

Subchronic Inhalation Toxicity Evaluation of silver nanoparticles

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Backgrounds

- Wide use in silver nano products due to its anti-microbial activity in Korea
- Increasing exposed population
- No definite data on silver nano aerosol inhalation
- Applicability of current occupational exposure levels of silver particles to silver nanoparticles

Silver nano applications in Korea

- Bedding
- Washer
- Water purification
- Tooth paste
- Shampoo
- Rinse
- Nipple & Nursing bottle
- Fabrics
- Socks
- Deodorant
- Filter
- Kitchen utensils
- Toy
- Mattress
- Humidifier

Numbers of products associated with specific materials

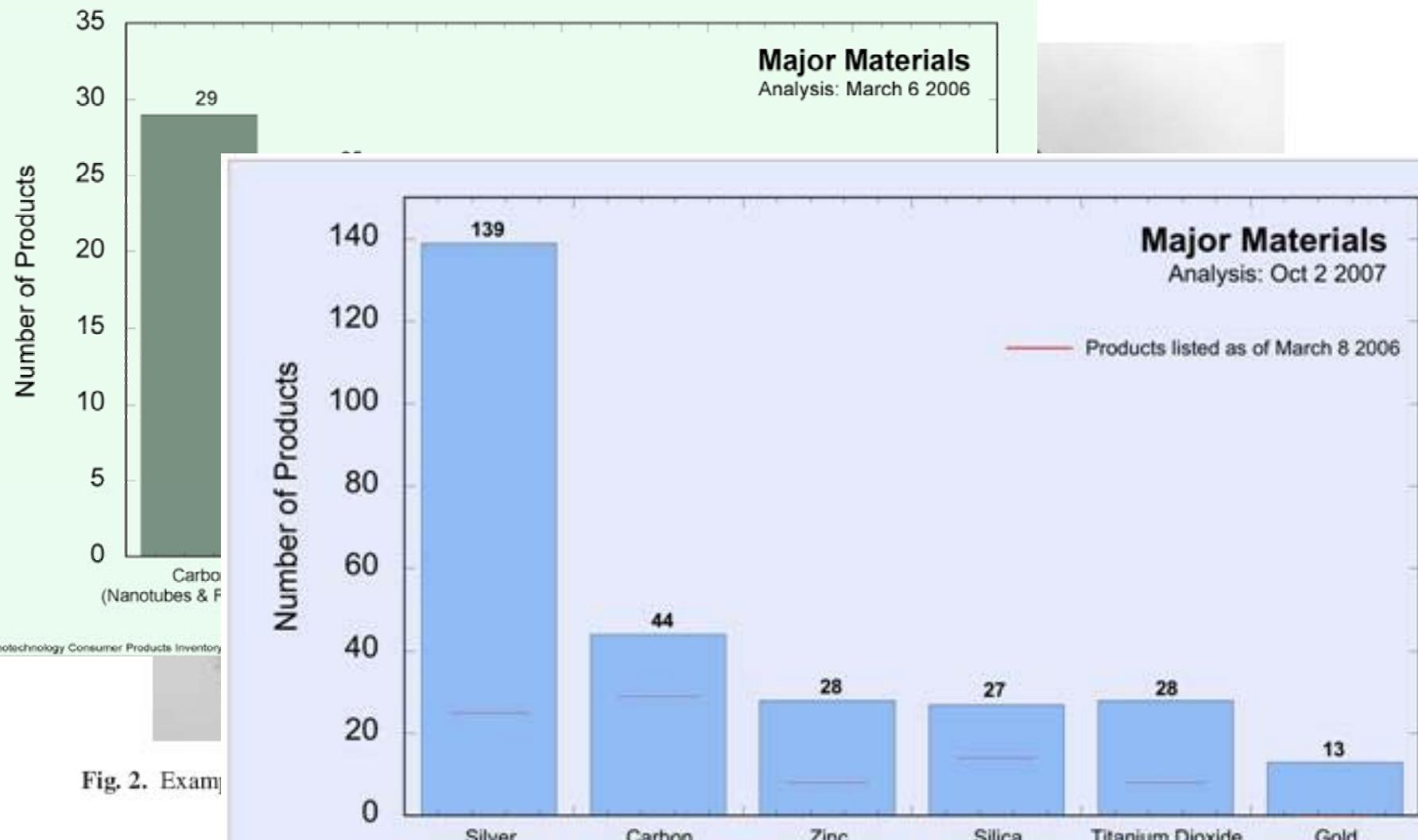
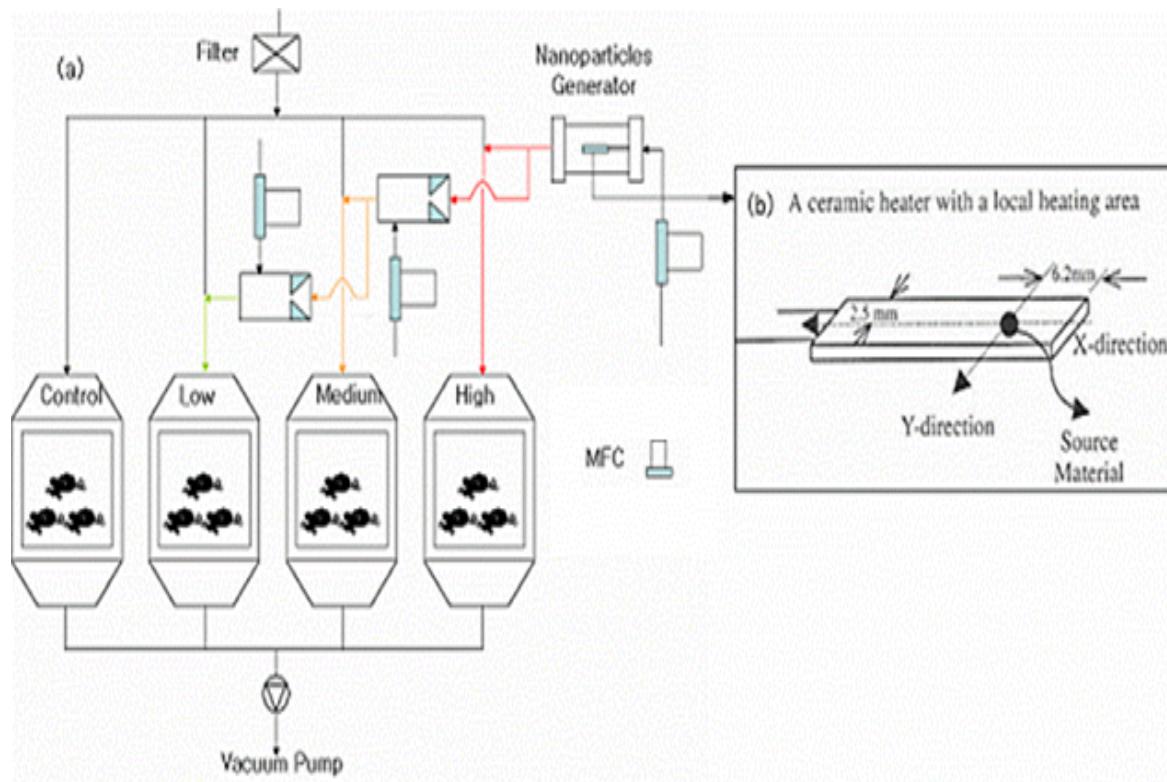


Fig. 2. Exam

Mayna

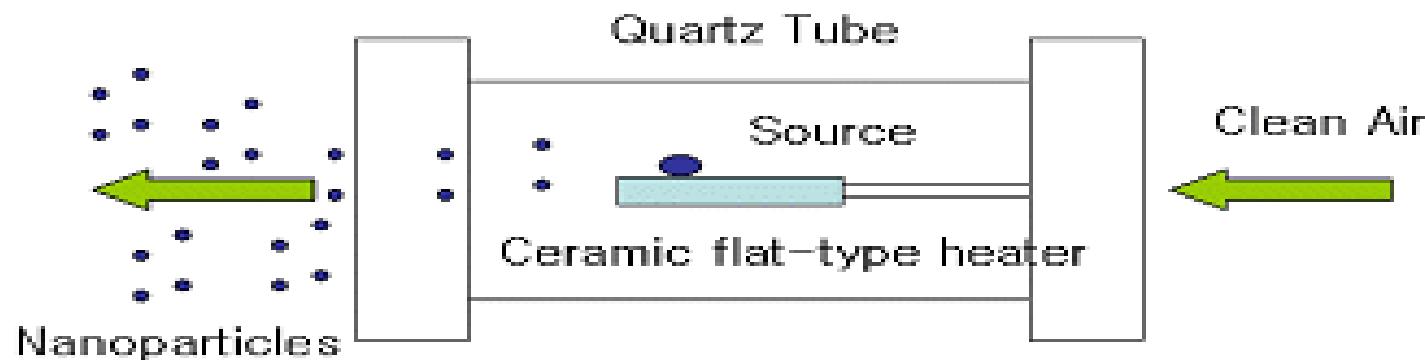
90 day inhalation toxicity
study

Silver nanoparticle generation method



Silver nanoparticle generation

- Silver nanoparticle generator (ISO 10801)



