Grant agreement number 2009 21 01

WP7
Toxicokinetics

Wim H. De Jong
RIVM (National Institute for Public Health and the Environment)
Bilthoven, The Netherlands

Co-funded by
the Health Programme
of the European Union

WP7 Objectives

- Determine feasible dose for in vivo studies
  - Toxicokinetics: to obtain organ levels above detection levels
  - Genotoxicity: highest dose possible without severe toxicity?
- Determine relevant target organs for possible genotoxic damage based on tissue distribution of MN.
- Determine time points for in vivo tissue sampling for in vivo studies.

Co-funded by
the Health Programme
of the European Union

Issues

- MN investigated TiO_2, SAS (SiO_2), MWCNT
  - For Ti determination an evaluation was done in 4 different laboratories with different ICP-MS equipment
- Toxicology
  - Dose range finding (identify tolerable dose)
    - IV and oral
- Toxicokinetics
  - Tissue distribution, kinetics after single dose (1x)
  - Tissue distribution after repeated dose (5x)
  - IV and oral

Co-funded by
the Health Programme
of the European Union

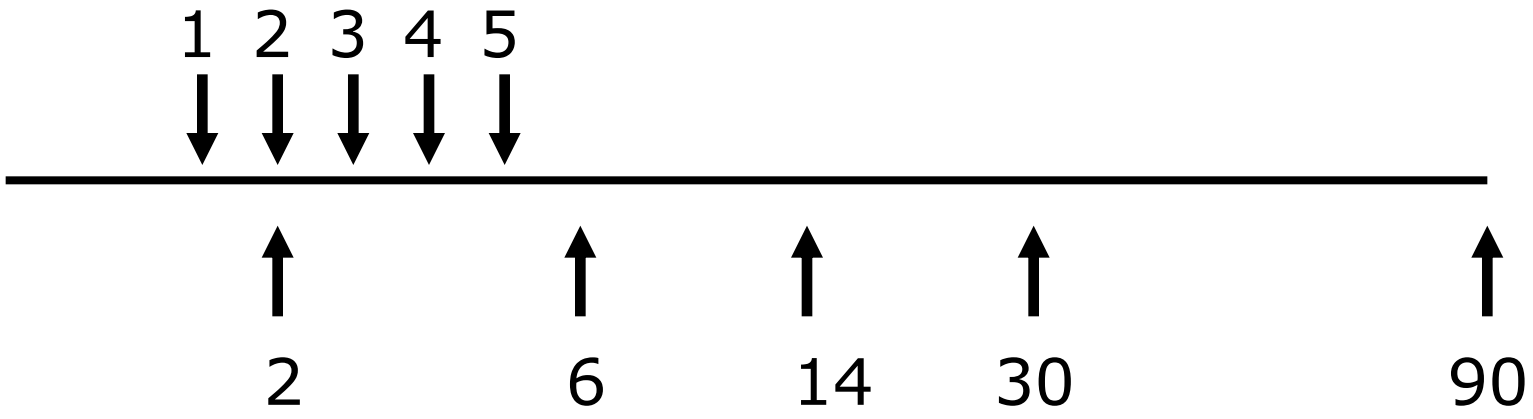
Toxicokinetic studies, nanomaterials

Nanomaterials		Route	Partner
TiO_2	NM-100	IV	RIVM
	NM-101	oral	NRCWE
	NM-102	oral, IV	NRCWE, RIVM
	NM-103	oral, IV	NRCWE, RIVM
	NM-104	oral, IV	NRCWE, RIVM
	NM-105	oral, IV	NRCWE, IMB-BAS
SAS	NM-200	oral, IV	ISS
	NM-203	oral, IV	
CNTs	NM-400	oral, IV	CEA
	NM-401	oral, IV	
	NM-402	oral, IV	
	NRCWE-006	oral, IV	

Co-funded by
the Health Programme
of the European Union

Toxicokinetic study design (oral and IV)

Treatment schedule



Blood was collected at (pretreatment, and on days 1 and 5)

Blood and organs were collected at days 2-6-14-30-90

Co-funded by
the Health Programme
of the European Union

Oral and IV administration of TiO_2

Co-funded by
the Health Programme
of the European Union

Oral administration of TiO_2

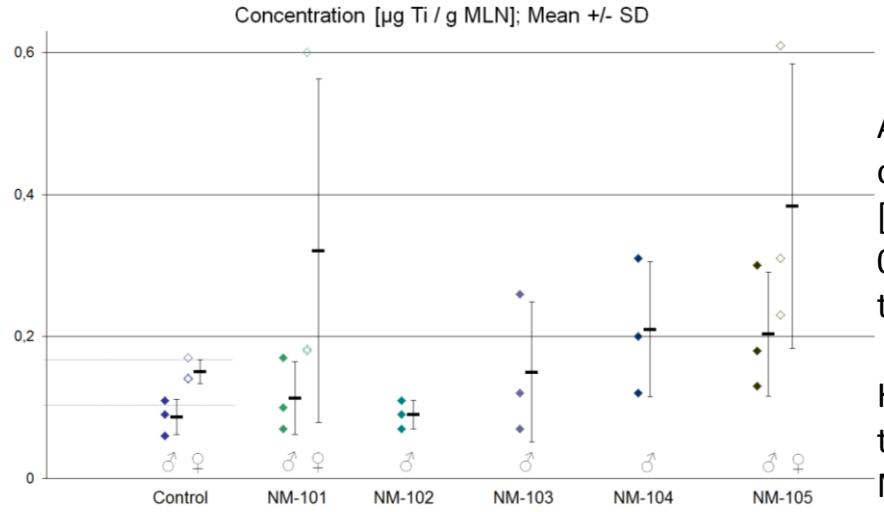
		Liver			Spleen		
		Animal 1	Animal 2	Animal 3	Animal 1	Animal 2	Animal 3
Control	5 x 0 mg ♂	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
NM-101	5 x 2.304 mg ♂	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
NM-102	5 x 2.304 mg ♂	< 0.03	**0,03**	< 0.03	< 0.03	< 0.03	< 0.03
NM-103	5 x 2.304 mg ♂	**0,08**	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
NM-104	5 x 2.304 mg ♂	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
NM-105	5 x 2.304 mg ♂	< 0.03	< 0.03	< 0.03	< 0.03	**0,12**	< 0.03
Control	5 x 0 mg ♀	< 0.03	< 0.03	**0,03**	< 0.03	< 0.03	**0,03**
NM-101	5 x 2.304 mg ♀	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
NM-105	5 x 2.304 mg ♀	< 0.03	< 0.03	< 0.03	**0,21**	< 0.03	**0,13**

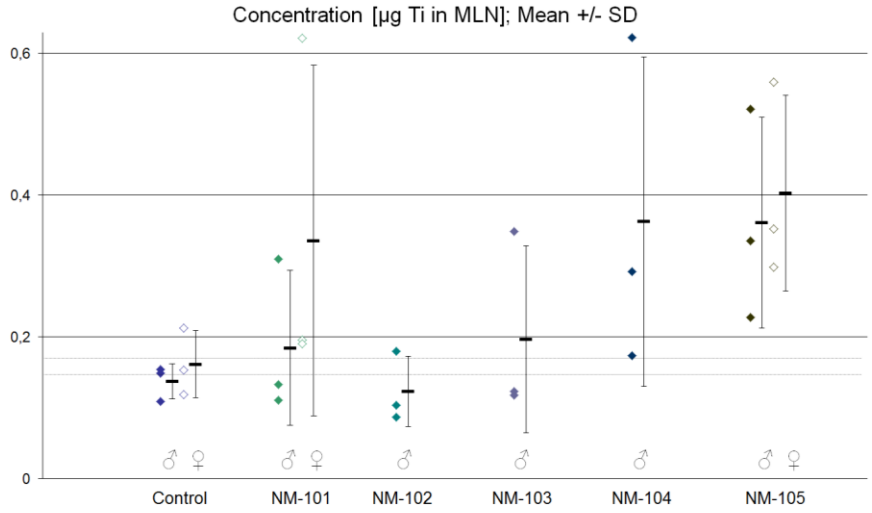
All concentration listed in [µg Ti / g tissue]

All liver and spleen tissue samples contained very low amounts of Ti.
Concentration were close to Limit of Detection (**n=4**), at Limit of Detection (**n=3**) or below the Limit of Detection (**n=47**) of 0.03 µg Ti / g tissue.
Of the 4 samples with concentrations above the LOD, 3 was in spleens of NM-105 exposed rats.

Co-funded by
the Health Programme
of the European Union

Oral administration of TiO_2 Uptake in Mesenteric Lymph Node





All groups of female rats have a slightly higher Ti concentration in MLN (per g/MLN). The difference (Female [Ti] - Male [Ti]) is:
0.064 for controls, 0.207 for NM-101 and 0.180 µg Ti / g tissue for NM-105.

However when adjusting for the lower weight of female MLN the difference decreased to: 0.024 for controls, 0.151 for NM-101 and 0.042 µg Ti in MLN tissue for NM-105.

The uptake is almost identical but the amount is present in a smaller organ in females

Co-funded by
the Health Programme
of the European Union

Oral administration of TiO_2 Translocation to mesenteric lymph nodes

Mean Ti-values for controls and for the MN leading to the highest concentration in MLN.

rats (NM-104):

Controls MLN : 0.137 µg

NM-104 exposed rats : 0.363 µg

Difference: 0.226 µg Total exposure: 11520 µg.

This means that (0.226/11520*100) **0.002%** were translocated to the mesenteric lymph nodes.

rats (NM-105):

Controls MLN : 0.162 µg

NM-105 exposed rats : 0.403 µg

Difference: 0.241 µg Total exposure: 11520 µg.

This means that (0.241/11520*100) **0.002%** were translocated to the mesenteria.

