National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Toxicokinetics and toxicity of nanosilver

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Content

1. Toxicokinetics Ag-NP
2. IV repeated (28 days) dose toxicity study of Ag-NP

Toxicokinetics

Kinetic properties are considered to be an important descriptor for potential human toxicity and thus for human health risks.

What kind of information do you get?

- Kinetics of a compound (blood clearance, internal exposure)
- Tissue distribution (target organs)
- Tissue clearance (biopersistence?)

- Local effective/toxic tissue concentration (medicines/substances)

Toxicokinetics

The particulate nature of nanomaterials influences the toxicokinetics

- ADME – absorption, distribution, metabolism?, excretion?
- Dependent on size, shape, material, etc…

Possible routes of exposure for absorption/uptake

- Skin contact (e.g. sunscreens)
- Oral (food, medicines)
- Inhalation (e.g. ambient air (pollution), consumer spray products (cosmetics), medicines)
- Intravenous (medicines)

Study: toxicokinetics and tissue distribution of Ag-NP of different sizes

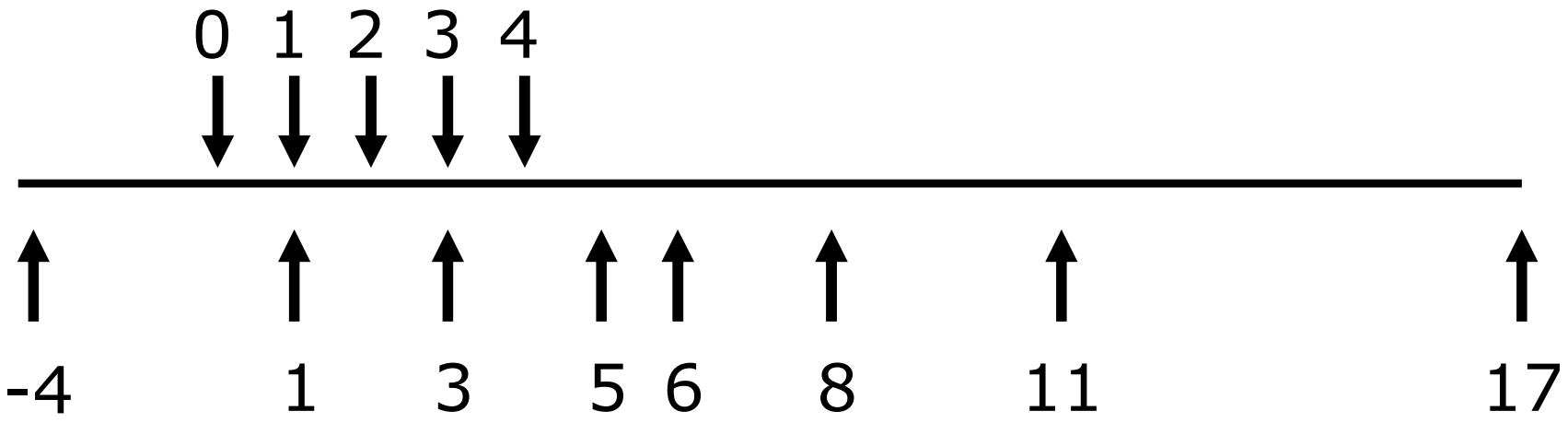
- Aim:
  - Identification of organs at risk for adverse effects (toxicity, genotoxicity)
- Methods:
  - IV administration in rats (to avoid barrier function in lung, GI-tract, skin)
  - Silver content in blood and organs was determined by inductively coupled plasma mass spectrometer (ICP-MS)
- Materials:
  - Ag nanoparticles of 20nm, 80 nm, 110 nm (nanoComposix, San Diego, USA)

Study: Tissue distribution and kinetics of nanosilver
Characterization of nanosilver of different sizes

Parameter	*20 nm*	*80 nm*	*110 nm*
Size ± SD (nm)	20.3 ± 1.9	79.8 ± 5.1	112.6 ± 7.8
Size distribution (%)	9.2	6.4	6.9
Number of particles in injection solution (ml^{-1})	5.0×10^{11}	9.4×10^{9}	3.5×10^{9}
Surface area per particle (nm^2)	6.5×10^{14}	1.9×10^{14}	1.4×10^{14}
Silver concentration in injection solution (µg/ml)	23.8	26.4	27.6

Treatment schedule Ag-NP toxicokinetics

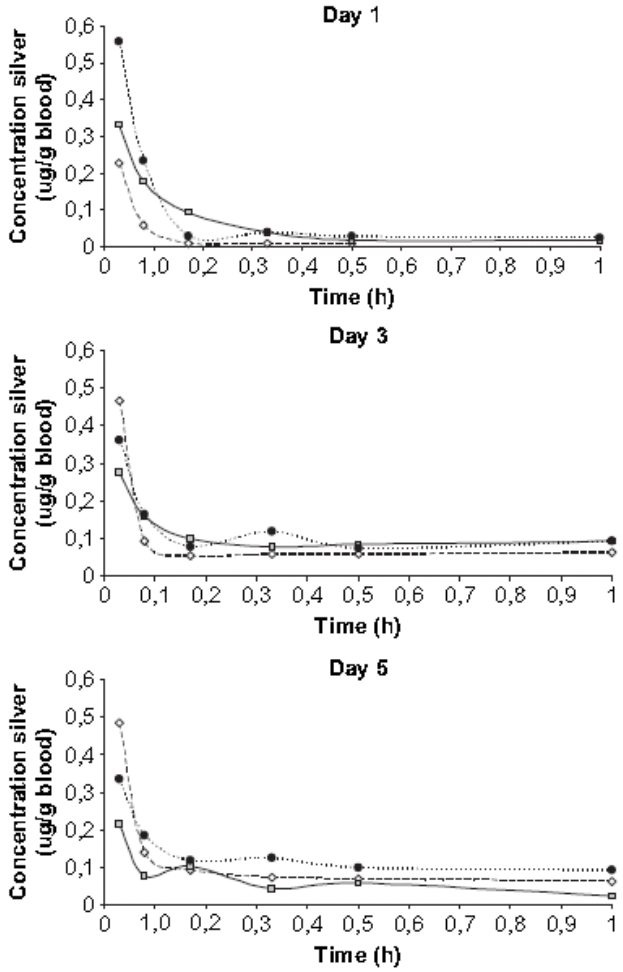
IV treatment



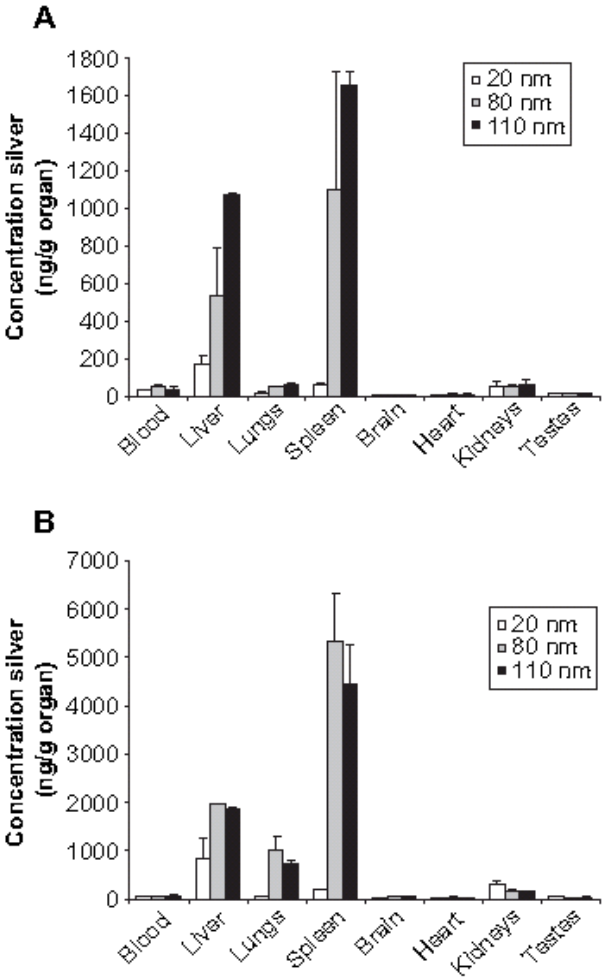
Blood was collected at (days -4, 1, 3, 5)

Blood and organs were collected at days 1-3-5-6-8-11-17

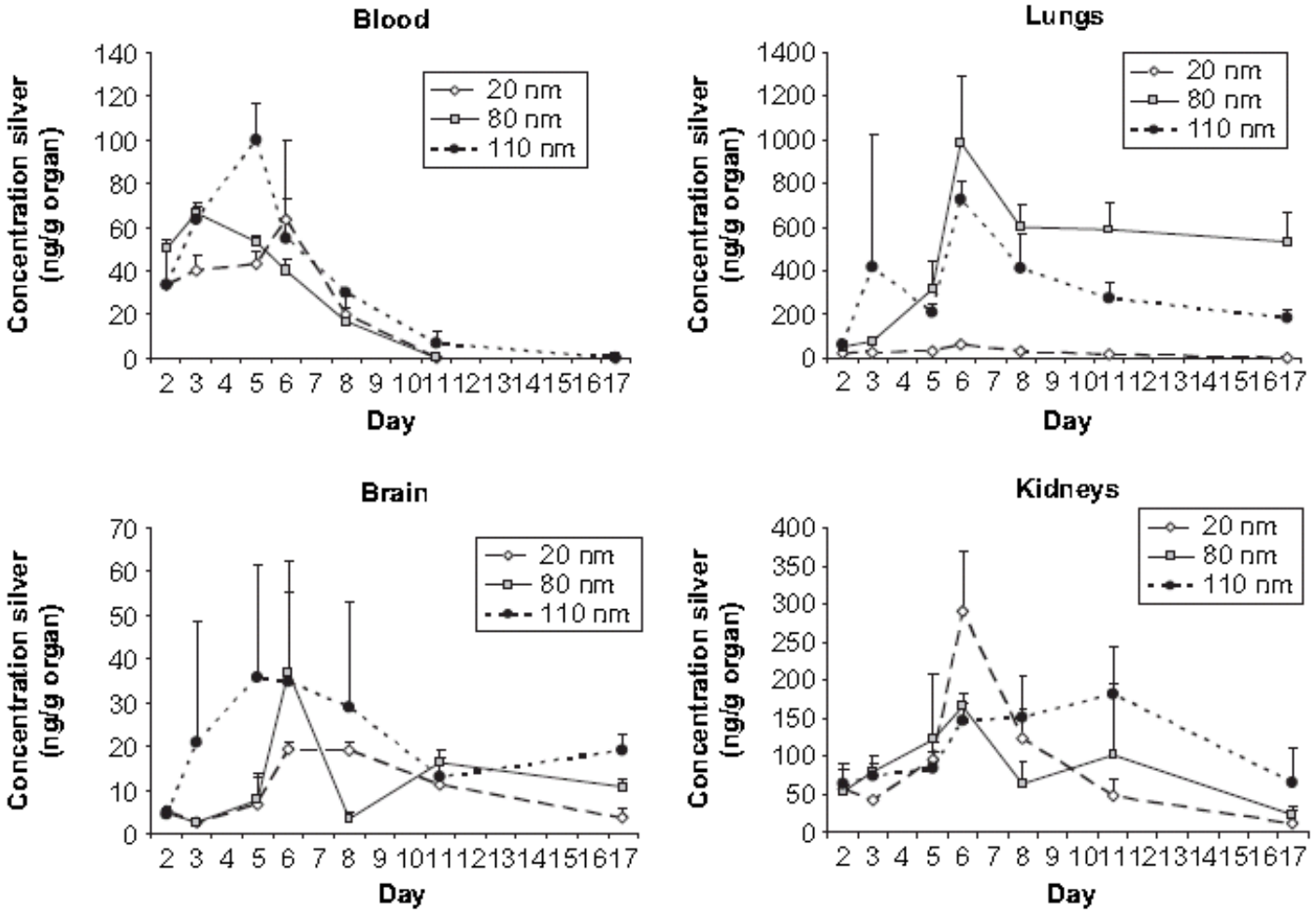
Blood kinetics at day 1-3 and 5 after IV Ag-NP



