

Programme 2009-2013

Toxicological properties of selected engineered nanomaterials (ENP)

In the course of the NANODEVICE project, several commercially available engineered nanomaterials (ENP) were investigated regarding their toxicological properties. Following the [evaluation of toxicology matrix](#), the results are summarized for each examined ENP:

Nanomaterials studied in the WP4 and their classification in data bank

I. Carbon nanomaterials

1. [Graphite NP \(NRCWE-005\)](#)
2. [Long, needle-like MCNT \(Mitsui-7; NRCWE-006\)](#)
3. [Long, tangled MCNT \(Cheaptubes 8-15 nm OD; NRCWE-007\)](#)

II. Metal nanoparticles

1. [Titanium dioxide \(TiO₂ Rutile NaBond 80 nm; NRCWE -004\)](#)
2. [Silicon oxide \(SiO_x, NanoAmor; NRCWE-008\)](#)
3. [Silver particles \(Ag, NANOGAP; NRCWE-009\)](#)

III. Calcium carbonate nanoparticles

1. [Calcium carbonate \(CaCO₃, NRCWE-011\)](#)
2. [Calcium carbonate for printing inks \(CaCO₃, functionalised; NRCWE-015\)](#)

IV. Iron oxide nanomaterials

1. [Alpha Fe₂O₃ particle \(NRCWE-018\)](#)
2. [Alpha Fe₂O₃ fibre \(NRCWE-019\)](#)
3. [Nickel-Zinc iron oxide \(NiO.5ZnO.5Fe₂O₄, NRCWE-020\)](#)
4. [Zinc iron oxide \(ZnFe₂O₄ 98%, NRCWE-021\)](#)
5. [Nickel iron oxide \(NiFe₂O₄, NRCWE-022\)](#)
6. [Fe-oxide \(FeyOx produced by WPI, NRCWE-023\)](#)


- Project summary
- [Timeline and work progress](#)
- [Public documents](#)
- **Toxicological properties**

Evaluation of toxicology is based on the following threshold values collected from the toxicological studies

	Immunotoxicology studies		
	Pro-inflammatory effects	Cytotoxicity	ROS production
Low	No secretion of cytokines	< 5 %	No ROS produced
Moderate	Some secretion of cytokines	5-15 %	Some ROS produced
High	Robust secretion of cytokines	> 15 %	Robust ROS

	Genotoxicology studies		
	Cytotoxicity (Trypan Blue/ATP assay)	Early genotoxic effects (DNA damage)	Micronuclei
Low	A clear (>50%) cytotoxicity not observed.	No induction of DNA damage.	No induction of micronuclei.
Moderate	No clear dose-dependency, >50% cytotoxicity at one or several doses.	Increase in DNA damage at one or several doses, dose-dependency not always observed.	Increase in micronuclei at one or several doses, dose-dependency not always observed.
High	Clear dose-dependent cytotoxicity, >50% cytotoxicity at several doses.	Clear dose-dependent increase in DNA damage, significant increase in DNA damage at several doses.	Clear dose-dependent increase in micronuclei, significant induction of micronuclei at the highest dose(s).

Graphene	Nanomaterial	Nomenclature	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)
NR/WE-005	Graphite NP	Graphite	n.d.	Length 3-4 nm	SS nano	TEM and SEM / WP1 (TKK, SP1)



TEM picture by WP1 /NANODEVICE

n.d. = Not detected

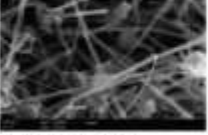
Dispersion status /WP	
Analysed in cRPM /WP3 (IFA, SP2)	

Specific surface area (m ² /g) /reference	
n.d.	

Immunotoxicology studies			
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects
6h	Human primary macrophages	Low	Low
24h	Mouse RAW 264.7 cell line	Low	Low
24h	A549 Alveolar epithelial cell line	Low	Low
24h	HaCaT keratinocyte cell line	Low	Low

Genotoxicity studies				
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei
4h	BEAS-2B	Moderate	n.d.	n.d.
24h	BEAS-2B	Low	Moderate	n.d.
48h	BEAS-2B	Low	n.d.	Low

Mitsui-7 MWCNT	Nanomaterial	Nomenclature	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)
NR/WE-305	Mitsui-7 MWCNT	Long, needle-like CNT	C > 99 %wt	Length 13 µm, OD > 50 nm	MitsuiCo, Ltd	TEM and SEM / WP1 (TKK, SP1)



SEM picture by WP1 /NANODEVICE

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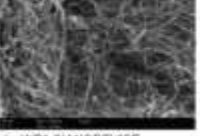
Dispersion status /WP	
Analysed in cRPM /WP3 (IFA, SP2)	

Specific surface area (m ² /g) /reference	
n.d.	