Since we also find NM-105 in the spleen and NM-103 in the liver of some rats, the total translocation is probably larger than shown above

Co-funded by
the Health Programme
of the European Union

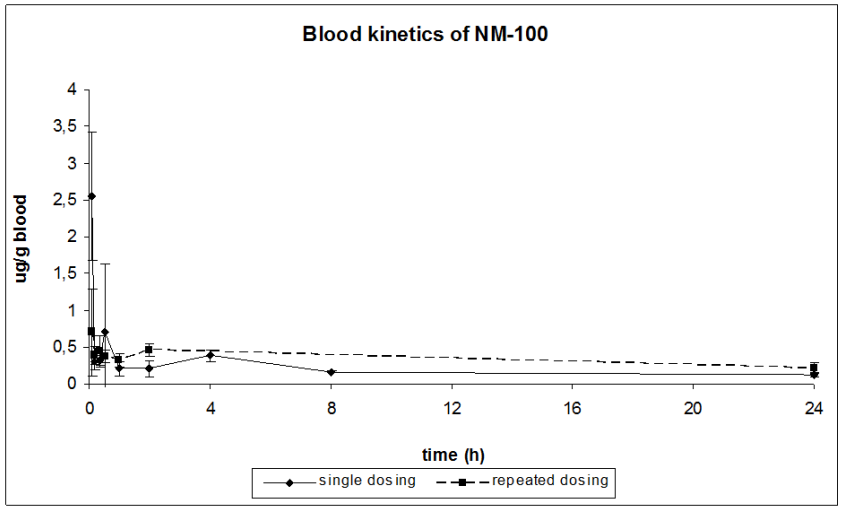
TiO_2 nanomaterials

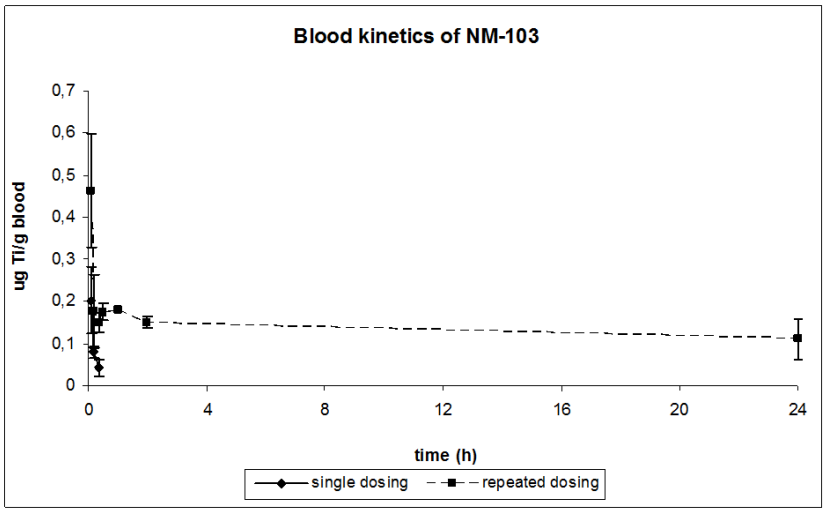
IV administration TiO_2

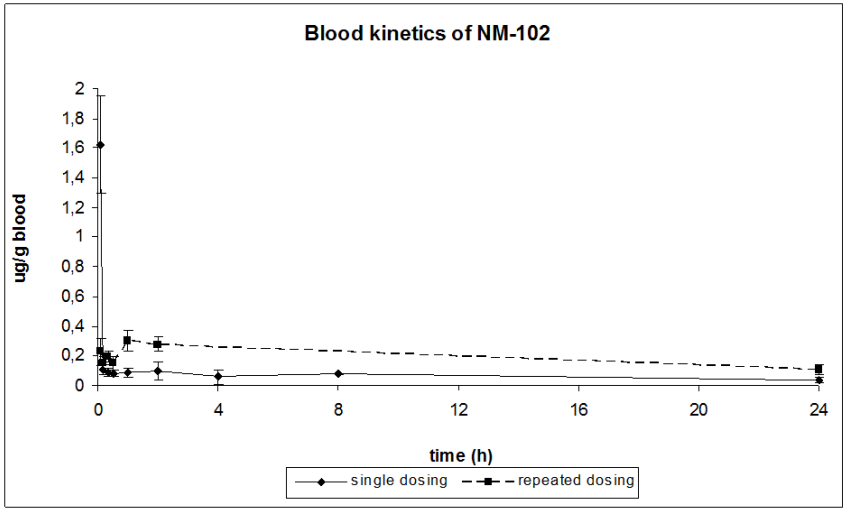
NM-100, 200-220 nm, anatase
NM-102, 15-25 nm, anatase
NM-103, 20 nm, rutile, hydrophobic
NM-104, 20 nm, rutile, hydrophilic
NM-105, 22 nm, 85% anatase, 15% rutile

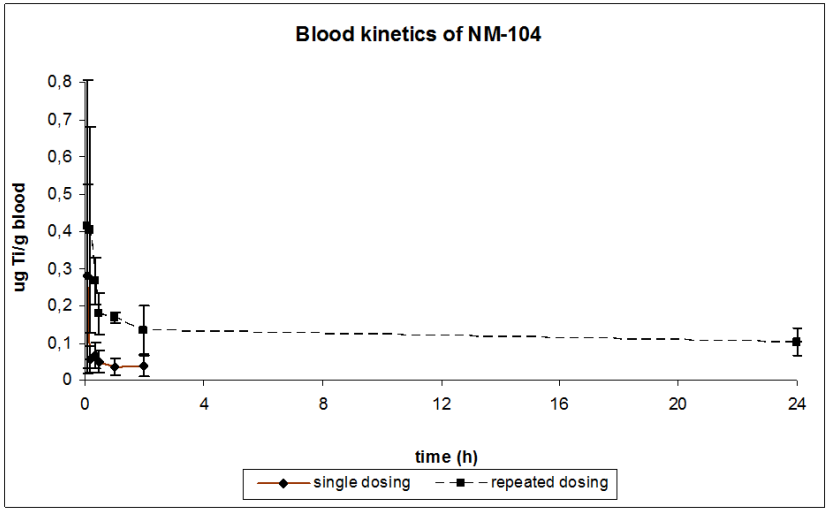
Co-funded by
the Health Programme
of the European Union

Blood kinetics of TiO_2 after IV administration NM-100, NM-102, NM-103, and NM-104





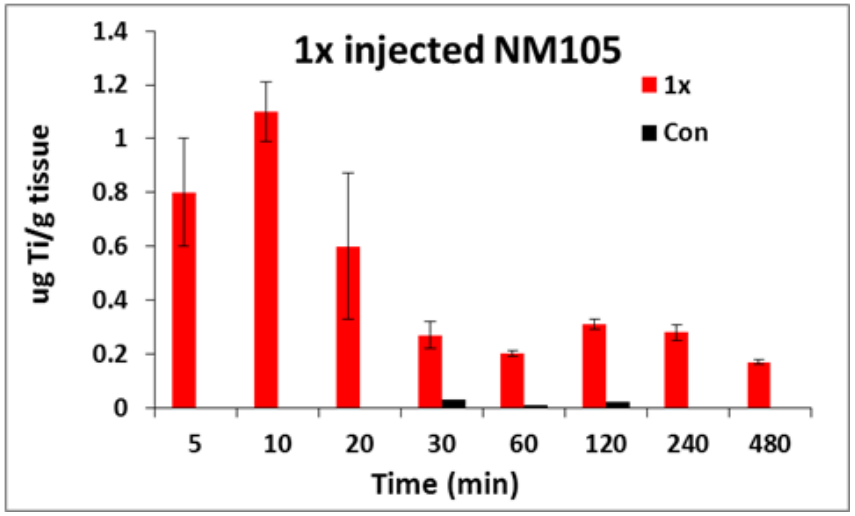


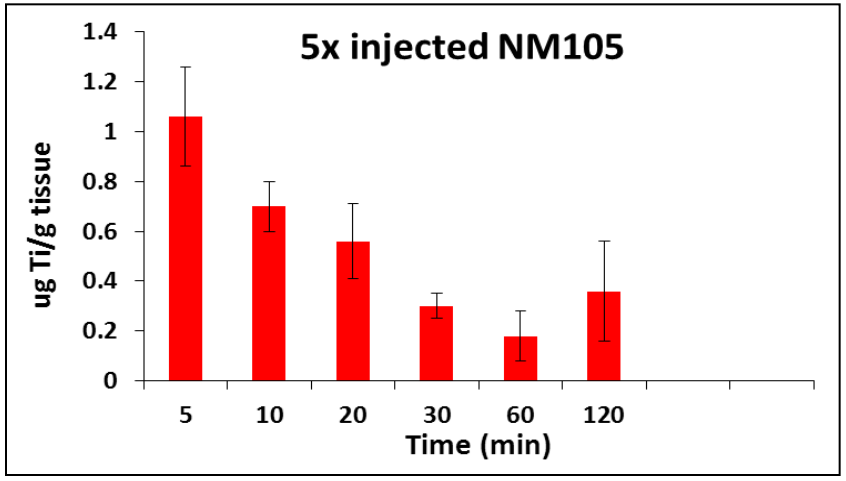


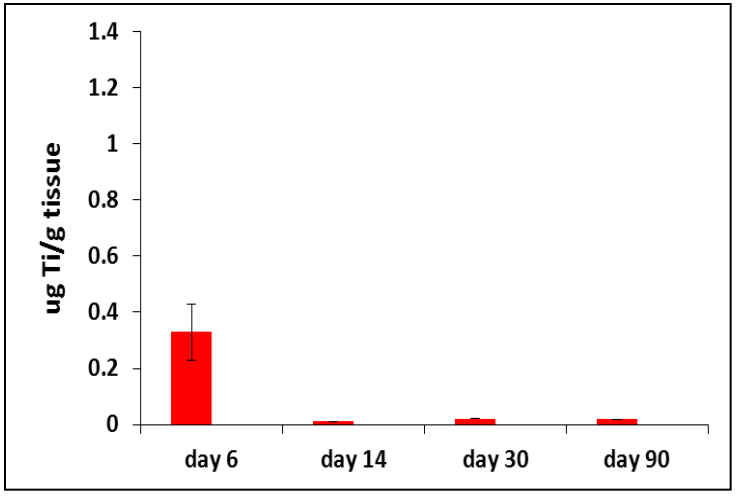
Co-funded by
the Health Programme
of the European Union