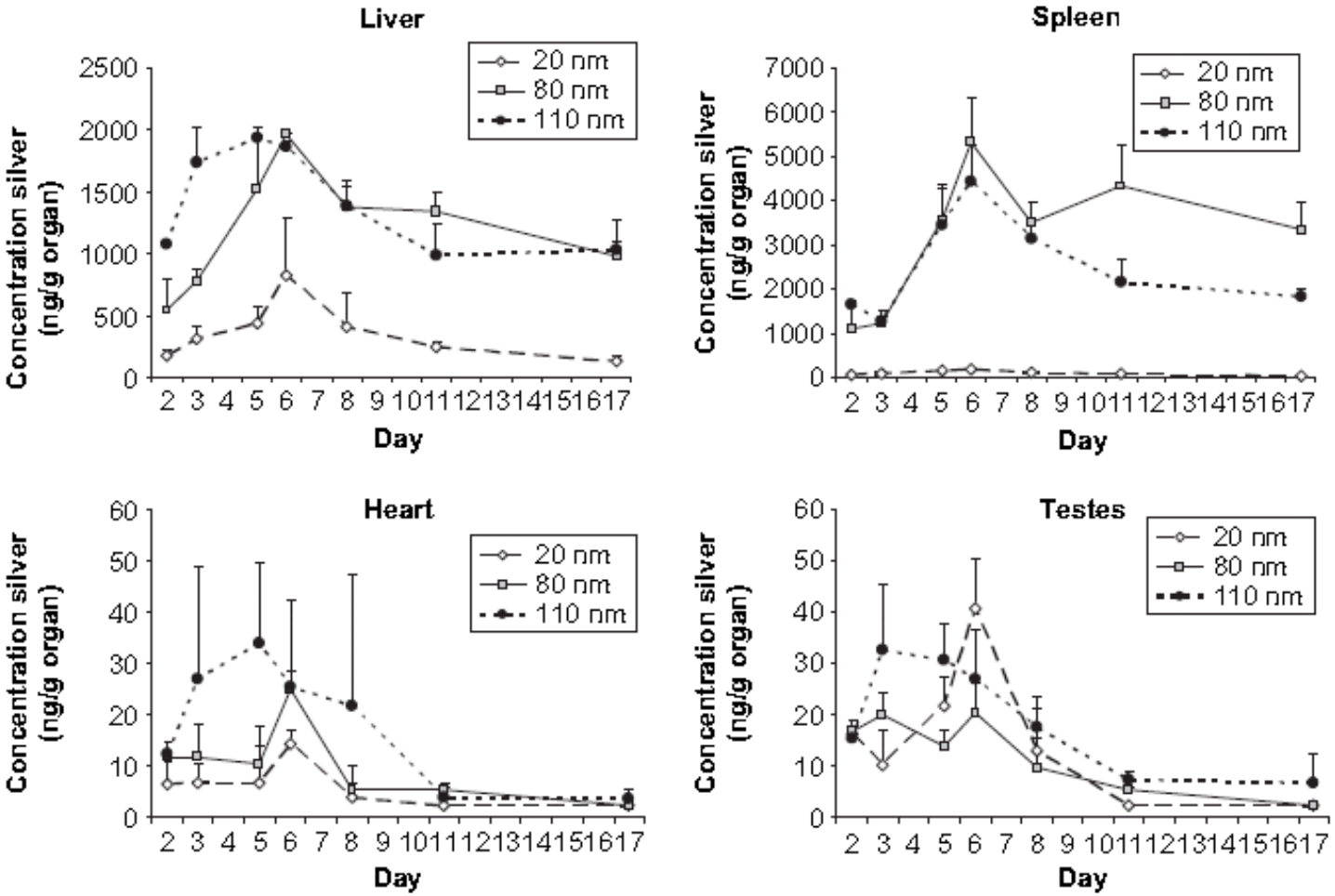
Tissue distribution after 1x and 5x IV Ag-NP



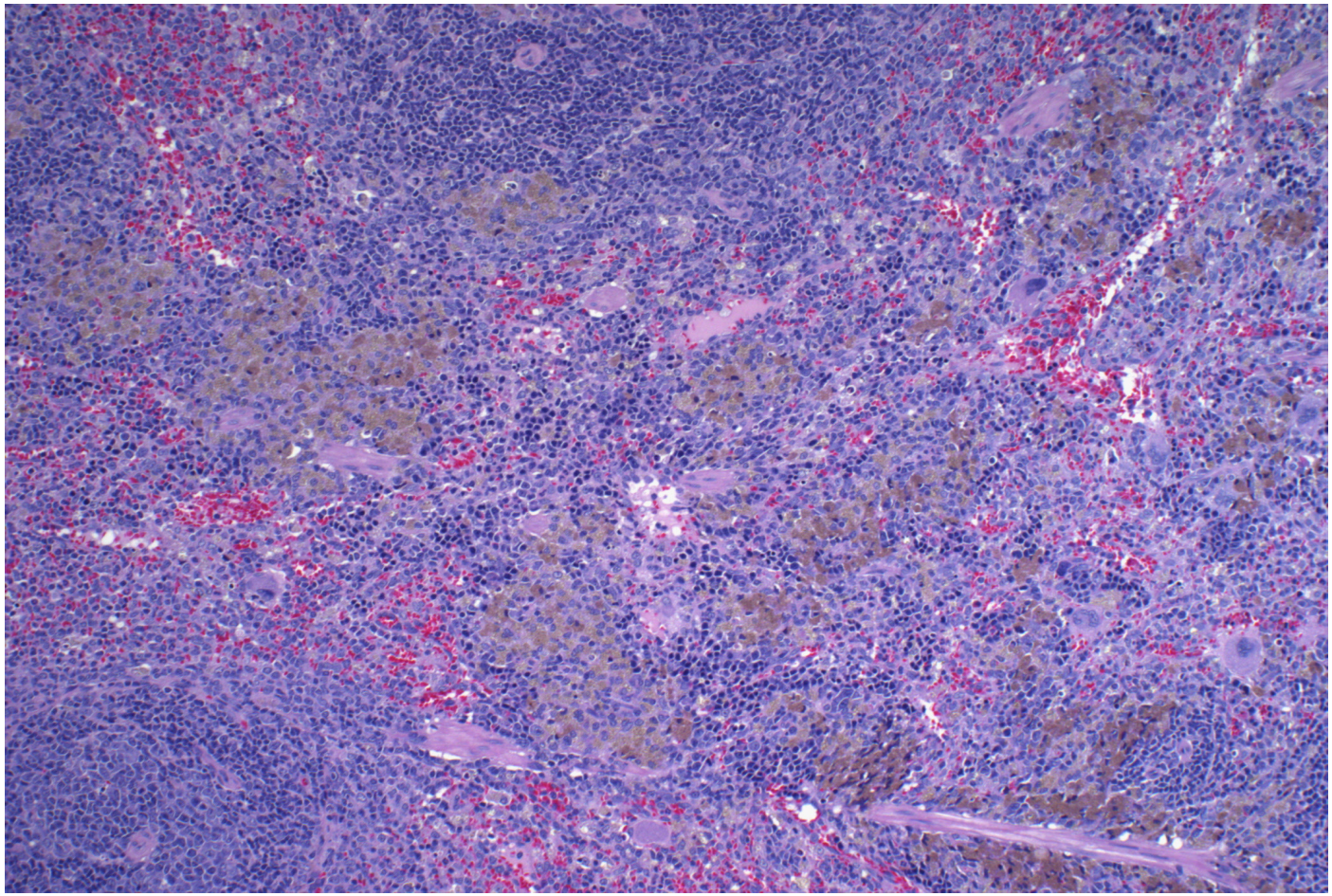
Ag-NP tissue distribution in time 1



Ag-NP tissue distribution in time 2



Detail of accumulation of silver nanoparticles in spleen



Summary Ag-NP toxicokinetic studies

- The particulate nature of nanomaterials influences the toxicokinetics
  - ADME – absorption, distribution, metabolism?, excretion ?
  - Dependent on size, shape, material, etc…

- High uptake in organs that are part of RES
  - distribution mainly to liver and spleen
- Uptake by other organs limited but there may be a risk for accumulation
- 20 nm Ag-NP low recovery (excretion/dissolution?)

- Questions
  - Does accumulation result in persistence (washing out)?
  - Long term effect of particle presence in organs? Also considering low level in non RES organs.

Study: Dermination of systemic toxicity of Ag-NP

Study design

- Intravenous administration (IV) of silver nanoparticles Ag-NP
  - 20 nm and 100 nm diameter
- IV for 28 consecutive days
- Bench mark approach
- Autopsy at day 29
- Parameters
  - Body weight (growth), organ weight, hematology, clinical chemistry, immune activity

Characterization of Ag-Np

Parameter	*20 nm CTH1359*	*100 nmCTH1409*
Size ± SD (nm)	21.0 ± 2.6	107 ± 7.6
Coefficient of Variation (%)	12.2	7.1
Size range (min-max diameter)	12.4 – 27.9	92.8 – 128.4
Number of particles (ml^{-1})	3.9×10^{13}	3.8×10^{11}
Surface area per particle (nm^2)	1.40×10^{3}	3.62×10^{4}
Surface area (nm^2/ml)	5.49×10^{16}	1.37×10^{16}
Silver concentration (mg/ml)	2	2.6
Zeta potential (mV)	-40.8	-38.7

a. Information provided by manufacturer nanoComposix, San Diego, USA.

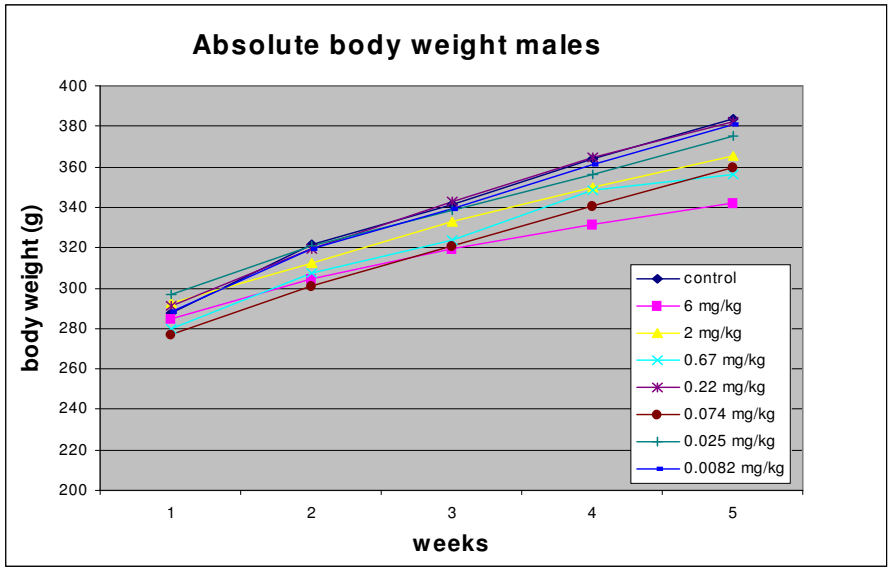
Treatment schedule

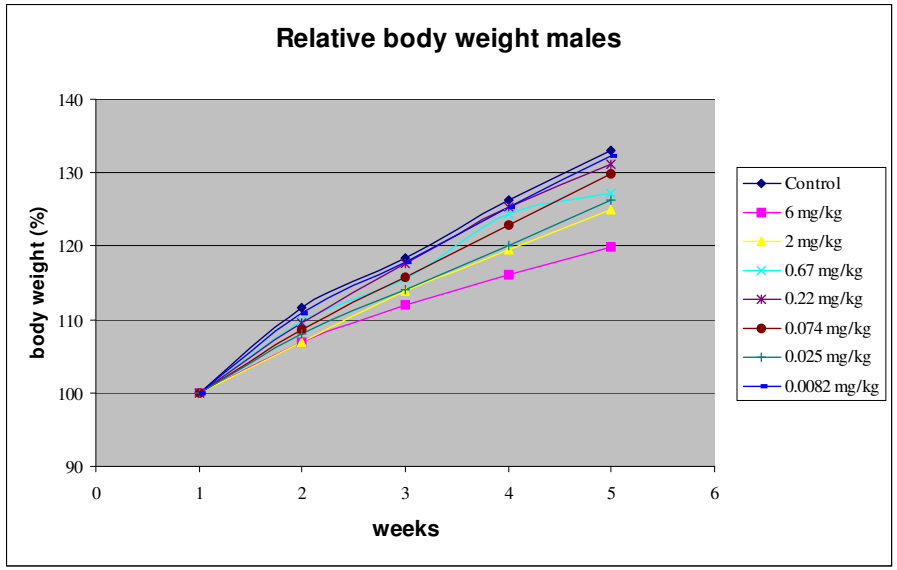
Treatment	*Dose (mg/kg bw per day)*	*M – F(n)*
Phosphate buffer	0	2 – 2
Phosphate buffer	0	2 – 2
20 nm nanosilver	0.0082	2 – 2
20 nm nanosilver	0.0025	2 – 2
20 nm nanosilver	0.074	2 – 2
20 nm nanosilver	0.22	3 – 3
20 nm nanosilver	0.67	3 – 3
20 nm nanosilver	2	3 – 3
20 nm nanosilver	6	3 – 3
Phosphate buffer	0	4 – 4
100 nm nanosilver	6	2 – 2

