Inhalation Toxicology

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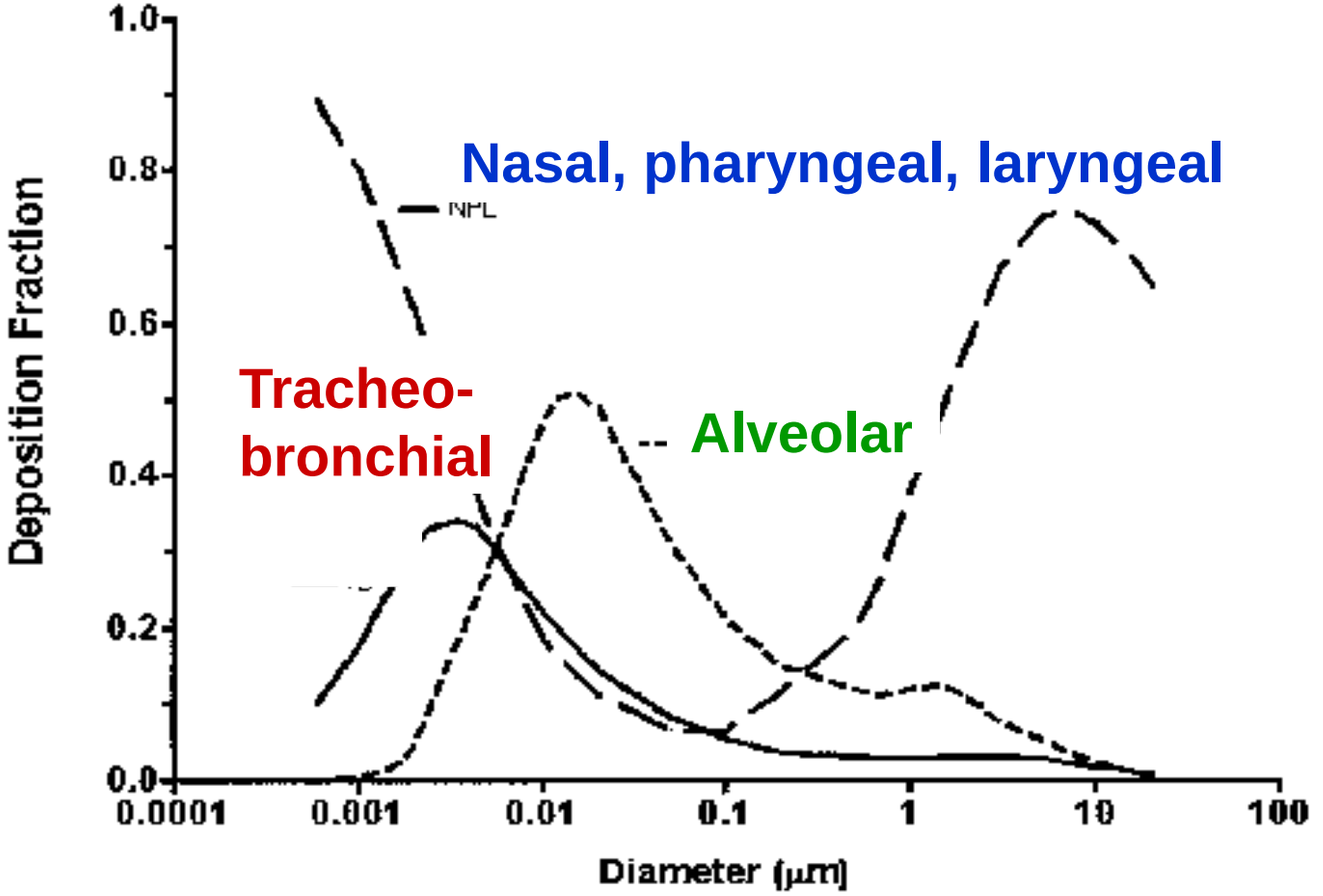
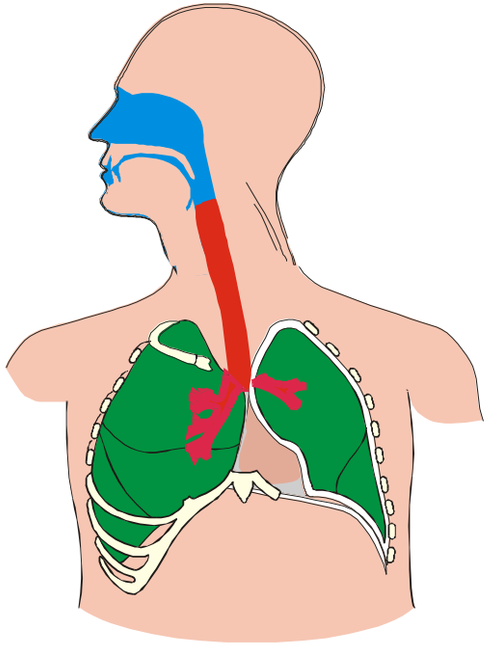
Deposition of Particles: Impaction, Sedimentation, Diffusion, (Interception)





A= Alveolar; TB=Tracheobronchial; NPL= Nasal, Pharyngeal, Laryngeal

International Commission on Radiological Protection (ICRP) 1994

Designing Inhalation Tests: Essential Issues I

Different particle shapes need different approaches

- **Granular nanoparticles → TiO_2, amorphous SiO_2**
- **Fibrous nanoparticles → CNT**

Dispersion modes

- **Dry, pressurized air**
- **Liquid, particle-specific formulation**

Designing Inhalation Tests: Essential Issues II

Determinants of biokinetic behaviour

Agglomeration status can be varied to focus on hazard or occupational risk scenarios

- Individual nanoparticles, by spark generator
- Agglomerates, tending to disintegration (nanoparticle/phosphate mixed type)
- Agglomerates, stable

Solubility in physiological fluids

- pH=7.4 →alveolar lumen
- pH=4.5 →lysosomes in macrophages
-

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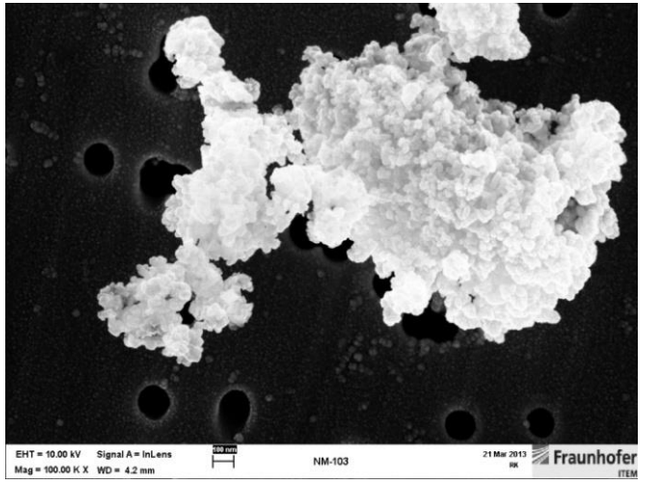
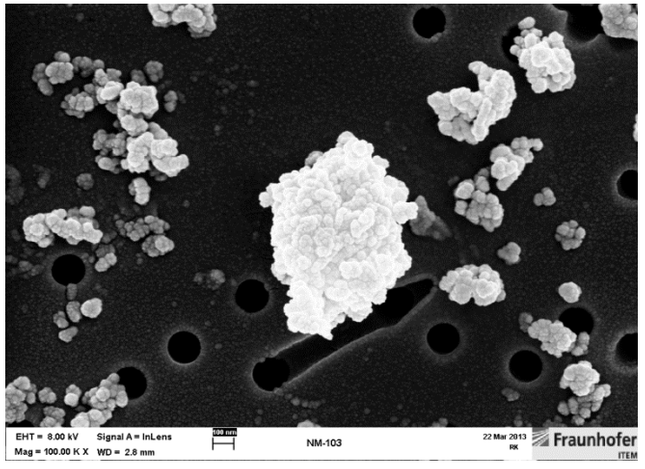
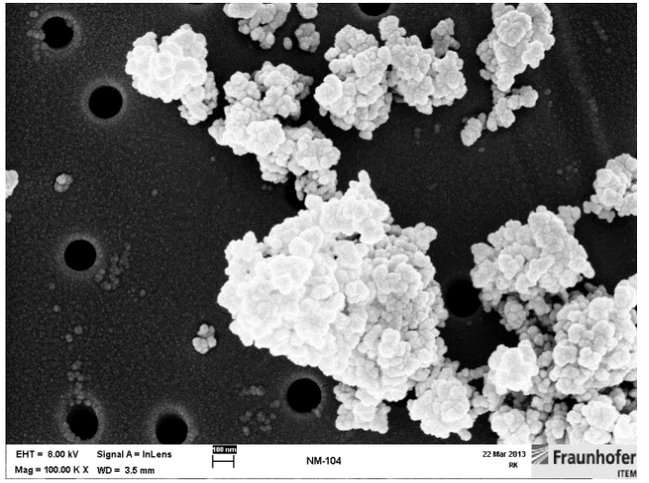
Test Items

