DISTRIBUTION AND BIOLOGICAL EFFECTS OF FULLERENE C_{60}, TITANIUM DIOXIDE, AND SILVER NANOPARTICLES AFTER SINGLE AND MULTIPLE INTRAGASTRICAL ADMINISTRATIONS TO RATS

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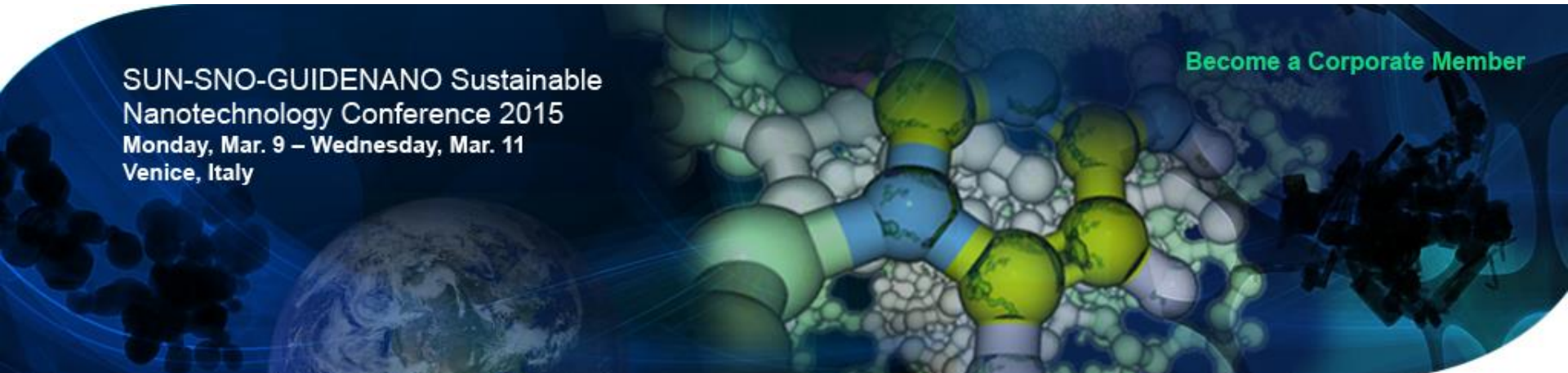


Contents

- ✓ ***Engineered nanomaterials*** **(ENM) used in the study**
- ✓ ***Analytical*** **methods for determination of NPs localization**
- ✓ ***Design*** **of *in vivo* acute and sub-chronic toxicological experiments**
- ✓ ***Biodistribution*** **of nanoparticles in organs and tissues**
- ✓ ***Biological effects*** **of ENMs:**
  - ❖ *animal lethality, state of the treated animals, general appearance, activity, behavior;*
  - ❖ *body weight, food and water intake;*
  - ❖ *dynamics of biochemical parameters and hematological indices;*
  - ❖ *pathomorphological analysis of the internal organs.*
- ✓ ***Conclusions***

Cooperation with EU in nanosafety studies

A.N. Bach Institute of Biochemistry, Moscow, Russia

→ «Toxicokinetics and organ toxicity and dose-response models using selected ENMs»

→ «Development of models for prediction of potential risks through SAR and QSAR, PBPK-PB and Monte Carlo»

↑

Project of the 7th Framework Program of EU

«Managing Risks of Nanomaterials» («MARINA»), 2011-2015

MARINA

MANAGING RISKS OF NANOMATERIALS

SEVENTH FRAMEWORK PROGRAMME

Engineered nanomaterials used in the study

OECD WPMN priority list of 13 ENMs as a representative set of reference compounds

- Single-walled & multi-walled carbon nanotubes
- Gold NPs
- Iron NPs
- Aluminium oxide
- **Fullerenes (C_{60})**
- **Silver NPs**
- **Titanium dioxide**
- Cerium oxide
- Zinc oxide
- Silicon dioxide
- Dendrimers
- Nanoclays