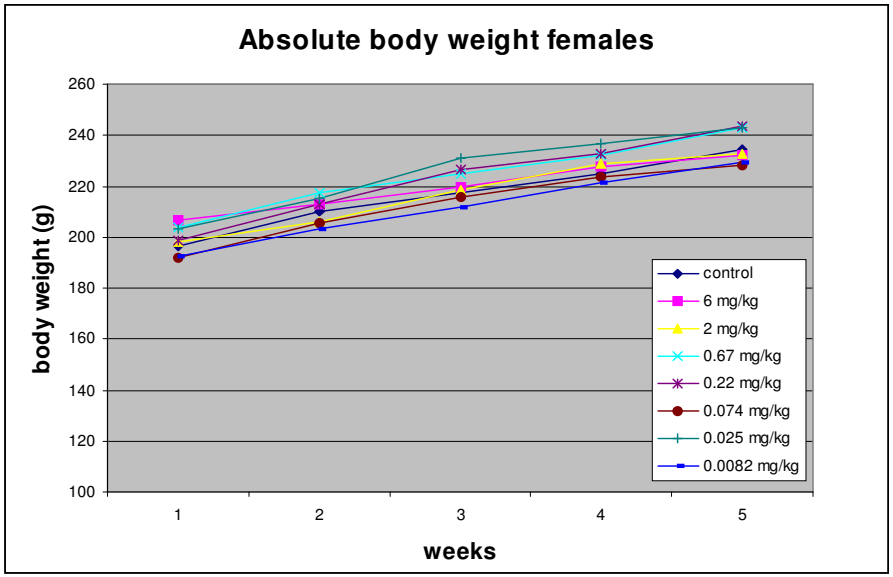
Treatment efficacy

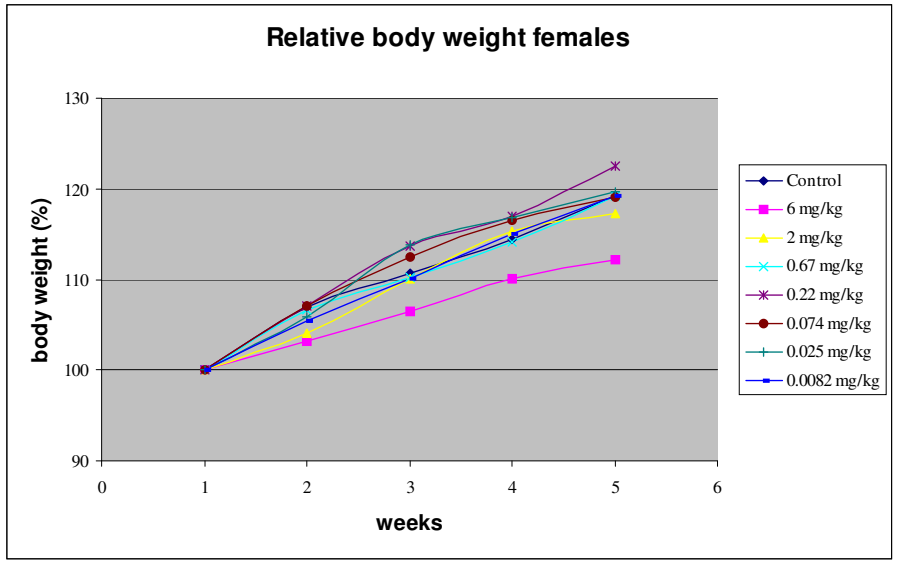
Treatment	*Dose mg/kg bw per day*	*Total dose mg/kg Mean ± SD*	*Total dose Administered Mean ± SD*	*% of intended dose (min – max)*
PB	0	0	0	-
PB	0	0	0	-
20 nm Ag-NP	0.0082	0.2	0.2 ± 0.01	98 (93 – 102)
20 nm Ag-NP	0.025	0.7	0.7 ± 0.02	99 (95 – 101)
20 nm Ag-NP	0.074	2.1	2.0 ± 0.1	97 (93 – 101)
20 nm Ag-NP	0.22	6.2	6.0 ± 0.2	96 (93 – 100)
20 nm Ag-NP	0.67	18.7	18.3 ± 0.5	98 (93 – 101)
20 nm Ag-NP	2	56	54.6 ± 2.1	98 (91 – 101)
20 nm Ag-NP	6	168	157.9 ± 7.6	94 (88 - 100)
PB	0	0	0	-
100 nm Ag-NP	6	168	155.7 ± 7.9	93 (86 – 98)