Blood clearance of NM-105 after IV administration

Grant agreement number 2009 21 01

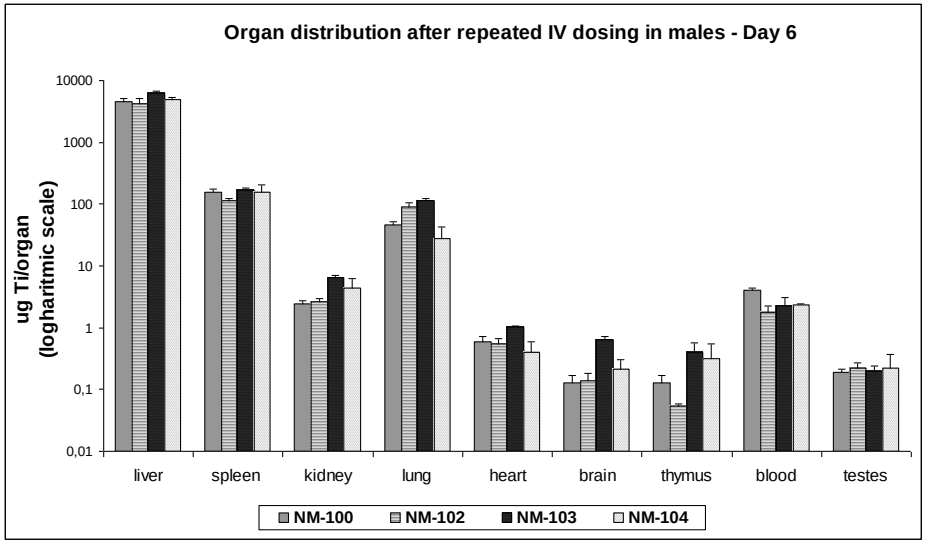


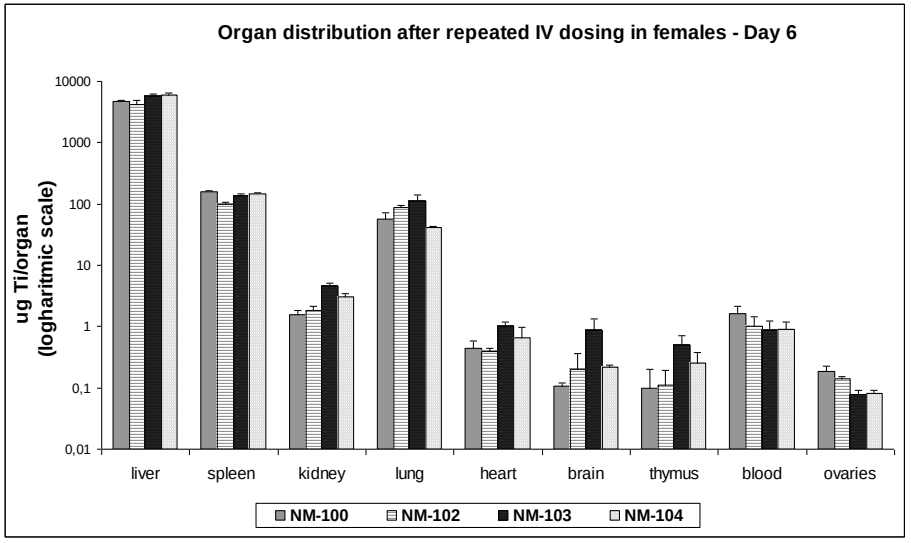




Co-funded by
the Health Programme
of the European Union

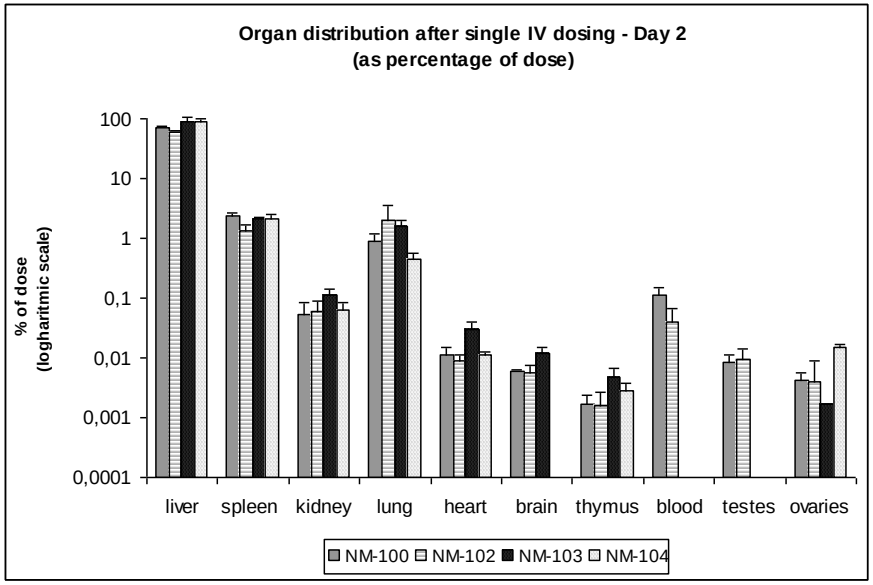
Organ distribution in µg Ti/organ after IV administration NM-100, NM-102, NM-103, and NM-104

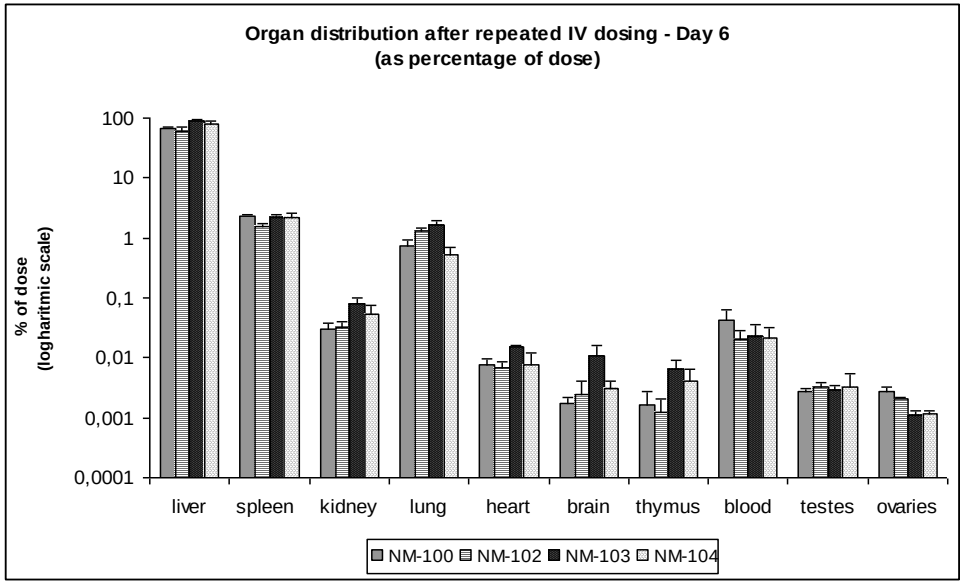




Co-funded by
the Health Programme
of the European Union

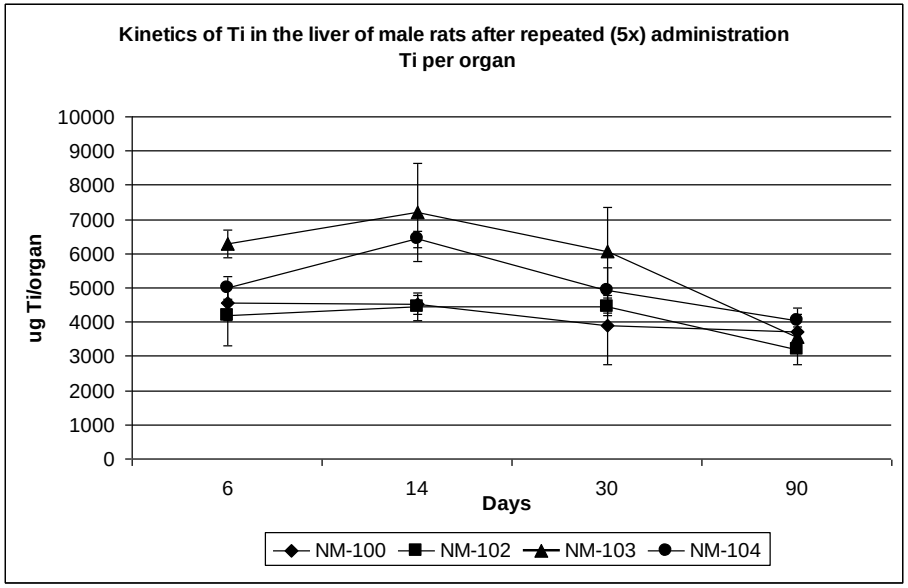
Organ distribution of Ti as % of dose after IV administration NM-100, NM-102, NM-103, and NM-104

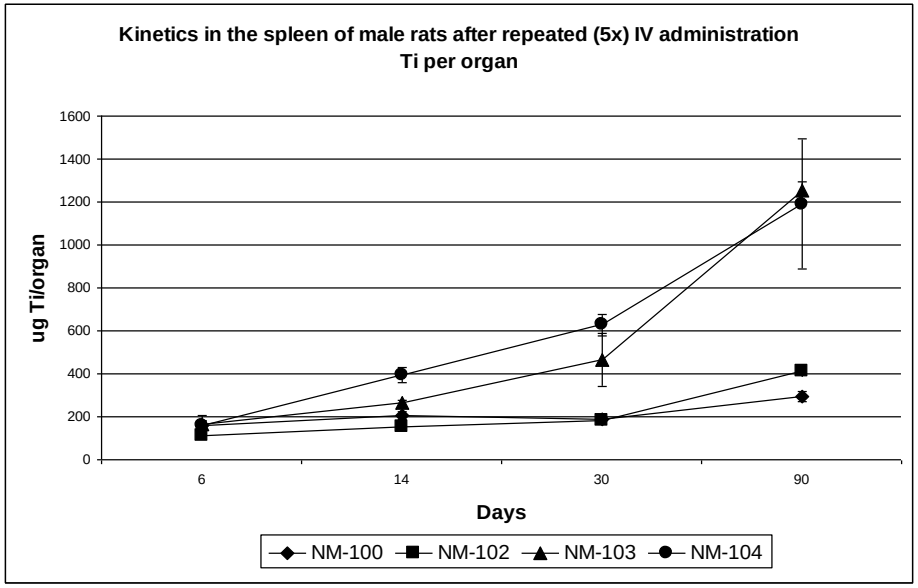




Co-funded by
the Health Programme
of the European Union

Kinetics of organ distribution of Ti day 6 – day 90 after repeated (5x) IV administration: NM-100, NM-102, NM-103, and NM-104





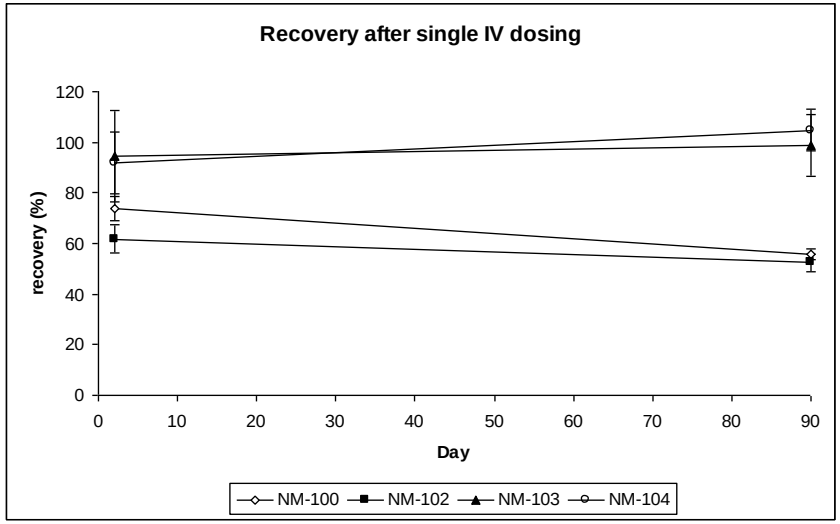
Decrease of Ti in time in liver
Increase of Ti in time in spleen