- Based on OECD Guideline for Testing of Chemicals
No. 413 'Subchronic Inhalation Toxicity: 90-day Study'

Silver nanoparticle exposure condition

Route	Inhalation
Dose	High : $3.0 \pm 0.2 \times 10^6$ particles/cm³ Middle : $1.5 \pm 0.2 \times 10^6$ particle/cm³ Low : $0.7 \pm 0.2 \times 10^6$ particle/cm³
Duration	6 hr/day, 5 day/week, 90 days
Animal	10 male SD rat / dose group 10 female SD rat / dose group
Test substance	Silver nanoparticles

Test substance characterization

- Dose monitoring : Nanoparticle monitoring method (ISO 10808)
- Nanoparticle distribution (ISO 10808) : diameter, number, surface area, volume : DMAS. CPC
- Shape, CMD, Purity: TEM-EDX (ISO 10808)

Evaluation items

- Body weight
- Food consumption : 1 / week
- Eye : Ophthalmoscope
- Urin : Urisachn strip 10 test, Urine analyzer
- Necropsy :
 - Organ weight : Brain, thymus, lung, heart, liver, spleen, kidney, adrenal, testis, ovary, olfactory bulb
- Blood biochemistry
- Hematology
- Coagulation test
- Histopathology

Lung Function Test

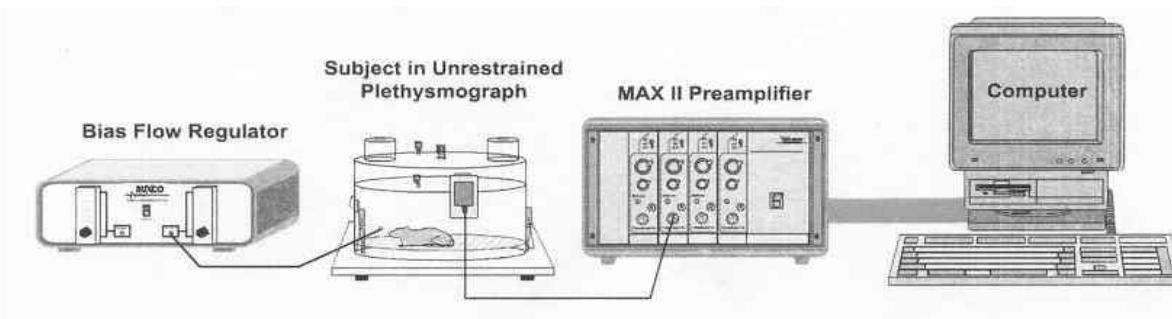
□ Pulmonary toxicity Biomarkers

BAL (bronchoalveolar lavage) cell distribution 평가

: total cell count, macrophage, PMN, lymphocyte 측정

: total protein, albumin 및 LDH

Lung function test : tidal volume, minute volume, frequency, inspiratory time, expiratory time, peak inspiratory flow, peak expiratory flow



Silver nanoparticle distribution

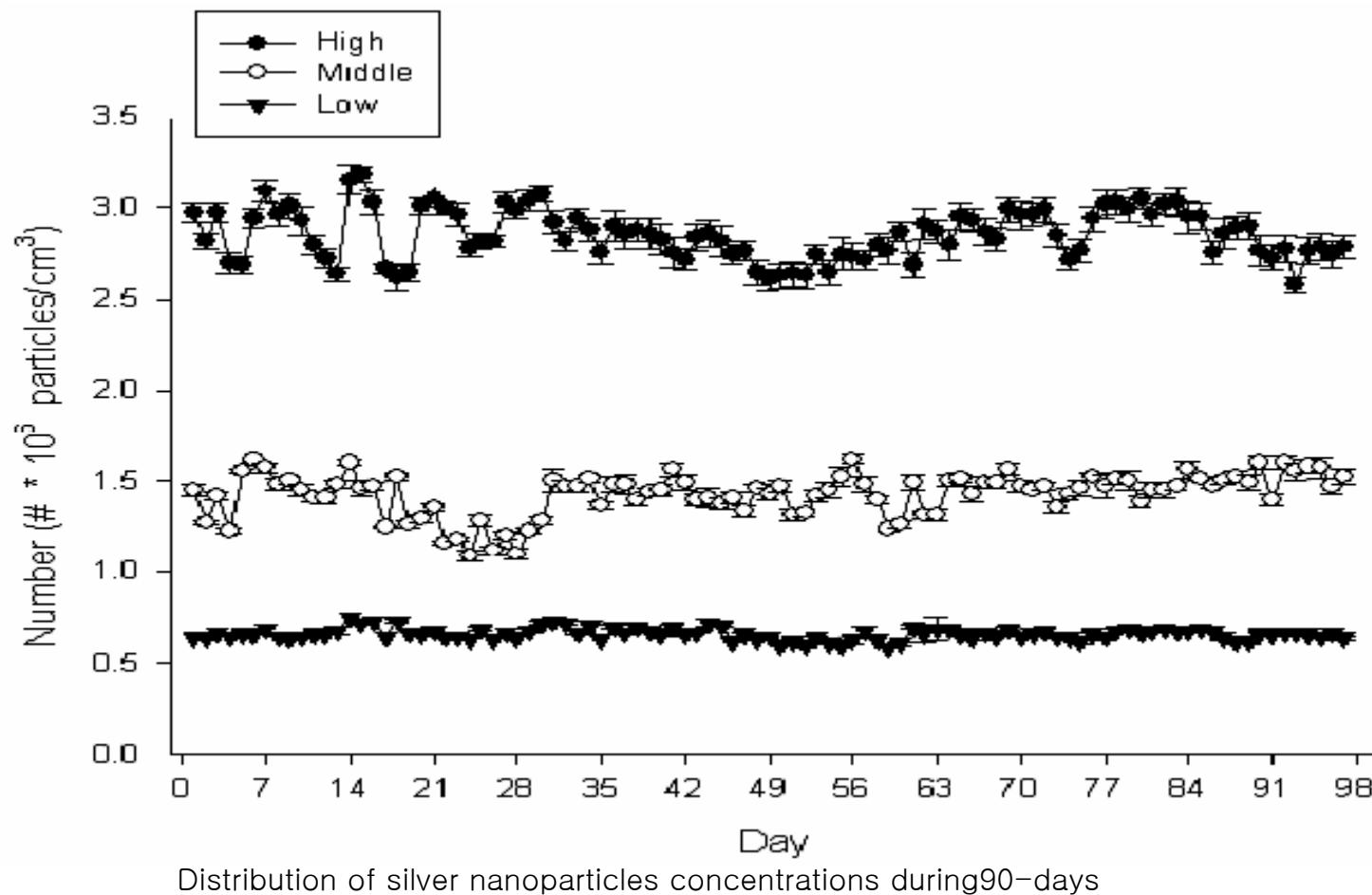
Distribution of silver nano particle

Group	Site	Diameter [†] (nm)	Number ($\times 10^6$ particles/cm ³)	Mean ± S.E	
				Surface ($\times 10^9$ nm ² /cm ³)	Mass ($\mu\text{g}/\text{m}^3$)
Control		0	0	0	0
Low	Up ^a	18.12(0.05)	0.67±0.00	1.08±0.03	49.36±0.67
	Down ^b	18.13(0.06)	0.66±0.00	1.08±0.04	48.53±0.66
	Total	18.12(0.04)	0.66±0.00	1.08±0.02	48.94±0.47
Middle	Up	18.30(0.05)	1.44±0.01	2.39±0.02	133.75±1.48
	Down	18.35(0.05)	1.42±0.01	2.36±0.02	132.51±1.49
	Total	18.33(0.03)	1.43±0.00	2.37±0.01	133.13±1.05
High	Up	18.72(0.07)	2.86±0.01	6.43±0.05	494.50±5.97
	Down	19.13(0.06)	2.85±0.01	6.78±0.04	535.06±4.35
	Total	18.93(0.05)	2.85±0.01	6.61±0.03	514.78±3.74