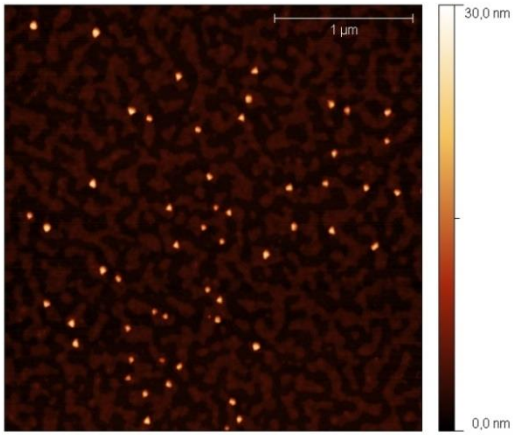
Selected due to:

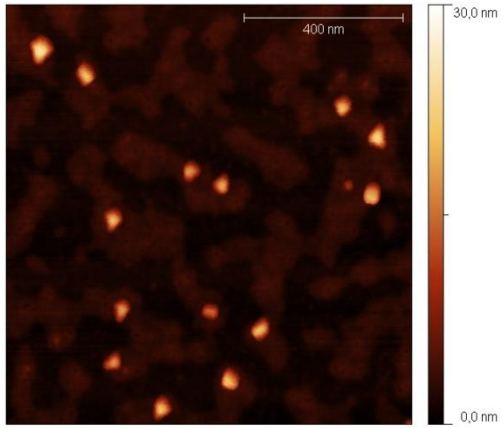
- large-scale production
- wide commercial use
- expected or demonstrated *in vivo* or *in vitro* biological effects

ENM characterization

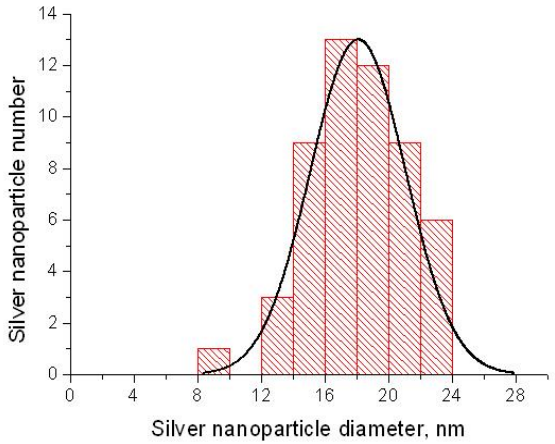
Silver NPs: by Atomic Force Microscopy

AFM images at scanning square 3 x 3 nm or 1 x 1 nm



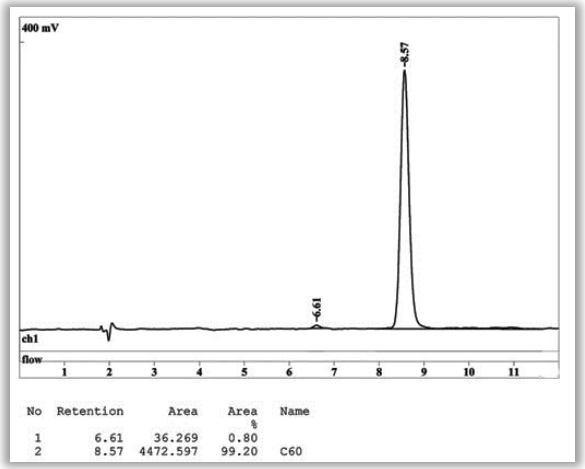


Size distribution of silver NPs:
Average diameter = 18.1± 3.0 nm



Silver NPs (20 nm) of 99.9 % purity from Nanocs, cat. no. SNP20-20.