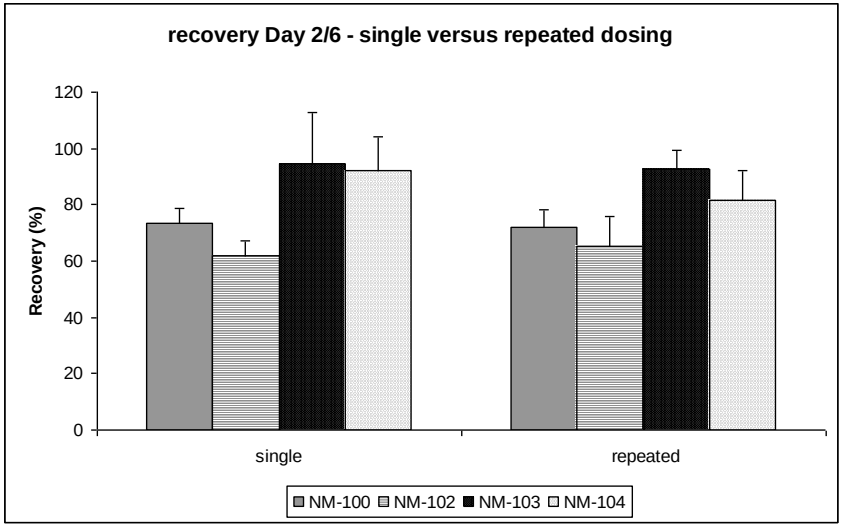
Redistribution between liver and spleen of Ti.

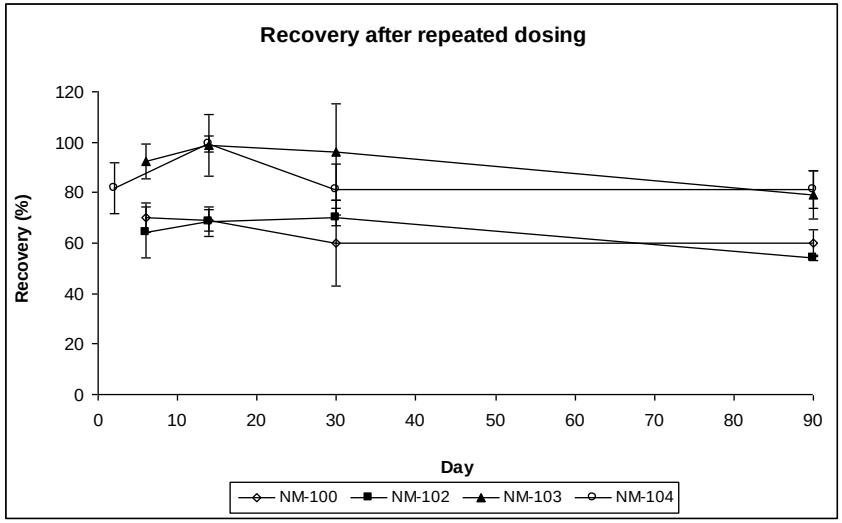
Co-funded by
the Health Programme
of the European Union

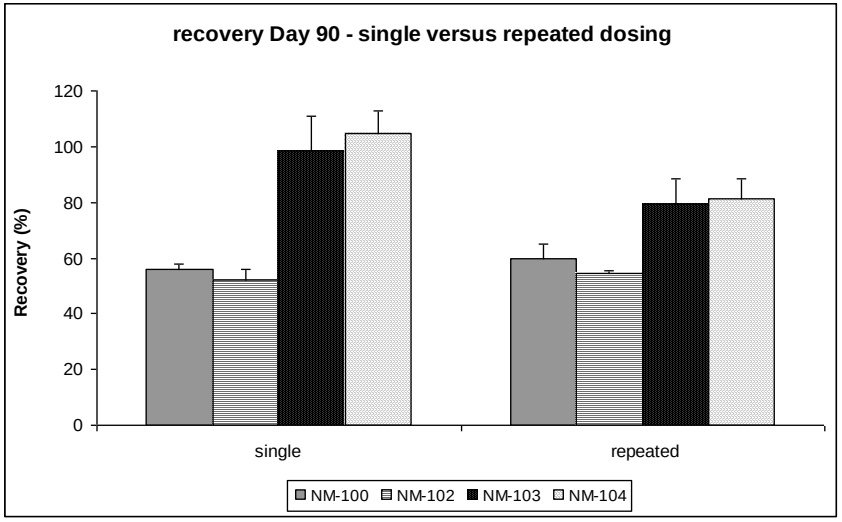
Recovery of Ti as percentage of dose after repeated (5x) IV administration NM-100, NM-102, NM-103, and NM-104

Grant agreement number 2009 21 01



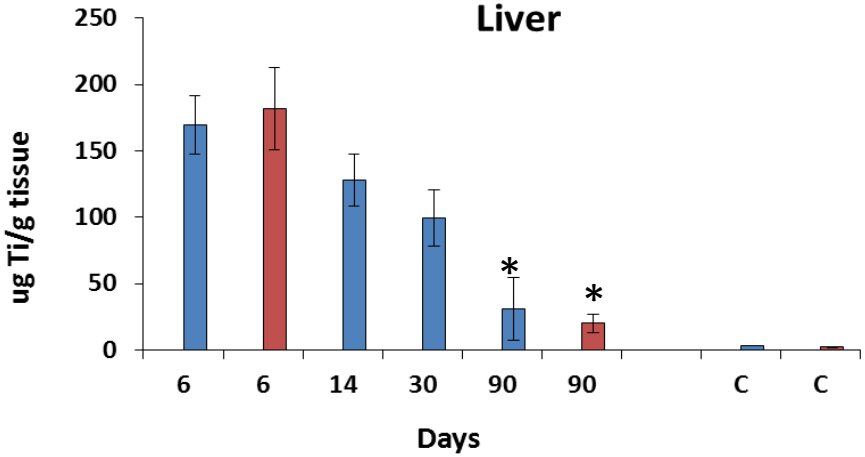


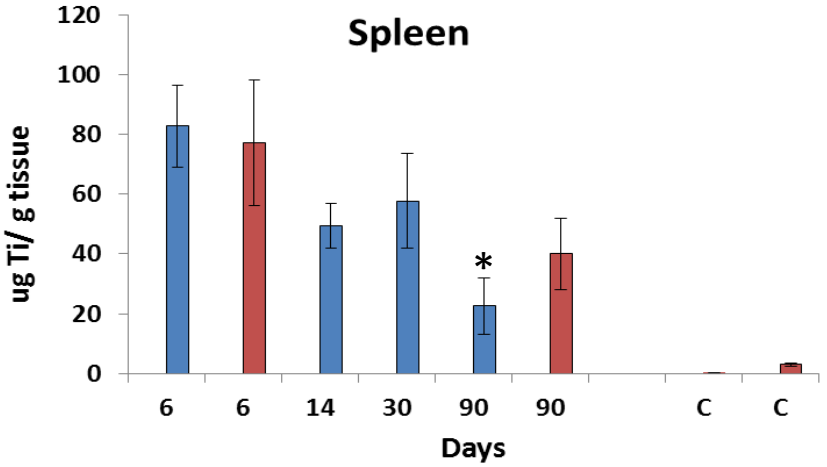


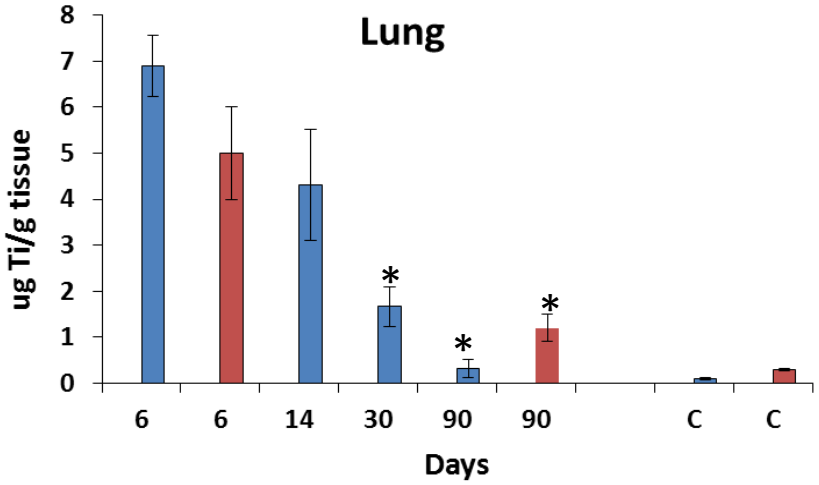


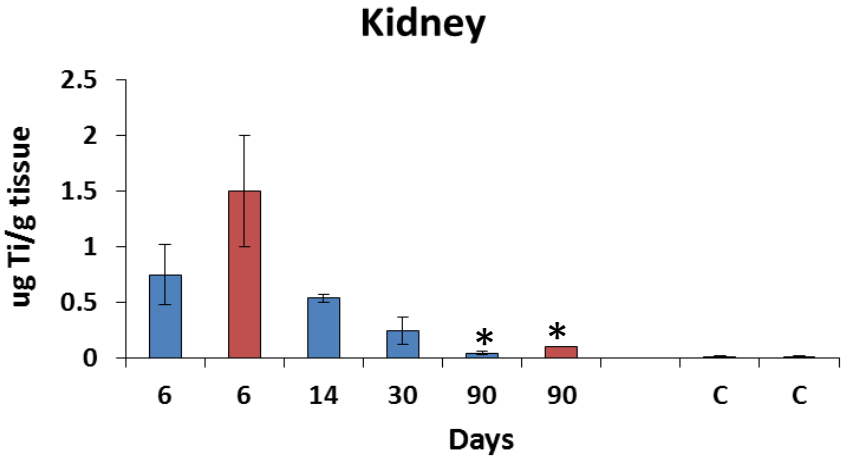
Co-funded by
the Health Programme
of the European Union