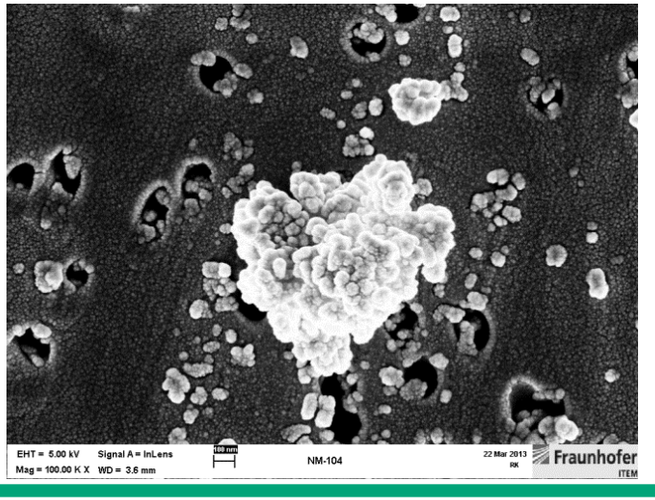
NM-103



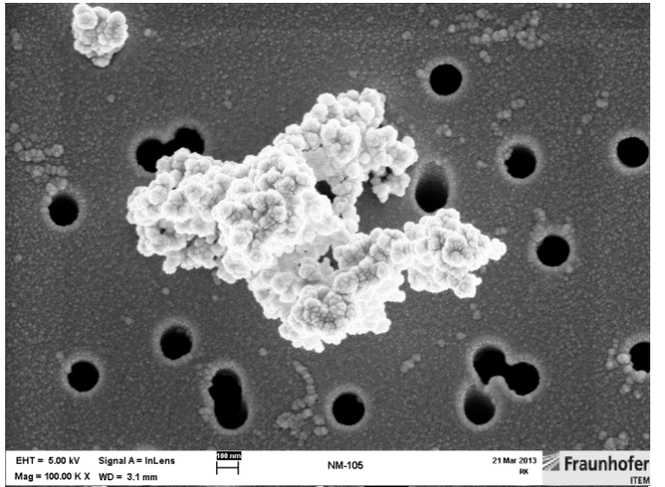


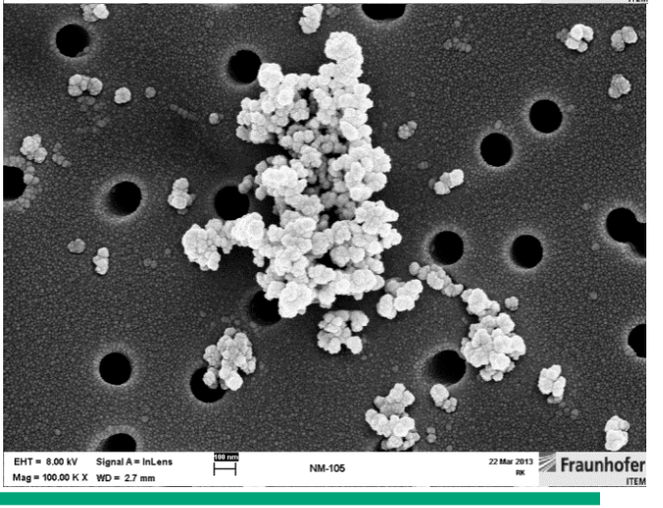
NM-104





NM-105





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Nanoparticles/ultrafine Particles

Properties of nanoparticle agglomerates (µ size):

- **Aerodynamic behaviour similar to microscaled particles**
- **Agglomerate density vs. material density**

  → MMAD ~ geometric diameter x $\rho^{1/2}$
- **Disintegration after deposition (instability)**
- **Formation of even larger agglomerates (macrophage activity)**

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in vivo Inhalation Tests - State of the Art and Future Approaches for Toxicity Testing of Nanoparticles

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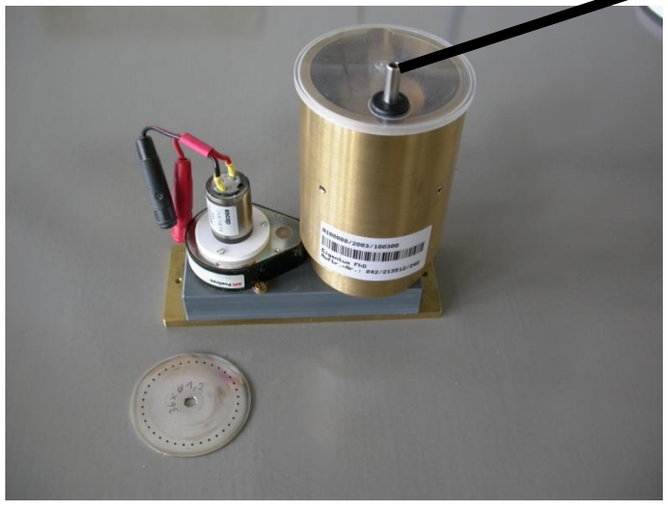
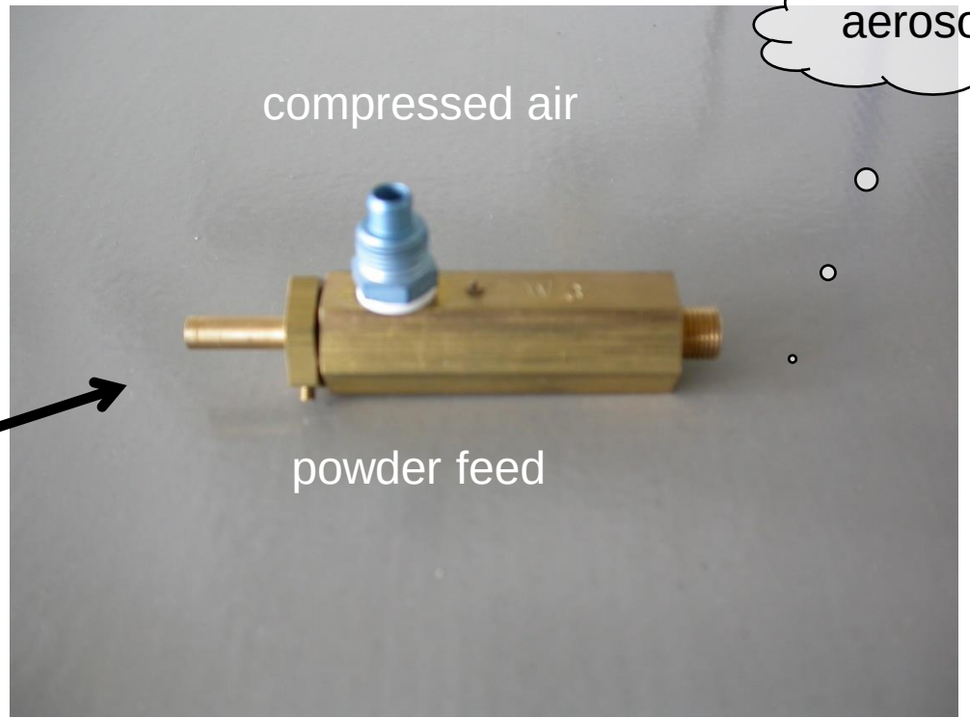
Aerosol Generation: Dry dispersion

Powder

- Generation by
  nozzles,
  brush generator,
  etc.
  (into reservoir e.g.)