Body weight 28 days 20nm

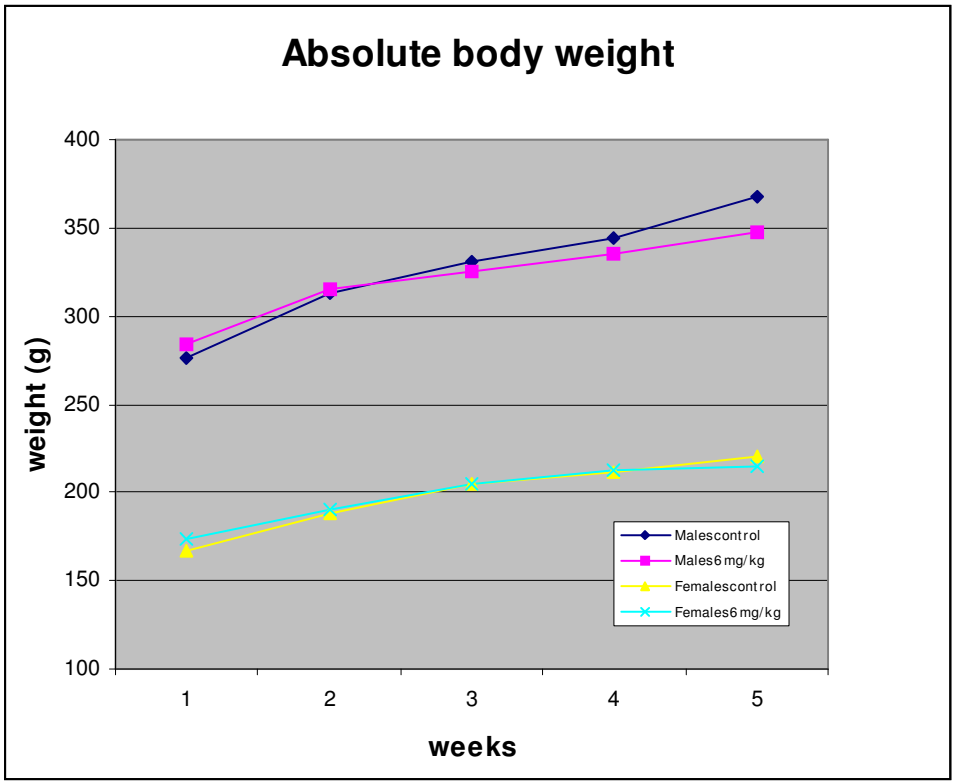


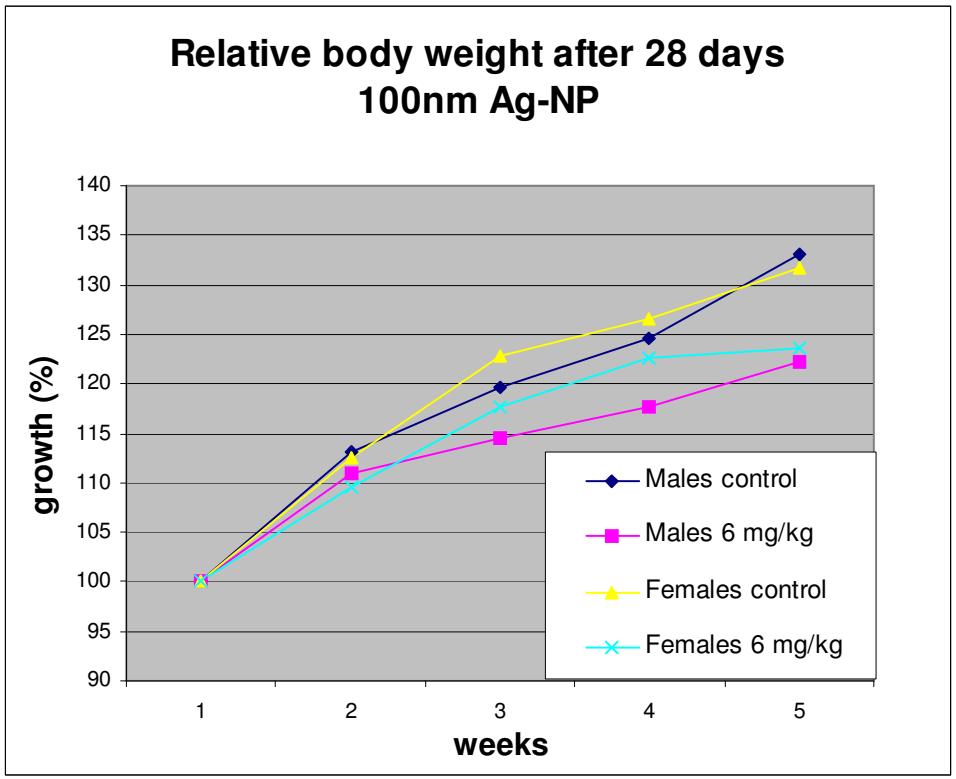




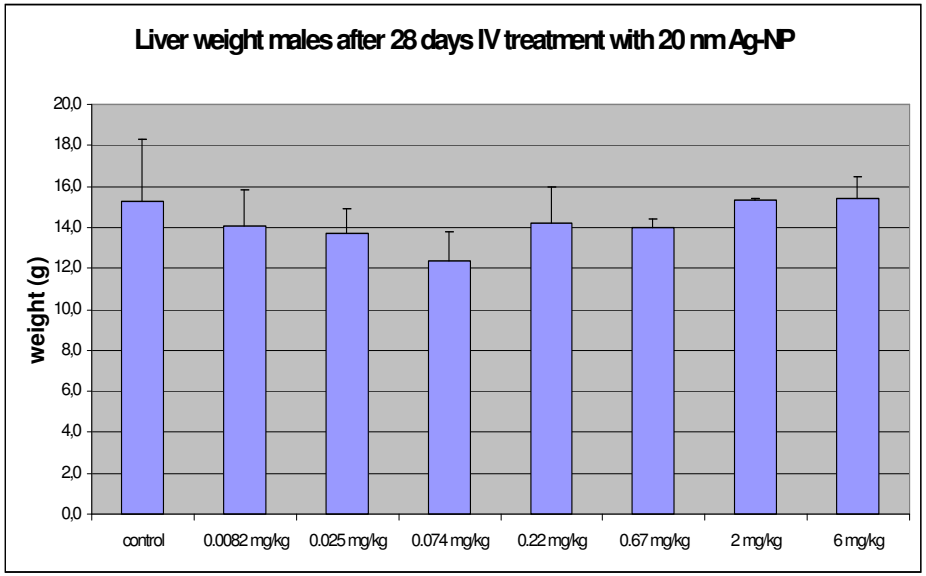


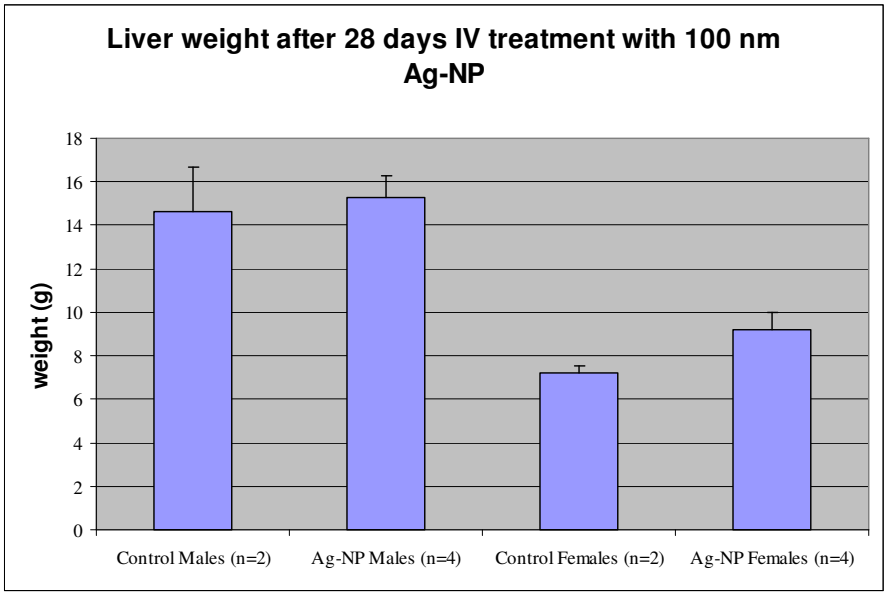
Body weight 28 days 100 nm

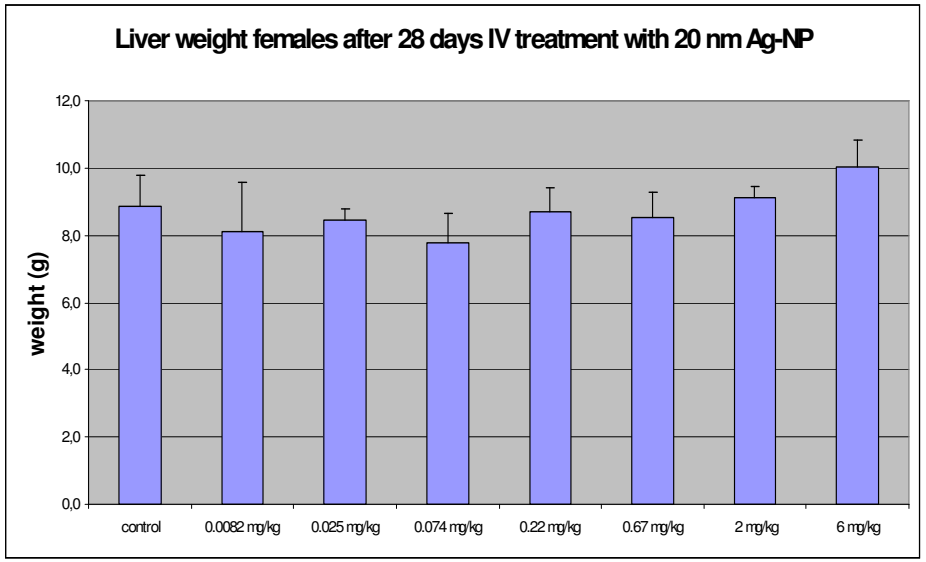




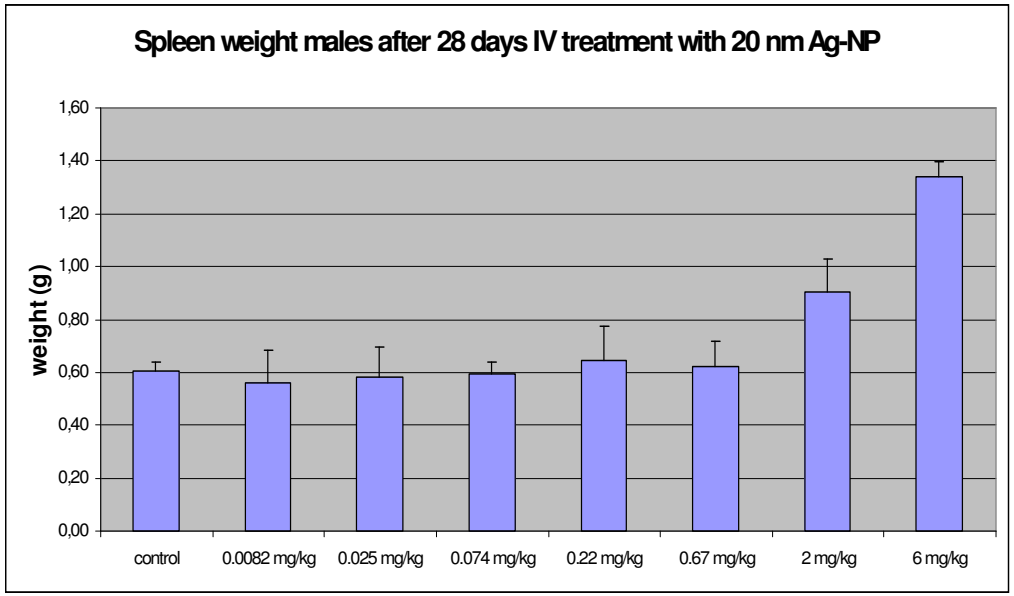
Organ weight: liver

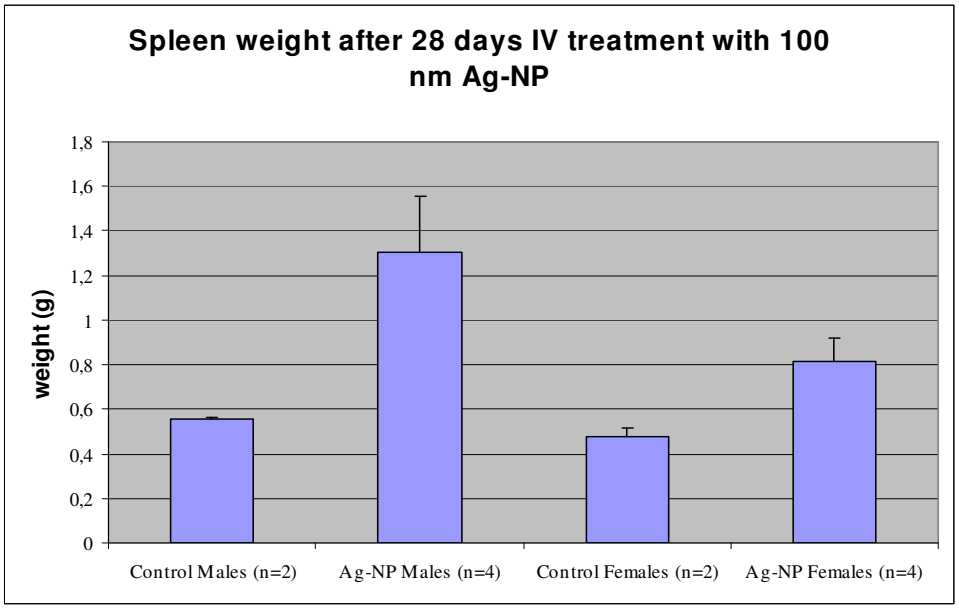


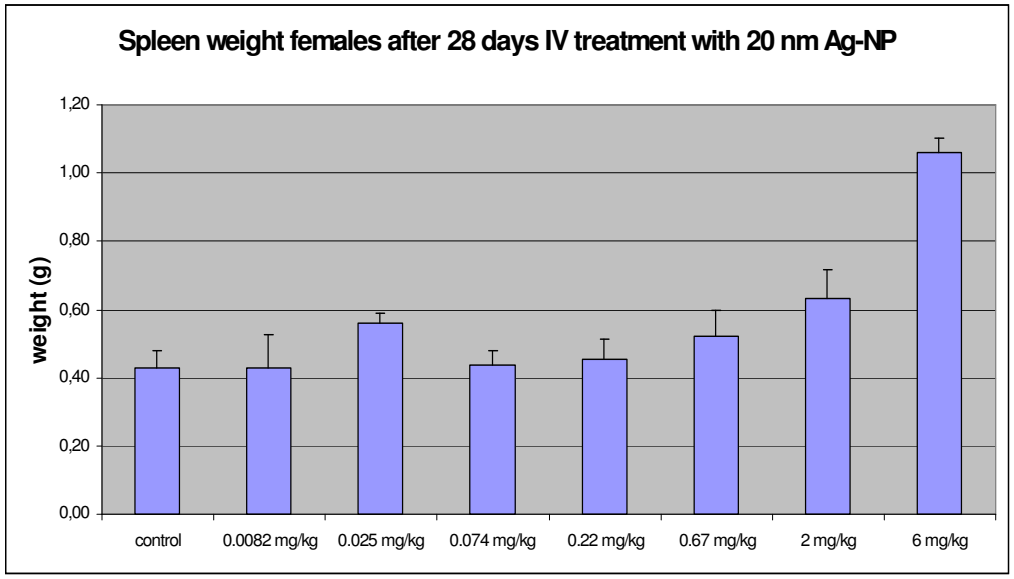