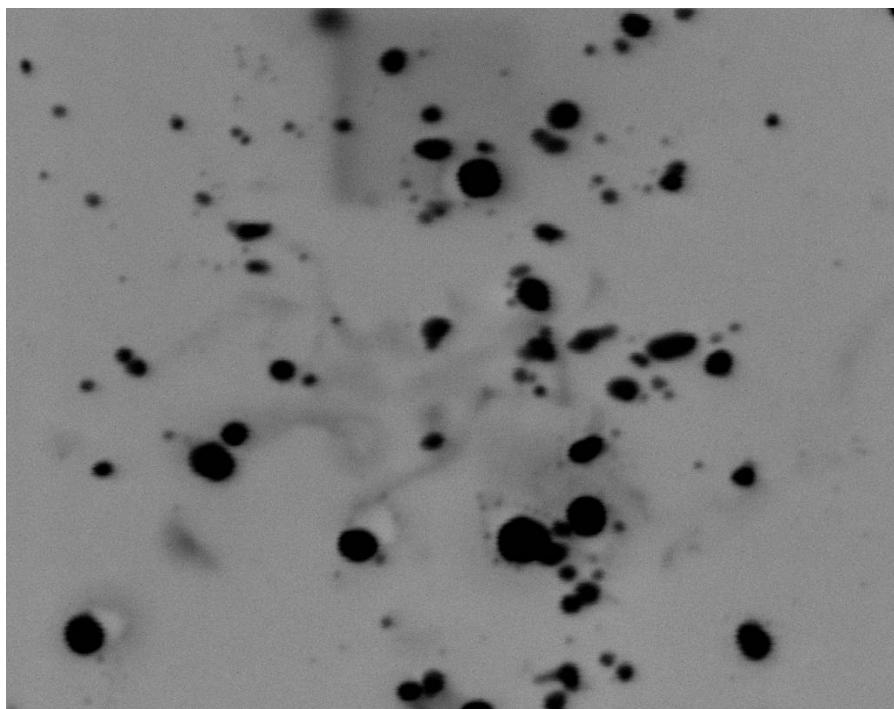
† : GM(GSD)

※ HEPA filter 를 통과한 공기 중 분진 수 : channel 1 – 1.62±0.07 개/min
channel 2 – 0.39±0.03 개/min

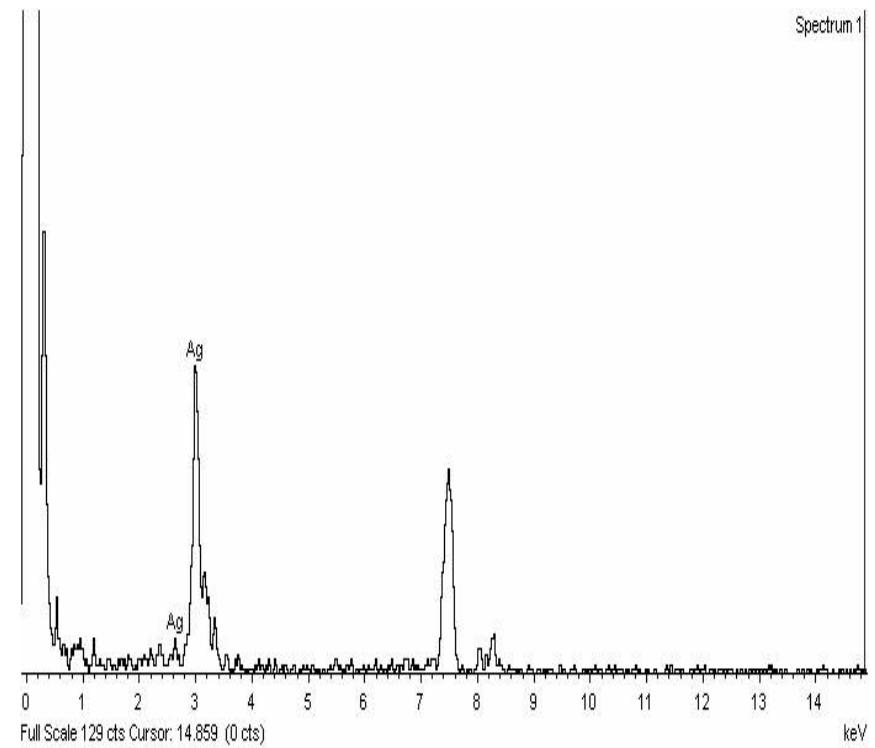
Maintenance of concentrations during 90 day exposure period



