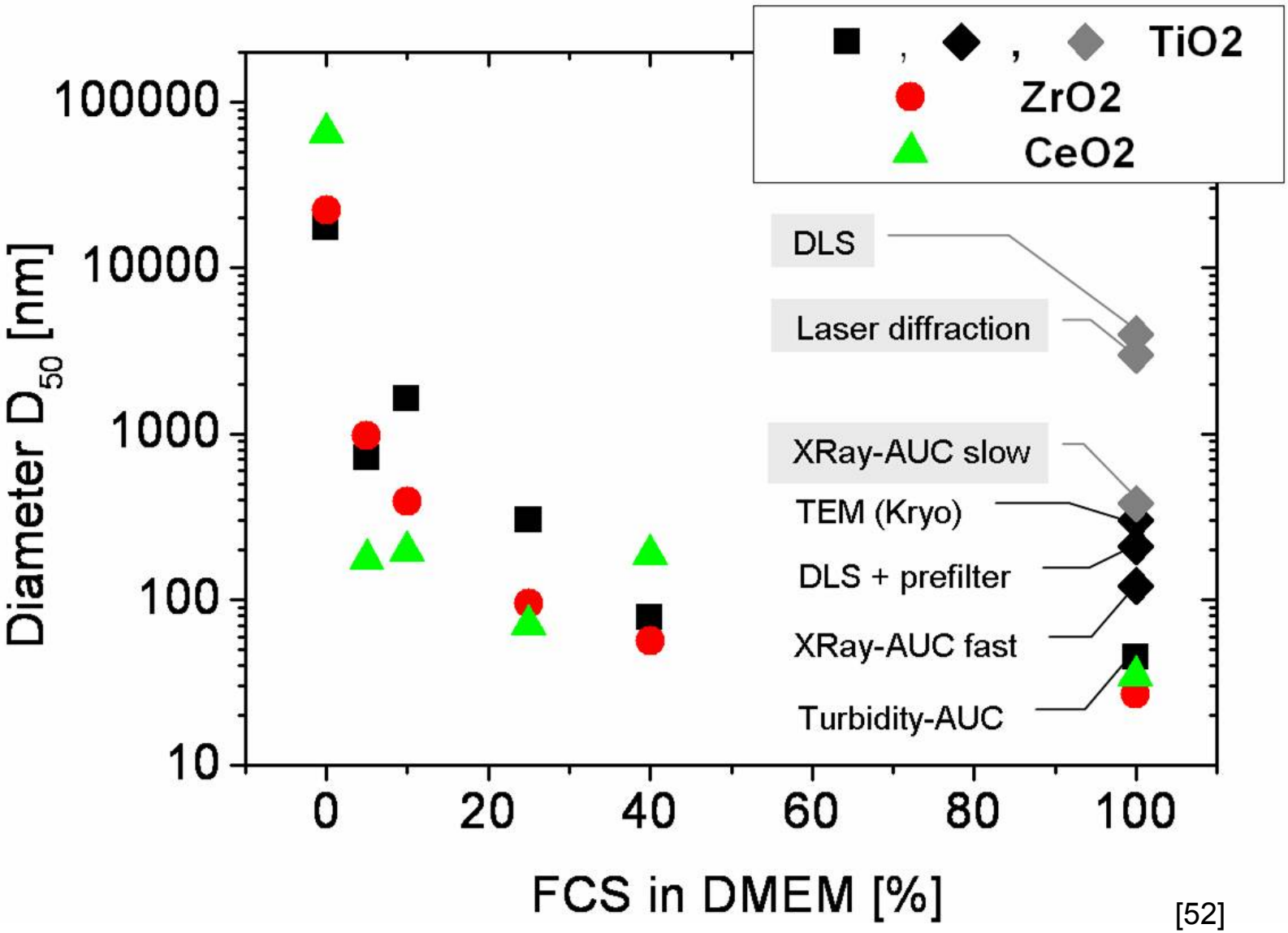
Significant amount of ultrafine particles

Sizes and ζ-Potentials of NPs in Different Dispersion Media



[52]

Diameters of NP in DMEM



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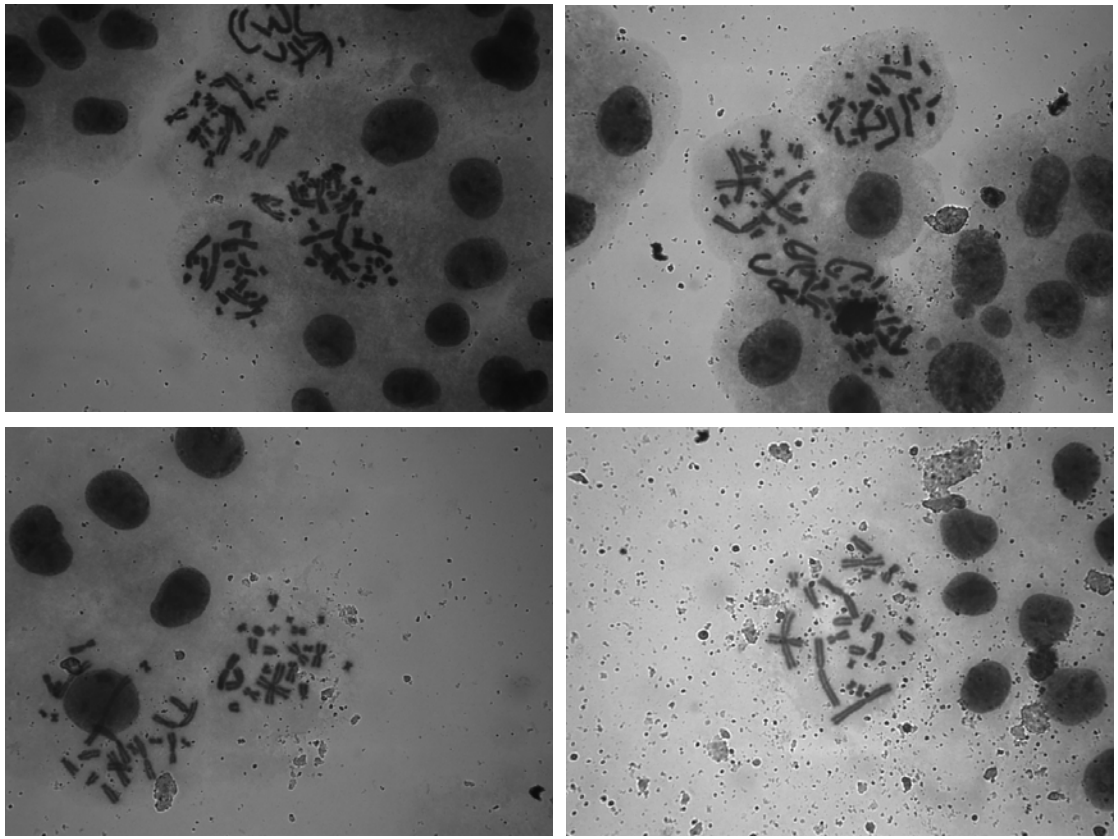
Agglomerates interfers with the scoring for cytogenetic damage