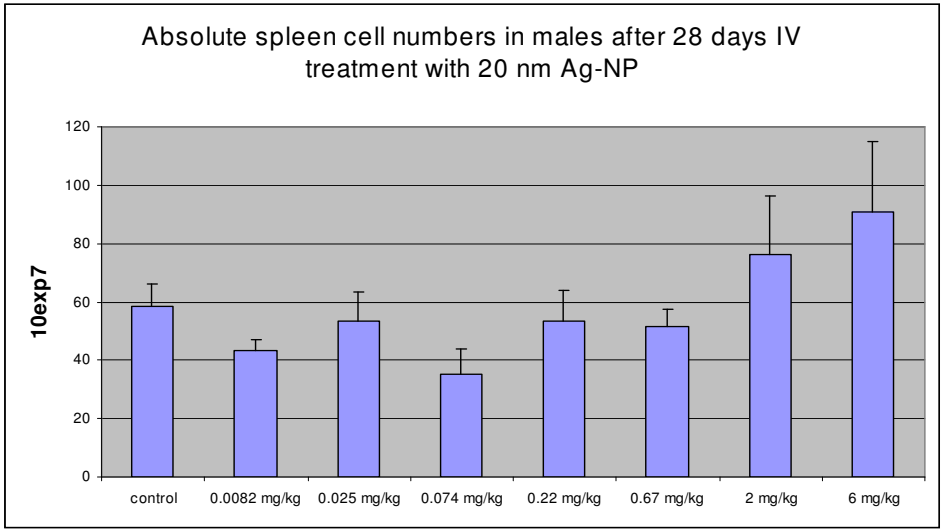
Organ weight: spleen

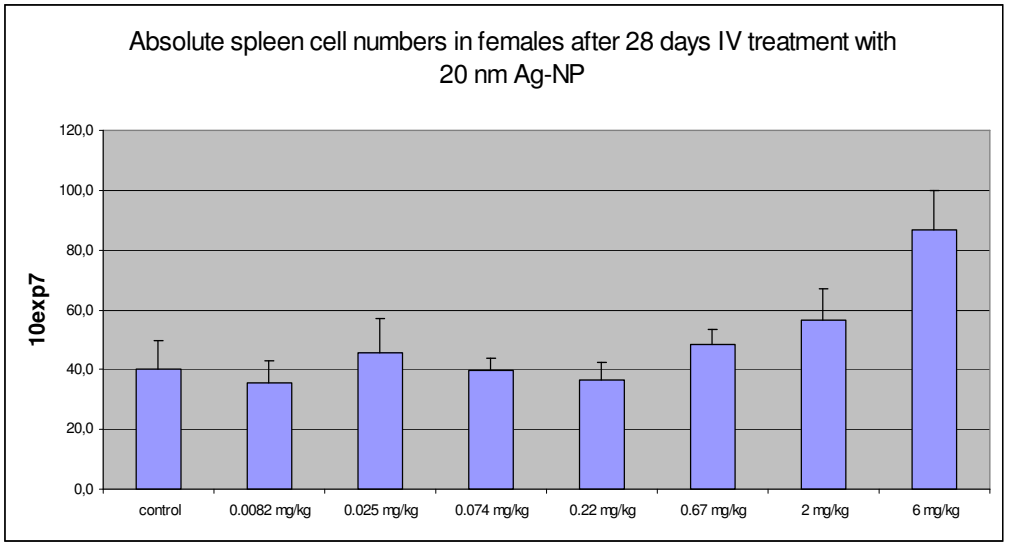


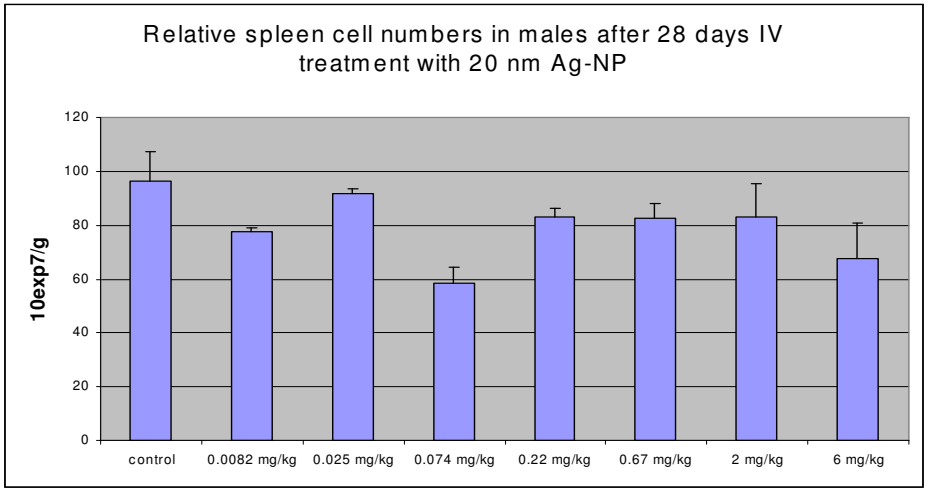


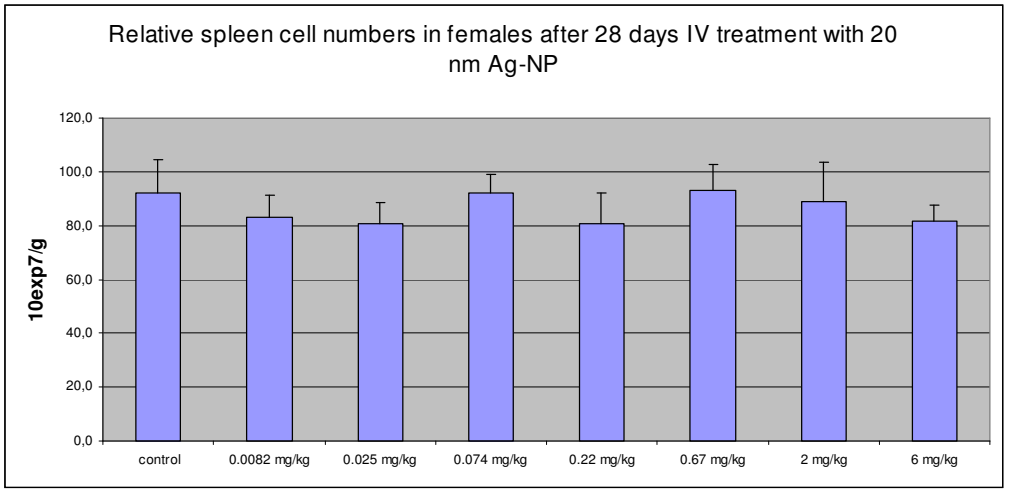


Spleen cell number

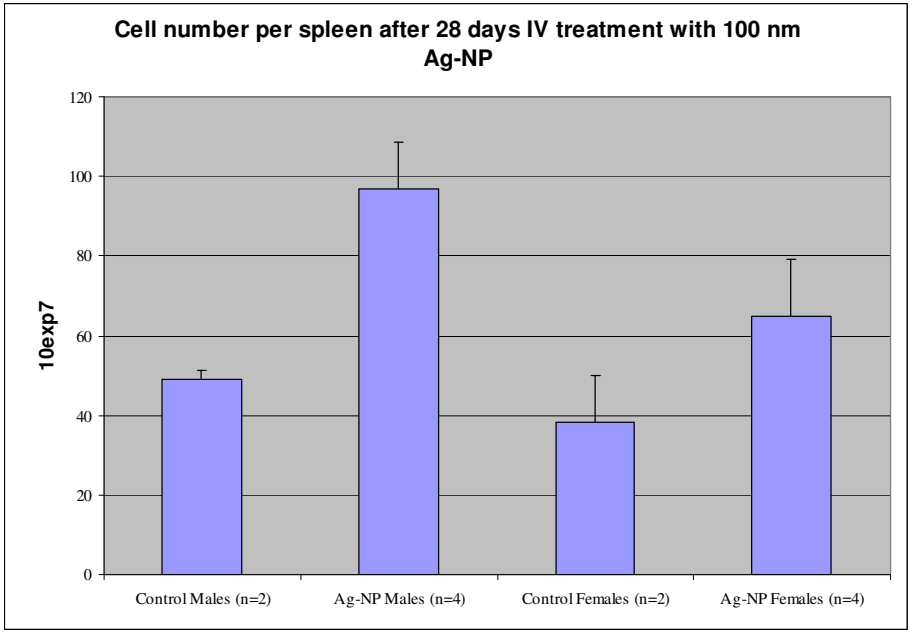


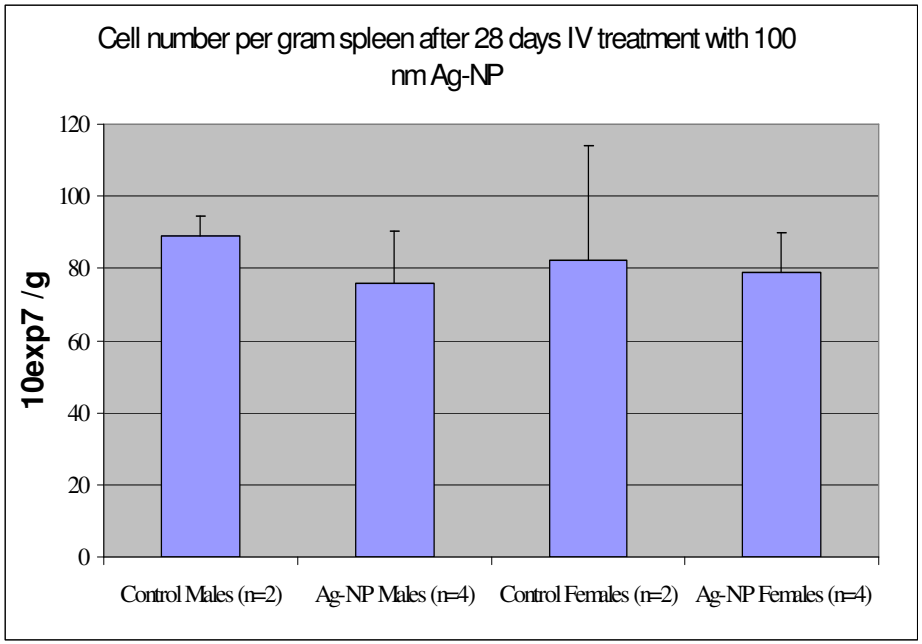




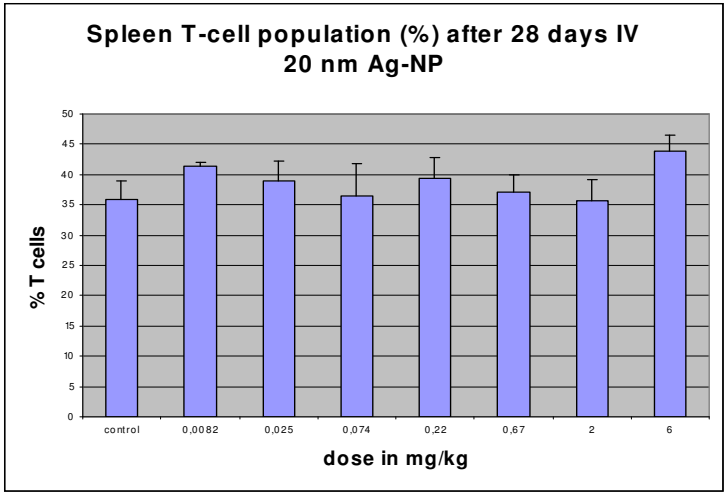


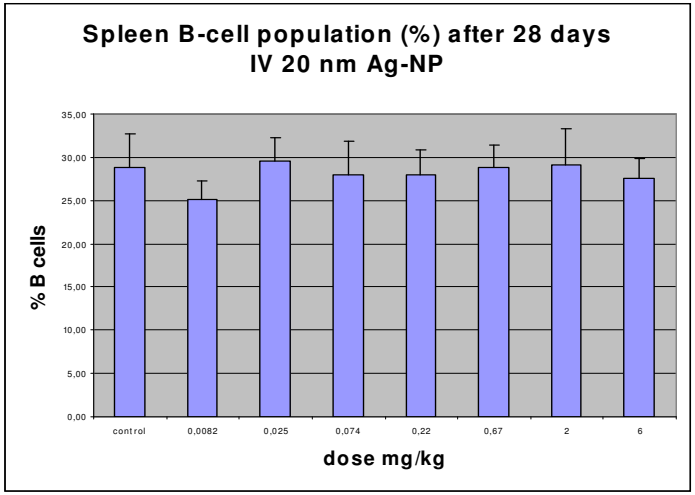
Spleen cell number