Tissue distribution of NM-105 after repeated (5x) IV administration









Co-funded by
the Health Programme
of the European Union

NM-105 Organ distribution as percentage of dose Repeated (5x) IV administration

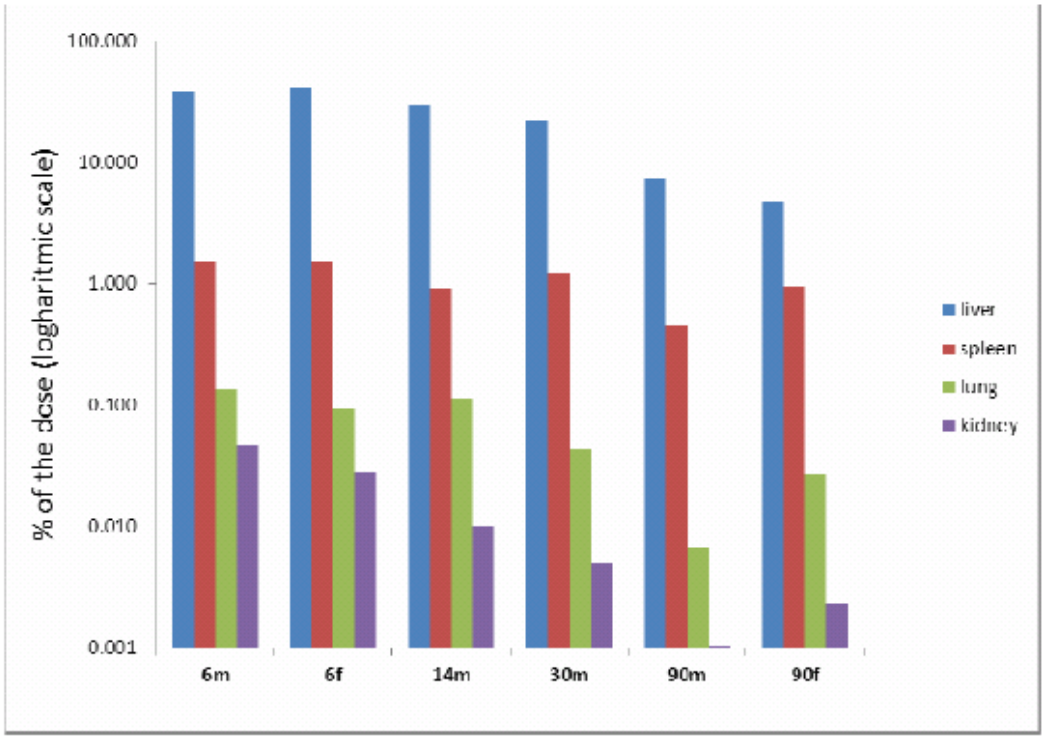
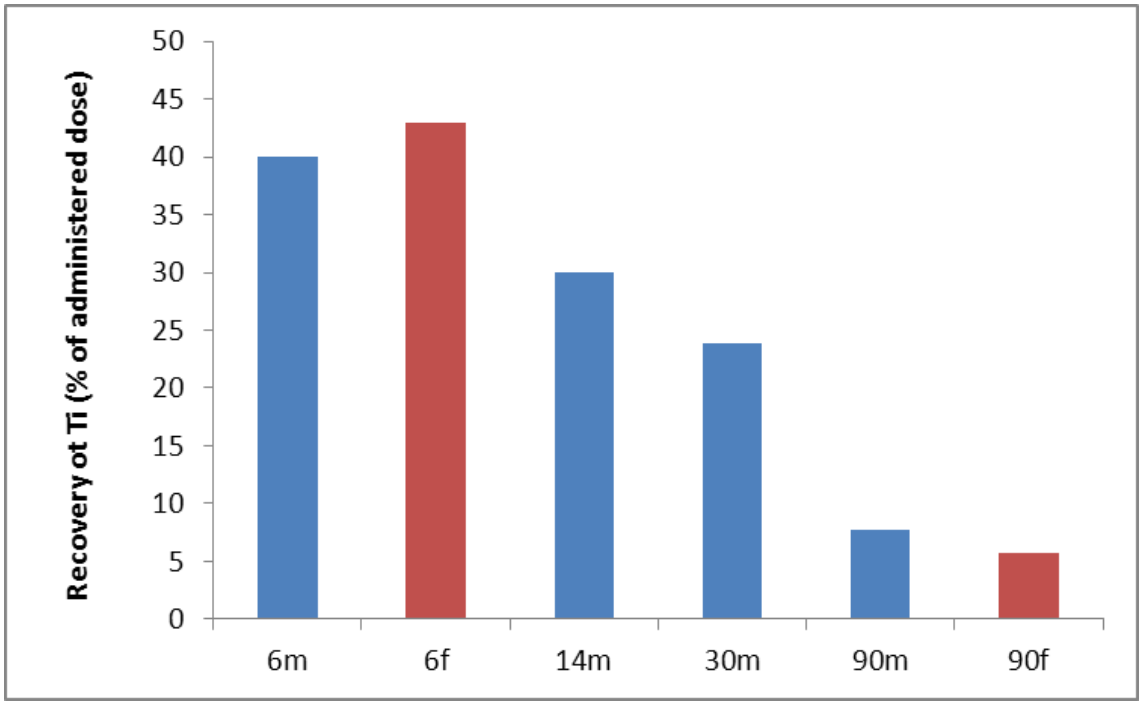


Figure 7. Organ distribution of NM-105 after 5 repeated i.v. dosing to male and female Wistar rats presented as percentage of dose measured on day 6, 14, 30, and 90. m: male rat; f: female rats.

Co-funded by
the Health Programme
of the European Union

NM-105 Recovery of Ti as percentage of dose

Grant agreement number 2009 21 01



Recovery of Ti in the investigated organs (liver, spleen, lung, and kidney) following 5 consecutive administrations of NM-105 to male and female Wistar rats presented as percentage of dose measured on day 6, 14, 30, and 90. m: male rat; f: female rats.

Co-funded by
the Health Programme
of the European Union

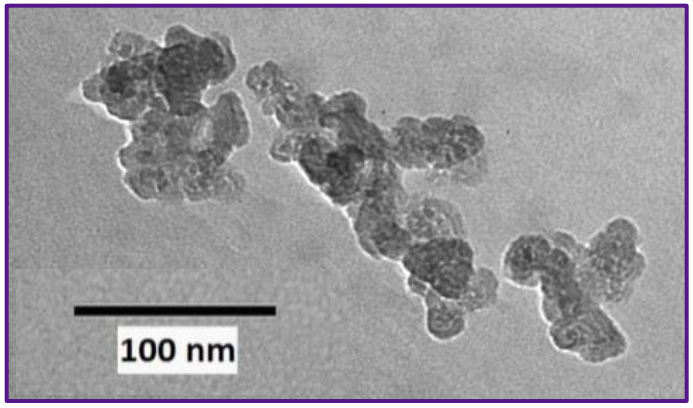
Conclusions TiO_2 tissue distribution measured as Ti

- Main target organs liver and spleen, and to a lesser extent lung and kidney
- Some reduction in Ti content in time, but Ti still present at day 90 after administration
- Repeated dosing (5x) results in fractional increase of Ti content
- NM-105 shows a clear decline in recovery at day 90, whereas for the other TiO_2 nanomaterials the decline at day 90 was limited.
- No excretion via faeces. Ti level in controls and IV treated animals similar (data not shown)

Co-funded by
the Health Programme
of the European Union

SAS (SiO_2)

Oral and IV administration of SAS



Co-funded by
the Health Programme
of the European Union

SiO_2 detection: novel analytical method

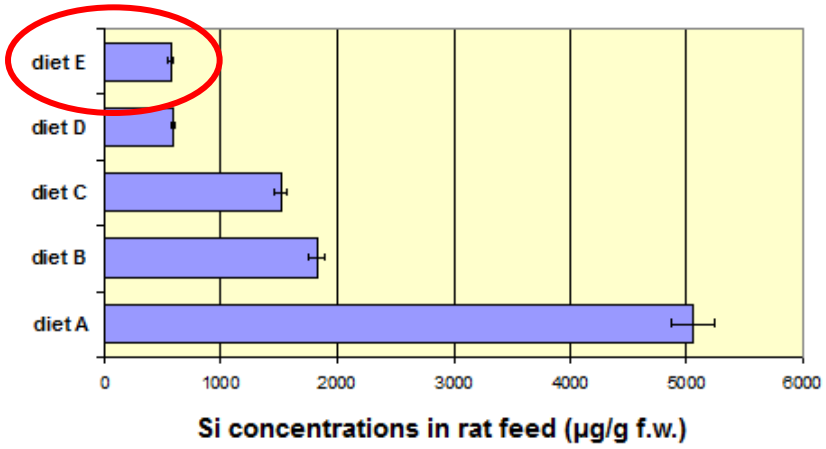
Detection method

- Sample preparation
  - Use of *clean room conditions*, *ultrapure reagents* and *non quartz (=SiO_2) vessels* to avoid Si contamination
- Analytical detection
  - SiO_2 MNs in tissues and biological fluids determined *as Si* by quadrupole ICP-MS, but Si determination by Q-ICP-MS *regarded as nearly impossible at sub-µg/g levels* owing to Si release from equipment and spectral interferences
  - Si release from equipment: the entire *sample introduction system* of the ICP mass spectrometer has been substituted with *non-quartz components*
  - Spectral interferences: ICP-MS measurements using *entirely novel analytical method* based on dynamic reaction cell technology (*J Anal Atom Spectrom* 27, 1540 2012)
- Quality control
  - No certified reference materials available → in house preparation at ISS of a *quality control material* to check accuracy of Si determination

Co-funded by
the Health Programme
of the European Union

SiO_2 detection: problems with background

- Lowering Si background in biological tissues
  - One of the main issues in measuring the concentration of administered SiO_2 MNs in tissues and biological fluids via Si determination is the high endogenous Si background in such matrixes
  - Si background concentration in tissues depends on the Si amount ingested *via* the diet. Different standard rat diets were analysed for their Si content



10-fold differences in Si content found

  - The diet with the lowest Si level was fed to the animals in the *in vivo* studies → Si background in rat tissues was reduced below the analytical LOQ

Co-funded by the Health Programme of the European Union

Tissue concentrations after repeated oral administration of SAS

- Cumulative dose 100 mg/kg body weight

Tissue distribution of Si in female rats after repeated oral dose of SAS nanoparticles (mg Si/kg fresh weight).

♀	Controls	NM-200		NM-203	
		Day 6	Day 14	Day 6	Day 14
Liver	0.6±0.1	1.3±0.3	1.3±0.2	0.8±0.2	0.3±0.1
Spleen	0.9±0.6	0.6±0.1	1.6±0.6	≤LOD	1.2 ±0.0
GI tract*	14.8±2.8	10.5±2.4	17.5±5.9	8.6±1.7	8.6 ±1.8
Mesenteric lymph nodes	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD

Tissue distribution of Si in male rats after repeated oral dose of SAS nanoparticles (mg Si/kg fresh weight).