Fullerene C_{60} NPs: by High Performance Liquid Chromatography



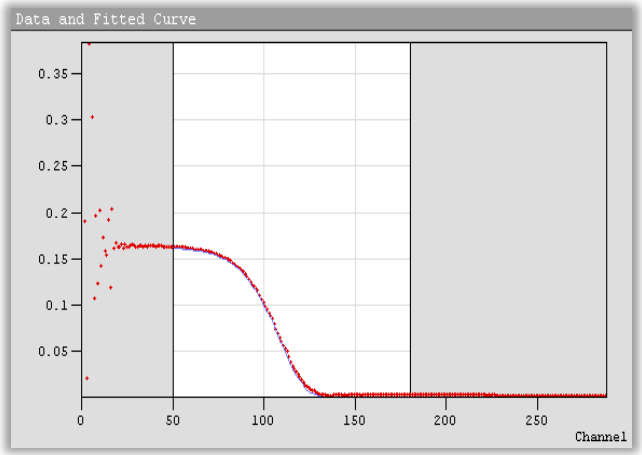
Fullerene C_{60} of 99.95 % purity from SES Research, cat. no. 600-9980

Main fraction (C_{60}) – 99.2%

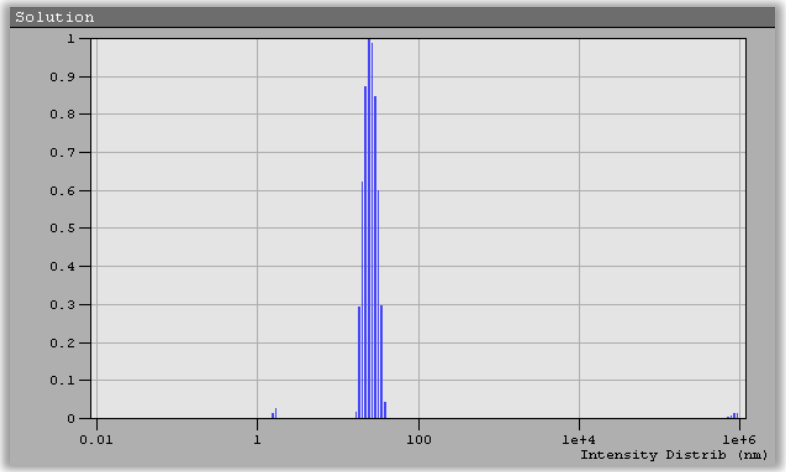
ENM characterization

Titanium dioxide NPs: by Dynamic Light Scattering and Transmission Electron Microscopy

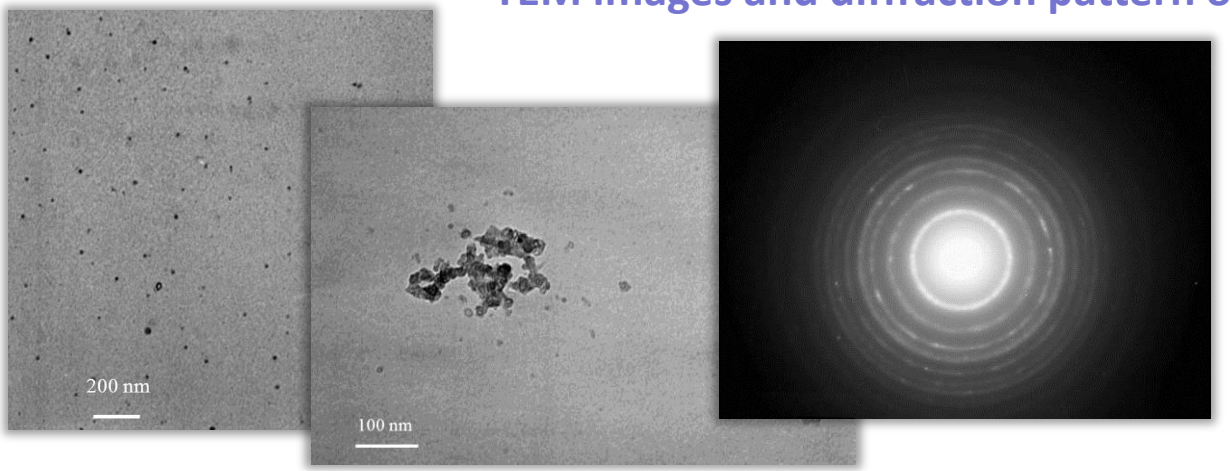
Autocorrelation function for DLS measurements of hydrodynamic radius



Size distribution of TiO_2 NPs



TEM images and diffraction pattern of TiO_2 NPs



Titanium dioxide NPs (anatase, <25 nm) of 99.7% purity from Sigma-Aldrich, cat. no. 637254.