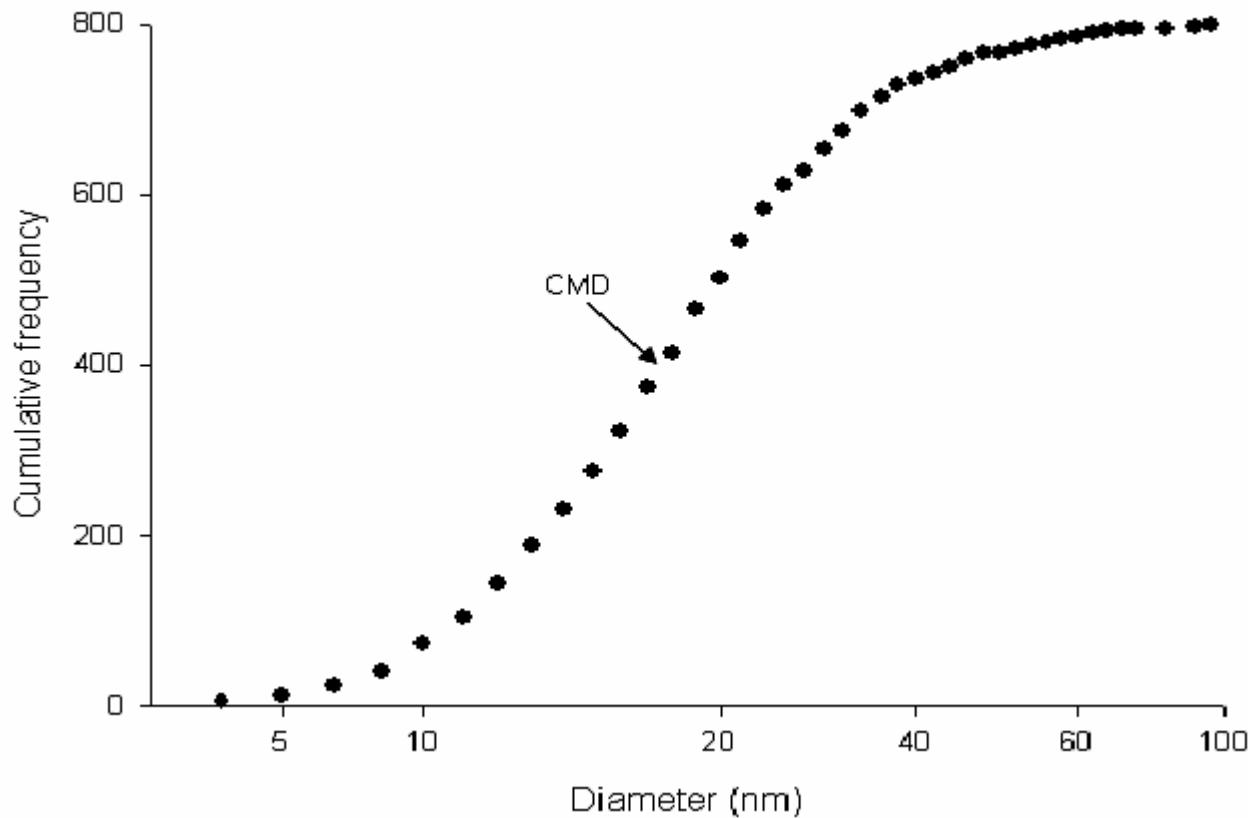
Particle shape and purity by TEM-EDX



400 nm



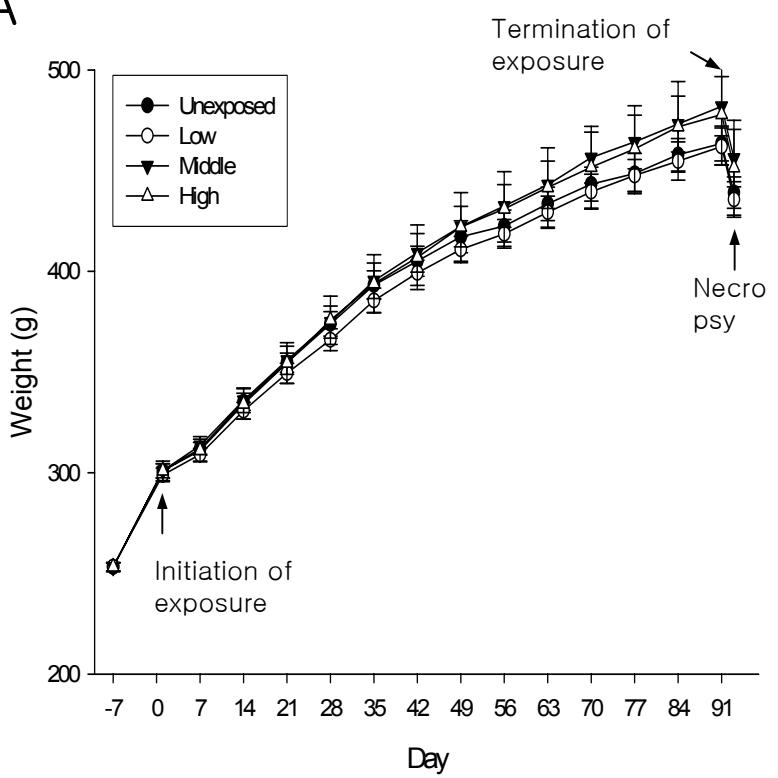
Particle distribution by TEM



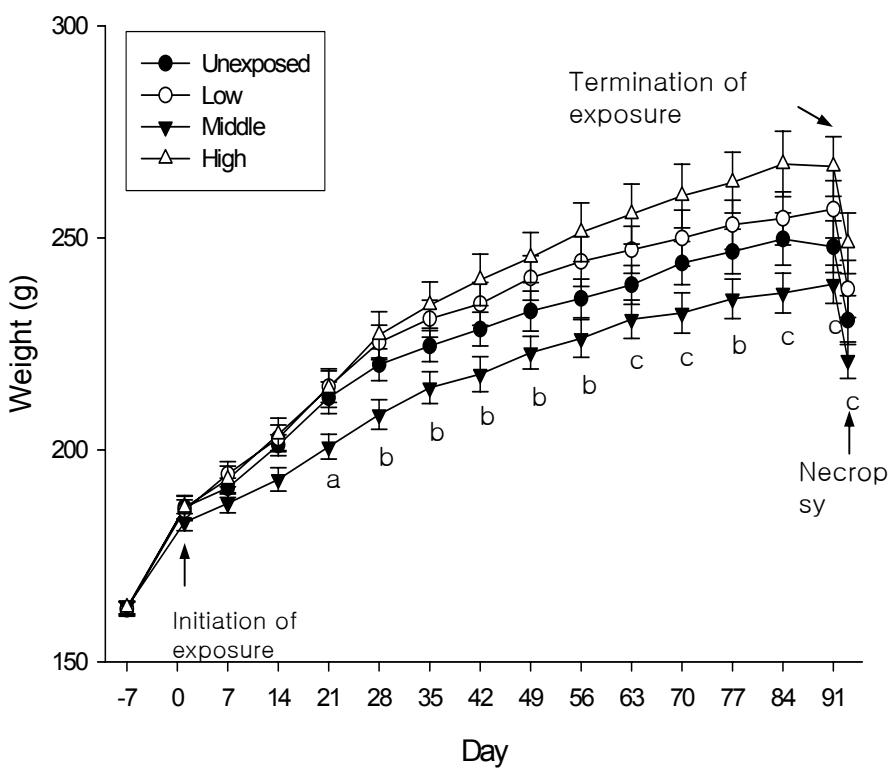
Log normal distribution of silver nanoparticles

Body weight changes

A



B



Body weight of rats exposed to silver nanoparticles. A : male, B : female

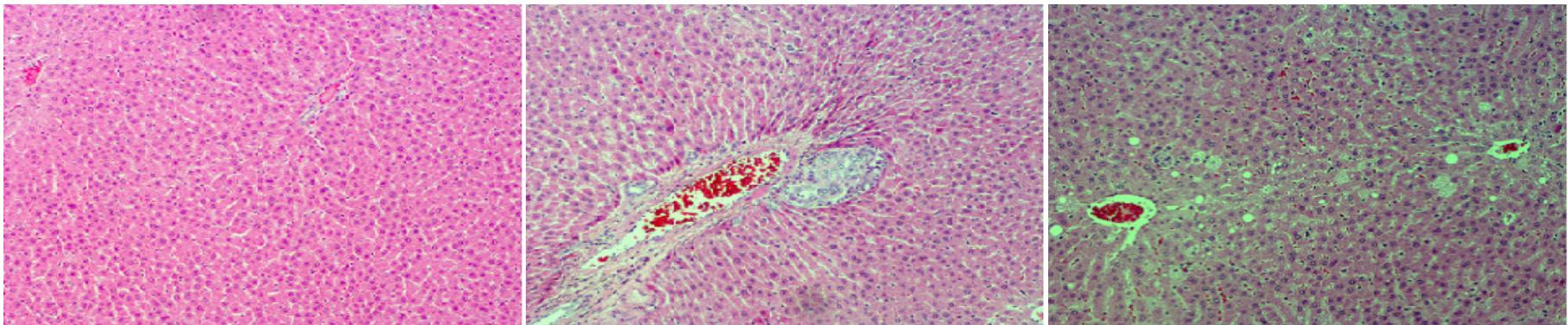
(a : $p < 0.05$, middle vs. other groups, b : $p < 0.05$, middle vs. low and high groups, c : $p < 0.05$, middle vs. high group)

Lung weight change

		Control	Low	Middle	High
Male	L	0.49 ±0.02	0.50 ±0.02	0.51 ±0.02	0.57 ±0.03
	R	0.92 ±0.04	0.95 ±0.05	0.94 ±0.04	1.10±0.06
Female	L	0.38 ±0.01	0.37 ±0.02	0.35 ±0.01	0.41 ±0.01
	R	0.68 ±0.03	0.70 ±0.01	0.67 ±0.04	0.77 ±0.03