disc feeder“

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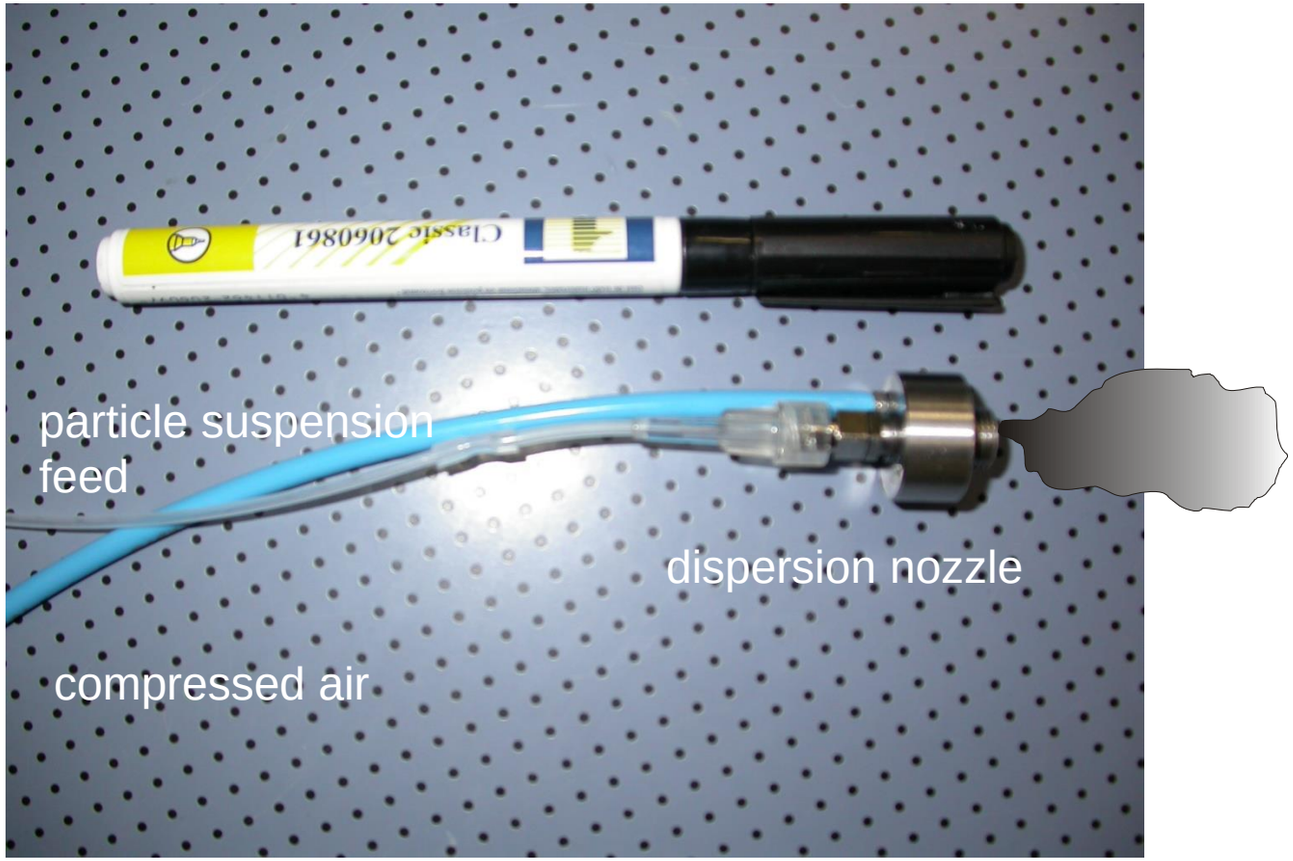
Aerosol Generation: Liquid dispersion

Particle suspension

- Generation by
  nozzles,
  ultrasonic nebulizers,
  evaporation/ recondensation,
  etc.
  (into reservoir e.g.)



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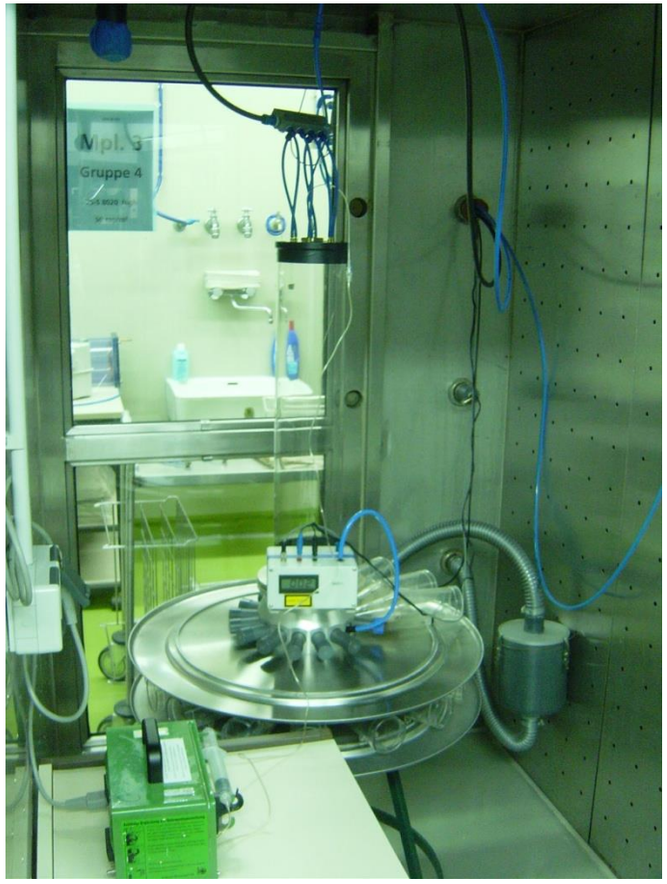
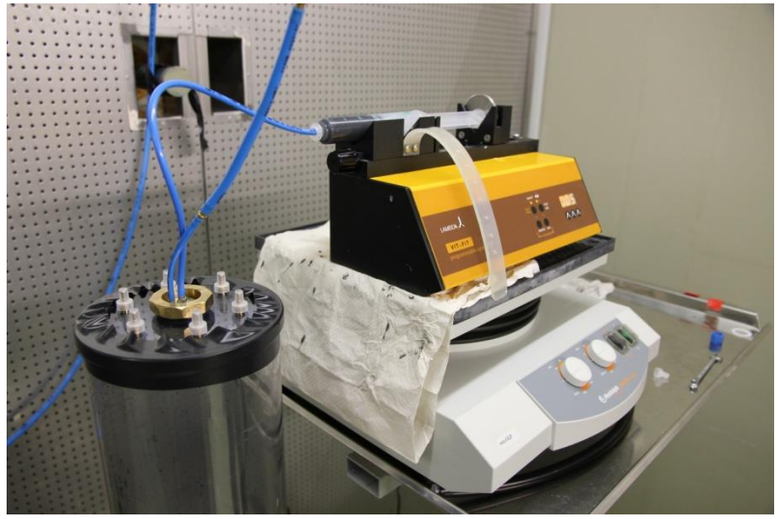
Inhalation Set-up: Liquid Formulation

Particle suspension stable over exposure period

Stable perfusor propulsion

Mixing chamber forming a homogeneous respirable aerosol before inhalation





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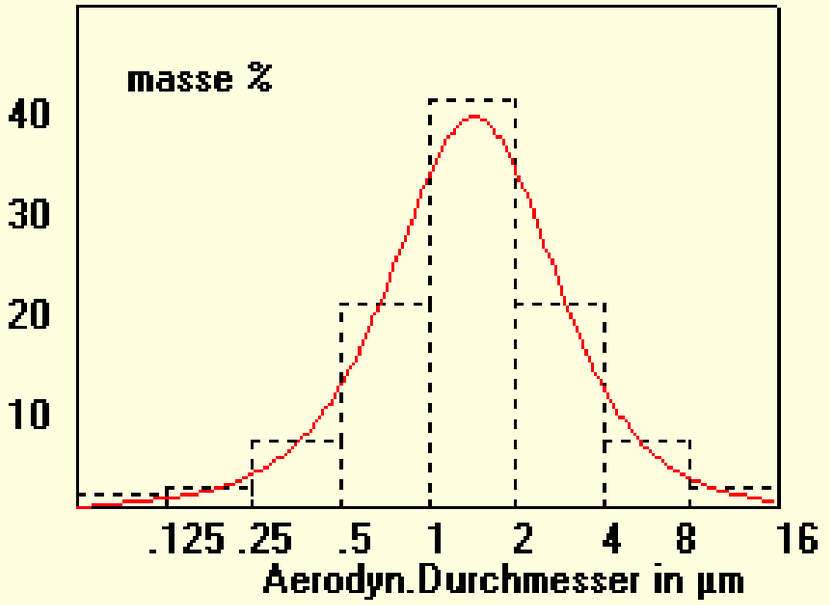
Essential → Respirability of the Aerosol

Size distribution: Cascade impactor → MMAD

- Mass Median Diameter (MMAD)
- Geometric Standard Deviation (GSD)

In addition needed for nanomaterials:
- **Scanning mobility particle sizer SMPS**
- **Aerodynamic particle sizer APS**



Inhalation Set-up: Carbon Nanotubes

CNT characterization

- Measurement of length and diameter
- FE-SEM Supra 55 (Zeiss Co.)
- ζ potential
- Endotoxin analysis
- e.g. ESR analysis: Acellular - Analysis of potential ROS release

CNT aerosol properties

- Low density
  ρ =0.005-0.02
- Clumping
- 40 mg/m^3 achievable

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Inhalation Set-up: Dispersion of CNT Suspension