Average diameter by DLS = 55.5 ± 4.7 nm

Analytical methods used in the study of ENM localization

Atomic absorption spectroscopy (AAS)

- **SILVER NPs**
- **TiO_2 NPs**

Atomic absorption spectrometer AAnalyst 800, «Perkin Elmer», USA

High performance liquid chromatography

- **FULLERENE**

Chromatograph STAYER, «Aquilon», Russia

Design of the *in vivo* experiment

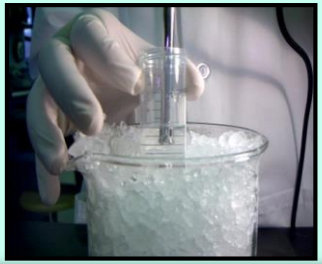
Male rats were daily intragastrically administered with TiO_2 NPs or Silver NPs or Fullerene C_{60} via gavage at dose of 2000 mg/kg of body weight during the period of 1 day (acute toxicity experiment) and 250 mg/kg b.w. during 30 days (sub-chronic toxicity experiment)

Determination of maximum tolerated dose (adult outbred mice)						
Group	Amount of mice, males/ females	Dose, mg/kg b.w.	Times of administra-tion	Recovery period	Day of euthanasia	Parameters studied
1	6/6	control	1	14	15	Lethality; State of animals; Body weight; Food and water intake; General appearance; Activity; Behavior; External manifestations of toxicity
2	6/6	1000				
3	6/6	2000				
4	6/6	3000				
5	6/6	4000				
6	6/6	5000				
Single administration (adult Sprague-Dawley rats)						
1	18/0	control	1	14	1^{st} 7^{th} 14^{th} of the recovery period	ENM content in organs and tissues + biochemical and hematological parameters
2	6/0	2000				
3	6/0	2000				
4	6/0	2000				
Multiple administrations (adult Sprague-Dawley rats)						
1	18/0	control	30	no	7^{st} 18^{th} 30^{th}	ENM content in organs and tissues + biochemical and hematological parameters
2	6/0	250				
3	6/0	250				
4	6/0	250				

Scheme of biomaterial treatment before AAS and HPLC detection of TiO_2, Ag and C_{60} nanoparticles