Histopathology of Liver

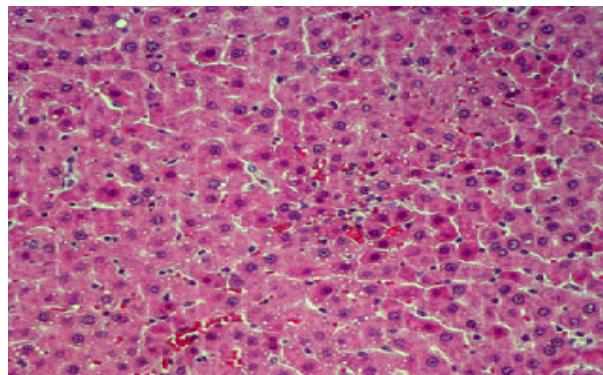
Liver



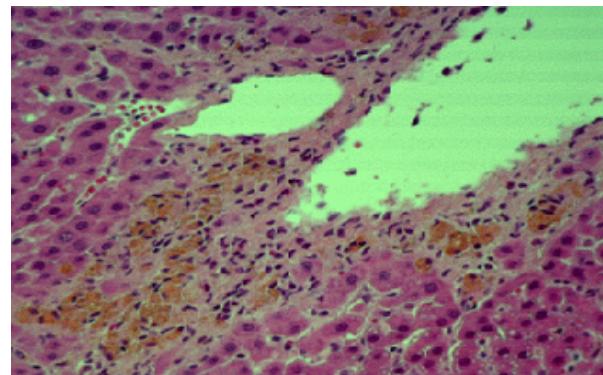
Control x100

Bile duct hyperplasia x100

Vacuolization x100



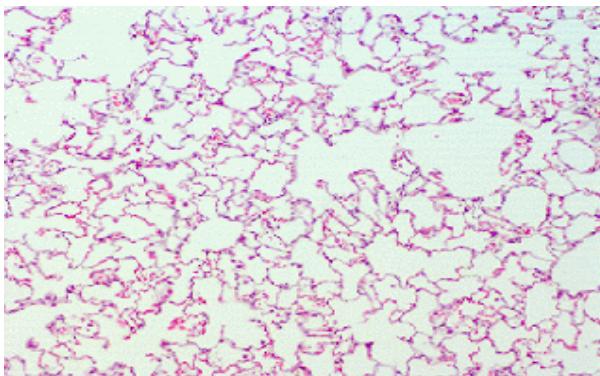
Hepatocytes swelling x200



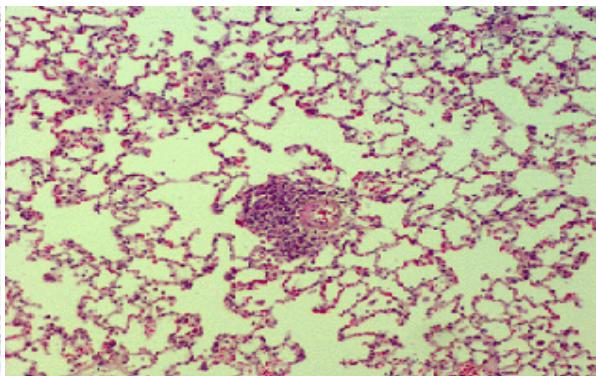
Siderotic near central vein x200

Histopathology of Lung

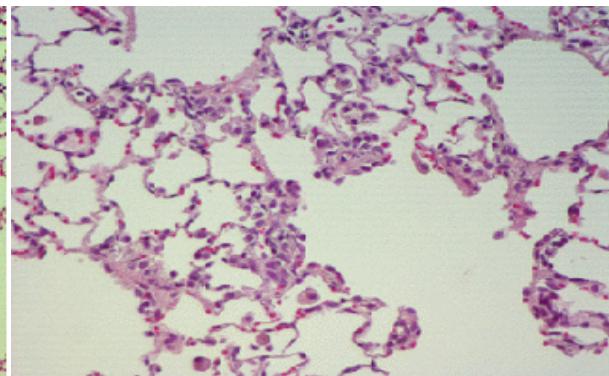
Lung



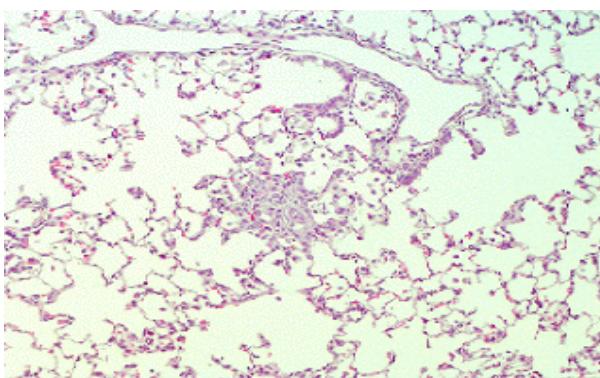
Control x100



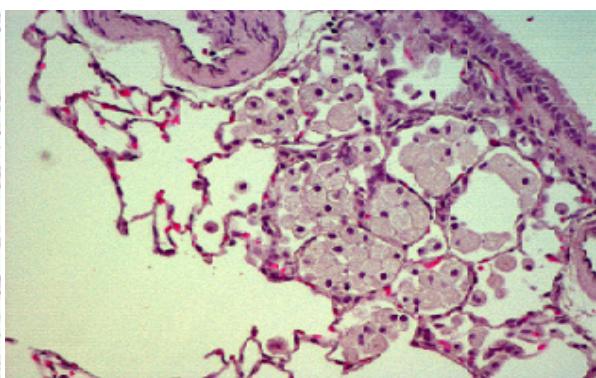
Perivasculitis x100



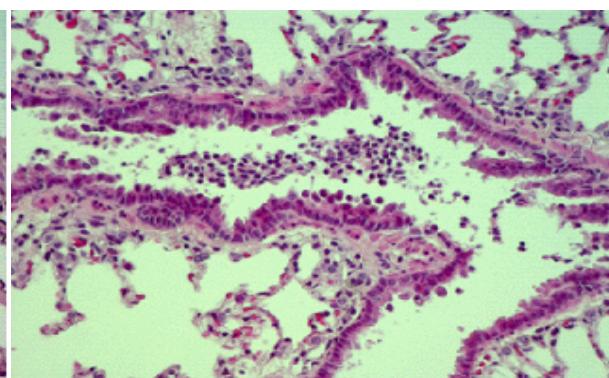
Alveolitis x200



Granulomatous lesions x100

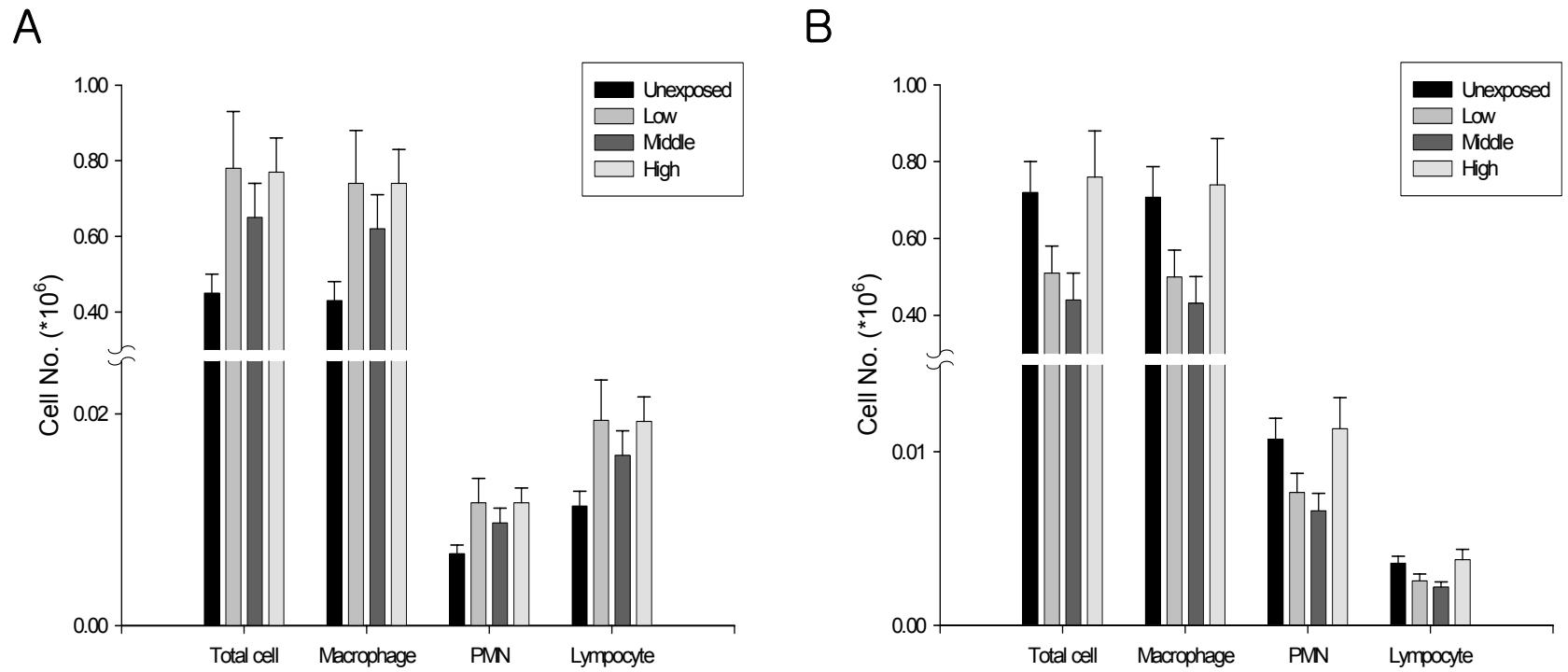


Histiocytosis x400