Immunotoxicology studies			
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects
3h	Human primary macrophages	n.d.	Moderate
6h	Human primary macrophages	Low	High
9h	Human primary macrophages	Moderate	High
24h	Mouse RAW 264.7 cell line	Moderate	Moderate
24h	A549 Alveolar epithelial cell line	Low	Low
24h	HaCaT keratinocyte cell line	Low	Low

Genotoxicity studies				
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei
4h	BEAS-2B	High	n.d.	n.d.
24h	BEAS-2B	High	Moderate	n.d.
48h	BEAS-2B	High	n.d.	Low

Cheaptubes OD 8-15 nm MWCNT	Nanomaterial	Nomenclature	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)
NR/WE-507	Cheaptubes MWCNT	Long, tangled CNT	C > 99 %wt	Length 10-50 µm, OD 8-15 nm	Cheaptubes Inc	TEM and SEM / WP1 (TKK, SP1)



SEM picture by WP1 /NANODEVICE

n.d. = Not detected

Dispersion status /WP	
Analysed in cRPM /WP3 (IFA, SP2)	

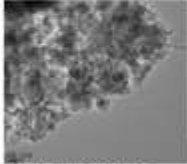
Specific surface area (m ² /g) /reference	
233 / Vippola et al. 2009 *	

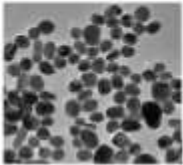
* Vippola et al. Nanotoxicol of selected engineered nanoparticles 2009

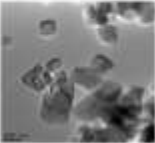
Immunotoxicology studies			
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects
3h	Human primary macrophages	n.d.	Moderate
6h	Human primary macrophages	Low	Low
9h	Human primary macrophages	n.d.	Low
24h	Mouse RAW 264.7 cell line	High	Moderate
24h	A549 Alveolar epithelial cell line	Low	Low
24h	HaCaT keratinocyte cell line	Low	Low

Genotoxicity studies				
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei
4h	BEAS-2B	Moderate	n.d.	n.d.
24h	BEAS-2B	Moderate	Moderate	n.d.
48h	BEAS-2B	Low	n.d.	Low

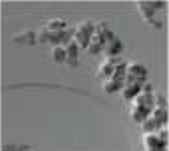
Rutile NaBond 80 nm (TiO ₂)							
Code	Nanomaterial	Nomenclature	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)	
NRCWE-004	Rutile NaBond 80 nm (TiO ₂)	rutile TiO ₂	n.d.	80 nm	NaBond	TEM and SEM / WP1 (TKK, SP1)	
			n.d. = Not detected		Dispersion status /WP n.d. Specific surface area (m ² /g) /reference n.d.		
TEM picture by WP1 /NANODEVICE							
Immunotoxicology studies							
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects				
6 h	Human primary macrophages	Low	Low				
24h	A549 Alveolar epithelial cell line	Low	Low				
24h	HaCaT keratinocyte cell line	Low	Low				
Genotoxicity studies							
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects		Micronuclei		
4h	BEAS-2B	Moderate	n.d.		n.d.		
24h	BEAS-2B	Low	High		n.d.		
48h	BEAS-2B	Low	n.d.		Low		

NanoAmor SiO ₂							
Code	Nanomaterial	Nomenclature	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)	
NRCWE-005	NanoAmor SiO ₂	SiO ₂	n.d.	15 nm	NanoAmor	TEM and SEM / WP1 (TKK, SP1)	
			n.d. = Not detected		Dispersion status /WP Analysed in cRPMI /WP3 (NFA, SP2) Specific surface area (m ² /g) /reference n.d.		
TEM picture by WP1 /NANODEVICE							
Immunotoxicology studies							
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects		ROS production		
6 h	Human primary macrophages	High	High		n.d.		
6 h	THP-1 cell line	High	High		Low		
24h	A549 Alveolar epithelial cell line	Moderate	Low		n.d.		
24h	HaCaT keratinocyte cell line	Moderate	Moderate		n.d.		
Genotoxicity studies							
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects		Micronuclei		
4h	BEAS-2B	High	n.d.		n.d.		
24h	BEAS-2B	High	High		n.d.		
48h	BEAS-2B	High	n.d.		Low		