Biological samples

Homogenization



Chemical decomposition of biomatrix: low pH; high t; high P

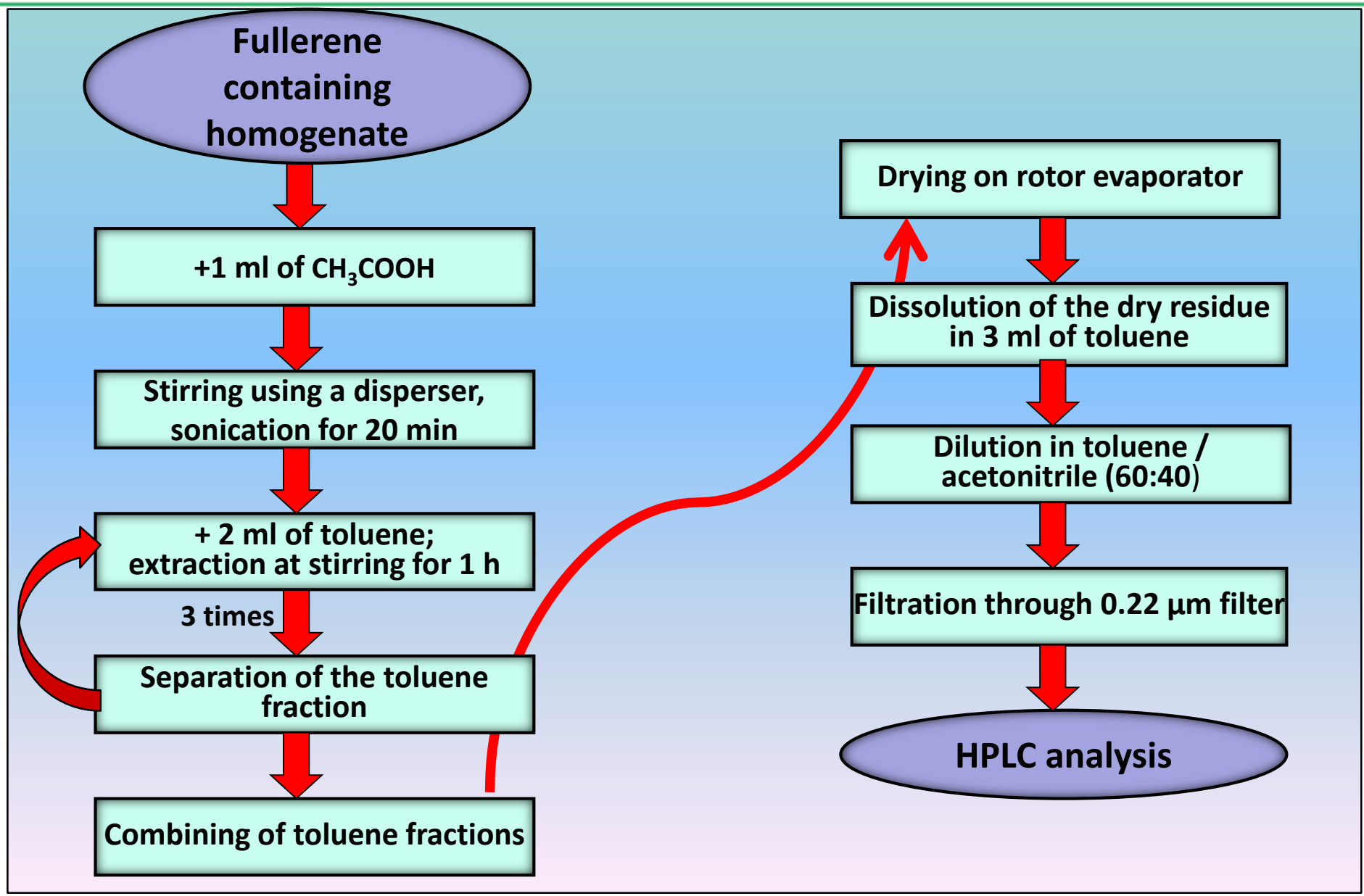
Liquid-liquid extraction

TiO_2 and silver detection by Atomic Absorption Spectroscopy

Fullerene C_{60} detection by High Performance Liquid Chromatography

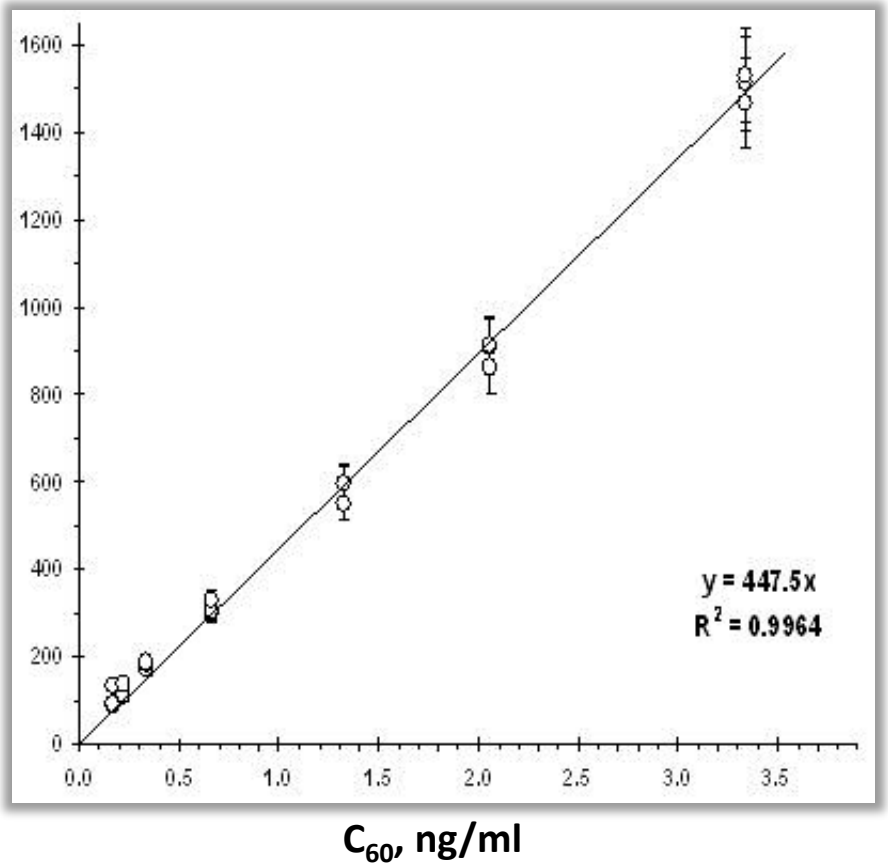
Procedure of C_{60} extraction from biological material:

to increase fullerene recovery & reduce the duration of biosample treatment



Percentage of NPs recovery in biomaterial prior to detection in real samples

C_{60} calibration curve by HPLC



Percentage of recovery by comparing the amount of NPs "pre-added" to homogenates and revealed by HPLC

Organ or tissue	Detected fullerene C_{60}, %
Lung	88.5±3.8
Liver	78.4±4.6
Kidneys	83.5±5.1
Spleen	84.5±4.3
Adrenal glands	87.1±4.4
Brain	85.7±4.4
Testicle	88.7±4.6
Stomach	91.4±5.2
Small intestine	91.3±5.5
Heart	89.3±3.4
Thymus	91.3±2.1
Skin	92.1±2.3
Adipose tissue	93.6±4.5
Muscle tissue	89.9±4.7
Blood serum	91.2±4.2

Biodistribution of silver nanoparticles in organs and tissues detected by AAS after single administration (dose – 2000 mg/kg b.w.) and multiple administrations (dose – 250 mg/kg b.w.)

ORGAN OR TISSUE	CONTENT OF SILVER NANOPARTICLES (µg/g of organ or tissue)					
	SINGLE EXPOSURE			MULTIPLE EXPOSURES		
	DAY OF SAMPLING					
	1st	7th	14th	7th	18th	30th
Lungs	*n/f**	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*
Liver	*n/f*	0.86±0.08	*n/f*	*n/f*	0.11±0.01	0.12±0.01
Kidneys	*n/f*	0.63±0.07	*n/f*	0.24±0.01	0.24±0.01	0.23±0.01
Spleen	*n/f*	0.07±0.01	*n/f*	*n/f*	0.18±0.01	0.18±0.01
Adrenal glands	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*
Brain	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*
Testicles	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*
Stomach	0.11±0.01	*n/f*	*n/f*	0.02 ±0.001	0.02±0.001	0.02±0.001
Small intestine	0.22±0.01	0.14±0.01	*n/f*	0.02 ±0.001	0.02 ±0.001	0.02 ±0.001
Heart	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*
Thymus	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*
Skin	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*
Adipose tissue	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*
Muscle tissue	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*	*n/f*

**n/f – not found*

The amount of the detected silver in comparison to the administered doses is the evidence of its efficient excretion