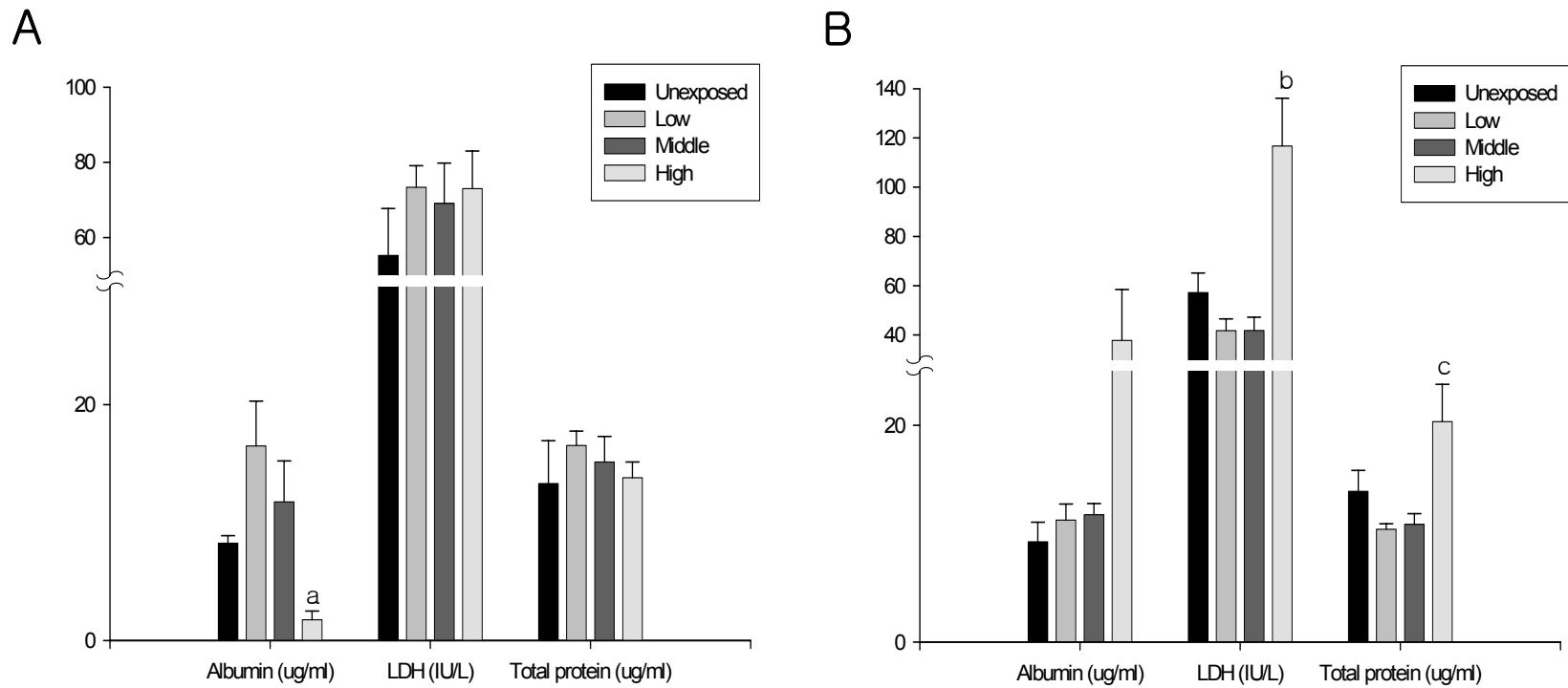
Inflammation x100

Bronchoalveolar lavage



Cell no. of total cell, macrophage, PMN, and lymphocyte of rats exposed to silver nanoparticles, A : male, B : female.

Bronchoalveolar lavage

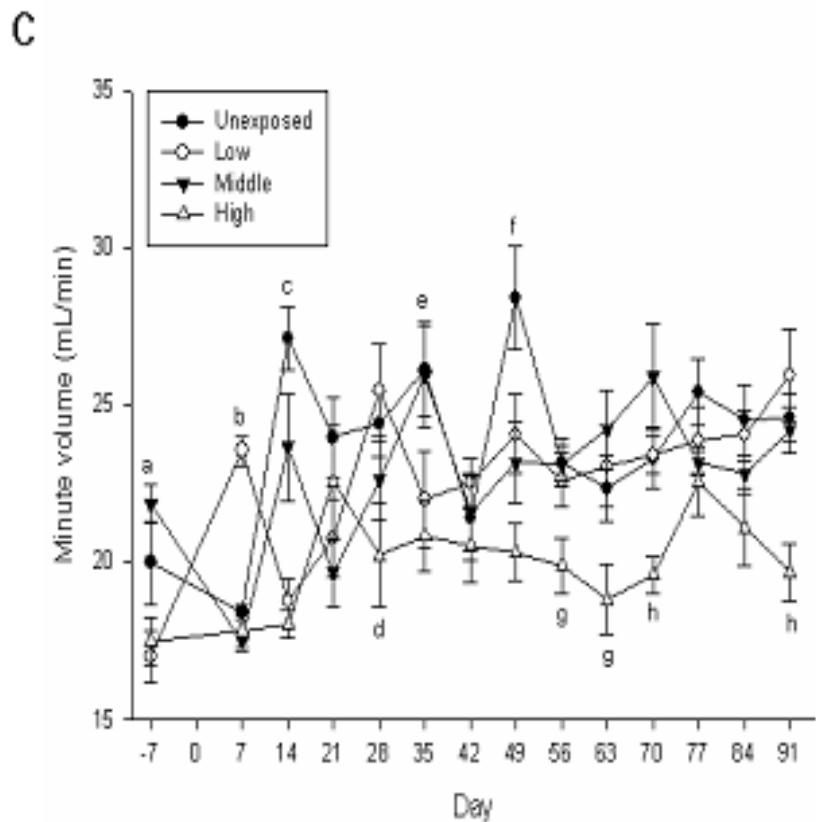


Values of albumin, LDH and total protein of rats exposed to silver nanoparticles, A : male, B : female.

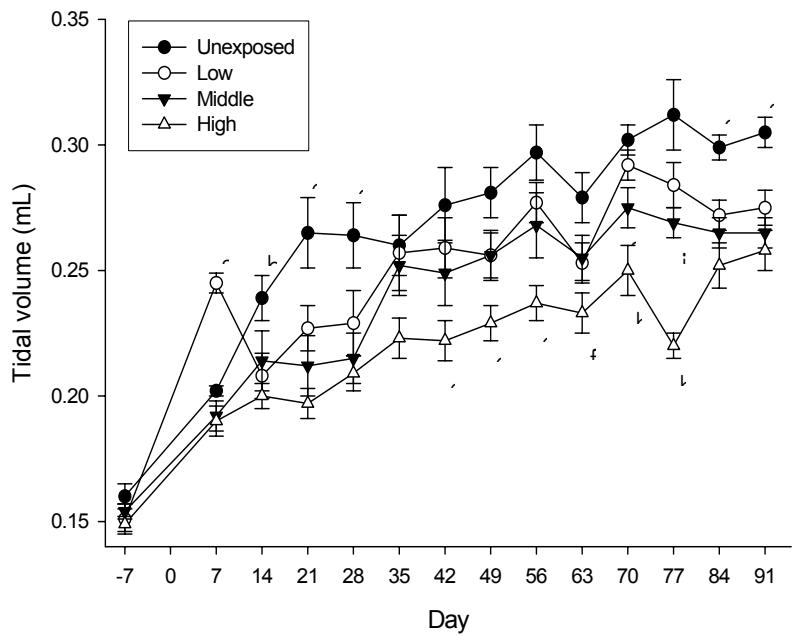
a : Significant different from high vs. low and middle groups, $p < 0.05$, b : Significant different from high vs. other groups, $p < 0.01$,
c : Significant different from high vs. other groups, $p < 0.05$

Lung function test (male)