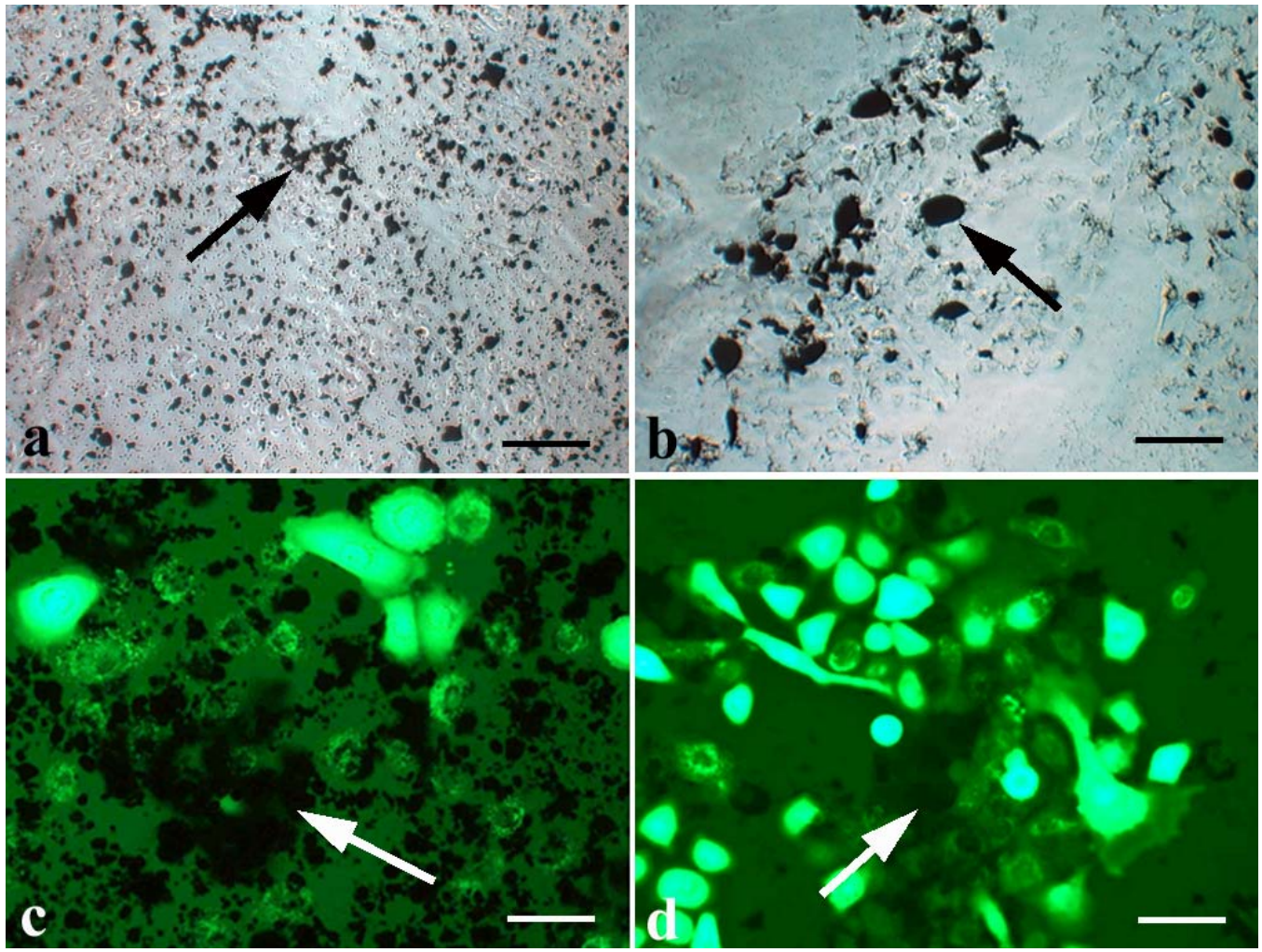


Metaphase plates of spread V79 Chinese Hamster cells: Agglomeration of Titanium dioxide, modified, on the slides (magnification 1000x) 78, 156, 312, 624 mg/mL TiO_2

HEK treated with 0.1 mg/ml CNM for 24h

(a) CB (b) SWCNT

(c) CB CAM staining of live cells (d) SWCNT; CAM staining of live cells.



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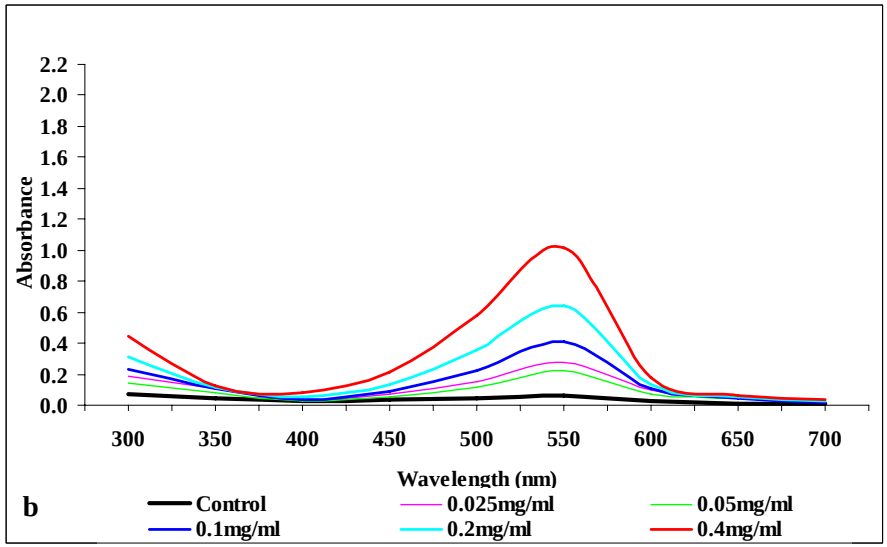
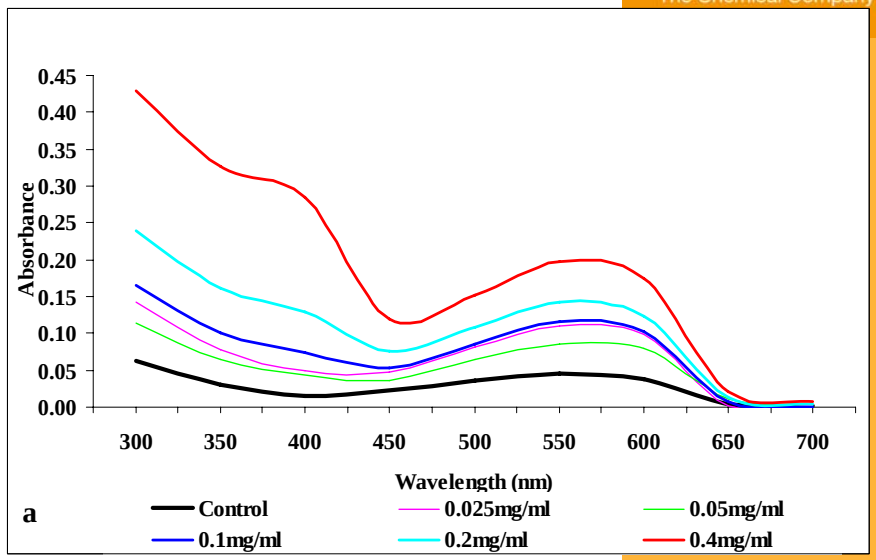
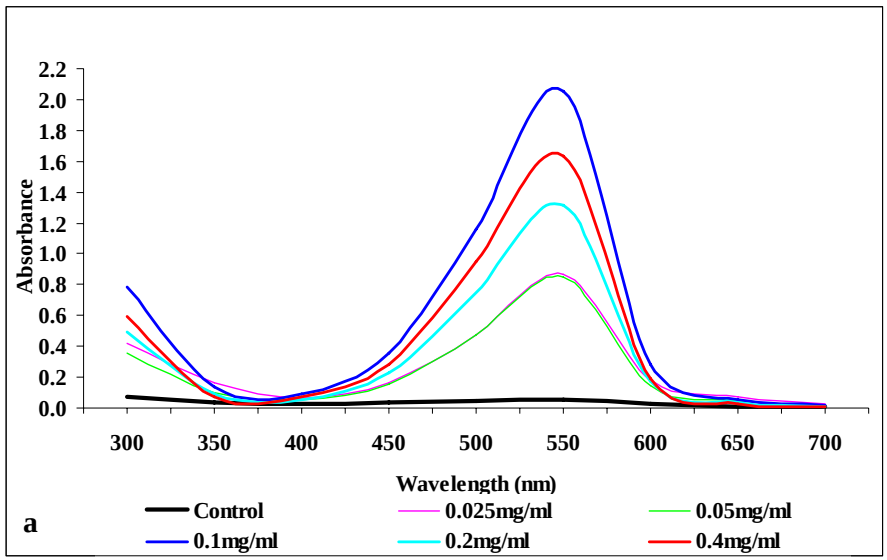
UV/Vis spectrum with NR and MTT

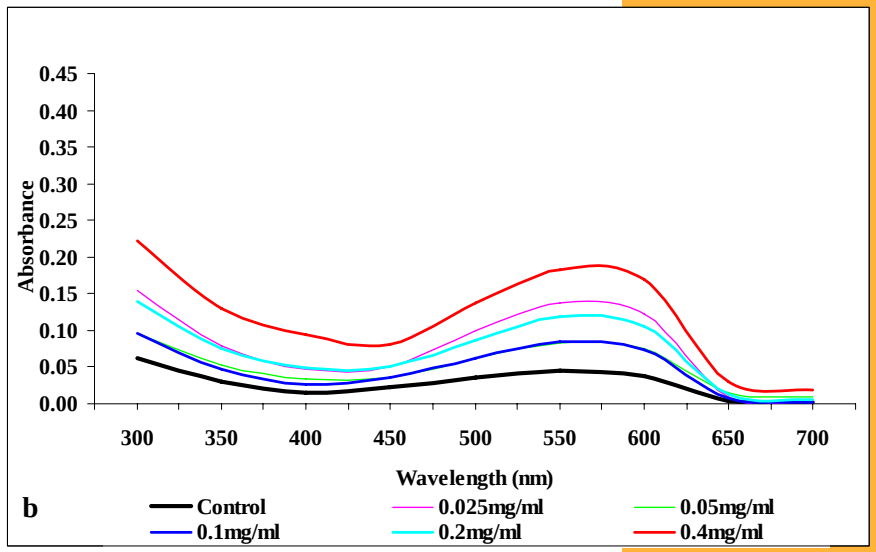
(a) CB

(b) SWCNT









Nanomaterials Interaction with Dyes used in Viability Tests

	CB (0.4 mg/ml)	SWCNT (0.4 mg/ml)	C_{60} (0.4 mg/ml)	nC_{60} (0.047 μg/ml)	QD-COOH (20 nM)
NR	+978.5*	+96.2*	+34.6*	+14.9	+3.0
MTT	+209.1*	+46.5*	+33.8*	+12.8	+3.9
96 AQ	−14.5*	+2.6	−1.1	−0.8	−1.1
aB	−88.3*	−33.5*	−1.3	+0.6	+4.5*
CTB	−30.1*	−20.3*	−1.5	+1.5*	−2.2
CTO	−100.0*	−100.0*	−63.5	+102.6	−62.6

* Significantly ($p < 0.05$) different from paired control.