Biodistribution of fullerene C_{60} and titanium dioxide nanoparticles in organs and tissues after acute and sub-chronic toxicity experiment

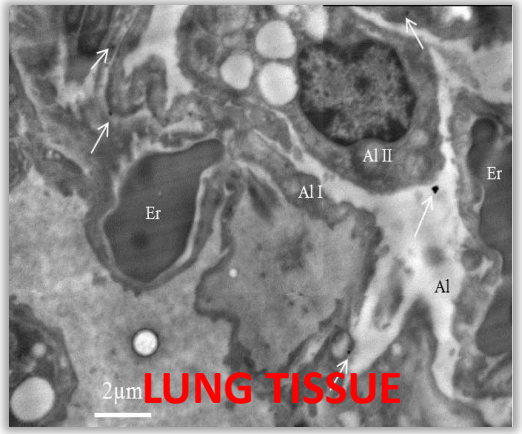
DAY OF THE RECOVERY PERIOD AFTER ADMINISTRATION	*SINGLE ADMINISTRATION* (2000 mkg/kg b.w.)	
	FULLERENE C_{60} NANOPARTICLES	TiO_2 NANOPARTICLES
1st	LUNGS; STOMACH; SMALL INTESTINE	SPLEEN; SMALL INTESTINE
7th	LIVER; KIDNEYS; SPLEEN	LIVER; KIDNEYS; SPLEEN; STOMACH; SMALL INTESTINE
14th	*NO (total excretion)*	NO *(total excretion)*
DAY OF ADMINISTRATION	*MULTIPLE ADMINISTRATIONS* (250 mkg/kg b.w.)	
7th	LIVER; KIDNEYS; SPLEEN; SMALL INTESTINE	LIVER; KIDNEYS; SPLEEN; SMALL INTESTINE
18th	LIVER; KIDNEYS; SPLEEN; SMALL INTESTINE	LIVER; KIDNEYS; SPLEEN; SMALL INTESTINE
30th	LIVER; KIDNEYS; SPLEEN; SMALL INTESTINE	LIVER; KIDNEYS; SPLEEN; SMALL INTESTINE

The majority of NPs (>99%) were not absorbed in gastrointestinal tract and excreted from rats!

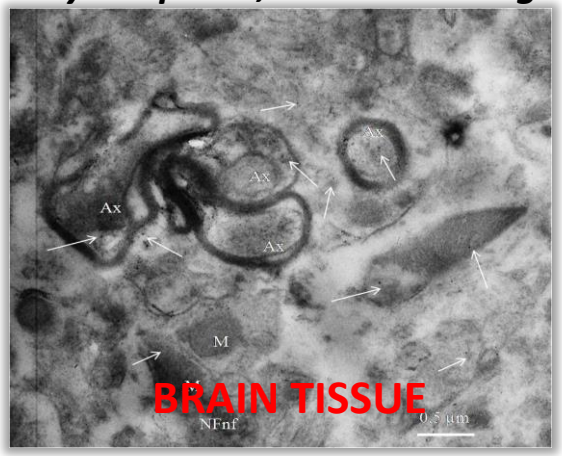
TEM observation of ultrathin sections of organs and tissues after exposure to TiO_2

After single administration NPs were examined:

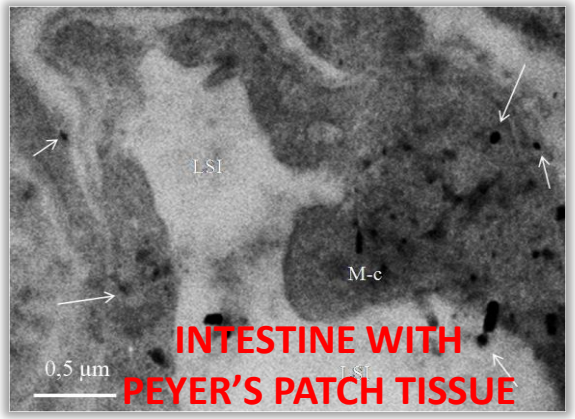
in mucosal epithelium of small intestine; in liver parenchymal tissue; in red and white pulp of spleen; in Payer's patch, brain and lung tissues