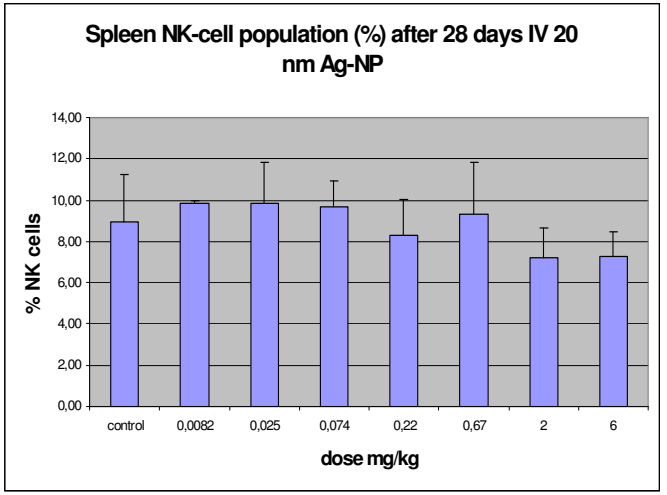


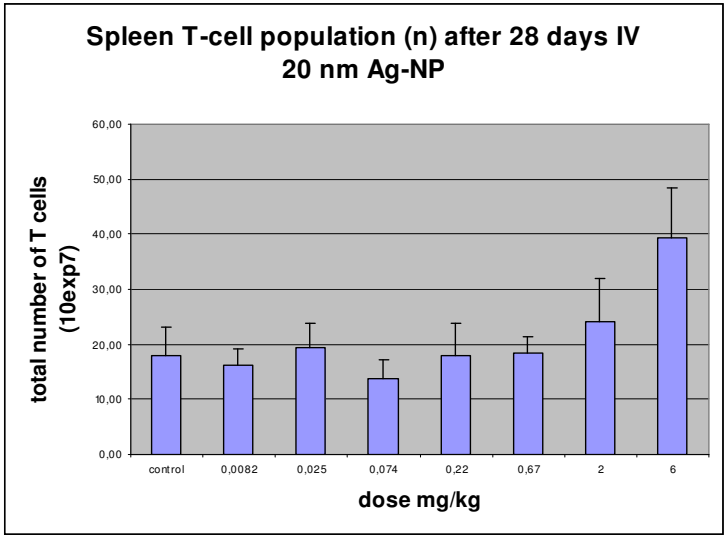


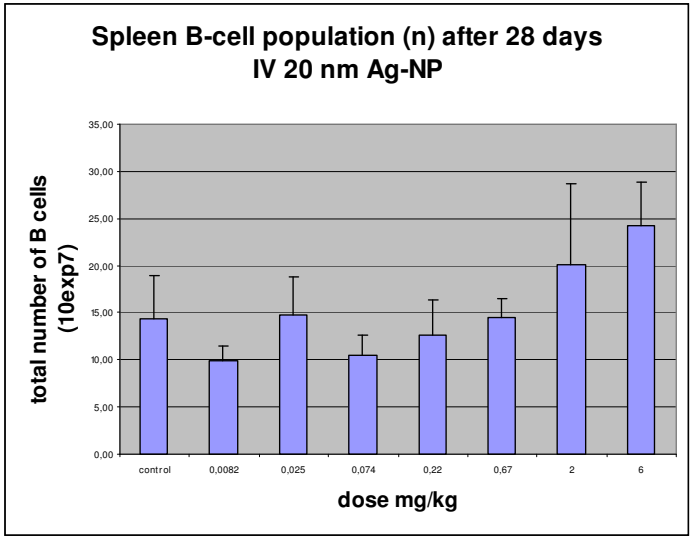
Spleen cell subsets 20 nm

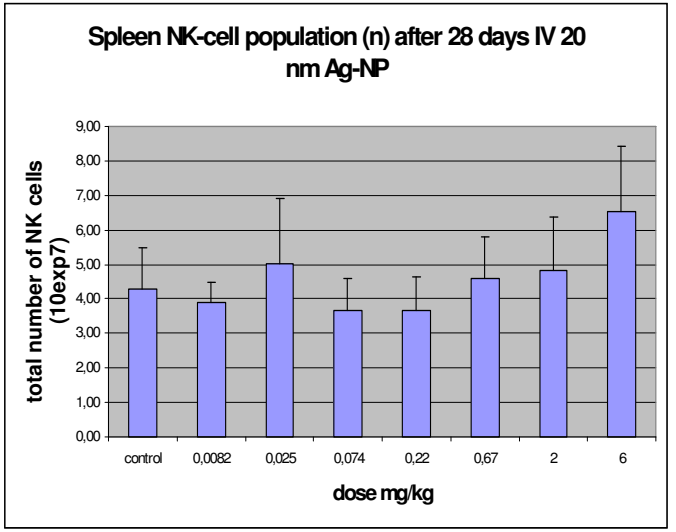






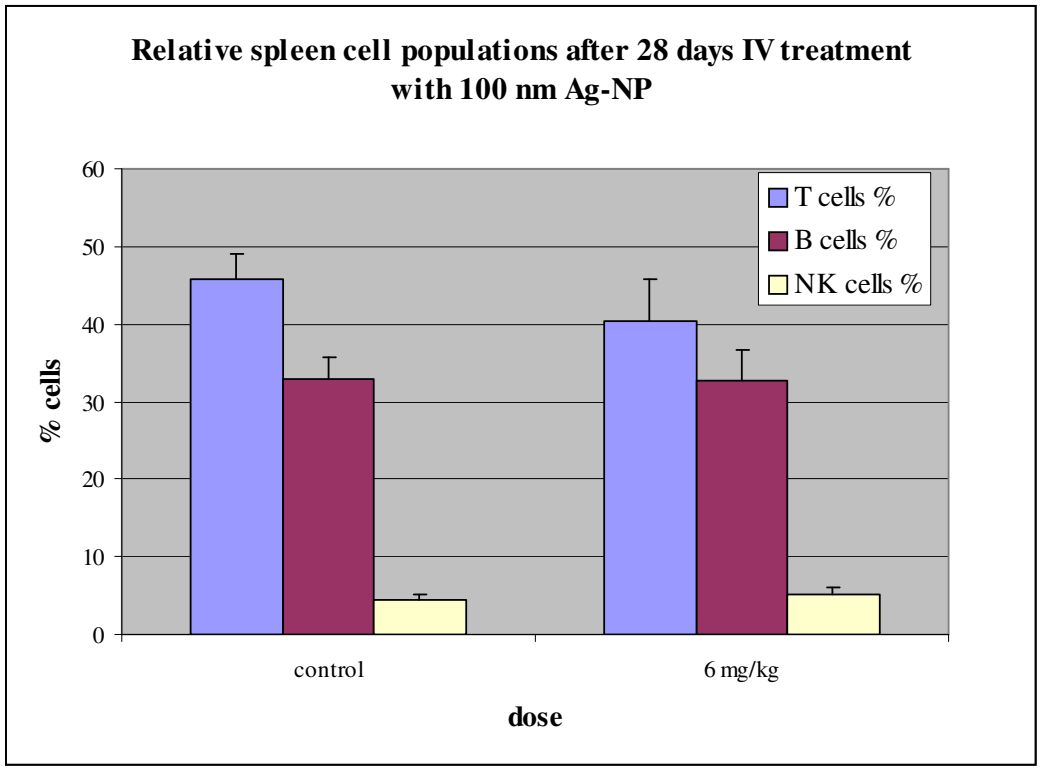


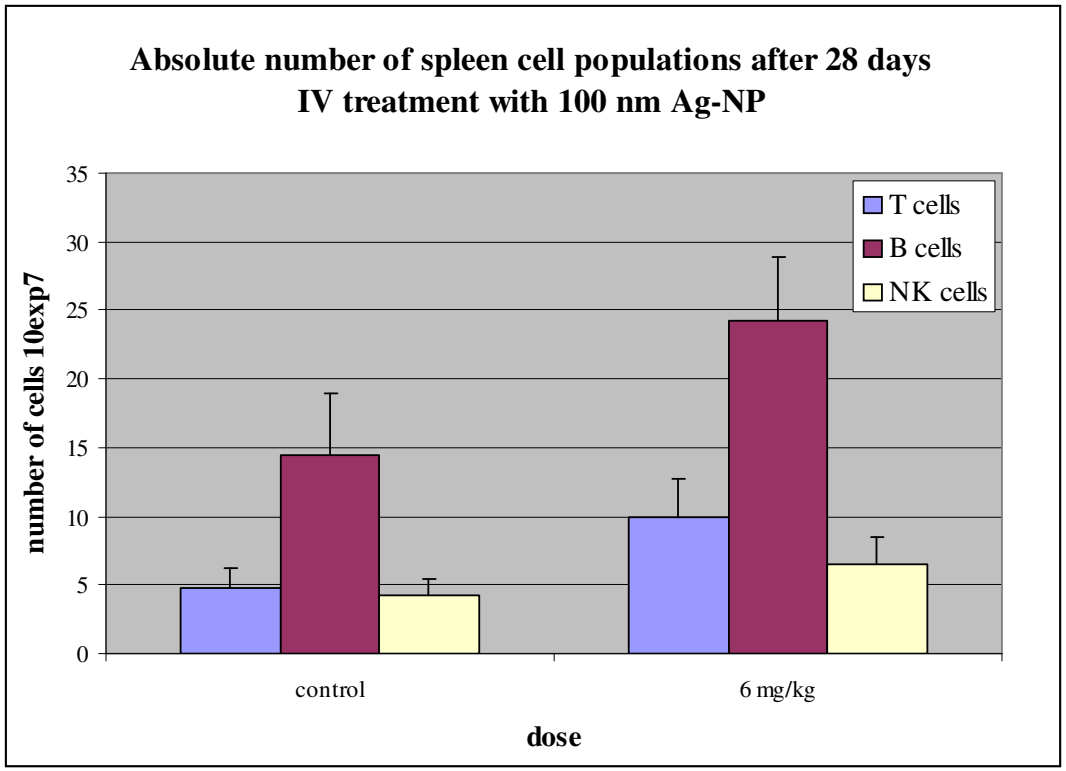




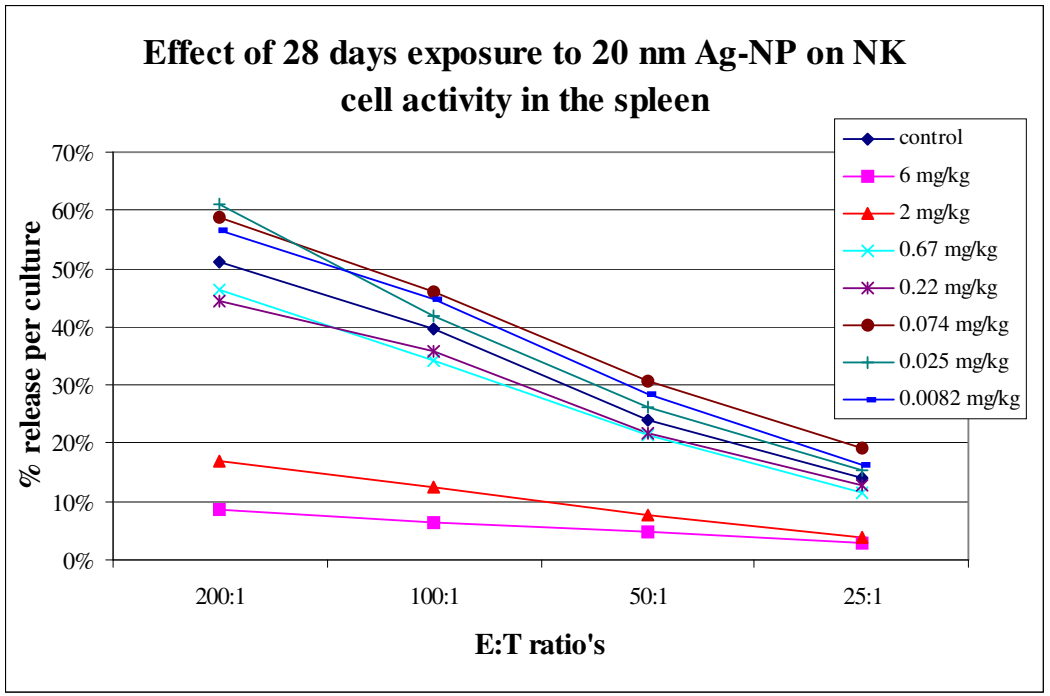
Increase in spleen weight is caused by increase in all major cell populations