Percent difference of nonspecific absorbance relative to blank well controls (no cell control) at the highest relative NM concentration

calcein AM (CAM), Live/Dead (LD), NR, MTT, Celltiter 96® AQueous One (96 AQ), alamar Blue, (aB), Celltiter-Blue® (CTB), CytoTox One™ (CTO), and flow cytometry

[58]

Comparison of Cell Viability Tests

Comparison of viability assays with nanomaterials

	CB	SWCNT	C_{60}	nC_{60}	QD-COOH
NR	F^P	F^P	D^P	B^P	A
MTT	F^P	D^P	D^P	B^P	A
96 AQ	C^N	A	A	A	A
aB	F^N	D^N	A	A	B^P
CTB	D^N	C^N	A	B^P	A
CTO	F^N	F^N	B^{N*}	B^{P*}	B^{N*}

A — Assay works well; data reliable.

B — Assay works well; nonsignificant difference ≥10% or significant difference ($p<0.05$) <10%.

C — Assay works; significant difference ($p<0.05$) from 10% to <20%.

D — Assay works poorly; significant difference ($p<0.05$) from 20% to <50%.

F — Assay fails; significant difference ($p<0.05$) ≥50%.

^{N}Data below blank controls; ^{P}data above blank controls; *highly variable data.

[58]

ICH Genotoxicity Guidelines

If no cytotoxicity is observed then the lowest precipitating concentration should be used as the top concentration but not exceeding 5mg/plate for bacterial tests and 5mg/ml or 10mM for mammalian cell tests. If dose-related cytotoxicity or mutagenicity is noted, irrespective of solubility, then the top concentration should be based on toxicity as described above. This may require the testing of more than one

Review of Published Genotoxicity Tests with Nanomaterials

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Reference	Material	Preparation/Characteristics	Test system/Concentration	Results/Problems
Ashikaga, T. et al., 2000	TiO_2	**Characteristics:** Crystal. str. P. size [µm] S. Area [m^2/g] 1 Anatase / 72.6 2 Anatase 0.4 18 3 Rutile 0.03-0.05 40 4 Anatase 0.021 50 5 Rutile 0.64 2.7 6 Rutile 5 - 7 Anatase 5 - 8 Amorphous 0.05 -	**Test system:** • Agarose gel electrophoresis: Super-coiled pBR 322 DNA (20 µg/mL) was mixed with 5 µL of an aqueous suspension of TiO_2 (80 µg/mL). The mixture was irradiated with UVA and then subjected to agarose gel electrophoresis.	Anatase-type TiO_2 showed strong photodynamic DNA strand-breaking activities. Rutile-type samples showed weak or no activities
Auffan, M. et al., 2006	DMSA-coated Maghemite Nanoparticles	**Characteristics:** Nanoparticles are roughly spherical with a mean coherent diameter of 6 nm. The specific surface area: 172 m^2/g	**Cell line:** normal human fibroblasts **Concentrations:** from 10^{-6} to 10^{-1} g/mL **Test systems:** • Cytotoxicity Assay • Comet Assay	Well-stabilized NmDMSA produced weak cytotoxic and non genotoxic effects.
Avogbe, P.H. et al., 2005	Ultrafine particles from three urban locations	Continuous Measurement of the number of particles with 10-1000 nm in diameter Number of particles per cm^3: 0-320000	**Test system:** • Comet Assay with FPG protein to detect FPG sensitive sites. **Cell line:** mononuclear blood cells (MNBC)	Urban air with high levels of benzene and UFP is associated with elevated levels of SB and FPG sites in MNBC.
Bräuner, E.V et al., 2007	Urban air particles	**Preparations:** Participants were exposed in exposure chamber for 24 h. **Characteristics:** Average diameters 12, 23, 57 and 212 nm	**Test system:** • Comet Assay (with FPG enzyme) **Cells:** Peripheral mononuclear blood cells **Time:** 6 and 24 h	Exposure for 6 and 24 h increased the level of SBs and FPG sites. The 57 nm fraction caused the highest yield of DNA damage.
Chen, G. et al., 2007	Nano-titanium dioxide	Final concentration of TiO_2: 0.1 mg/mL Irritation under UV light for 90 min. Immersing the electrode in Resveratrol solution (0.5 mmol/L) for 30, 60, 90, 120, 140 s, 9, 20, 30, 60 min	**Test system:** • Electrochemical Method: Substrate electrode: DNA and nano-TiO_2 were co-modified onto the surface of the gold electrode. Reference electrode: Calomel electrode (SCE) Counter electrode: Platinum wire electrode	The ROS produced from TiO_2 nanoparticles can oxidatively damage DNA and the herb resveratrol has a repairing effect to the oxidized DNA.
Dufour, E.K. et al., 2006	Microfine uncoated Zinc oxide (ZnO) Particle size <200 nm	**Preparation:** Micronised uncoated ZNO formulated as a 10% emulsion for Ames Test and CHO cells. Aqueous suspension of micronised uncoated ZnO for V-79 cells and human keratinocytes.	**Test systems:** • (Photo) Ames test: **Srains:** TA98, 100, 1573 and *E.coli* WP2 • Chromosome aberration: Cell Line: CHO and V79 cells **Concentrations:** 0, 54, 84, 105 131, 164, 256, 320 µg/mL • Comet Assay: Cells: V-79 and human keratinocytes (HaCaT cells)	Non-mutagenic in Ames test Clastogenic in vitro (CHO cells, V-79 cells) Photo clastogenic in vitro (CHO cells V-79 cells) Equivocal photo-genotoxicity in vitro (weakly positive in V-79, clearly negative in HaCaT cells).
Dunford, R. et al. 1997	Titanium Dioxide and ZnO from sunscreens.	**Characterization:** Commercial TiO_2 samples (20-50 nm in	**Test systems:** • Agarose Gel electrophoresis:	The results demonstrate that sunscreen TiO_2 and ZnO can

DNA Damage Tests with Positive Outcome

Comet assay:

14 of 19 studies were positiv (*in vitro* unless stated otherwise)

Carbon Black [7,9], SWCNT [4]

Cobalt chrome alloy [5]

TiO_2 [6,8,14,15], V_2O_3 and V_2O_5 (Krug, personal communication)

Diesel exhaust particles (*in vitro* and *ex vivo*) [10], general traffic vehicle exhaust (*ex vivo*) [11], urban and rural air pollution (*ex vivo*) [12], urban air particles of defined size ranges (*ex vivo*) [13]

Other DNA damage

6 studies were positive

photovoltaic TiO_2 [21]

CdSe/ZnS quantum dots [16]

Gold nanoparticles [17], nickel powder [18]

wildfire smoke samples [19]

SWCNT (*ex vivo*) [20]

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Gene Mutation Tests with Positive Outcome

1 of 6 **Ames** test was weakly positive in a single strain

water-soluble FePt with capping [22]

5 of 7 **Mammalian gene mutation** assays were positive (all *in vitro* unless stated otherwise):

SiO_2 [23,24], TiO_2 [6]

MWCNT [25]

Carbon Black [26,27] (*ex vivo* and *in vitro*)

Chromosome Mutation Tests with Positive Outcome

12 of 14 **MNT** (all *in vitro* unless stated otherwise)

- TiO_2 [6,8,29], cerium-doped TiO_2 [30], TiO_2 + irradiation [14]
- SiO_2 [23,24], zinc oxide [31]
- CoCr [5], magnetite (*ex vivo*) [33,34]
- MWCNT (*in vitro* and *ex vivo*) [32],
- diffusion flame system as particle generator doped with iron or without iron ex vivo, the main hydrocarbons of the non-iron and iron-doped flame being toluene, butane, styrene, benzene and xylene [35].