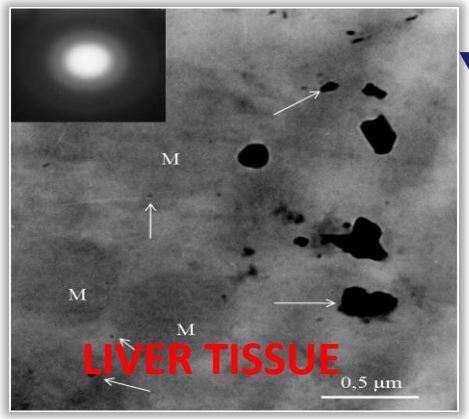
- in alveolocytes; - in alveolar cavities;
-in blood capillaries;
-in erythrocytes;
- in alveolar macrophages



-In myelinated axons;
- in nerve fibers;
-in blood capillaries;
-in erythrocytes;
- in lymphocytes

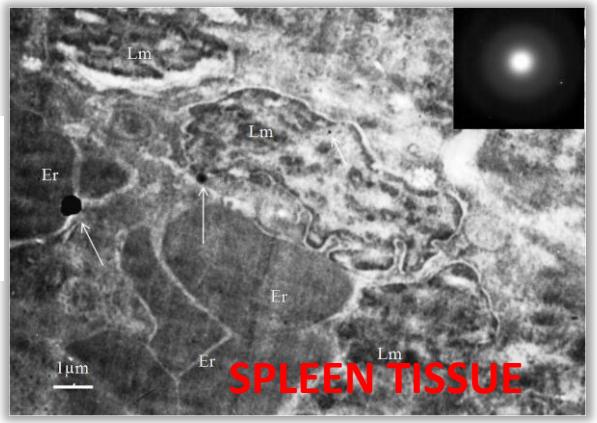


-in enterocyte microvilli;
- in blood vessel;
-in erythrocytes;
-in underlying mucosal tissue



- in hepatocytes;
-In blood vessel;
-- in erythrocytes

- in blood elements;
-In blood vessel;
- in hyaloplazm



Aggregates of ≥100 nm

Observation of ultrathin sections by TEM: morphological changes in cells

✓ **NPs** localized in intracellular environment: in nuclei, on endoplasmic reticulum membranes, in mitochondria, lysosomes, in cytoplasm, etc.;

✓ **NPs** caused morphological changes in all structures (except those of brain and lung tissues);

✓ **Cell** structures responsible for the energy metabolism and the protein-synthesis function were the most vulnerable and susceptible to negative effects

Summary of ENMs biological action

After single and repeated-dose toxicity experiment:

- **no** animal mortality, toxicity signs, substantial behavior or motor deviations was recorded throughout the observation periods;
- **the** body weights, food and water consumption, absolute weights of internal organs did not vary for the treated and control animals;
- **no** statistically significant differences in biochemical parameters and hematological indices were found for control and treated rats;
- **necropsy** of internal organs revealed no visible pathomorphological changes.

Overall conclusions

- **singly or multiply administered silver, TiO_2 and C_{60} NPs absorbed from gastrointestinal tract with infiltration into the bloodstream and translocation into secondary organs (liver, spleen, kidneys, etc.) with *no pronounced toxic effects on the macroorganism level;***
- **the amounts of NPs accumulated in tissues were comparable for acute and sub-chronic experiments;**
- **the amounts of NPs detected in organs and tissues were far smaller than the administered doses that was the indication of their efficient excretion;**
- **NPs localized in many intracellular structures and were responsible for some morphological changes in them.**

Acknowledgements

MARINA
MANAGING RISKS OF NANOMATERIALS
SEVENTH FRAMEWORK PROGRAMME

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