→ **Acute Toxicity Tests**

Aerosolization from liquid formulation („dispersion medium“)

glucose - BSA – DPPC

Aerosol concentration: filters
MMAD: Evaluation of Nuclepore® filter sample (SEM)

Literature: Porter et al., Nanotox 2, 144 (2008); Porter et al., Tox 269, 136 (2010)

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Inhalation Set-up: Dry Dispersion of CNT

28-Day/90-day toxicity tests with MWCNT:

- Use/adaption of a system developed at CDC/NIOSH (USA)
- Dry aerosolization by acoustical feeder system
- Automated computer-controlled system
- Aim: To generate a respirable aerosol from the bulk

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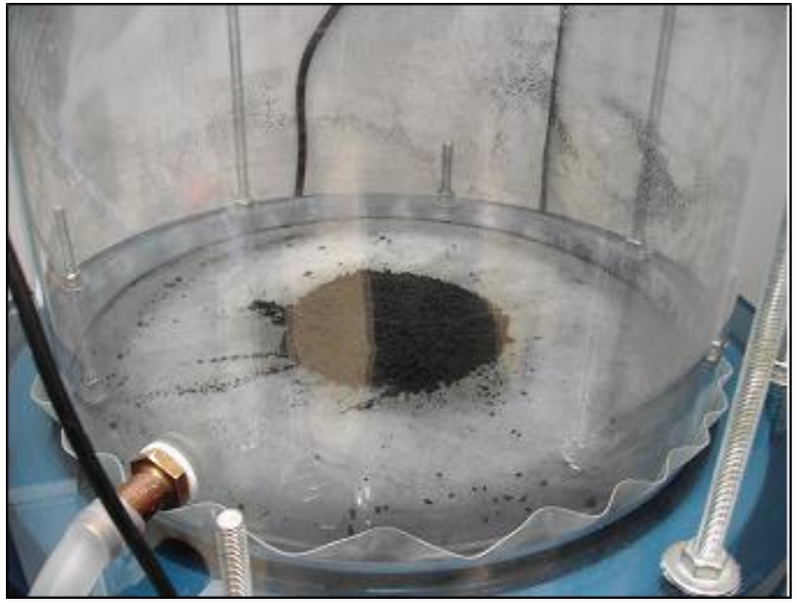
Inhalation Set-up: Dispersion of CNT Suspension

28-Day/90-Day toxicity tests: Methodology

Literature: McKinney et al., Inhalation Tox 21, 1053 (2009); Porter et al., Tox 269, 136 (2010)



CNT aerosol generator system constructed at Fraunhofer ITEM

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Nanoparticle Projects at Fraunhofer ITEM
Funded by Authorities

Federal Ministry for Education and Research (BMBF)

→ CNT; CeO_2; $BaSO_4$ - Carbon black

Federal Environmental Agency (UBA) → CNT

Federal Institute for Risk Assessment (BfR) → Ag

Federal Institute for Occupational Safety and Health (BAuA) → TiO_2

European Commission – 2nd SIINN → Graphene Nanoplatelets

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→ Results from Various Inhalation Studies

- **Acute test: Eu_2O_3**
- **Acute test: ^{60}Co-CNT**
- **28-day test: 3 TiO_2**
- **90-day test: ZnO and SiO_2**

Just started: 90-day test - CeO_2 and $BaSO_4$

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Biokinetics of Nanoscaled Eu_2O_3 Particles Following an Acute Inhalation in Rats

Experimental design

- **Liquid dispersion technique**
- **Hazard-driven exposure scenario**

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Acute inhalation test with Eu_2O_3

- 6-hr exposure period
- Aerosol concentration: 9.0 mg/m^3
- MMAD: 1.35 µm; GSD: 1.65
- Deposition fractions: 6.1% P (MPPD model v 2.11)
- Estimated deposited mass: approx. 39.5 µg in lungs

Solubility of Eu_2O_3 particles at various pH values

(mg/g)

Medium	pH	1 hr	24 hrs
Gamble‘s solution	4.5	1.3	11.4
Gamble‘s solution	7.4	0.0	0.2
Artificial lysosomal fluid	4.5	619	906
Artificial alveolar fluid	7.4	0.0	0.3

Analytical data after 2, 3, 4 or 5 days resulted in data equal to those after 24 hrs

Data of Chemical Analysis

	Retained europium oxide per organ - means (n=3)					
	ng/organ	% of lungs	ng/organ	% of lungs	ng/organ	% of lungs
Preserved organs	1 hour		1 day		5 days	
Lungs	36,779		34,467		35,047	
Brain	**2.2**	**0.006**	**3.8**	**0.011**	**3.1**	**0.009**
Spleen	**8.3**	**0.023**	**2.9**	**0.008**	**3.9**	**0.011**
Kidneys	**5.7**	**0.016**	**5.5**	**0.016**	**10.8**	**0.031**
Adrenals	<1	<0.003	<1	<0.003	<1	<0.003
Thymus	1.3	0.004	1.6	0.005	37.7	0.108
Liver	**32.3**	**0.088**	**93.8**	**0.272**	**294**	**0.854**
Heart	1.9	0.005	1.6	0.005	22.9	0.065
LALN	4.7	0.013	276	0.801	536	1.529
MLN	<1	<0.003	6.5	0.019	<1	<0.003
Testes	2.5	0.007	3.1	0.009	16.0	0.046
Epididy-mides	1.2	0.003	<1	0.003	<1	<0.003
Blood	4.8	0.013	<1	0.003	<1	<0.003
Urine	n.m.	n.m	32.5	0.094	13.9	0.040
Feces	n.m.	n.m	70,472		5200	
Normalized data for spleen, kidney, liver (ng/g Organ)						
Spleen	23.7		8.5		10.8	
Kidney	3.0		3.1		6.0	
Liver	4.6		13.2		42.0	
Drinking water: < 0.001 µg/l						
Food: < 0.001 µg/g						

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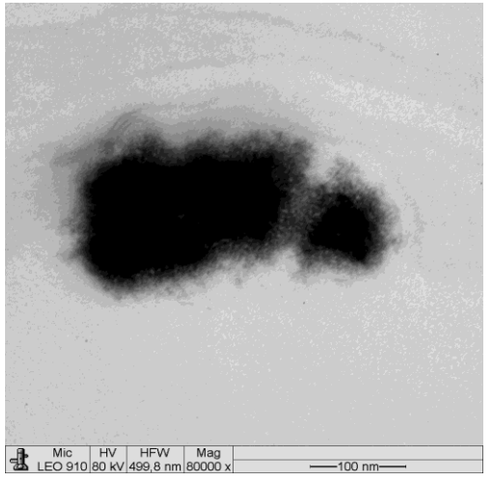
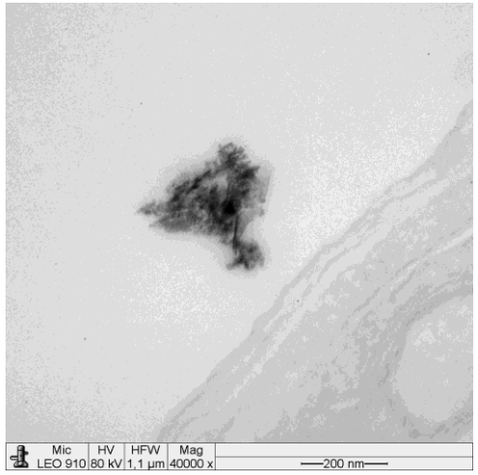
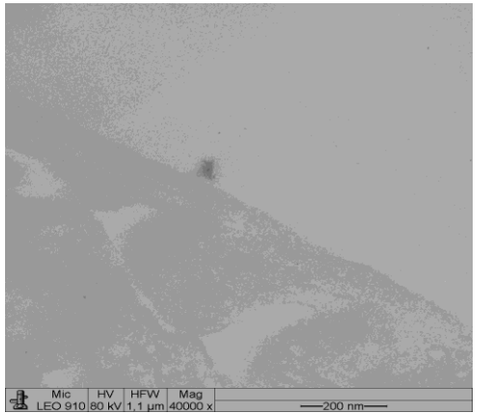


Attached to surfactant after 1 hr

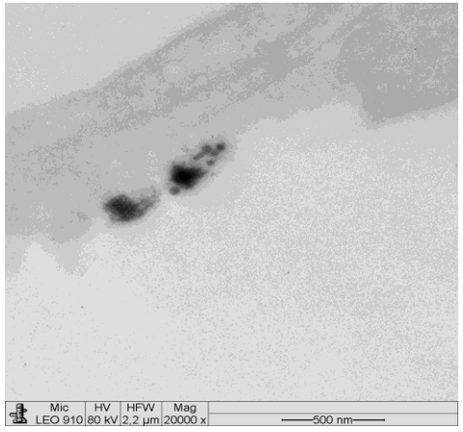


Attached to cellular surface after 1 day

Examples of Eu_2O_3 particles in lungs detected after acute inhalation



Attached to cellular surface after 1 hr



Attached to cellular surface after 1 day

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Conclusions

- **Liver → main translocation site of Eu (elemental) (approx. 0.1-1% of dose) detected).**
- **Very small amounts of Eu were detected in other organs suggesting a very low elemental translocation effect to remote organs.**
- **TEM analysis on Eu_2O_3 particles: Lungs → yes; Liver → no**
- **The Eu_2O_3 dissolution behavior in various fluids suggests that alveolar macrophages did not effectively internalize Eu_2O_3 particles.**