♂	Controls	NM-200		NM-203	
		Day 6	Day 14	Day 6	Day 14
Liver	0.5±0.1	≤LOD	0.4±0.1	≤LOD	≤LOD
Spleen	0.6±0.4	≤LOD	0.7 ±0.2	0.9±0.2	0.7±0.1
GI tract*	18.9±6.9	10.5±2.4	19.8 ±1.6	9.6±4.7	14.3±10.0
Mesenteric lymph nodes	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD

Found concentration >LOD and ≤LOQ

* Small intestine

Co-funded by the Health Programme of the European Union

Tissue concentrations after single IV administration of SAS

Grant agreement number 2009 21 01

- Single IV dose 20 mg/kg body weight

Tissue distribution of Si in **female rats** (n=3) after single IV dose of SAS nanoparticles (mg Si/kg fresh weight).

♀	Controls	NM-200		NM-203	
		Day 2	Day 90	Day 2	Day 90
Liver	0.4 ±0.1	97.4±14.8	1.6±0.8	97.7±19.1	1.2±0.8
Spleen	≤LOD	39.9±6.1	0.6±0.1	78.7±22.0	0.7±0.5
Lungs	0.7±0.3	41.8±8.0	1.2±0.1	11.2±7.3	≤LOD
Heart	0.5±0.4	0.9±1.0	0.4±0.2	0.4±0.3	0.5±0.4
Brain	0.4±0.3	0.4±0.1	0.3±0.0	0.4±0.1	0.6±0.2
Kidneys	0.5±0.1	1.2±0.3	0.9±0.5	0.8±0.2	0.6±0.1
Ovaries	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD

Tissue distribution of Si in **male rats** (n=3) after single IV dose of SAS nanoparticles (mg Si/kg fresh weight).

♂	Controls	NM-200		NM-203	
		Day 2	Day 90	Day 2	Day 90
Liver	0.5 ±0.1	105.3±10.3	4.1±2.7	97.8±20.3	1.6±1.7
Spleen	≤LOD	39.0±15.0	1.1±1.5	237.3±28.9	≤LOD
Lungs	0.7±0.3	43.4±10.2	≤LOD	24.0±1.2	≤LOD
Heart	0.6±0.2	0.8±0.2	1.2±0.4	2.1±0.4	0.5±0.1
Brain	0.5±0.2	0.4±0.1	0.4±0.1	0.5±0.1	0.8±0.6
Kidneys	0.4±0.1	1.1±0.2	0.4±0.1	1.4±0.1	0.6±0.3
Testis	1.6±1.0	1.1±0.2	0.9±0.1	1.2±0.2	0.8±0.3

Found concentration >LOD and ≤LOQ

Co-funded by the Health Programme of the European Union

Tissue concentrations after repeated IV administration of SAS

- Cumulative IV dose 20 mg/kg body weight

Table 5-13. Tissue distribution of Si in male rats (n=3) after repeated IV dose of SAS nanoparticles (mg Si/kg fresh weight).

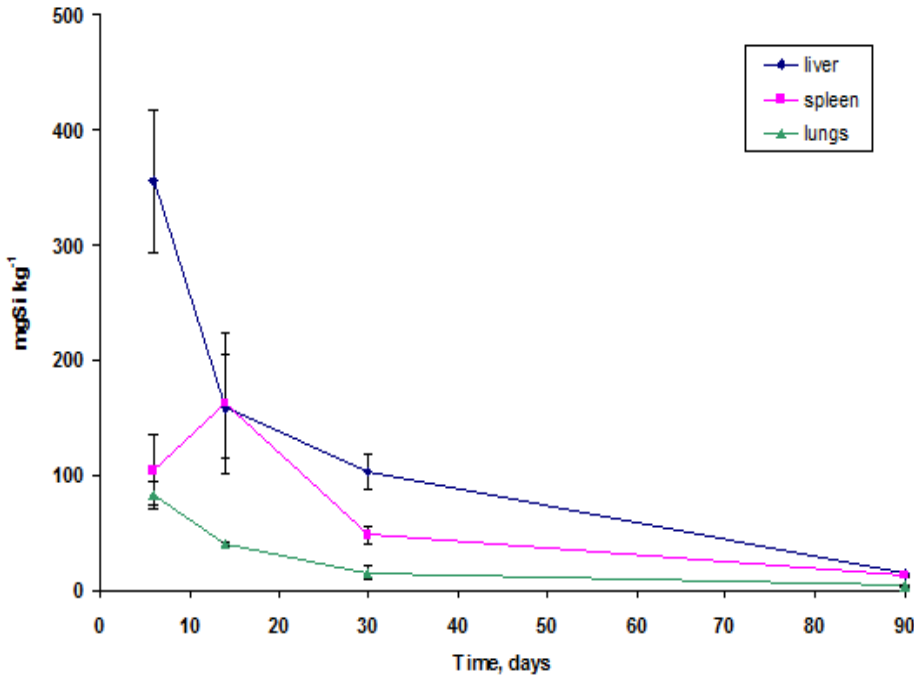
	Controls	NM-200				NM-203			
		Day 6	Day 14	Day 30	Day 90	Day 6	Day 14	Day 30	Day 90
Liver	0.4 ± 0.0	355 ± 62	159 ± 45	103 ± 15	14 ± 2	250 ± 29	100 ± 16	62 ± 10	11 ± 4
Spleen	≤LOD	105 ± 30	162 ± 61	48 ± 8	13 ± 1	357 ± 69	146 ± 27	77 ± 4	28 ± 9
Lungs	0.8 ± 0.3	82 ± 11	40 ± 2	16 ± 6	3.6 ± 0.8	66 ± 14	25 ± 3	11 ± 5	2.0 ± 0.4
Heart	0.6 ± 0.2	3.1 ± 1.2	2.7 ± 1.5	1.0 ± 0.3	0.9 ± 0.3	5.4 ± 3.5	3.7 ± 3.9	1.6 ± 1.3	1.8 ± 1.3
Brain	0.5 ± 0.2	0.5 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.6 ± 0.2	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Kidneys	0.4 ± 0.1	2.5 ± 0.4	0.7 ± 0.1	0.6 ± 0.0	0.5 ± 0.7	6.2 ± 1.2	3.3 ± 1.0	1.4 ± 0.4	0.6 ± 0.1
Testis	1.3 ± 0.3	1.4 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	1.7 ± 0.6	1.0 ± 0.1	0.7 ± 0.0	0.7 ± 0.1

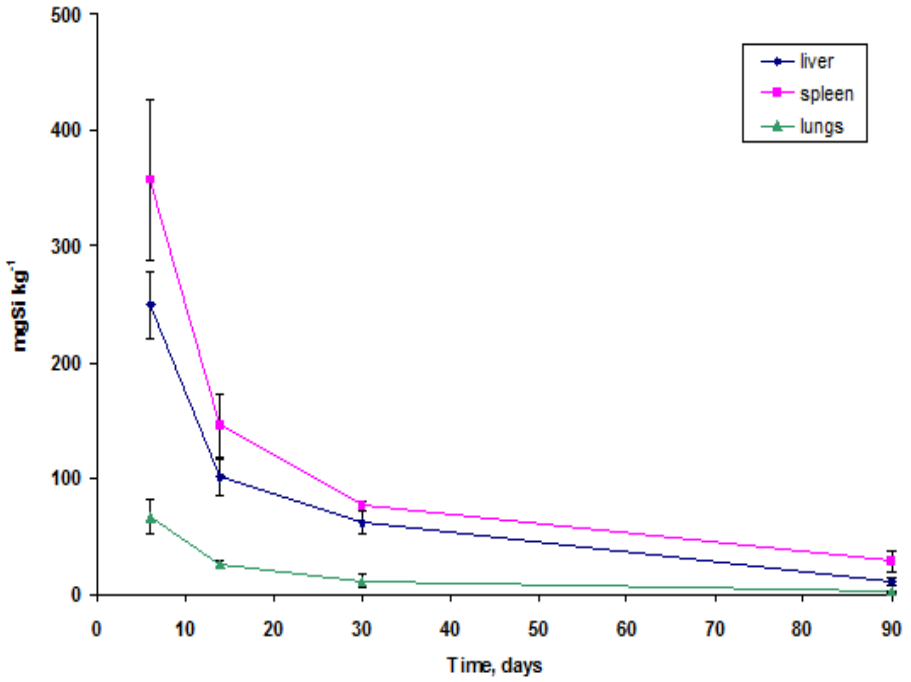
Found concentration >LOD and ≤LOQ

Co-funded by
the Health Programme
of the European Union

Repeated IV administration: differences between MNs

Grant agreement number 2009 21 01





Organ distribution after IV repeated dosing of **NM-200** (*left*) and **NM-203** (*right*) for 5 days to male Sprague-Dawley rats. Si levels in major organs are shown.

Co-funded by
the Health Programme
of the European Union

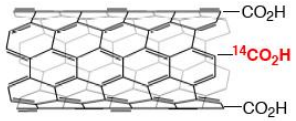
Conclusions: Si tissue distribution studies after oral or IV administration of SAS (SiO_2) nanoparticles

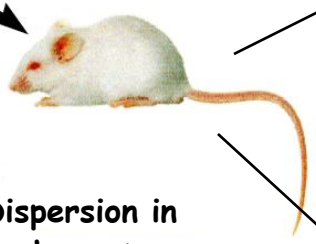
- Negligible to no accumulation of Si after **repeated oral administration**
- Single and repeated **IV administration** resulted in accumulation in various organs
  - After IV administration main target organs liver, spleen, and lung
  - Gender and particle differences were noted (NM-200 highest in liver, NM-203 highest in spleen)
  - Liver and spleen pathology after NM-203 administration
- Gradual decrease in organ levels over time

Co-funded by
the Health Programme
of the European Union

Biopersistence of MWCNT in rat after i.v. exposure (previous studies)

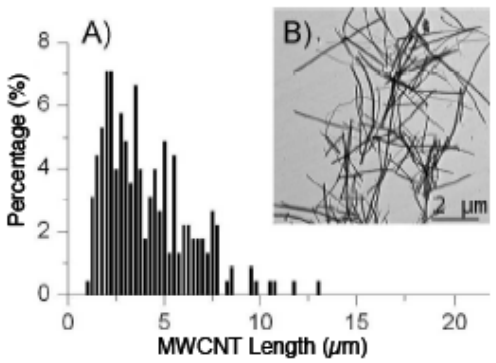
Dose: 0.35 mg (1.75mg/kg)

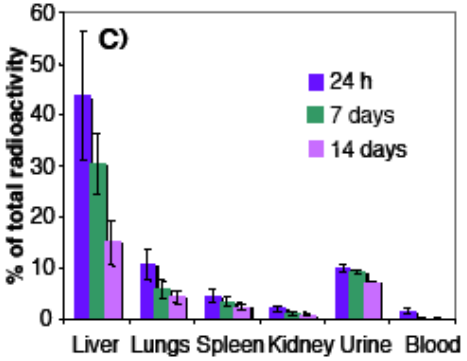


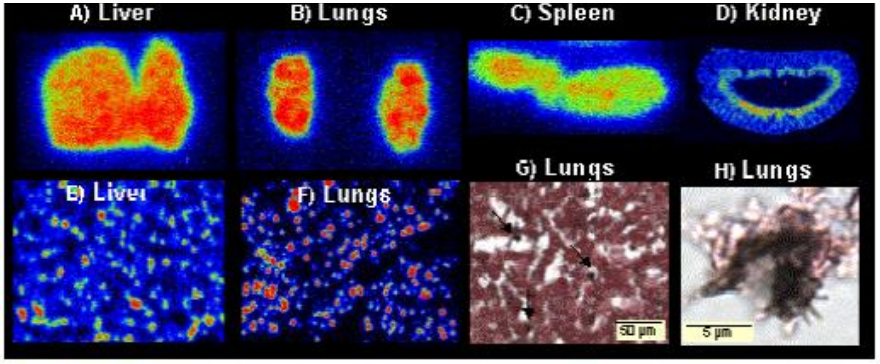


Dispersion in rat serum,

iv inj







Conclusions :

1°the sensitivity threshold will make possible to detect CNT 3 to 6 months after injection, biopersistence can be assessed.