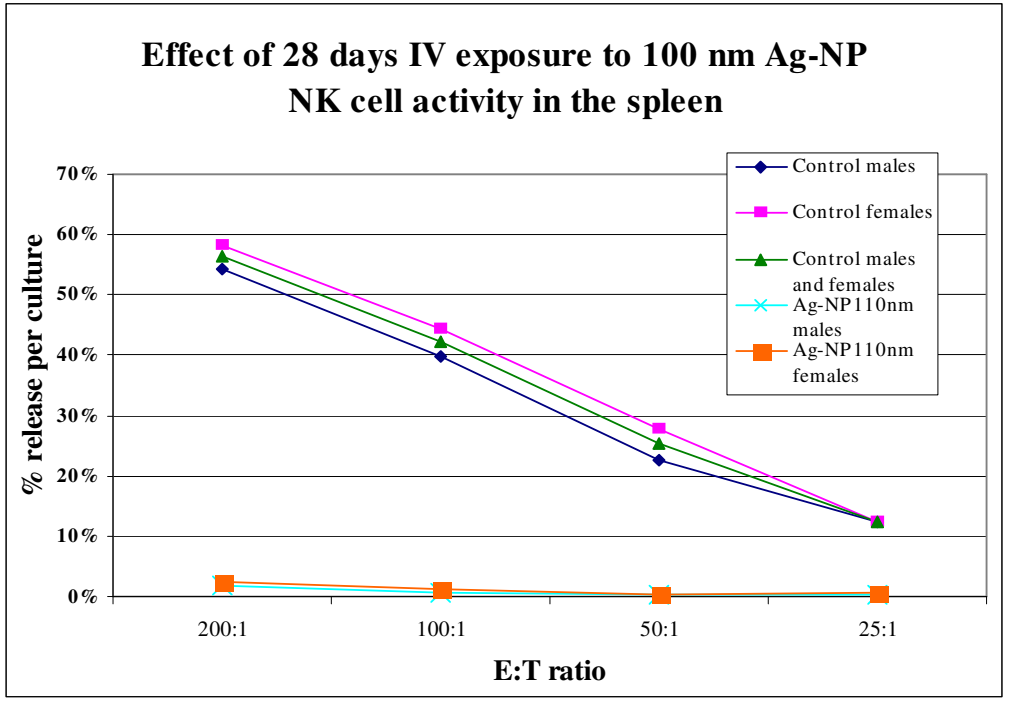
Spleen cell subsets 100 nm



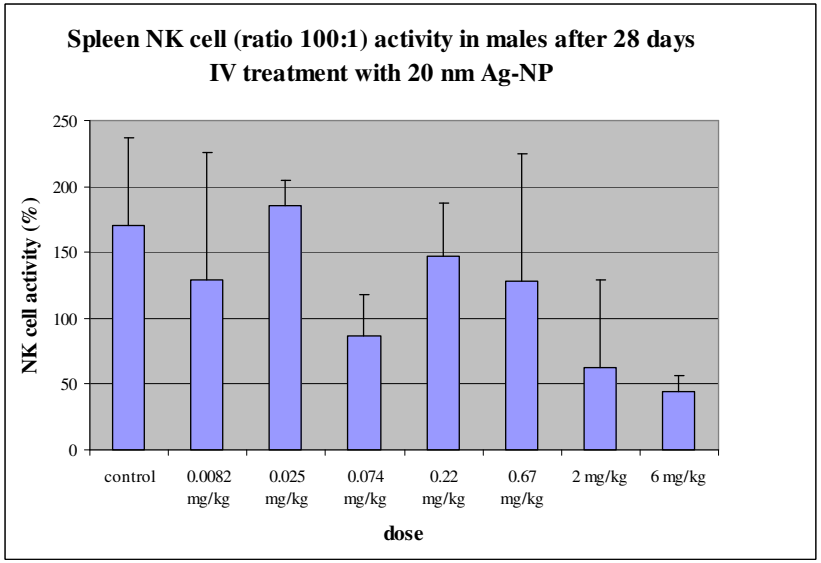


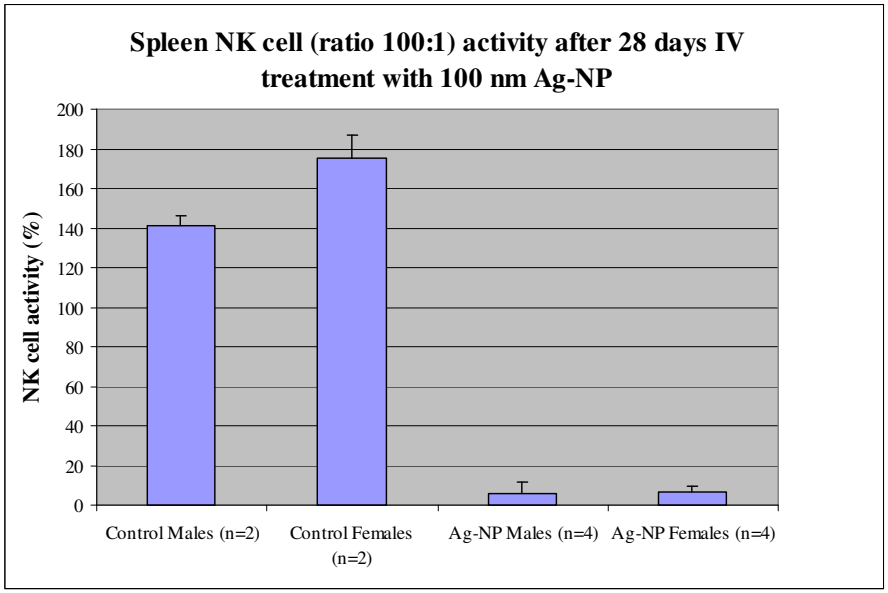
Spleen NK cell activity

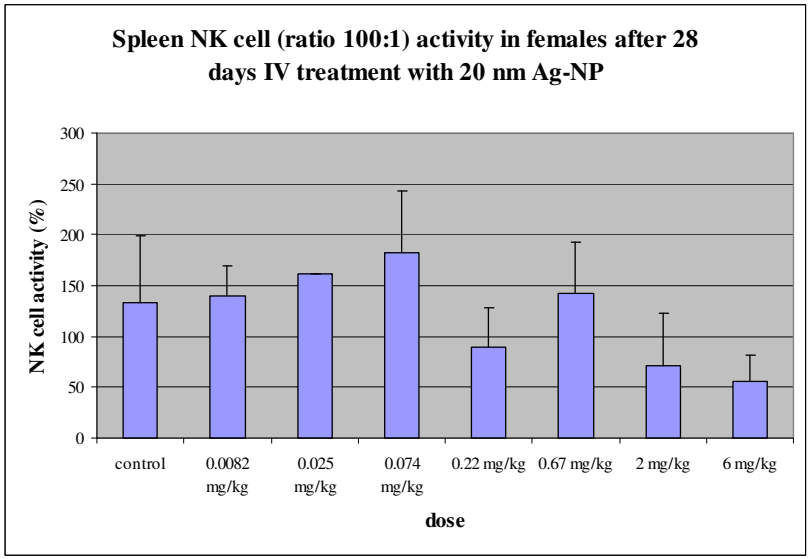




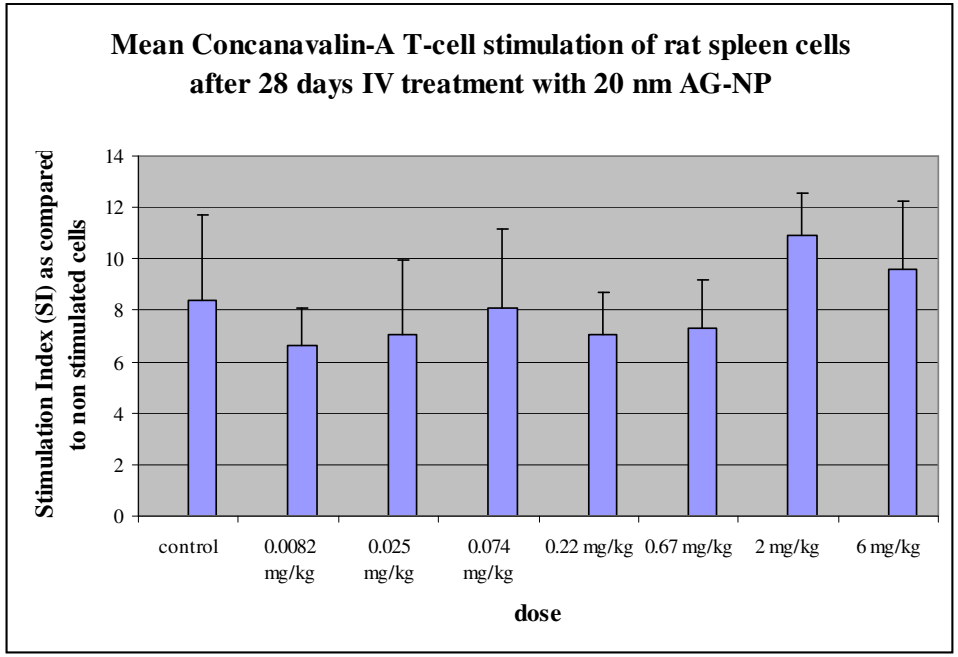
Spleen NK cell activity

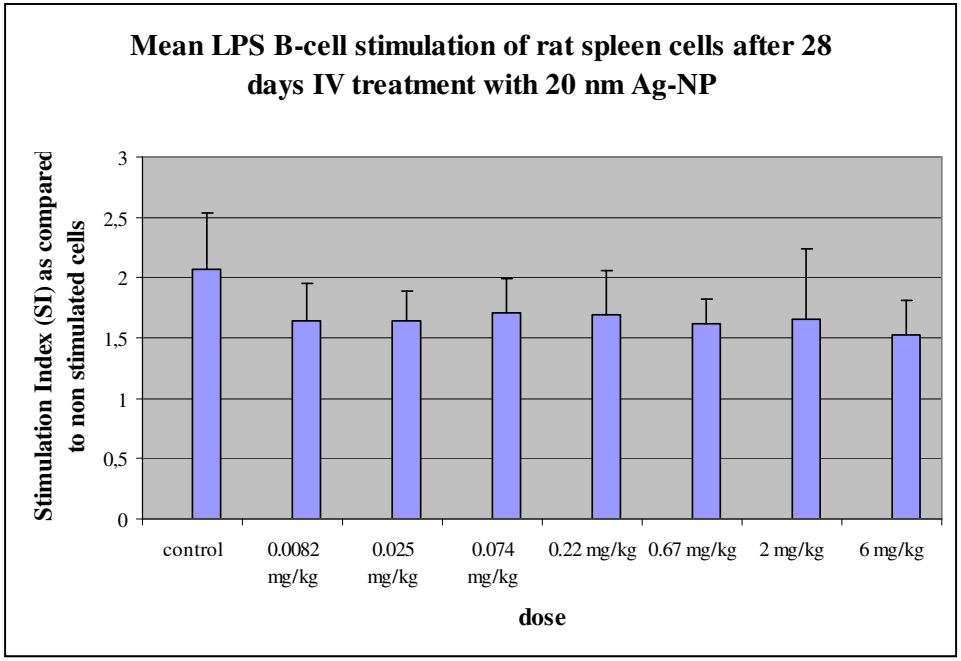






Spleen mitogen activity





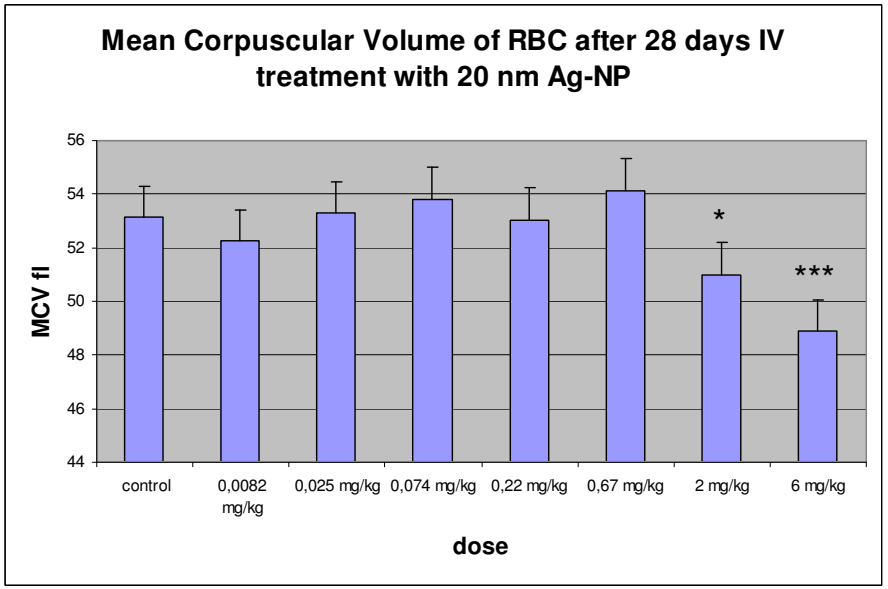
Blood values 20 nm Ag-NP

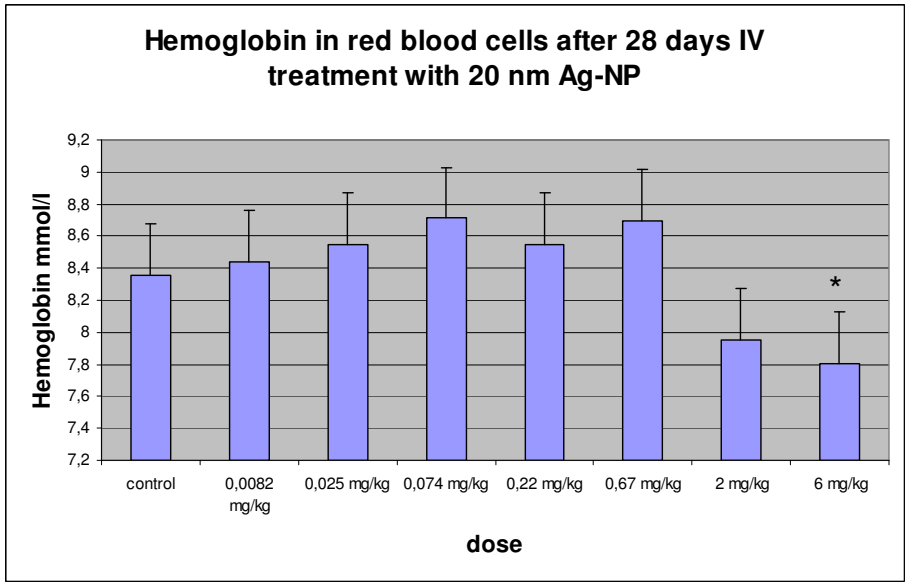
Group/dose Mg/kg (n)	WBC 109/L	PMN abs	Lymphocytes abs	PMN %	Lymphocytes %
Control (7)	5.23	0.28	4.76	5.96	90.36
0.008 (4)	4.53	0.42	3.92	9.64	86.10
0.025 (4)	4.78	0.35	4.25	7.86	88.20
0.074 (4)	4.92	0.91	4.29	7.86	87.73
0.22 (5)	6.06	0.57	5.26	8.67	87.53
0.67 (5)	6.79	0.69	5.83	10.00	86.04
2 (4)	6.49	0.78	5.39	12.00	83.11
6 (5)	5.90	**1.09**	4.52	**17.67**	**77.62**

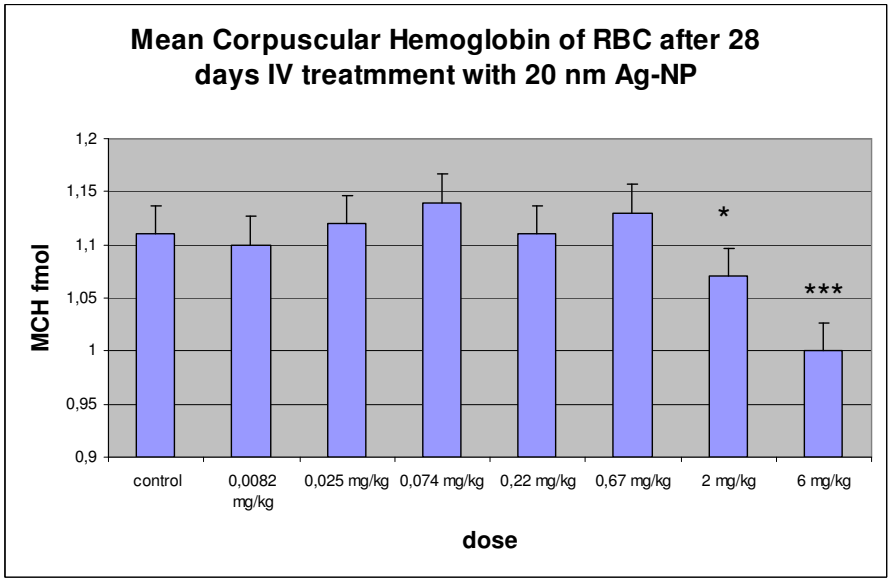
Blood values 100 nm Ag-NP

Group/dose Mg/kg (n)	WBC 10^9/L	PMN abs	Lymphocytes abs	PMN %	Lymphocytes %
Control M (2)	6.93	0.76	5.97	11.23	85.88
Ag-NP 110 nm (3)	8.74	**2.21**	6.17	**25.73**	**70.17**
Control F (1)	6.57	0.40	6.06	6.05	92.35
Ag-NP 110 nm (4)	6.42	**1.08**	4.87	**16.95**	**76.20**