3 of 6 **CA** (all *in vitro*)

- TiO_2 (increase of chromosome aberrations only + irradiation)
- zinc oxide [31], [14]
- diffusion flame system as particle generator (*vide supra*) [35]

DNA-damage-dependent Signalling, Biomarkers and Special Methods

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Carbon Black Printex 90 in A549 type II [7]

- p53 phosphorylation
- phosphorylated p53BP1
- single-strand DNA breaks (Comet assay)
- phosphorylated BRCA1.
- Carbon black particles of larger size showed none of the responses

TiO_2 (P25) dispersed with calf thymus DNA and irradiated [36]

- DNA and RNA damage visualized by scanning micrographs

DNA Damage Tests with Negative Outcome

5 of 19 Comet assays were negative (all *in vitro* unless stated otherwise)

- TiO_2 [14]
- Carbon Black [38]
- SiO_2 [23,24]
- Maghemite coated with DMSA [37]
- vehicle exhaust (*ex vivo*) (no increase in DNA strand breaks as determined by Comet assay, but oxidative DNA damage in terms of FPG-sensitive sites) [11]

1 Test on DNA damage (8-oxoguanine) was negative

after intratracheal instillation in rats (*ex vivo*)

- TiO_2 [39]

Gene Mutation Tests with Negative Outcome

5 of 6 **Ames** test were negative

- TiO_2 [14,40]
- zinc oxide [31]
- SWCNT [4]
- silica-coated magnetic nanoparticles labeled with rhodamine B isothiocyanate “MNPs@SiO2(RITC)” [41]

2 of 7 **Mammalian gene mutation** tests were negative

- TiO_2 *in vitro* [14]
- diesel exhaust particles *ex vivo* (*cII* mutation frequency in lung tissue of transgenic MutaTMMice exposed by inhalation

Chromosome Mutation Tests with Negative Outcome

3 of 6 **CA** were negative (all *in vitro*)

TiO_2 [40,44]

"MNPs@SiO2(RITC)" [41]

2 of 14 **MNT** were negative (all *in vitro*)

TiO_2 [42]

V_2O_3 and V_2O_5 [Krug, personal communication]

Positive versus Negative Test Results depending on the Test System

TiO_2 [14]

Particle size 21 nm, anatase

UV irridiation

Positive Comet assay in Chinese hamster lung CHL/IU cells

Positive CA in Chinese hamster lung CHL/IU cells

Negative in Ames test

Negative Mouse lymphoma L5178 tk+/- gene mutation assay

SiO_2 [24]

Particle size 7 - 123 nm

Positive MNT (cytokinesis block version) WIL2-NS human B-cell lymphoblastoid cells

Positive HPRT assay WIL2-NS human B-cell lymphoblastoid cells

Negative Comet assay WIL2-NS human B-cell lymphoblastoid cells

SWCNT [4]

Diameters from 0.4 to 1.2 nm, a length of 1-3 µm

Positive Comet assay V79 cells

Negative MNT V79 cells (limited but not stat. sign.MN induction)

Negative Ames test (in the Salmonella strains YG1024 or YG1029)

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Positive versus Negative Test Results depending on the Particle Size

TiO_2 [8]
- human bronchial epithelial cells (BEAS-2B)
- Comet assay (with FPG) and MNT in the absence of light
  - Positive: primary particle size 10 nm and 20 nm, anatase
  - Negative: primary particle size 200 nm and >200 nm

TiO_2 [31]
- Syrian hamster embryo fibroblasts
- MNT
  - Positive: primary particle size < 20 nm
  - Negative: primary particle size >200 nm

Carbon Black [7]
- A549 cell line
- Comet assay
  - Positive : Printex 90 (primary particle size 14 nm)
  - Negative : Coarse carbon black (primary particle size 260 nm)

Cobalt chrome alloy [5]
- Primary human dermal fibroblasts
- Comet assay and MNT
  - Positive : primary particle size 29.5±6.3 nm
    - tail moment about 17-fold incresed
    - centromer-positive micronuclei
  - Positive, but less pronounced: primary particle size 2.904±1.064 µm
    - tail moment about 4-fold increased
    - less centromer-positive micronuclei

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Apparently Surprising Positive versus Negative Test Results with respect to the Test Substance

Comet Assay and **lacZ gene Mutation** [9]

MutaMouse lung epithelial cell line

Positive : Carbon Black (primary size 14 nm)

Negative : Quartz (mean particle size 1.59 µm)

Comet Assay [38]

Hel 2999 human embryonic lung fibroblast cell line

Positive : Quartz (α-quarz, <5 µm)

Negative : Carbon Black (37 nm)

Comet assay

Human lung alveolar type II adenocarcinoma cells

V_2O_3

Positive nanosized

Negative bulk sized

V_2O_5

Positive bulk sized

Negative: nanosized

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Perspectives

What can we learn?

1. **Know what nanomaterial has been tested and in what form !**
2. **Consider uptake and distribution of the nanomaterial !**
3. **Use standardized methods !**
4. **Recognize that nanomaterials are not all the same !**
5. **Use *in vivo* studies to correlate *in vitro* results !**
6. **Take nanomaterials specific properties into account !**
7. **Learn about the mechanism of genotoxic effects !**

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Conclusions

Experiences with other, non-nano, substances (molecules and larger particles) taught us, that mechanisms of genotoxic effects can be diverse and their elucidation can be demanding, while there often is an immediate need to assess the genotoxic hazard.

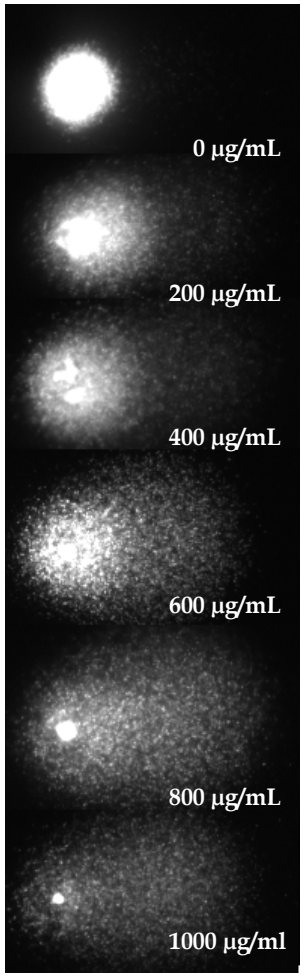
Thus a practical and pragmatic approach is the use of a battery of standard genotoxicity testing methods covering a wide range of mechanisms.

Application of these standard methods to nanomaterials demands, however, several adaptations and the interpretation of results from the genotoxicity tests may need additional considerations.

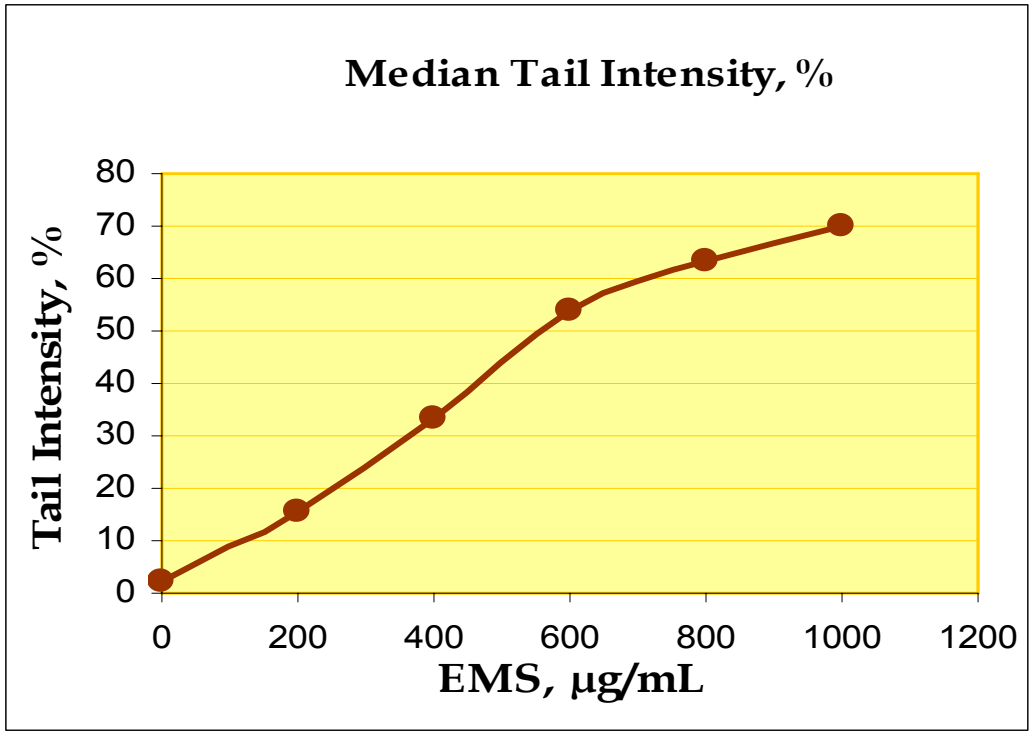
EXAMPLE:

Inhalation study with *ex vivo* COMET Assay

Comet Assay Method



V79 cells treated with EMS for two hours



Inhalation of Aerosols from Nanomaterials by Rats

- Head/Nose only
- Brush-Generator
- Analysis of concentrations
- Particle size measurement
  - Impactor
  - OPC
  - SMPS

From "Short-term inhalation tests of 8 nanomaterials". Landsiedel et al., March 2008

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5-Day Inhalation Study

Male Wistar rats

1	2	3	4	5	6	7	8	9 – 28*	29
x	**x**	**x**	**x**	**x**	**R**	**R**	**R**	**R**	**R**
				H			**e**		**H + e**

X Head-nose exposure to aerosols for 6 hours per day on 5 consecutive days

R Post-exposure time (only 2 weeks after TiO_2 exposure)

H Histology of selected organs including cell proliferation and apoptosis

e Examinations of blood and broncho-alveolar lavage fluid

[57]

Biological Parameters

Histopathology

Proliferation and Apoptosis

Clinical chemistry
Protein
lactate dehydrogenase (LDH)
Alkaline phosphatase (ALP)
γ-Glutamyltransferase (GGT)
N-acetyl-β-Glucosaminidase (NAG)
total cell count
cell differential analysis
- -macrophage (MPH)
- -polymorph nuclear granulocytes (PMN)
- -lymphocyte (LYMPH)

Troponin I

Parameters of oxidative stress
Carboxymethyllysin (CML)
Malondialdehyd (MDA)
8-OHdG

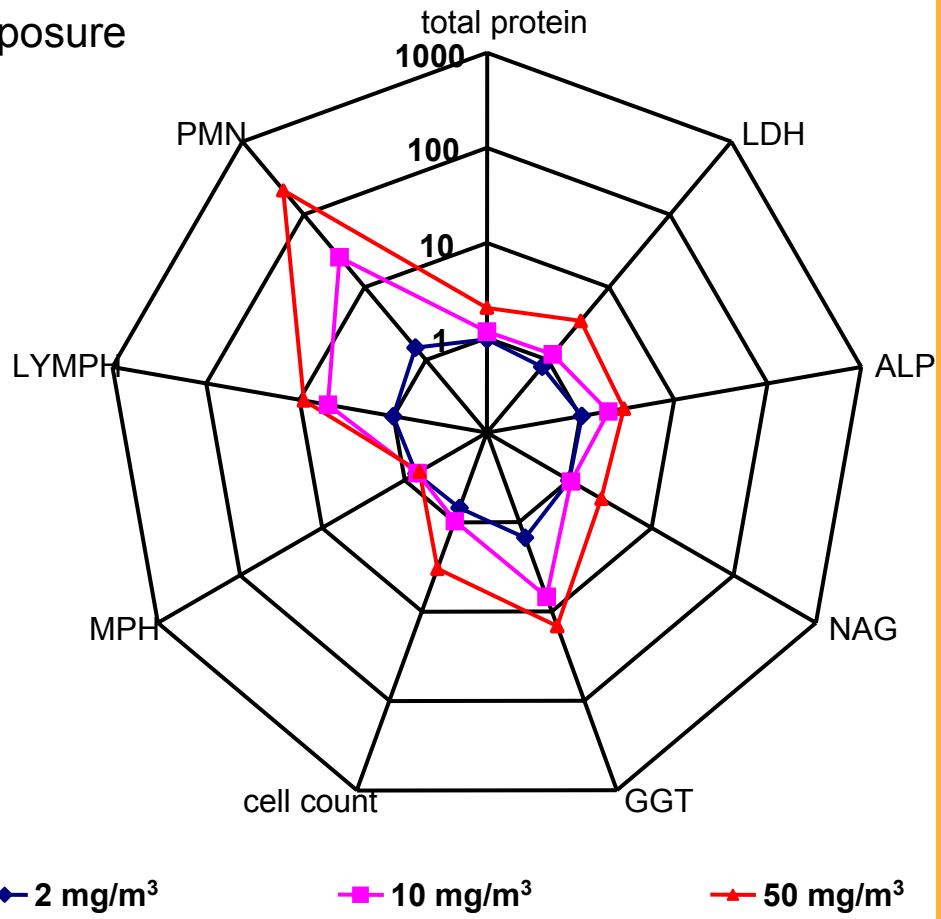
Cytokines *et al.*

1. Apolipoprotein A1
2. ß-2 Microglobulin
3. Calbindin
4. CD40
5. CD40L
6. Clusterin
7. C-Reactive Protein
8. Cystatin
9. EGF
10. Emdothelin-1
11. Eotaxin
12. Factor VII
13. FGF-basic
14. FGF-9
15. Fibrinogen
16. GCP-2
17. GM-CSF
18. Growth Hormone
19. GST-α
20. GST-1 Yb
21. Haptoglobin
22. IFN-γ
23. IgA
24. IL-1α
25. IL-1ß
26. IL-2
27. IL-3
28. IL-4
29. IL-5
30. IL-6
31. IL-7
32. IL-10
33. IL-11
34. IL-12p70
35. IL-17
36. Insulin
37. IP-10
38. KC/GROα
39. Leptin
40. LIF
41. Lipocalin-2
42. MCP-1
43. MCP-2
44. MCP-3
45. MCP-5
46. M-CSF
47. MDC
48. MIP-1α
49. MIP-1ß
50. MIP-1γ
51. MIP-2
52. MIP-3ß
53. MMP-9
54. Myoglobulin
55. OSM
56. Osteopontin
57. RANTES
58. SCF
59. Serum Amyloid P
60. SGOT
61. TIMP-1
62. Tissue Factor
63. TNF-α
64. TPO
65. VCAM-1
66. VEGF
67. von Willebrand Factor

PULMONARY TOXICITY TiO_2

Concentration-Effect Diagram

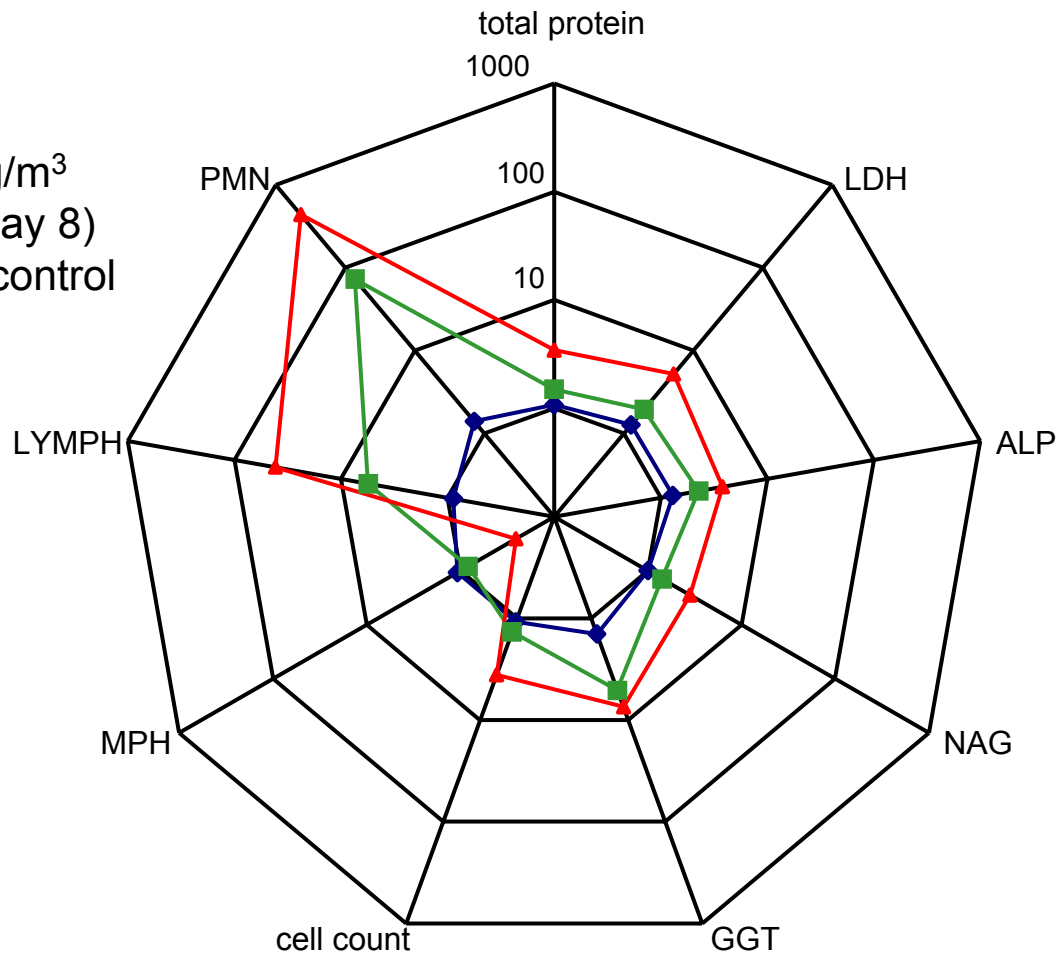
- Rats exposed to 2, 10 and 50 mg/m³ nano-TiO_2
- Immediately after the last exposure
- Relative increase vs. control