2°aggregates are observed : formed during their dispersion in solution or after ?

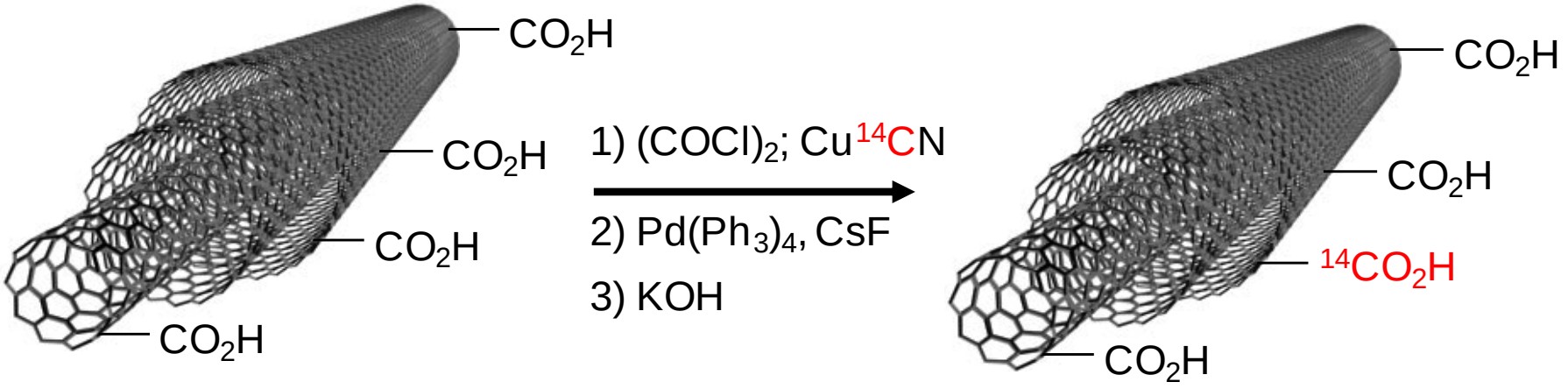
→

3° after 14 days, 10% of the injected dose can be detected in liver.

Georgin and al, JACS 2009, 131, 14658

Co-funded by the Health Programme of the European Union

Method CNT Specific labeling



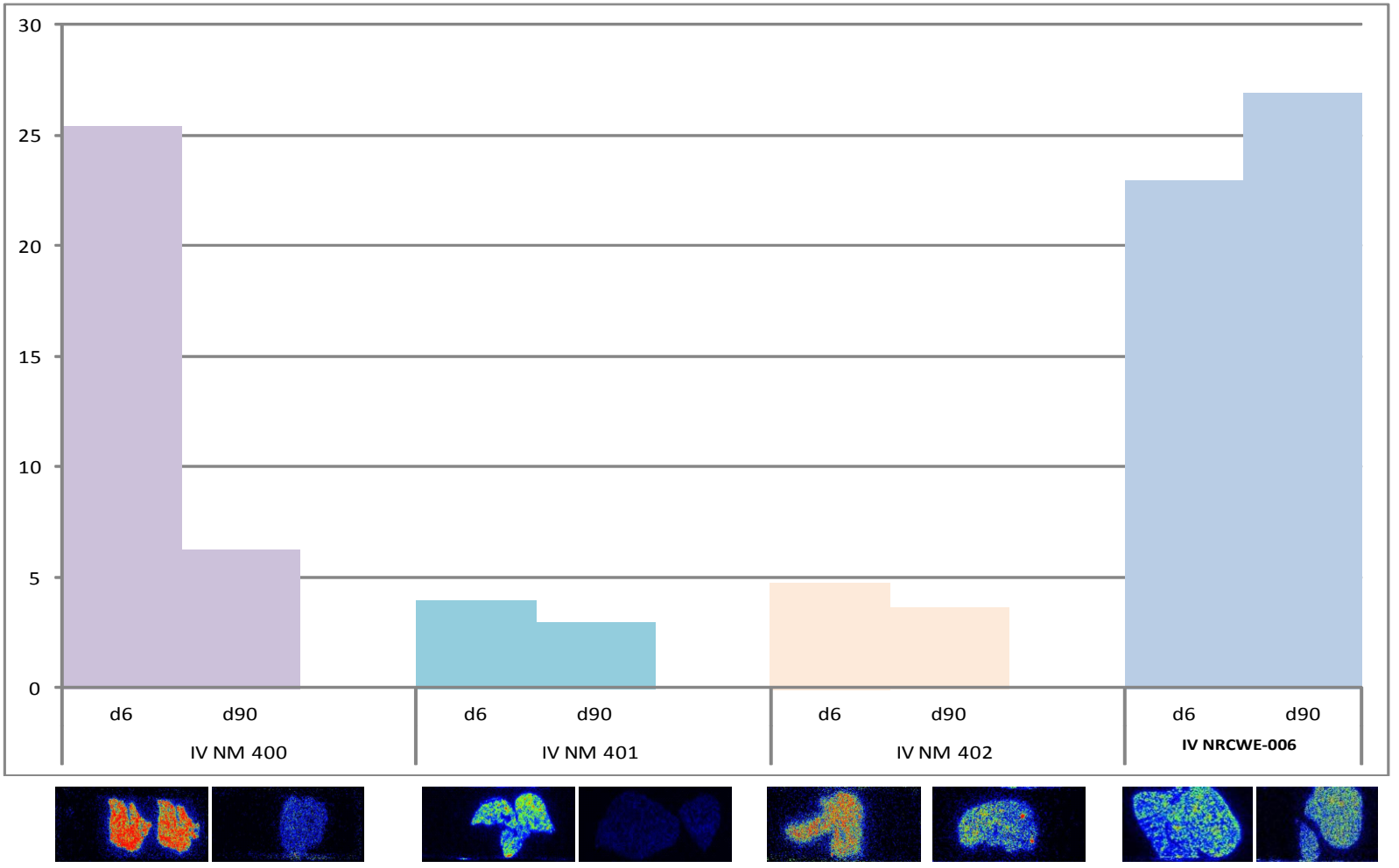
Scheme. Radiolabelling of NT batches

Co-funded by
the Health Programme
of the European Union

NANOGENOTOX

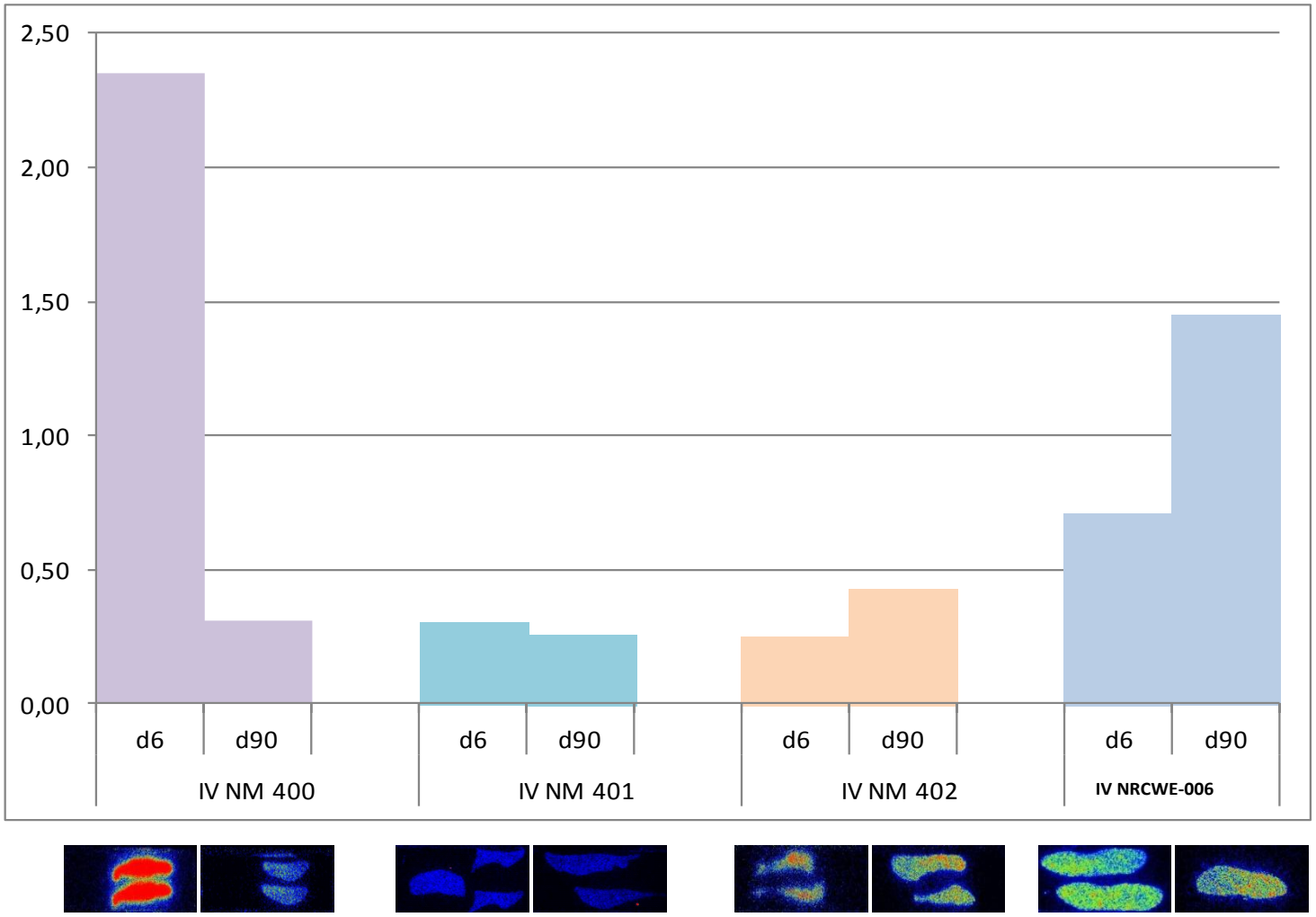
Grant agreement number 2009 21 01

^{14}C as % of dose in **LIVER** after IV administration of CNT in rats (males repeated dose)