NanoGAP; NGAP NP (Ag)							
Code	Nanomaterial	Nomenclature	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)	
NRCWE-006	NanoGAP; NGAP NP (Ag)	Ag	n.d.	42.5 ± 14.5nm	NanoGAP	TEM and SEM / WP1 (TKK, SP1)	
			n.d. = Not detected		Dispersion status /WP Analysed in cRPMI /WP3 (NFA, SP2) Specific surface area (m ² /g) /reference n.d.		
TEM picture by WP1 /NANODEVICE							
Immunotoxicology studies							
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects				
6 h	Human primary macrophages	Low	Low				
24h	A549 Alveolar epithelial cell line	Moderate	High				
24h	HaCaT keratinocyte cell line	Low	High				
Genotoxicity studies							
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects		Micronuclei		
4h	BEAS-2B	Moderate	High		n.d.		
24h	BEAS-2B	Low	High		n.d.		
48h	BEAS-2B	High	n.d.		Low		

Calcium carbonate (CaCO ₃)							
Code	Nanomaterial	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)		
NRCWE-011	CaCO ₃	n.d.	15-40 nm	SS nano	TEM and SEM / WP1 (TKK, SP1)		
			n.d. = Not detected		Dispersion status /WP Analysed in cRPMI /WP3 (NFA, SP2) Specific surface area (m ² /g) /reference n.d.		
TEM picture by WP1 /NANODEVICE							
Immunotoxicology studies							
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects		ROS production		
6 h	Human primary macrophages	Low	Moderate		n.d.		
6 h	THP-1 cell line	Low	Low		Moderate		
24h	A549 Alveolar epithelial cell line	Low	Low		n.d.		
24h	HaCaT keratinocyte cell line	Low	Low		n.d.		
Genotoxicity studies							
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects		Micronuclei		
4h	BEAS-2B	High	n.d.		n.d.		
24h	BEAS-2B	High	Moderate		n.d.		
48h	BEAS-2B	High	n.d.		Low		

Calcium carbonate (CaCO ₃) for printing inks					
Code	Nanomaterial	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)
NRCWE-015	CaCO ₃ for printing inks	n.d.	15-40 nm	SS Nano	TEM and SEM / WP1 (TKK, SP1)



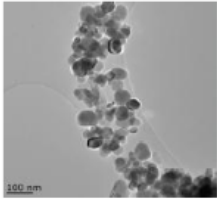
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Dispersion status /WP				
Analysed in cRPMI /WP3 (NFA, SP2)				
Specific surface area (m2/g) /reference				
n.d.				

Immunotoxicology studies				
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects	ROS production
6 h	Human primary macrophages	Low	Moderate	n.d.
6 h	THP-1 cell line	Low	Low	Moderate
24h	A549 Alveolar epithelial cell line	Low	Low	n.d.
24h	HaCaT keratinocyte cell line	Low	Low	n.d.

Genotoxicity studies				
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei
4h	BEAS-2B	Moderate	n.d.	n.d.
24h	BEAS-2B	High	Low	n.d.
48h	BEAS-2B	High	n.d.	High

α Fe ₂ O ₃ particle					
Code	Nanomaterial	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)
NRCWE-018	α Fe ₂ O ₃ particle	n.d.	20-60 nm	NanoAmor	TEM and SEM / WP1 (TKK, SP1)




TEM picture by WP1 /NANODEVICE n.d. = Not detected

Dispersion status /WP				
Analysed in cRPMI /WP3 (NFA, SP2)				
Specific surface area (m2/g) /reference				
n.d.				

Immunotoxicology studies			
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects
6 h	Human primary macrophages	Low	Low
24h	A549 Alveolar epithelial cell line	Low	Low
24h	HaCaT keratinocyte cell line	Low	Low

Genotoxicity studies				
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei
4h	BEAS-2B	Low	n.d.	n.d.
24h	BEAS-2B	Low	High	n.d.
48h	BEAS-2B	Low	n.d.	Moderate

α Fe ₂ O ₃ fiber					
Code	Nanomaterial	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)
NRCWE-019	α Fe ₂ O ₃ fiber	n.d.	40-150 nm x 250-600 nm	NanoAmor	TEM and SEM / WP1 (TKK, SP1)

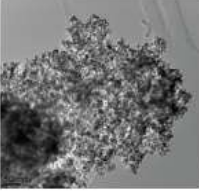


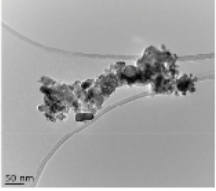
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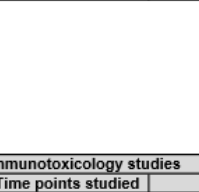
Dispersion status /WP				
Analysed in cRPMI /WP3 (NFA, SP2)				
Specific surface area (m2/g) /reference				
n.d.				