PULMONARY TOXICITY MWCNT

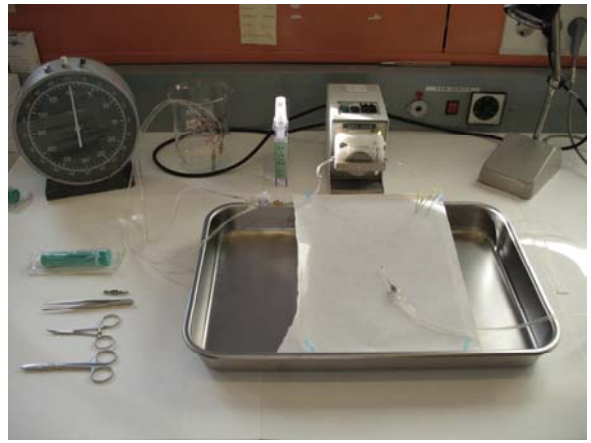
Concentration-Effect Diagram

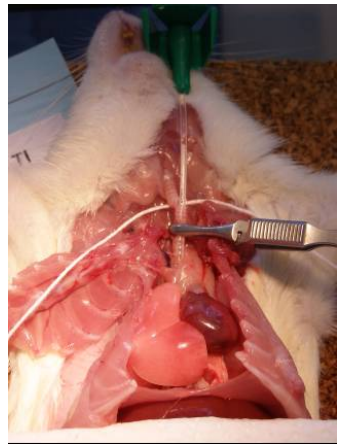
- Rats exposed to 0.1; 0.5 and 2.5 mg/m^3
- Lavage parameters (day 8)
- Relative increase vs. control



Preparation of Lung cells

- **Perfusion**
- **Lavage**
- **Enzyme instillation**
- **Enzymatic digestion**
  - Collagenase IV
  - Trypsin
  - DNAse I
- **Cell isolation**
  - 230 µm and 73.7 µm MESH
  - Percoll 1.040 g/ml gradient
  - Viability measurement by Trypan Blue dye exclusion method





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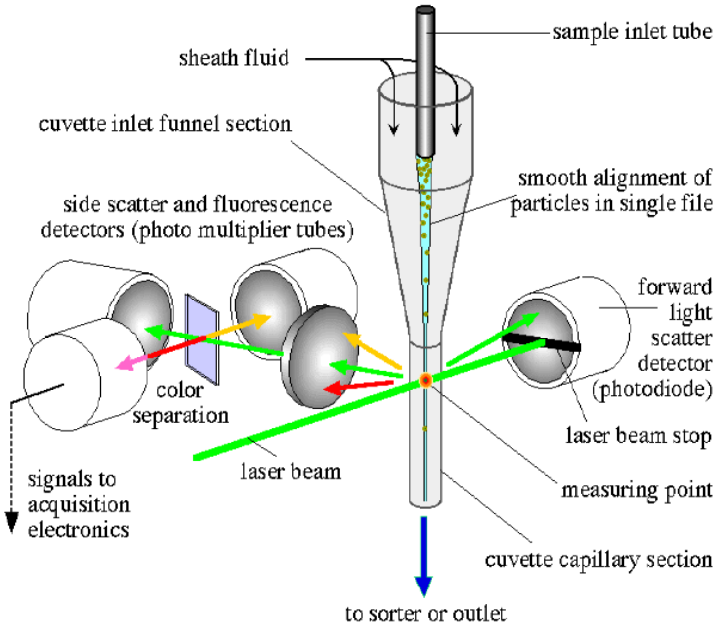
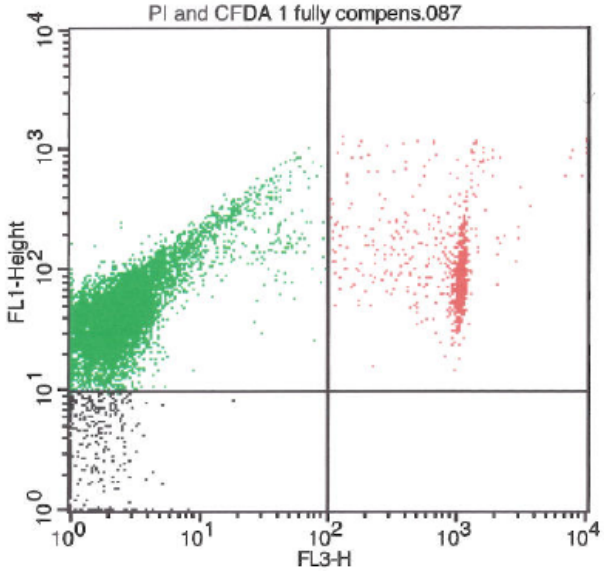
Characterization of Lung cells

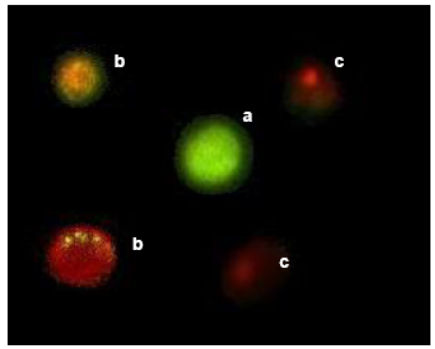
Cell viability assessment

- Staining cells with carboxyfluorescein (CFDA) and ethidium bromide
- FACS analysis with CFDA and propidium iodide
- Trypan Blue dye exclusion method





[a]Green (viable)
[b]Red and green (compromised but viable)
[c]Red (dead)



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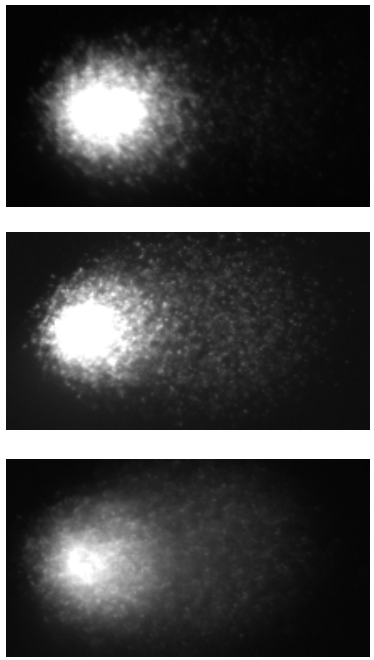
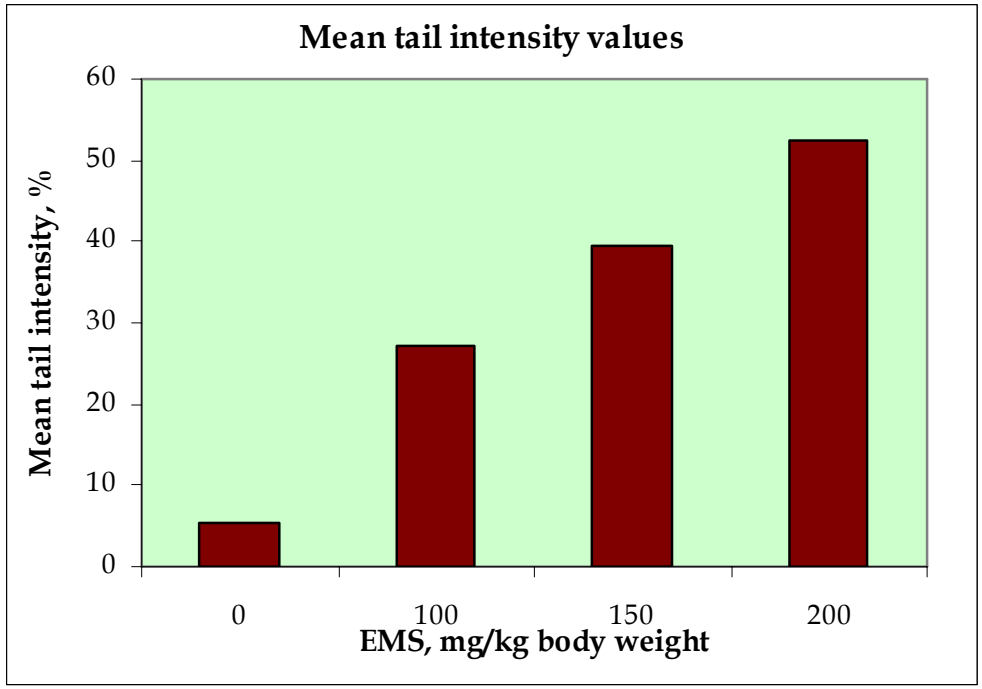
Positive control EMS at 150 mg/kg b.w.