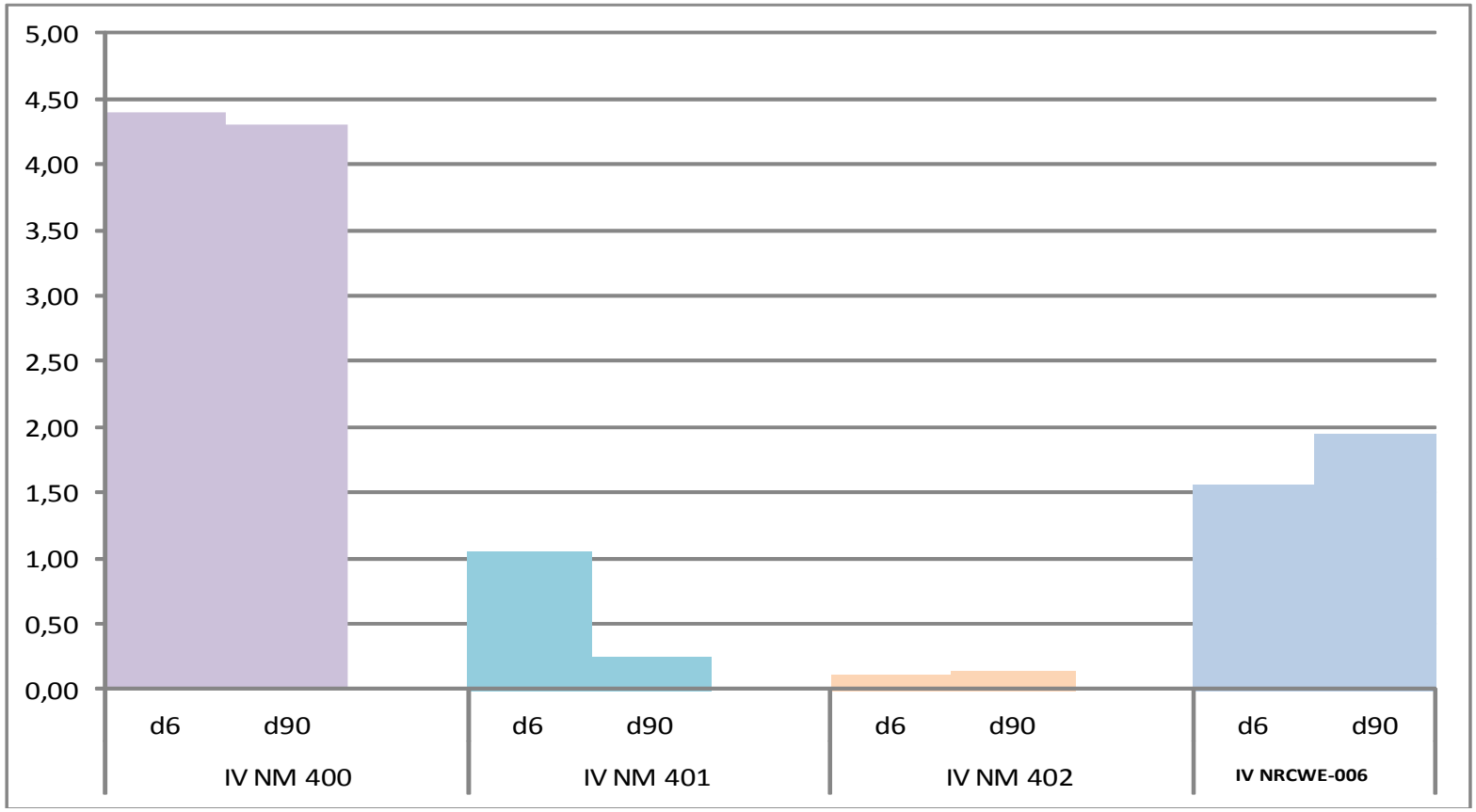
Co-funded by
the Health Programme
of the European Union

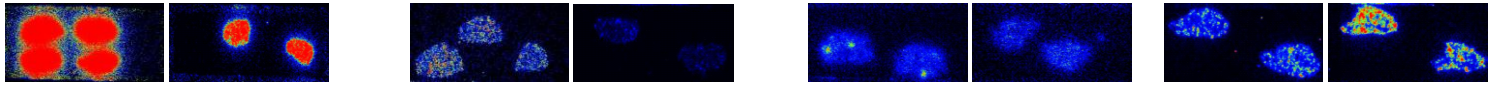
^{14}C as % of dose in **SPLEEN** after IV administration of CNT in rats (males repeated dose)



Co-funded by
the Health Programme
of the European Union

^{14}C as % of dose in **LUNG** after IV administration of CNT in rats (males repeated dose)





Co-funded by
the Health Programme
of the European Union

Recovery ^{14}C in major organs as % of dose (males repeated dose)

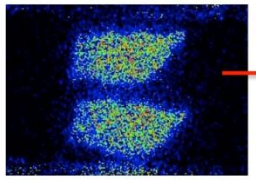
MWCNT	Day 6	Day 14	Day 30	Day 90
NM-400	39 ± 10	45 ± 21	20 ± 8	11 ± 4
NM-401	5.6 ± 3	6.9 ± 1.5	4.4 ± 0.6	3.6 ± 0.6
NM-402	5.3 ± 1.9	4.7 ± 0.5	4.1 ± 1.1	4.2 ± 1.9
NRCWE-006	26 ± 20	38 ± 6	42 ± 91	31 ± 10

Recovery is expressed as % of total dose administered.

Co-funded by
the Health Programme
of the European Union

Identification of ^{14}C radiolabelled MWCNT in spleen

Extraction of MWCNT (NRWE006) from organs and analyses by high resolution TEM

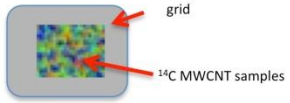


Indirect detection of MWCNT in spleen tissue sections by radioimaging

Extraction of ^{14}C MWCNT (NRWE006) from homogenized spleen

Samples spotting on TEM grid

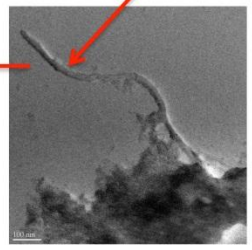
Radioimaging of grid



Red spots confirm the presence of « radioactive materials »

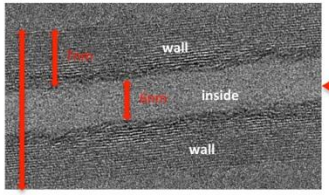
TEM

MWCNT ?????



High resolution TEM

MWCNT Yes



∅ = 20 nm

high resolution TEM analyses identify MWCNT:
CNT walls are clearly visible, as well as the inside of CNT, giving access to diameter size of CNT (20 nm)

Co-funded by the Health Programme of the European Union

Conclusions CNT tissue distribution

- No uptake after oral administration
- Liver, spleen and lung were the main target organs (IV administration)
  - Presence of radiolabelled MWCNT identified with TEM
- Major differences were noted between the 4 investigated CNT nanomaterials
  - NM-400 decrease in liver and spleen day 6-day 90, not in lung
  - NM-401 minimal decrease day 6-day 90
  - NM-402 and NRCWE-006 no decrease day 6 - day 90

Co-funded by
the Health Programme
of the European Union

General conclusions tissue distribution TiO_2, SAS (SiO_2) and CNT nanomaterials

- Maximum dose as prepared according to dispersion protocol of WP (from 10 to 20 mg/kg bw) is generally well tolerated by the animals
- Main target organs liver and spleen, followed by lungs and kidney after IV administration
- Low if any absorption of MN from the GI-tract
- In general there is a decrease in organ levels over time, but for some TiO_2 MN it is a rather minimal decrease with suggestion for persistence
- Differences between TiO_2 MNs investigated are minimal with the exception of the decrease in organ concentrations of NM-105
- For SAS (SiO_2) there is a clear decrease in time in liver, spleen and lungs
- Differences between SAS MNs investigated were noted (toxicity)
- Some clear differences can be noted between the different MWCNT

Co-funded by
the Health Programme
of the European Union

WP7 Partners involved

- AFFSA-MA (ANSES), France
  - Thierry Guérin, Laurent Noël, Yacine Nia
- AFFSA-F (ANSES), France
  - Michel Laurentie
- BfR, Germany
  - Jutta Tentschert
- CEA, France
  - Vincent Dive, Frédéric Taran, Olivier Spalla, Bertrand Czarny
- IMB-BAS, Bulgaria
  - Margarita Apostolova, Irina Karadjova, Julian Kirilov, Nina Kaneva
- INERIS, France
  - Benedicte Trouillier
- ISS, Italy
  - Francesco Cubadda, Francesca Maranghi, Federica Aureli
- NRCWE, Denmark
  - Nicklas Raun Jacobsen, Håkan Wallin
- RIVM, The Netherlands
  - Wim De Jong (WP leader), Esther Brandon, Agnes Oomen

Co-funded by
the Health Programme
of the European Union

Grant agreement number 2009 21 01

Discussion regarding WP 7

Elias Fattal
Institut Galien Paris-Sud/UMR CNRS 8612
Université Paris Sud
Châtenay-Malabry

Co-funded by
the Health Programme
of the European Union

Main Observations

- The choice of IV administration raises the question of relevance of the route of administration and the choice of the dispersing medium the question of impact on distribution
- Liver and Spleen are always target tissues whatever the nanoparticles (this is classical but bone marrow should have been explored as well)
- Silica nanoparticles are the only particles that are cleared whereas accumulation and persistence seems to occur for all other types
- Oral administration show low level of uptake but Peyers patches should be explored

Co-funded by
the Health Programme
of the European Union

Recommandations

- Explore deeply cellular fate of the diverse nanoparticles after liver uptake (Küpffer cells or hepatocytes)
- Explore if hepatocytes functions are modified in, case of hepatocytes uptake
- The persistence of materials raises the question of carcinogenicity

Co-funded by
the Health Programme
of the European Union