Biokinetics of CNT Following an Acute Inhalation in Rats Using ^{60}Co Labelling

Experimental design

- **Liquid dispersion technique**
- **Hazard-driven exposure scenario**

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Test System - Exposure

- **Wistar rats, males**
- **Aerosolisation of an aqueous formulation („dispersion medium“)**

  glucose - BSA – DPPC (Porter et al.)
- **Acute inhalation study: 1 x 4 h**
- **Aerosol concentration: 3.7 mg/m³ (filter)**
- **MMAD: < 3 µm (evaluation of nuclear pore filters; SEM)**

Literature: Porter et al., Nanotox 2, 144 (2008); Porter et al., Tox 269, 136 (2010)

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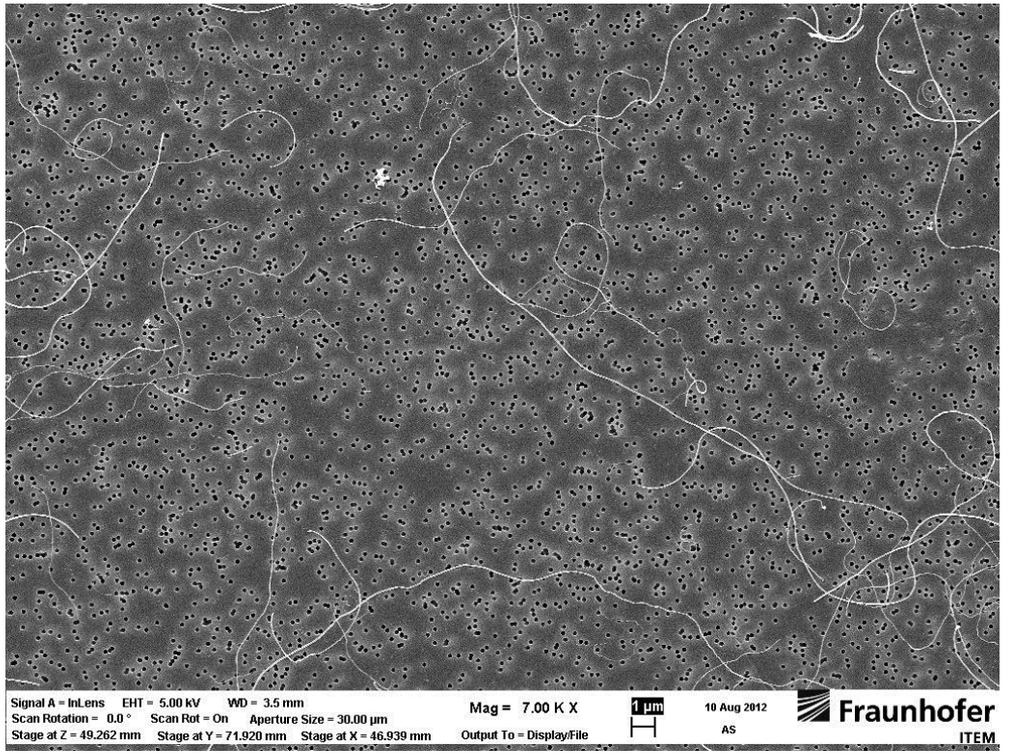
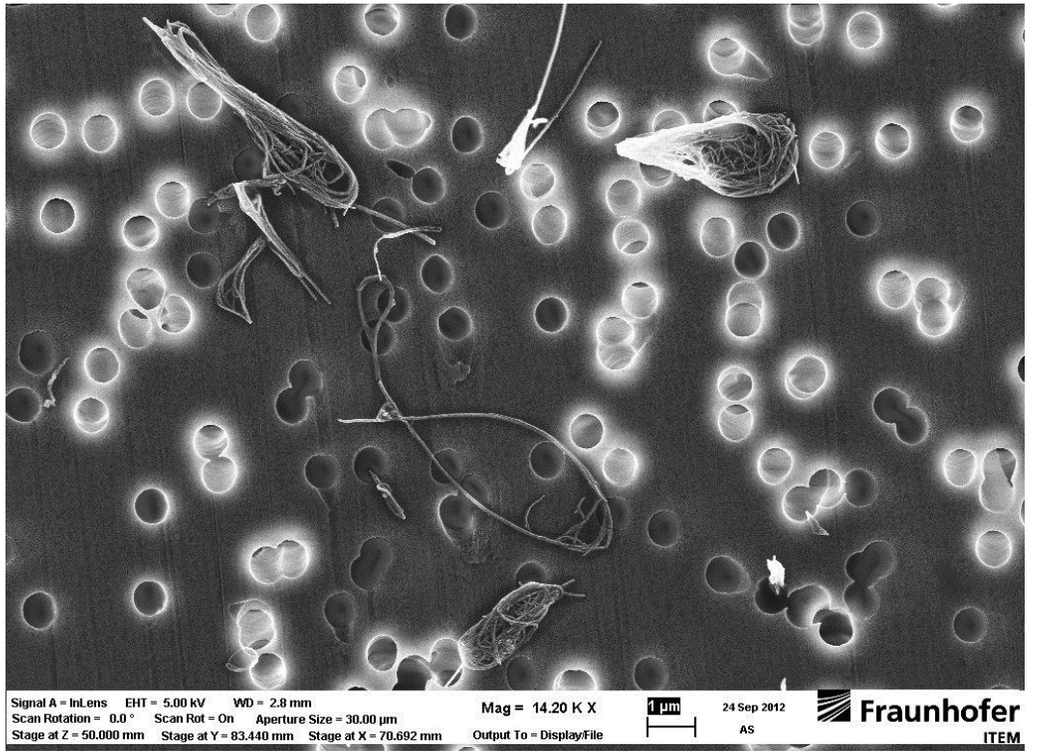
SEM Photographs of MWCNT



Liquid formulation for aerosol generation



Filter sample from aerosol

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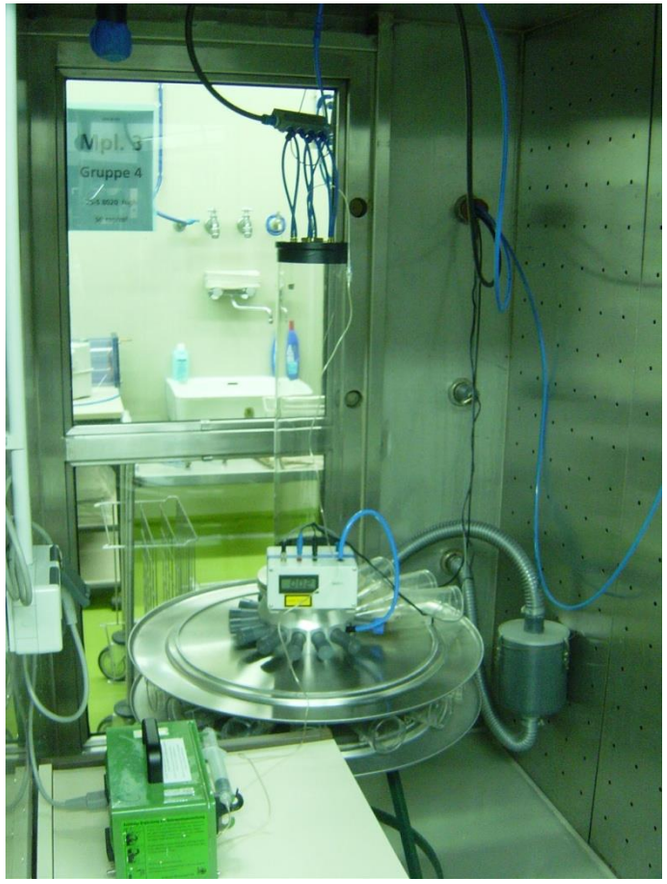
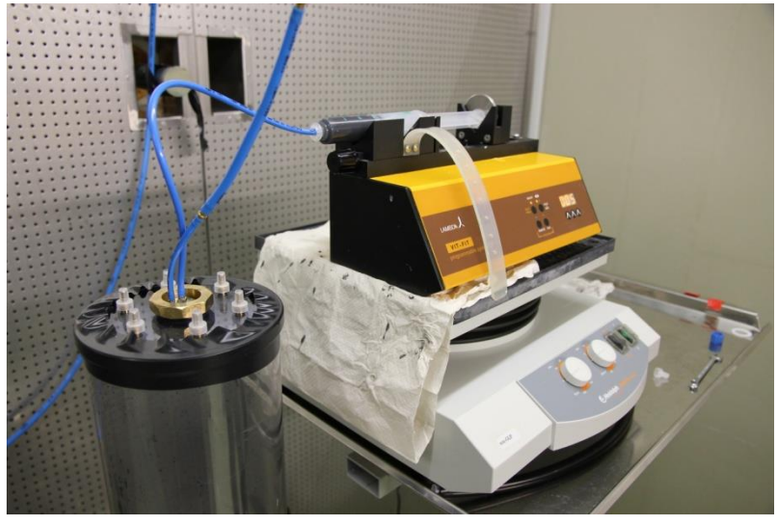
Inhalation Set-up: Liquid Formulation