Inhalation study with T-Lite SF

No DNA damage was detected
by Comet assay
in the rat lung cells 24 days after 4 day inhalation exposure
to T-Lite SF™ (titanium dioxide)

Conc. mg/m^3	Average Viability, %	Mean comet tail moment	Median comet tail moment	Mean comet tail intensity	Median comet tail intensity	Mean tail length in µm	Median tail length in µm
0	93	0.89	0.19	5.15	1.39	38.28	34.94
0	95	2.13	0.26	8.97	2.49	40.22	32.64
0	98	0.83	0.11	5.39	1.21	27.00	25.94
Average	95	1.28	0.19	6.50	1.70	35.17	31.17
10	92	0.76	0.07	4.42	0.69	26.28	23.85
10	78	0.88	0.06	2.99	0.34	34.20	30.54
10	96	0.16	0.02	1.70	0.26	24.48	24.27
Average	88.7	0.60	0.05	3.04	0.43	28.32	26.22

Thank you !

REFERENCES

[1] D.B .Warheit, C.M. Sayes, K.L. Reed, K.A. Swain, Health effects related to nanoparticle exposures: Environmental, health and safety considerations for assessing hazards and risks, Pharmacol. Ther. 120 (2008) 35-42.

[2] V.L. Colvin, The potential environmental impact of engineered nanomaterials, Nature Biotechnol. 21 (2003) 1166-1170.

[3] Landsiedel R, Schulz M, Kapp MD, Oesch F: "Genotoxicity Investigations on Nanomaterials: Methods, Preparation and Characterization of Test Material, Potential Artifacts and Limitations - Many Questions, Some Answers", *Mutation Research* (in press)

[4] E.R. Kisin, A.R. Murray, M. J. Keane, X.C. Shi, D. Schwegler-Berry, O. Gorelik, S. Arepalli, V. Castranova, W.E. Wallace, V.E. Kagan, A.A. Shvedova, Single-walled carbon nanotubes: Geno- and cytotoxic effects in lung fibroblast V79 cells, J. Toxicol. Environ. Health, Part A, 70 (2007) 2071–2079.

[5] I. Papageorgiou, C. Brown, R. Schins, S. Singh, R. Newson, S. Davis, J. Fisher, E. Ingham, C.P. Case, The effect of nano- and micron-sized particles of cobalt–chromium alloy on human fibroblasts in vitro, Biomaterials 28 (2007) 2946-2958.

[6] J.J. Wang, B.J. Sanderson, H Wang, Cyto- and genotoxicity of ultrafine TiO2 particles in cultured human lymphoblastoid cells, Mutat. Res. 628 (2007) 99-106.

[7] R.M. Mroz, R.P. Schins, H. Li, L.A. Jimenez, E.M. Drost, A. Holownia, W. MacNee, K. Donaldson, Nanoparticle-driven DNA damage mimics irradiation-related carcinogenesis pathways, Eur. Respir. J. 31 (2008) 241-251.

[8] J.R. Gurr, A.S. Wang, C.H. Chen, K.Y. Jan KY, Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells, Toxicology 213 (2005) 66-73.

[9] N.R. Jacobsen, A.T. Saber, P. White , P. Møller, G. Pojana, U. Vogel, S. Loft, J. Gingerich, L. Soper, G.R. Douglas, H. Wallin, Increased mutant frequency by carbon black, but not quartz, in the lacZ and cII transgenes of muta mouse lung epithelial cells, Environ. Mol. Mutagen. 48 (2007) 451-461.

[10] M. Dybdahl, L. Risom, J. Bornholdt, H. Autrup , S. Loft , H. Wallin, Inflammatory and genotoxic effects of diesel particles in vitro and in vivo, Mutat. Res. 562 (2004) 119-131.

[11] P.S. Vinzents, P. Møller, M Sørensen, L. E. Knudsen, O. Hertel, F. P. Jensen, B. Schibye, S. Loft, Personal exposure to ultrafine particles and oxidative DNA damage, Environ. Health Perspect. (2005) 1485-1490.

[12] P.H. Avogbe, L. Ayi-Fanou, H. Autrup, S. Loft, B. Fayomi , A. Sanni , P. Vinzents, P. Møller, Ultrafine particulate matter and high-level benzene urban air pollution in relation to oxidative DNA damage, Carcinogenesis 26(2005) 613-620.

[13] E.V. Bräuner, L. Forchhammer, P. Møller, J. Simonsen, M. Glasius, P. Wåhlin, O. Raaschou-Nielsen, S. Loft, Exposure to ultrafine particles from ambient air and oxidative stress–induced DNA damage, Environ. Health Perspect. 115 (2007) 1177-1182.

[14] Y. Nakagawa, S. Wakuri, K. Sakamoto, N. Tanaka, The photogenotoxicity of titanium dioxide particles, Mutat. Res. 394 (1997) 125-132.

[15] R. Dunford, A. Salinaro, L. Cai, N. Serpone, S. Horikoshi, H. Hidaka, J. Knowland, Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients, FEBS Lett. 418 (1997) 87-90.

[16] M. Green, E. Howman, Semiconductor quantum dots and free radical induced DNA nicking, Chem. Commun. 121 (2005) 121–123.

[17] Y. Zheng, D.J. Hunting, P. Ayotte, L. Sanche, Radiosensitization of DNA by gold nanoparticles irradiated with high-energy electrons, Radiat. Res. 169 (2008) 19-27.

[18] Q. Zhang, Y. Kusaka, K. Sato, K. Nakakuki, N. Kohyama, K. Donaldson, Differences in the extent of inflammation caused by intratracheal exposure to three ultrafine metals: role of free radicals, J. Toxicol. Environ. Health, Part A, 53 (1998) 423–438.

[19] S.S. Leonard, V. Castranova, B.T. Chen, D. Schwegler-Berry, M. Hoover, C. Piacitelli, D.M. Gaughan, Particle size-dependent radical generation from wildland fire smoke, Toxicology 236 (2007) 103–113.

[20] Z. Li, T. Hulderman, R. Salmen, R. Chapman, S.S. Leonard, S.H. Young, A. Shvedova, M.I. Luster, P.P. Simeonova, Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes, Environ. Health Perspect. 115 (2007) 377-382.

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REFERENCES continued

[21] G. Chen, J. Zhao, X. Liu, G. Gao, J. Huang , G. Li, Electrochemical sensing DNA damage with nano-titanium dioxide and repair with a medicinal herb species resveratrol. J. Biotechnol. 127 (2007) 653-656.

[22] S. Maenosono, T. Suzuki, S. Saita, Mutagenicity of water-soluble FePt nanoparticles in Ames test, J. Toxicol. Sci. 32 (2007) 575-579.

[23] J.J.Wang, B.J.S. Sanderson, H. Wang, Cytotoxicity and genotoxicity of ultrafine crystalline SiO2 particulate in cultured human lymphoblastoid cells, Environ. Mol. Mutagenesis 48 (2007) 151-157.

[24] J.J. Wang, H. Wang, B.J.S. Sanderson, Ultrafine quartz-induced damage in human lymphoblastoid cells in vitro using three genetic damage end-points, Toxicol. Mechanisms Methods 17 (2007) :223–232.

[25] L. Zhu, D.W. Chang, L. Dai, Y. Hong, DNA damage induced by multiwalled carbon nanotubes in mouse embryonic stem cells, Nano Lett. 7 (2007) 3592-3597.

[26] H.G. Claycamp, Phenol sensitization of DNA to subsequent oxidative damage in 8-hydroxyguanine assays. Carcinogenesis 13 (1992) 1289–1292.

[27] E. Driscoll, L.C. Deyo, J.M. Carter, B.W. Howard, G. Hassenbein, T.A. Bertram, Effect of particle exposure and particle-elicited inflammatory cells on mutation in rat alveolar epithelial cells, Carcinogenesis 18 (1997) 423-430.

[28] G. Oberdörster, A. Maynard, K. Donaldson, V. Castranova, J. Fitzpatrick, K. Ausman, J. Carter, B. Karn, W. Kreyling, D. Lai, S. Olin, N. Monteiro-Riviere, D. Warheit, H. Yang, Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Particle Fibre Toxicol. 2 (2005) 8-43.