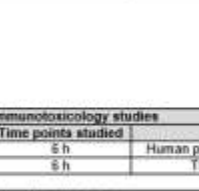
Immunotoxicology studies			
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects
6 h	Human primary macrophages	Low	Low
6 h	THP-1 cell line	Moderate	Moderate
24h	A549 Alveolar epithelial cell line	Low	Low
24h	HaCaT keratinocyte cell line	Low	Low

Genotoxicity studies				
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei
4h	BEAS-2B	Moderate	n.d.	n.d.
24h	BEAS-2B	Low	Moderate	n.d.
48h	BEAS-2B	Moderate	n.d.	Low

Nickel-Zinc Iron Oxide (Ni _{0.8} Zn _{0.2} Fe ₂ O ₄)										
Code	Nanomaterial	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)					
NRCWE-020	Nickel-Zinc Iron Oxide (Ni _{0.8} Zn _{0.2} Fe ₂ O ₄)	n.d.	10-30 nm	NanoAmor	TEM and SEM / WP1 (TKK, SP1)					
										
						Dispersion status /WP				
						Analysed in cRPMI /WP3 (NFA, SP2)				
Specific surface area (m ² /g) /reference										
n.d.										
TEM picture by WP1 /NANODEVICE n.d. = Not detected										
Immunotoxicology studies										
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects	ROS production						
6 h	Human primary macrophages	Low	Low	n.d.						
6 h	THP-1 cell line	n.d.	n.d.	Moderate						
24h	A549 Alveolar epithelial cell line	Low	Low	n.d.						
24h	HaCaT keratinocyte cell line	Low	Low	n.d.						
Genotoxicity studies										
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei						
4h	BEAS-2B	Moderate	n.d.	n.d.						
24h	BEAS-2B	High	Low	n.d.						
48h	BEAS-2B	High	n.d.	Moderate						

ZnFe ₂ O ₄ 98%+										
Code	Nanomaterial	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)					
NRCWE-021	ZnFe ₂ O ₄ 98%+	n.d.	15-30 nm	NanoAmor	TEM and SEM / WP1 (TKK, SP1)					
										
						Dispersion status /WP				
						Analysed in cRPMI /WP3 (NFA, SP2)				
Specific surface area (m ² /g) /reference										
n.d.										
TEM picture by WP1 /NANODEVICE n.d. = Not detected										
Immunotoxicology studies										
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects							
6 h	Human primary macrophages	Low	Low							
24h	A549 Alveolar epithelial cell line	Moderate	Low							
24h	HaCaT keratinocyte cell line	Low	Low							
Genotoxicity studies										
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei						
4h	BEAS-2B	High	n.d.	n.d.						
24h	BEAS-2B	High	Moderate	n.d.						
48h	BEAS-2B	High	n.d.	Moderate						

Nickel Iron Oxide (NiFe ₂ O ₄)										
Code	Nanomaterial	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)					
NRCWE-022	Nickel Iron Oxide (NiFe ₂ O ₄)	n.d.	20-30 nm	NanoAmor	TEM and SEM / WP1 (TKK, SP1)					
										
						Dispersion status /WP				
						Analysed in cRPMI /WP3 (NFA, SP2)				
Specific surface area (m ² /g) /reference										
n.d.										
n.d. = Not detected										
Immunotoxicology studies										
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects							
6 h	Human primary macrophages	Low	Low							
24h	A549 Alveolar epithelial cell line	Low	Low							
24h	HaCaT keratinocyte cell line	Low	Low							
Genotoxicity studies										
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei						
4h	BEAS-2B	Moderate	n.d.	n.d.						
24h	BEAS-2B	Moderate	High	n.d.						
48h	BEAS-2B	High	n.d.	Low						

Fe ₃ O ₄										
Code	Nanomaterial	Impurities	Size data from vendor	Source	TEM/SEM /work package (WP)					
NRCWE-023	Fe ₃ O ₄	n.d.	data pending	TUT	TEM and SEM / WP1 (TKK, SP1)					
										
						Dispersion status /WP				
						Analysed in cRPMI /WP3 (NFA, SP2)				
Specific surface area (m ² /g) /reference										
n.d.										
n.d. = Not detected										
Immunotoxicology studies										
Time points studied	Cell type	Cytotoxicity	Pro-inflammatory effects	ROS production						
6 h	Human primary macrophages	Low	Low	n.d.	n.d. = Not detected					
6 h	THP-1 cell line	n.d.	n.d.	Low						
Genotoxicity studies										
Time points studied	Cell type	Cytotoxicity	Early genotoxic effects	Micronuclei						
4h	BEAS-2B	Low	n.d.	n.d.						
24h	BEAS-2B	Low	High	n.d.						
48h	BEAS-2B	Moderate	n.d.	Moderate						