Particle suspension stable over exposure period

Stable perfusor propulsion

Mixing chamber forming a homogeneous respirable aerosol before inhalation





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Distribution 0, 1, 14 and 28 Days after Exposure

Parameter	MWCNT (%)											
	Sacrifice date (days after inhalation)											
	0			1			14			28		
	Mean	Std	N	Mean	Std	N	Mean	Std	N	Mean	Std	N
Lung	**16.0**			**10.3**			**6.7**			**5.2**		
LALN	0.02			0.02			<0.01			<0.01		
Liver	2.2			1.1			0.3			0.1		
Kidneys	0.8			0.2			0.05			0.03		
Brain	<0.01			<0.01			<0.01			<0.01		
Heart	0.03			0.02			<0.01			<0.01		
Spleen	0.02			<0.01			<0.01			<0.01		
Blood	0.9			0.02			<0.01			<0.01		
Pleural cast	0.3			0.05			<0.01			<0.01		
Carcass	80.3			7.4			0.7			0.4		
Total	100			19.1			7.7			5.8		
Urine				9.9								
Head	4.3			0.7			.	.	.	0.1		
GI tract	63.5			7.1								

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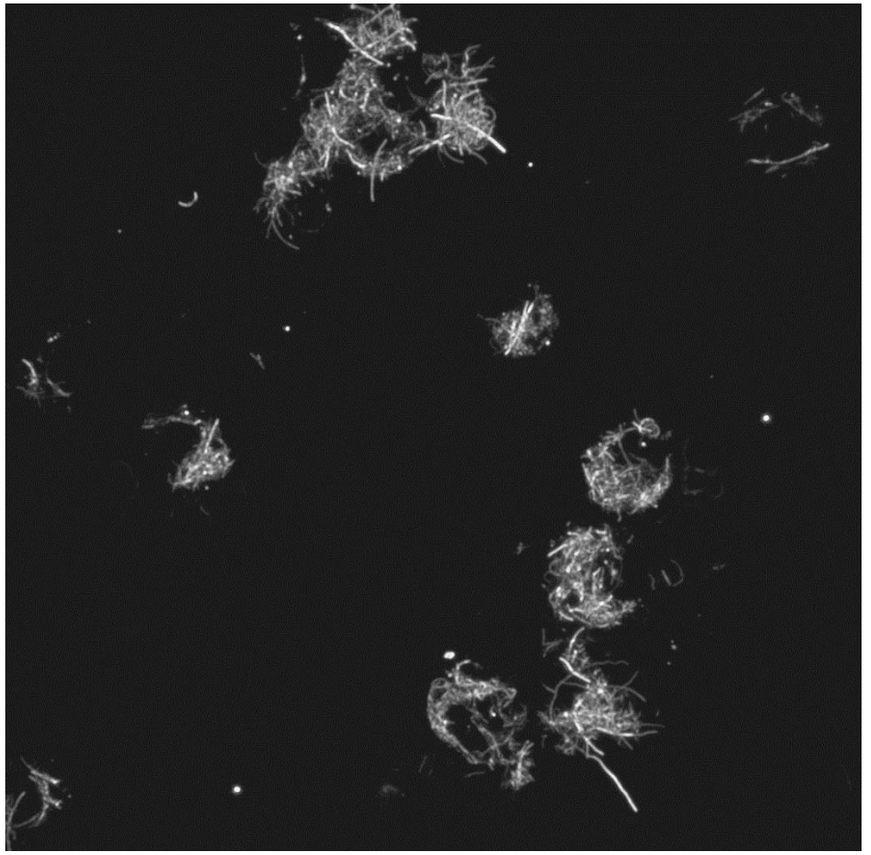
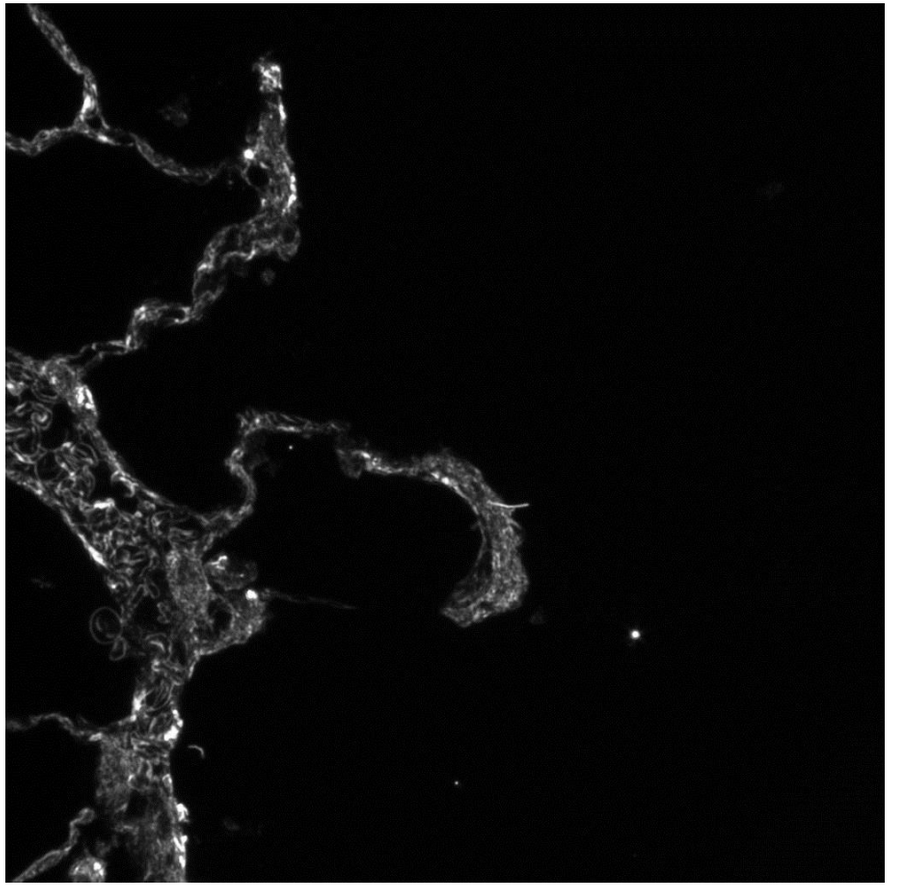
4-Hour Inhalation Test + 0/1/14/28-day recovery

Name NM	Mass balance / Recovery Bioavailability	Biodistribution Tissue levels	C,t-curve(s) T1/2, ke
CNT 'Designer'	No evident translocation of CNT detected – With darkfield-microscopy single CNT visible in liver, kidney and pleural cleft	**Lungs:** approx. 10% deposition (day 1) Remote organs (1 hr): Considerable amounts in liver , kidneys, blood, pleural cleft; up to 2.2% (liver) Cave: Dissolved Co ! Microscopy: no clear detection of CNT in urine and blood	**Lungs** **$T_{1/2}$ = approx. 1 mth**

Enhanced-Darkfield Light Microscopy Imaging (Mercer et al., 2011)



MWCNT in-/outside MPh in BALF



MWCNT sticking in lung septum

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Triple of nano-TiO_2

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Biokinetic Study to Compare Three TiO_2 (NM-103, NM-104, NM-105) in a 28-Day Inhalation Test in Rats

Experimental design

- **Dry dispersion technique**
- **Occupational exposure scenario**

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Objectives

- To mimic an occupational exposure scenario (dry dispersion technique)
- Dosing scheme: non-overload, partial and complete lung overload in the low, mid and high dose groups
- Toxicokinetic fate of TiO_2 agglomerates
- Identification of the respiratory cell types responsible for uptake of these particles