Minute volume

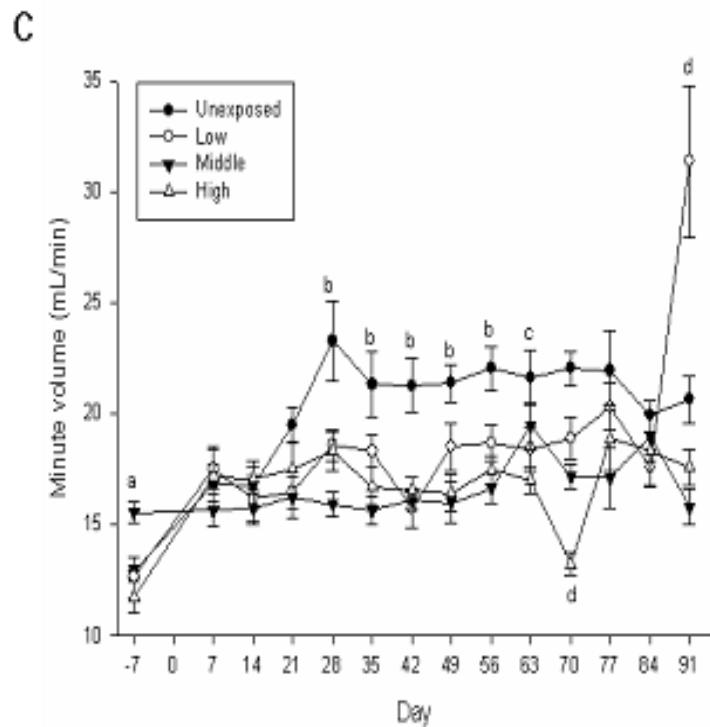


Tidal volume

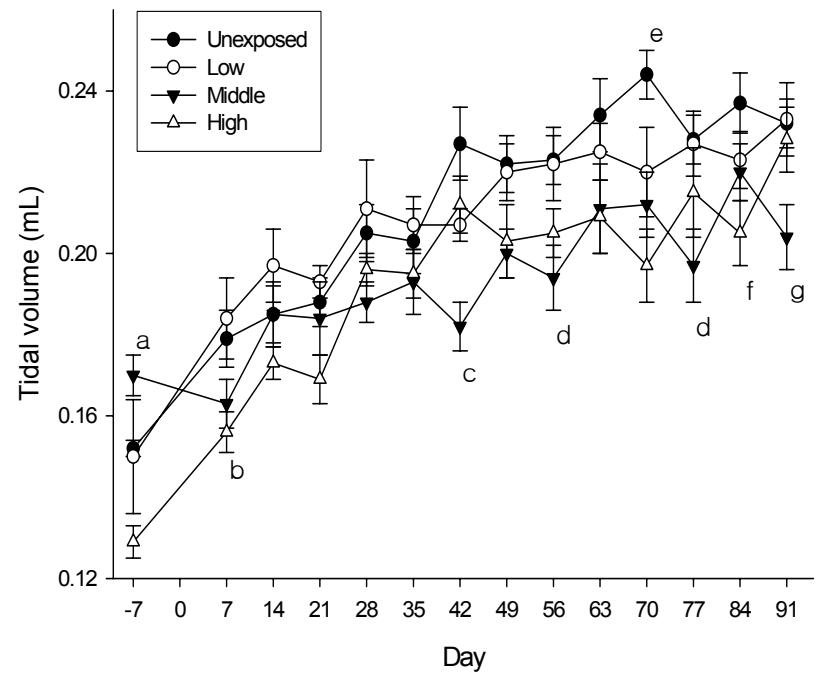


Lung Function Test (Female)

Minute volume



Tidal volume



Tissue distribution of silver nanoparticles (Male)

TESTS:	Liver	Kidney	Olfactory bulb	Brain	Lung	Blood
Control :						
MEAN	0.70	0.85	0.51	1.12	0.77	0.09
S.E.	0.20	0.20	0.38	0.34	0.25	0.02
Low :						
MEAN	3.52	1.63	6.44	3.45	613.57 [†]	0.68
S.E.	0.98	0.33	0.77	0.73	66.03	0.08
Middle :						
MEAN	13.75	3.58 ^b	17.10	7.89 ^b	5450.29 ^b	1.82 ^b
S.E.	2.88	0.41	1.61	0.95	904.17	0.20
High:						
MEAN	132.97 ^a	9.49 ^a	30.48 ^c	18.63 ^a	14645.42 ^a	4.31 ^a
S.E.	22.87	0.86	2.15	1.24	2630.24	0.37

a : p<0.01, high vs. other groups, b : p<0.01, middle vs. control and low group, c : p<0.01, high vs. other groups (dose-dependant),

† : p<0.05, male vs. female in low group. Unit ng/g of wet tissue

Tissue distribution of silver nanoparticles (Female)

	Female					
TESTS:	Liver	Kidney	Olfactory bulb	Brain	Lung	Blood
Control						
MEAN	0.90	0.94	2.26	0.66	1.01	0.05
S.E.	0.31	0.18	0.74	0.26	0.10	0.01
Low						
MEAN	4.55	2.61	7.43	4.09	295.92	0.85
S.E.	1.40	0.57	0.75	0.46	75.50	0.14
Middle						
MEAN	12.07	11.81	13.75	10.22 ^b	4241.17 ^b	2.10 ^b
S.E.	2.50	4.27	1.32	1.19	641.10	0.22
High						
MEAN	71.08 ^a	37.66 ^{a,†}	32.84 ^c	19.97 ^a	20585.63 ^a	6.86 ^{a,‡}
S.E.	24.50	7.04	2.74	2.41	1880.31	0.60

a : p<0.01, high vs. other groups, b : p<0.01, middle vs. control and low group, c : p<0.01, high vs. other groups (dose-dependant),

† : p<0.05, female vs. male in high group, ‡ : p<0.01, female vs. male in high group. Unit ng/g of wet tissue

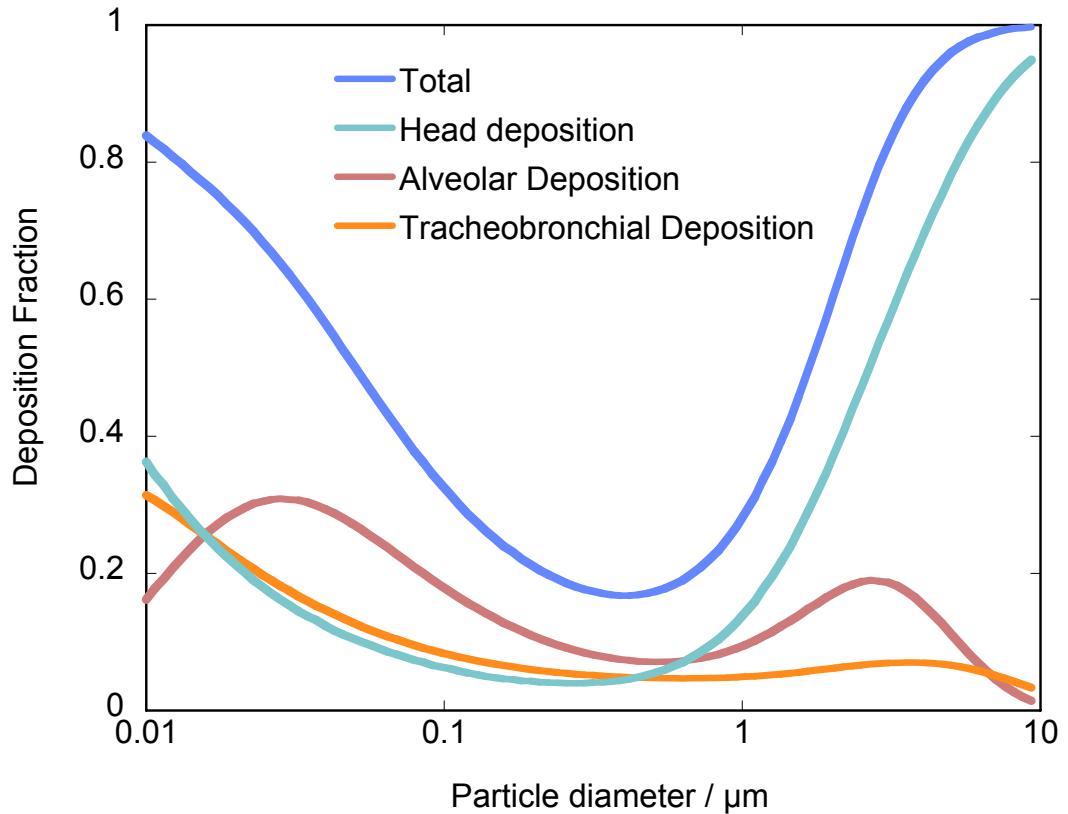
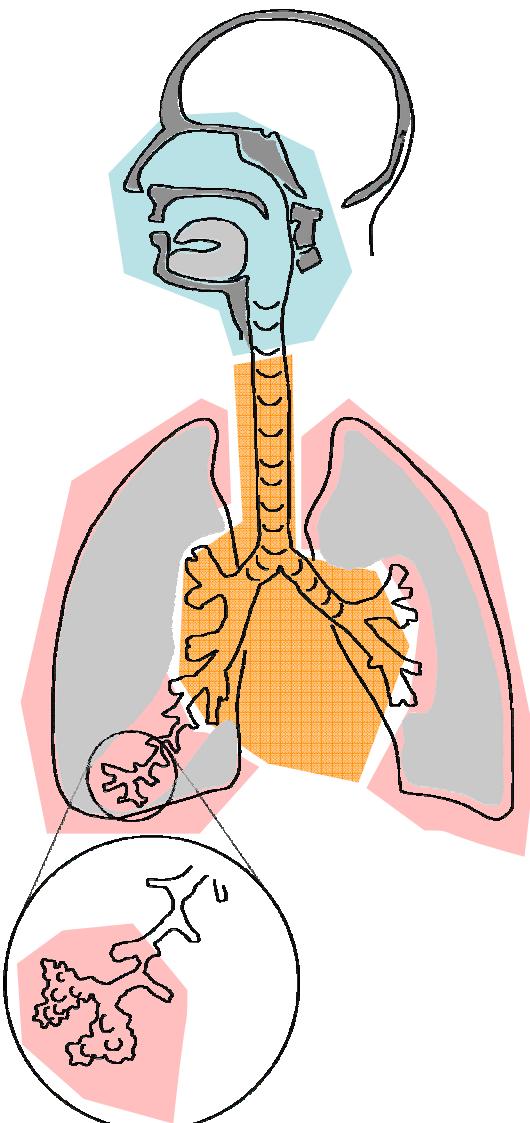
Applicability of existing OECD Test guidelines to Nanoparticle toxicity testing