[29] Q. Rahman, M. Lohani, E. Dopp, H. Pemsel, L. Jonas, D.G. Weiss, D. Schiffmann, Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in syrian hamster embryo fibroblasts, Environ. Health Perspect. 110 (2002) 797-800.

[30] L. Wang, J. Mao, G.H. Zhang, M.J. Tu, Nano-cerium-element-doped titanium dioxide induces apoptosis of Bel 7402 human hepatoma cells in the presence of visible light, World J. Gastroenterol. 13(2007) 4011-4014.

[31] E.K. Dufour, T. Kumaravel, G.J. Nohynek, D. Kirkland, H. Toutain, Clastogenicity, photo-clastogenicity or pseudo-photo-clastogenicity: Genotoxic effects of zinc oxide in the dark, in pre-irradiated or simultaneously irradiated Chinese hamster ovary cells, Mutat. Res. 607 (2006) 215-224.

[32] J. Muller, I. Decordier , P.H. Hoet, N. Lombaert, L. Thomassen, F. Huaux, D. Lison, M. Kirsch-Volders, Clastogenic and aneugenic effects of multi-wall carbon nanotubes in epithelial cells, Carcinogenesis 29 (2008) 427-433.

[33] M.L.L. Freitas, L.P. Silva, R.B. Azevedo, V.A.P. Garcia, L.M. Lacava, C.K. Grisolia, C.M. Lucci, P.C. Morais, M.F. Da Silva, N. Buske, R. Curi, Z.G.M. Lacava, A double-coated magnetite-based magnetic fluid evaluation by cytometry and genetic tests, J. Magnetism Magnetic Materials 252 (2002) 396–398.

[34] N. Sadeghiani, L.S. Barbosa, L.P. Silva, R.B. Azevedo, P.C. Morais, Z.G.M. Lacava, Genotoxicity and inflammatory investigation in mice treated with magnetite nanoparticles surface coated with polyaspartic acid, J. Magnetism Magnetic Materials 289 (2005) 466–468.

[35] J.H. Park, K.T. Han, K.J. Eu, J.S. Kim, K.H. Chung, B. Park, G.S.Yang, K.H. Lee, M.H. Cho, Diffusion flame-derived fine particulate matters doped with iron caused genotoxicity in B6C3F1 mice, Toxicol. Ind. Health 21(2005 57-65.

[36] H. Hidaka, S. Horikoshi, N. Serpone, J. Knowland, In vitro photochemical damage to DNA. RNA and their bases by an inorganic sunscreen agent on exposure to UVA and UVB radiation, J. Photochem. Photobiol. A: Chem. 111 (1997) 205-213.

[37] M. Auffan, L. Decome, J. Rose, T. Orsiere, M. Demeo, V. Briois, C. Chaneac, L. Olivi, J.L. Berge - Lefranc A. Botta, M.R . Wiesner, J.Y. Bottero, In Vitro Interactions between DMSA-coated maghemite nanoparticles and human fibroblasts: a physicochemical and cyto-genotoxical study, Environ. Sci. Technol. (2006) 40 4367-4373.

[38] B.Z. Zhong, W.Z. Whong, T.M. Ong, Detection of mineral-dust-induced DNA damage in two mammalian cell lines using the alkaline single cell gel/comet assay, Mutat Res. 393 (1997) 181-187.

[39] B. Rehn, F. Seiler, S. Rehn, J. Bruch, M. Maierd, Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: untreated and surface treated, Toxicol. Appl. Pharmacol. 189 (2003) 84–95.

[40] D.B. Warheit, R. A. Hoke, C. Finlay, E. M. Donner,K. L. Reed, C. M. Sayes, Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management, Toxicol. Lett. 171 (2007) 99–110.

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REFERENCES continued

[41] J.S. Kim, T.J. Yoon, K.N. Yu, B.G. Kim, S.J. Park, H.W. Kim, K.H. Lee, S.B. Park, J.K. Lee, M.H. Cho, Toxicity and tissue distribution of magnetic nanoparticles in mice, Toxicol. Sci. 89 (2005) 338–347.

[42] K. Linnainmaa, P. Kivipensas, H. Vainio, Toxicity and cytogenetic studies of ultrafine titanium dioxide in cultured rat liver epithelial cells, Toxicol. in Vitro 11 (1997) 329-335.

[43] D.R. Haynes, S.D. Rogers, D.W. Howie, M.J. Pearcy, B. Vernon-Roberts, Drug inhibition of the macrophage response to metal wear particles in vitro, Clin. Orthop. Relat. Res. 323 (1996) 316-326.

[44] E. Theogaraj, S. Riley, L. Hughes, M. Maier, D. Kirkland, An investigation of the photo-clastogenic potential of ultrafine titanium dioxide particles, Mutat. Res. 634 (2007) 205-19.

[45] K. Donaldson, C.L. Tran CL, An introduction to the short-term toxicology of respirable industrial fibres, Mutat Res. 553 (2004) 5-9.

[46] L.K. Duncan, J.R. Jinschek, P.J. Vikesland, C60 colloid formation in aqueous systems: effects of preparation method on size, structure, and surface charge, Environ. Sci. Technol. 42 (2008) 173-178.

[47] J.A. Brant, J. Labille, J.Y. Bottero, M.R. Wiesner, Characterizing the impact of preparation method on fullerene cluster structure and chemistry, Langmuir. 22 (2006) 3878-3885.

[48] Z. Markovic, B. Todorovic-Markovic, D. Kleut, N. Nikolic, S. Vranjes-Djuric, M. Misirkic, L. Vucicevic, K. Janjetovic, A. Isakovic, L. Harhaji, B. Babic-Stojic, M. Dramicanin, V. Trajkovic, The mechanism of cell-damaging reactive oxygen generation by colloidal fullerenes, Biomaterials 28 (2007) 5437-5448.

[49] L.K. Limbach, Y. Li, R.N. Grass, T.J. Brunner, M.A. Hintermann, M. Muller, D. Gunther, W.J. Stark, Oxide nanoparticle uptake in human lung fibroblasts: effects of particle size, agglomeration, and diffusion at low concentrations, Environ. Sci. Technol. 39 (2005) 9370-9376.

[50] R.S. Kane, A.D. Stroock, Nanobiotechnology: protein-nanomaterial interactions, Biotechnol. Prog. 23 (2007) 316-319.

[51] T. Cedervall, I. Lynch, S. Lindman, T. Berggard, E. Thulin, H. Nilsson, K. A. Dawson, S. Linse, Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, Proc. Natl. Acad. Sci. U S A, 104 (2007) 2050-2055.

[52] C. Schulze, A. Kroll, C.M. Lehr, U.F. Schäfer, K. Becker, J. Schnekenburger, C.Schulze Isfort, R. Landsiedel, W. Wohleben, Not ready to use - overcoming pitfalls when dispersing nanoparticles in physiological media, Nanotoxicology, 2 (2008) 51-61.

[53] L. Guo, A. Von Dem Bussche, M. Buechner, A. Yan, A.B. Kane, R.H. Hurt, Adsorption of essential micronutrients by carbon nanotubes and the implications for nanotoxicity testing, Small, 4 (2008) 721-727.

[54] A. Isakovic, Z. Markovic, B. Todorovic-Markovic, N. Nikolic, S. Vranjes-Djuric, M. Mirkovic, M. Dramicanin, L. Harhaji, N. Raicevic, Z. Nikolic, V. Trajkovic, Distinct cytotoxic mechanisms of pristine versus hydroxylated fullerene, Toxicol. Sci. 91 (2006) 173-183.

[55] R. Singh, D. Panatarotto, D. McCarthy, O. Chaloin, J. Hoebeke, C.D. Partidos, J.P. Briand, M. Prato, A. Bianco, K. Kostarelos, Binding and condenstation of plasmid DNA onto functionalized carbon nanotubes: toward the construction of nanotube-based gene delivery vectors, J. Am. Chem. Soc. 127 (2005) 4388-4396.

[56] Y. Pan, S. Neuss, A. Leifert, M. Fischler, F. Wen, U. Simon, G. Schmid, W. Brandau, W. Jahnen-Dechent, Size-dependent cytotoxicity of gold nanoparticles, Small 3 (2007) 1941-1949.

[57] Ma-Hock L, Burkhardt S, Strauss V, Gamer A, Wiench K, van Ravenzwaay B, Landsiedel R: Development of a short-term inhalation test in rats using nano-titanium dioxide as a model substance, *Inhalation Toxicology*, 21:102–118, 2009

[58] N.A. Monteiro-Riviere , A.O. Inman, L.W. Zhang: Limitations and relative utility of screening assays to assess engineered nanoparticle toxicity in a human cell line, Toxicology and Applied Pharmacology 234 (2009) 222–235

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