Characterisation of Test Items

TiO_2 Samples: Characterised and provided by the EU Commission/Joint Research Center (Ch. Klein; H. Stamm)				
EU/JRC-Code	**PPD (nm)**	**Surface - character - modified with - specific (m^2/g)**	**Name**	**Modification**
NM-103	**20**	**Hydrophobic Dimethicone (Silicone) 60 (56.2)**	**UV TITAN M262**	**Rutile**
NM-104	**20**	**Hydrophilic Glycerol 60 (46.0)**	**UV TITAN M212**	**Rutile**
NM-105	**22**	**Hydrophilic untreated 60 (56.3)**	**TiO_2 P25 Commercial sample, purchased and characterised by EU/JRC**	**Anatase/Rutile 80%/20%**

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LUNGS → P = particulate; S = soluble; T = Total; S(%) = soluble moiety in %

	Day 3				Day 45				Day 94			
	P	S	T	S (%)	P	S	T	S (%)	P	S	T	S (%)
NM-103, low	358	8.5	366	2.3	179	5.8	185	3.1	122	4.8	135	3.5
NM-103, mid	1625	9.6	1635	0.6	1530	10.7	1541	0.7	1107	13.2	1120	1.2
NM-103, high	7081	40.0	7121	0.6	7664	37.0	7701	0.5	6028	16.9	6045	0.3
NM-104, low	436	12.1	448	2.7	370	7.2	377	1.9	209	11.4	220	5.5
NM-104, mid	1698	11.2	1710	0.7	1674	17.4	1691	1.0	1344	9.8		0.7
NM-104, high	3782	34.6	3817	0.9	3928	16.1	3944	0.4	2860	17.9	2878	0.6
NM-105, low	477	21.8	499	4.4	255	7.9	262	3.0	121	4.3	125	3.5
NM-105, mid	1819	17.4	1836	0.9	1806	39.9	1846	2.2	1345	18.1	1363	1.3
NM-105, high	5879	54.0	5933	0.9	6679	51.8	6731	0.8	5527	44.4	5531	0.8

JRC Science and Policy Report

Titanium Dioxide,NM-103, NM-104, NM-105:

Characterisation and Physico-chemical Properties

Report EUR 26637 EN, 2014

Dissolution: <1 mg/l **(0.05% BSA/Gambles solution)**

2-3 mg/l **(Caco2)**

"TiO_2 NMs are categorised as highly durable nanomaterials with regard to the TiO_2 core."

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28-Day Inhalation Test + 1.5/3-mth recovery

Name NM	C,t-curve(s) T1/2, ke	Biodistribution Tissue levels
NM-103 low mid high	**(days)** **$T_{1/2}$ = 59** **$T_{1/2}$ = 162** **$T_{1/2}$ = 373**	**Lungs:** particulate: 96-97.5% Soluble: 2.5-5.5% in low dose groups particulate: > 98.7% Soluble: ≤ 1.3% in mid/high dose groups
NM-104 low mid high	**$T_{1/2}$ = 85** **$T_{1/2}$ = 267** **$T_{1/2}$ = 315**	**Liver:** test items generally below the limit of detection (but in some individual rats masses of up to 200 µg/liver) **Brain:** below the limit of detection
NM-105 low mid high	**$T_{1/2}$ = 48** **$T_{1/2}$ = 204** **$T_{1/2}$ = 485**	**Solubility of the test items limited by a given maximum under the conditions of the lung ambience**

Biokinetics of nano-ZnO and nano-SiO_2 after 90-Day Inhalation in Rats

Experimental design

- **Dry dispersion technique**
- **Occupational exposure scenario**

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Zinc Oxide

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Test/Reference Items - Exposure

BASF Z-Cote; zincite coated with triethoxycaprylylsilane, 130 nm

- **Z-COTE® HP1** **(nano-ZnO; coated with triethoxycaprylylsilane; BASF)** → **Cosmetics sector**
  NM-111

- **Z-COTE®** **(nano-ZnO; uncoated; BASF)**
  NM-110

- **ZnO 205532** **(µ-ZnO; Sigma-Aldrich)**

- **Risk-related exposure scenarios** → **Dry dispersion/agglomerates**

Fraunhofer ITEM

Solubility of Test Items in Various Media

test item	Matrix	pH	Solubility (%)
blank	Gambles S	4.5	< 0,01
		7.4	< 0,01
	Artificial LF	4.5	< 0,01
	Artificial AF	7.4	< 0,01
Z-COTE HP1	Gambles S	4.5	< 20
		7.4	< 0,05
	Artificial LF	4.5	> 90
	Artificial AF	7.4	< 0,05
Z-COTE	Gambles S	4.5	< 10
		7.4	< 0,05
	Artificial LF	4.5	> 90
	Artificial AF	7.4	< 0,05
Microscaled ZnO	Gambles S	4.5	< 20
		7.4	< 0,05
	Artificial LF	4.5	> 90
	Artificial AF	7.4	< 0,05

Fraunhofer ITEM

90-Day Study: Absolute Zn content in organs, blood, urine and feces

Day 1 post-exposure

	Clean air		Z-Cote® HP1		Z-Cote® HP1		**Z-Cote® HP1**		microscaled ZnO	
	control		0.3 mg/m³		1.5 mg/m³		**4.5 mg/m³**		4.5 mg/m³	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	(µg/Organ)		(µg/Organ)		(µg/Organ)		(µg/Organ)		(µg/Organ)	
LALN	2.60	1.55	2.86	0.78	2.01	0.40	2.53	0.70	2.98	1.68
MSLN	2.62	0.77	3.52	0.60	2.72	1.79	2.20	0.52	4.43	2.74
Brain	23.2	0.9	22.9	0.7	21.4	1.3	21.4	1.4	*20.8	1.4
Kidneys	58.1	3.5	56.2	5.4	*49.4	3.4	*49.5	4.1	51.7	6.7
Liver	276	19	320	29	287	27	287	20	302	80
Lung	19.9	0.2	20.4	0.6	22.2	2.0	**35.8	1.7	22.4	1.9
Blood	327	37	278	15	291	17	368	15	355	61
	(µg/16h)		(µg/16h)		(µg/16h)		(µg/16h)		(µg/16h)	
Urin	2.90	1.91	5.68	5.04	3.63	1.92	2.40	0.72	4.46	1.84
Feces	565	185	472	111	466	259	673	104	475	210

Fraunhofer ITEM

90-Day Study: Summary ZnO

Toxicokinetics

Zn chemical analysis:

Detectable only at day 1 post-exposure;
statistically significant in lungs for NM-111

Not longer increased at day 29 post-exposure

ZnO particles in tissues not detectable by TEM

Amorphous SiO_2

Fraunhofer ITEM

Test Item - Exposure

NM-200 **(nano-SiO2; precipitated synthetic amorphous silica; CAS # 112926-00-8; Master-Batch: JRC)**

→ Food sector

- **Risk-related exposure scenarios → Dry dispersion/agglomerates**

Fraunhofer ITEM

Structure / Solubility of NM-200 in Water and Media

- Structure:
  nanostructured material consisting of nanoscaled primary particles that are sintered to aggregates in the micrometer range

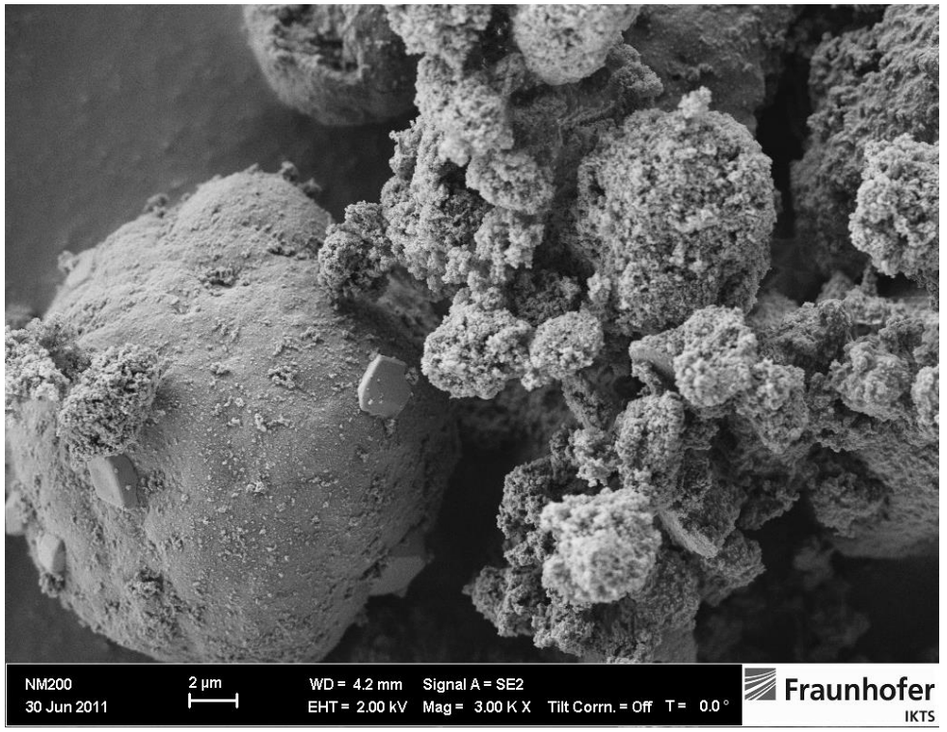
- Specific surface
  199 m^2/g

- Dissolution
  in water: approx. 5% over 2 wks.
  in physiological media: same magnitude as in water/ a bit reduced