Items	OECD TG 412/413	Applicability to nanoparticle
Mode of exposure	Head/nose-only or whole body	OK
Air change/hr, Oxygen	12-15hrs, 19-24%	OK
Animal volume	Less than 5% of test chamber	OK
Exposure time	6hrs/day/5-7day/week	OK
Group	1 control + 3dose	OK
Particle size	Mass median aerodynamic diameter (1-4 μm) Geometric standard deviation (1.5-3.0)	NO
Monitoring of exposure condition	Particle size distribution (1 time/concentration level/week)	need to be improved
Concentration	Within $\pm 20\%$ of the mean concentration	OK
Characterization of test atmosphere	Gravimetric and chemical analysis	Particle number, Surface area, Mass, Composition, CMD etc.
Temperature, Humidity	19-25°C, 30-70%	OK

Sex difference in tissue distribution of silver nanoparticle (kidney) (ng/g of wet tissue)

Sex		Control	Low	Middle	High
Inh	M	0.85± 0.20	1.63± 0.33	3.58 ± 0.41	9.49 ± 0.86
	F	0.94 ±0.18	2.61 ±0.57	11.81 ±4.27	37.66 ±7.04
Oral	M	0.02 ±0.01	1.3±0.59	5.98±2.36	24.32±10.63
	F	0.02 ±0.0	2.82±0.85	16.77±2.81	69.32±22.34

Particle deposition in the lungs



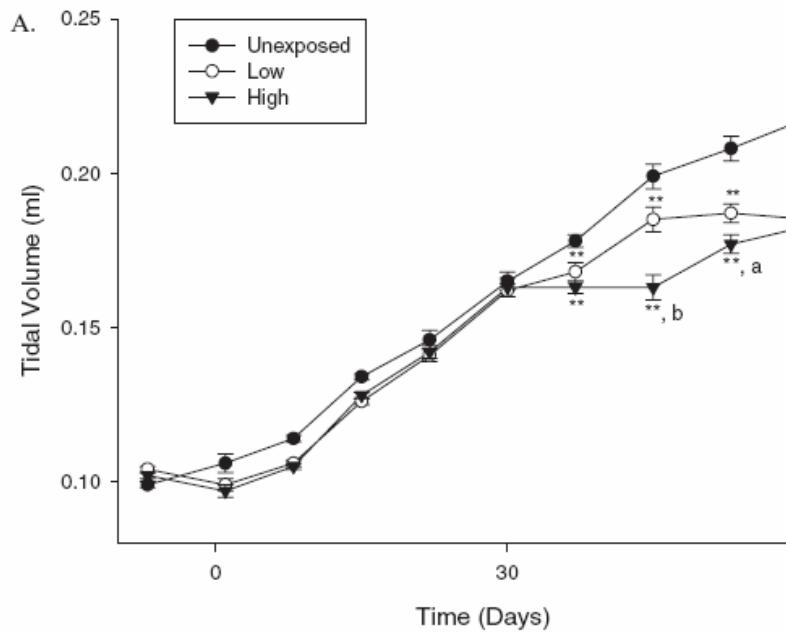
Modeled lung deposition. Mouth and nose breathing, person at rest.

Permission from
Maynard

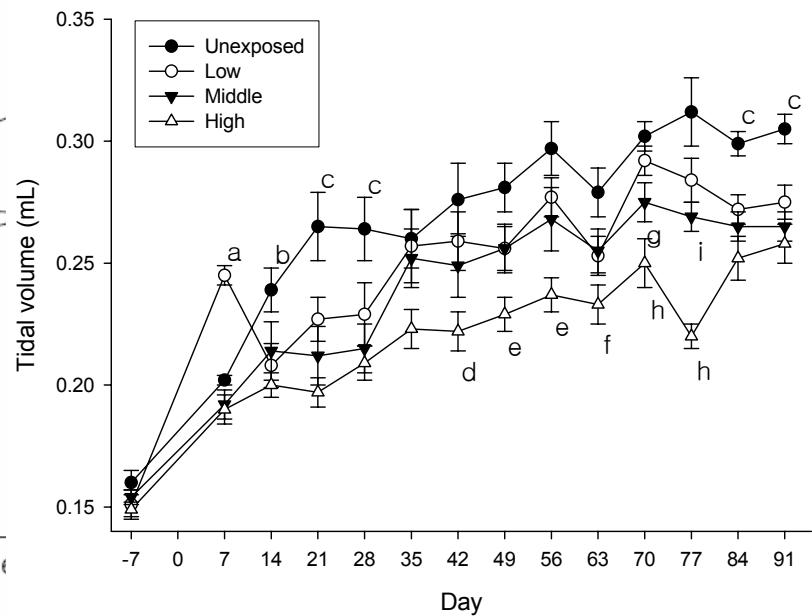
Source: Multiple Pathway Deposition Model (MDEP), CIIT

LFT decrease

Welding fume



Silver nanoparticle



Deposit dose

	WF (100 nm)	Ag nano (20 nm)
Exp. dose	65 mg/m ³ , 108 mg/m ³	0.049 (L), 0.133 (M), 0.515 mg/m ³ (H)
Inh. Vol/d	12 m ³	36 m ³
Total inh volume	540 m ³ (60 d),	2340 m ³ (90 d),
Total dose	35100 mg (L), 58230 mg (H)	117 mg (L), 311 mg (M), 1205 mg (H)
Deposition fraction	30%	80%
Deposit dose	10530 mg (L) 17469 mg (H)	94 mg (L), 275 mg (M), 1141 mg (H)
LFT decrease	~20%	~20%

Target organs for silver nanoparticles and NOAEL

Target organs: Liver and Lung

NOAEL

	rat
Number (particle/cm ³)	1.43×10^6
Surface area (nm ² /cm ³)	2.37×10^9
Mass (µg/m ³)	133

Acceptable concentration

	Workers	General population
Number (particle/cm ³)	1.75×10^6	9.1×10^5
Surface area (nm ² /cm ³)	2.9×10^9	1.5×10^9
Mass (μg/m ³)	163	85

※ ACGIH or MOL Silver TLV : 100 μg/m³ (Argyria),

Nano specific inhalation toxicity study

Particle generation	Nano specific particle generation Dispersed vs aggregated, monodispersed vs polydisperse
Dosimetry	Mass, number, surface area,
Particle characterization	Shape, diameter, purity, dispersion, surface chemistry, hygroscopicity, aggregation/agglomeration, purity
Tissue distribution	Target organ determination, toxicokinetics, durability, clearance
Lung function test	Obstructive disease, restrictive disease
Pathology	Nano specific pathology (TEM, Raman, Confocal),
Evaluation	BAL, Coagulation, etc

Conclusions

- Collaboration between toxicologist and engineer is strongly recommended to study the health and environmental effects of nanoparticle exposure.
- New dosimetry is required for inhalation toxicity testing.
- Nano-specific parameters are required for nanotoxicity evaluation.