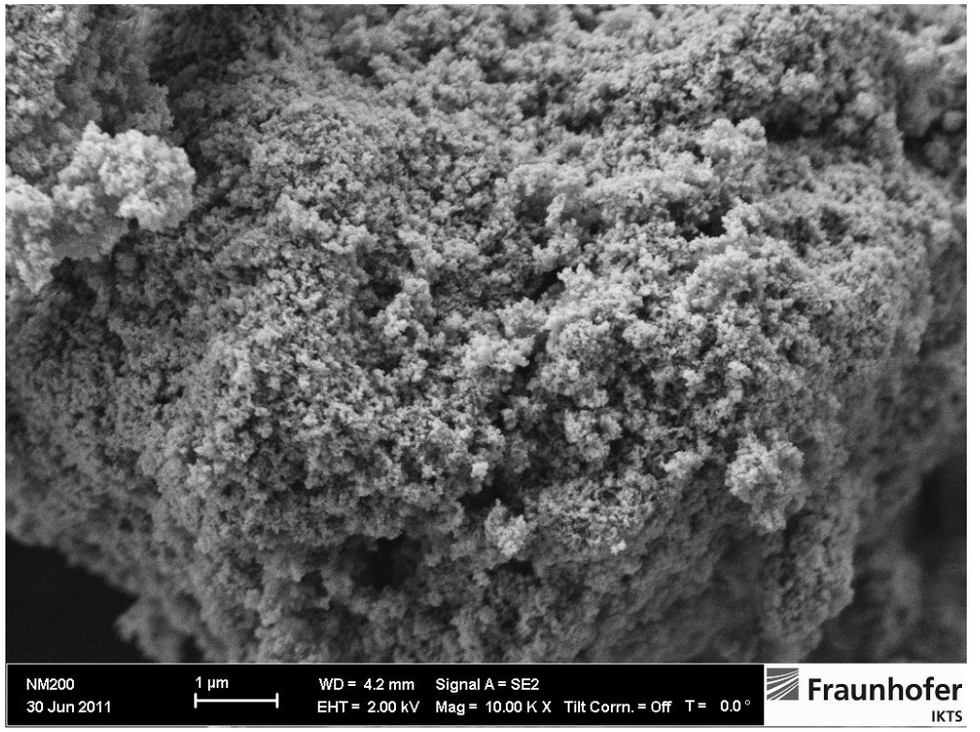
Fraunhofer ITEM

NM-200: SEM Photographs

SEM / 3K magnification



SEM / 10K magnification



Fraunhofer ITEM

90-Day Study: Retention of Test Item in Lungs

Retention µg/lung	90 + 1 day	90 + 30 days	90 + 90 days	$t_{1/2}$ (days)
NM-200 low	91	35	12	**32**
NM-200 mid	172	79	21	**31**
NM-200 high	307	150	34	**28**
Controls: <5µg/lung				

True density excluding voids: 2.19 g/cm^3
Bulk density: 0.12 g/cm^3
Tap density: 0.16 g/cm^3

Agglomerate density: approx. 0.5 - 1 g/cm^3

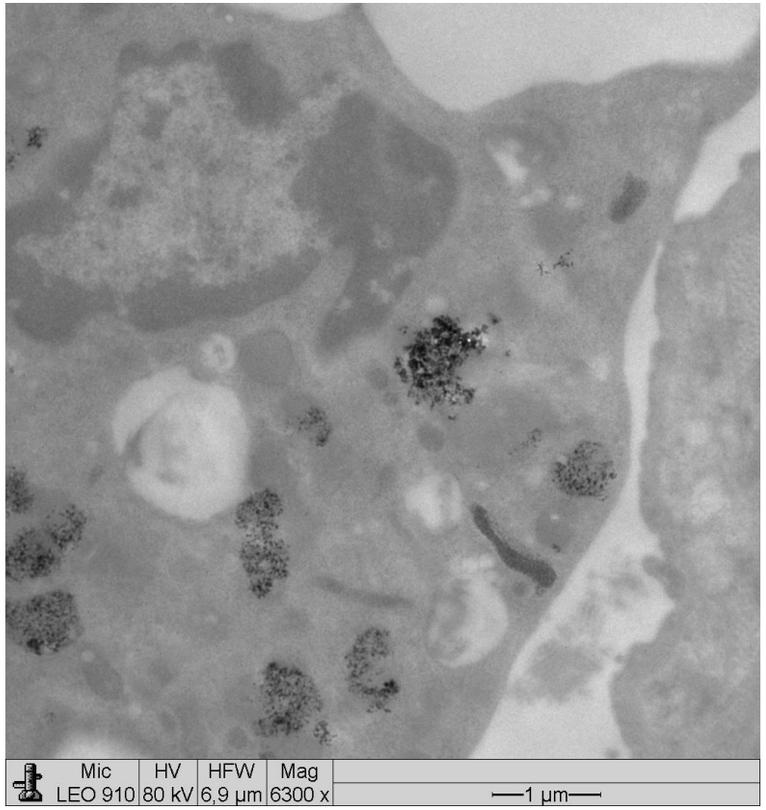
→ No overload in the high dose group at day 1

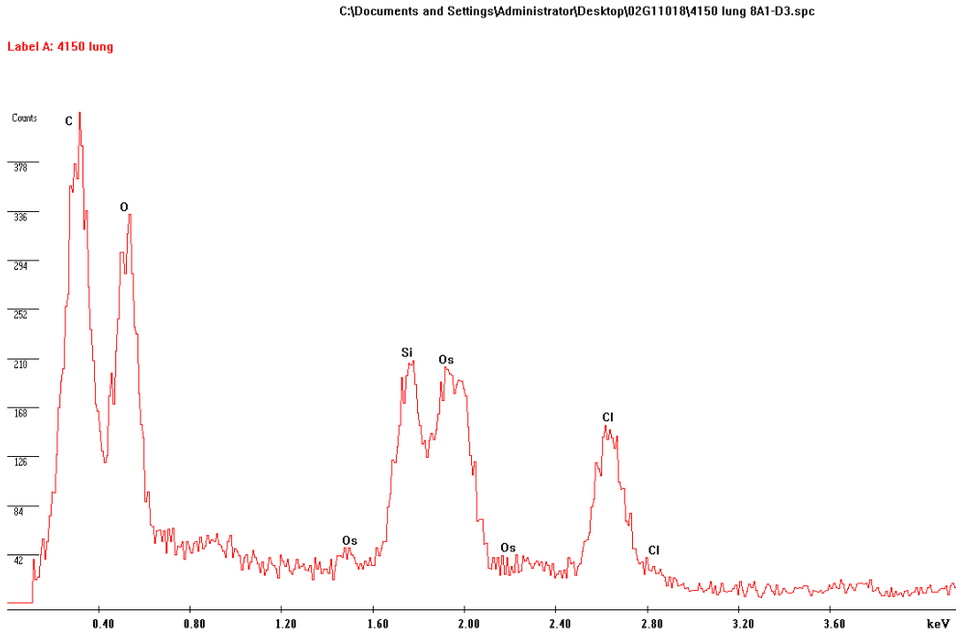
→ Evident dissolution effect , in addition to the physiological clearance

Fraunhofer ITEM

90-Day Study: TEM Analysis SiO_2

Animal 4150; high dose; day 91 of recovery → SiO_2 particles in lung intraalveolar macrophages





90-Day Study: Summary SiO_2

Toxicokinetics

- Si analysis: Detectable only in lungs - Day 1, 29 and 91 post-exposure
- SiO_2 particles detectable in lungs/LALN up to 91 day post exposure

  – not detectable in remote organs by TEM

  (nasal epithelium, trachea, larynx, liver, spleen, kidney and mesenteric lymph node)

Biokinetic fate of nanoparticles dependent on:

- **Status: Individual vs. agglomerated particles**

  → Deposition efficiency

- **Analytical vs. toxic lung load**

  → Lung clearance efficiency

- **Range of substance solubility (pH 7.4 - pH 4.5)**
- **Surface modification chemistry (without/with)**