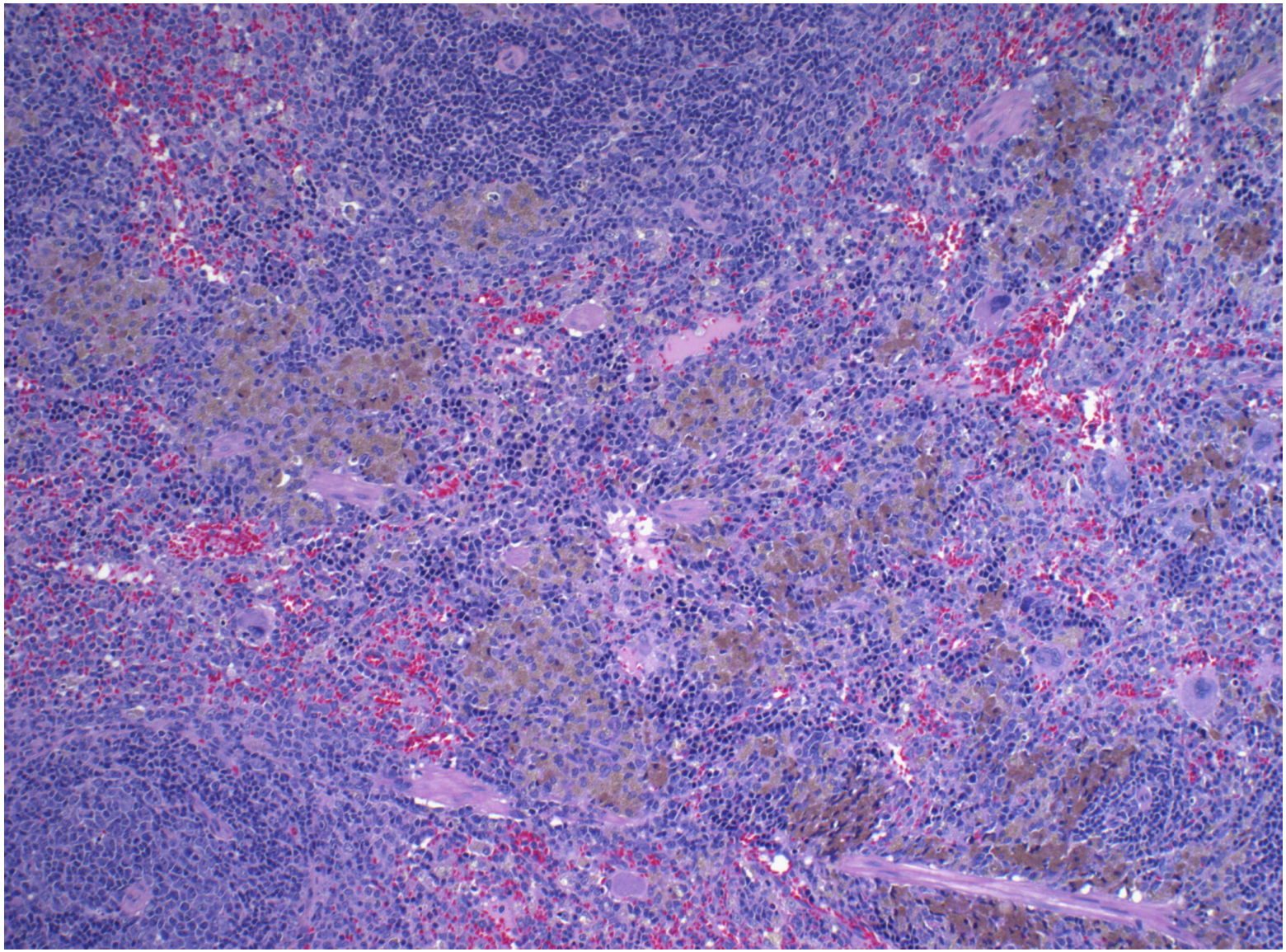
Hematology: 20 nm Ag-NP



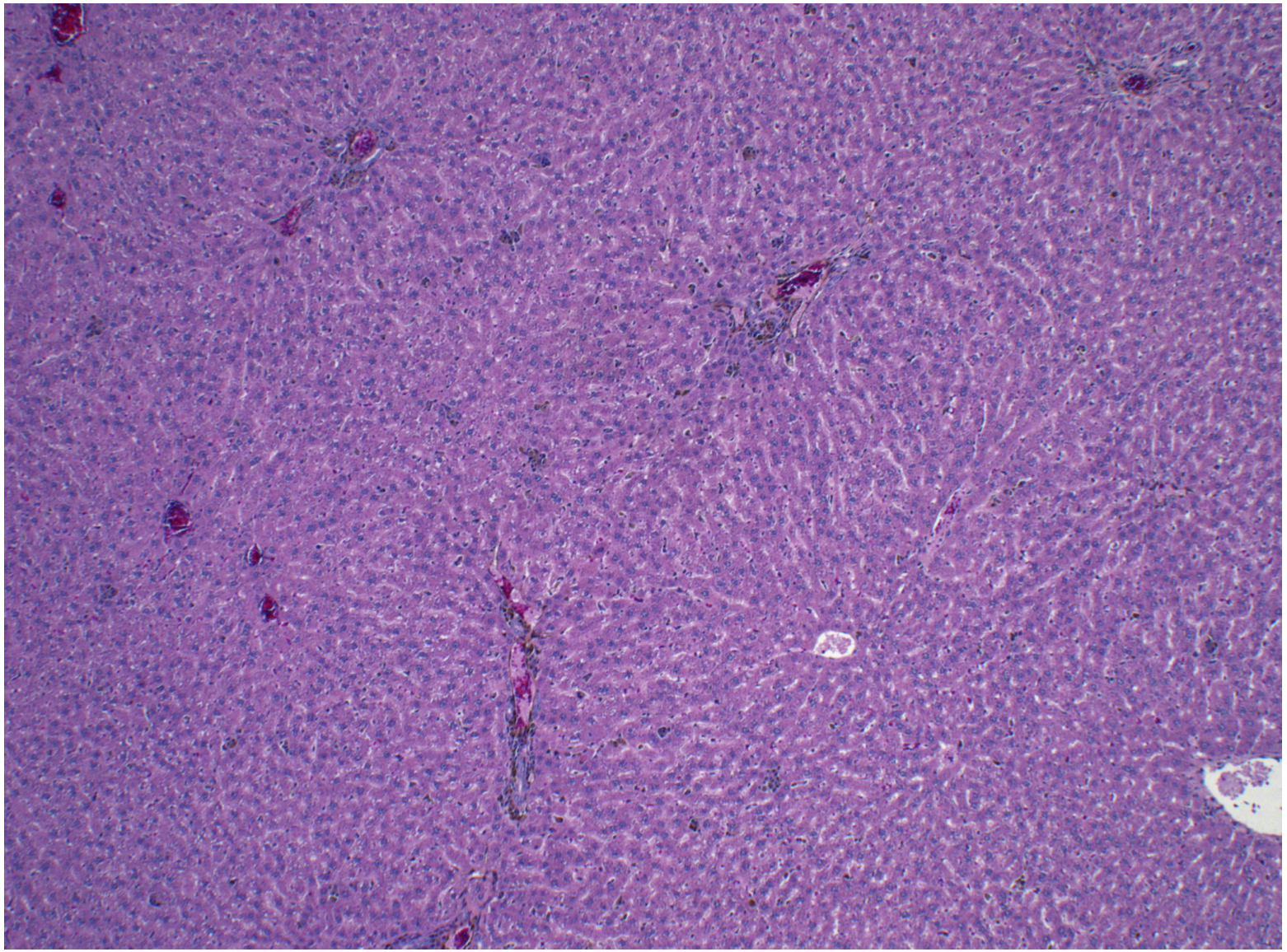




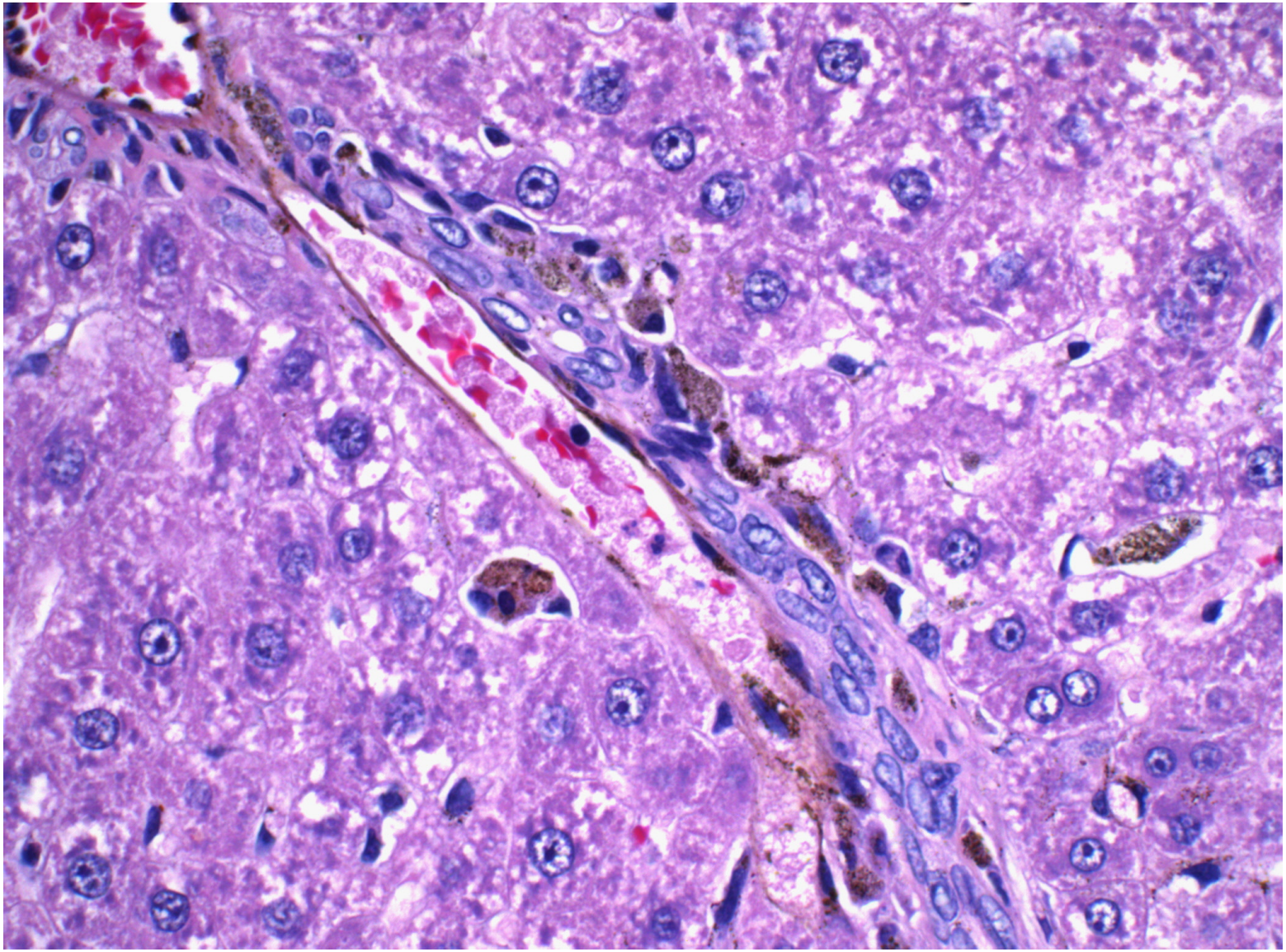
Spleen presence of pigment



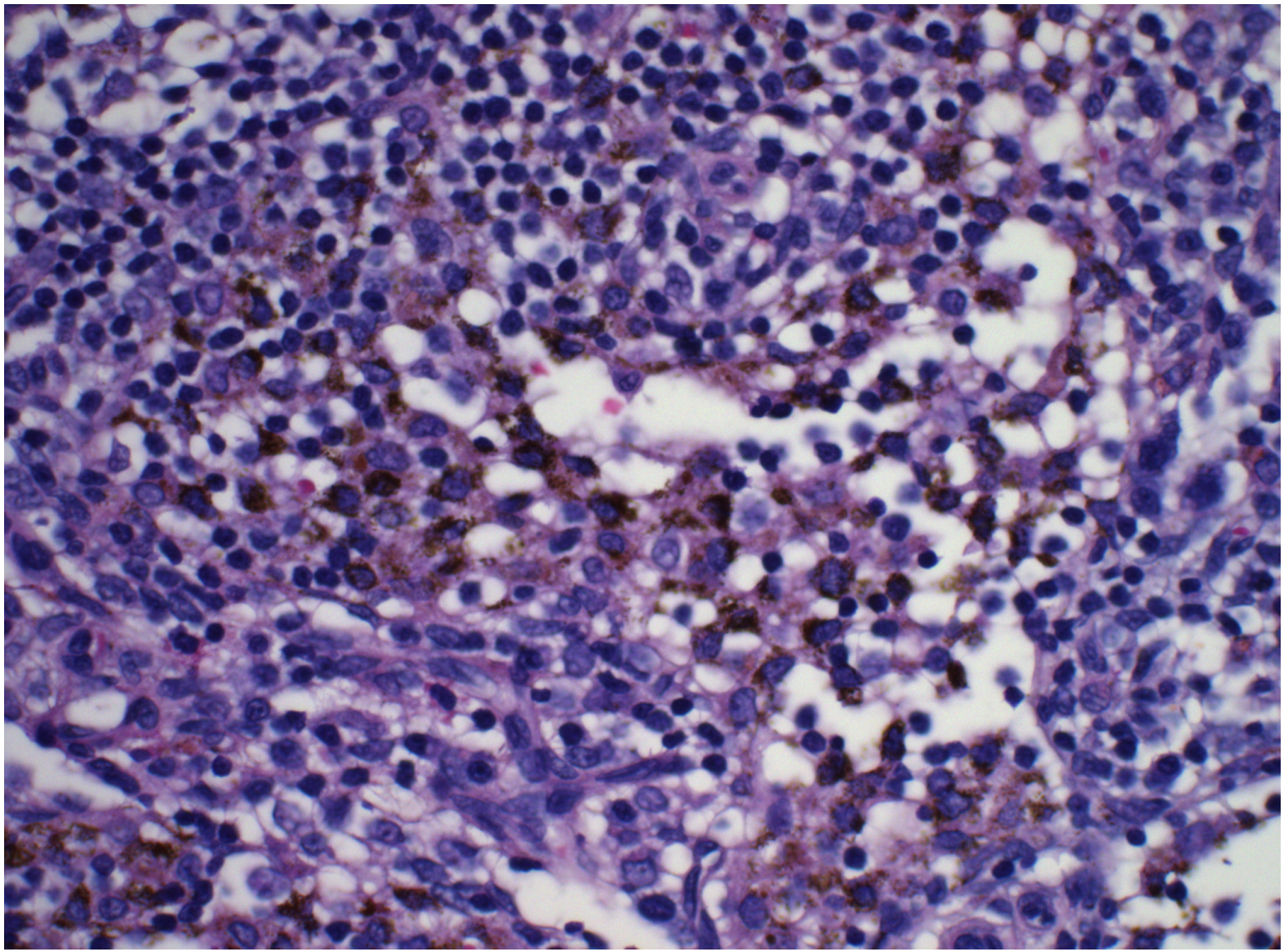
Liver presence of pigment



Detail spleen pigment



Pigment in lymph node



Summary of results in IV toxicity study with Ag-NP

20 nm Ag-NP				100 nm Ag-NP			
Dose	*Sex*	*Effect*	*Result*	*Dose*	*Sex*	*Effect*	*Result*
General							
2 – 6 mg/kg	M/F	Body weight gain	↓	6 mg/kg	M/F	Body weight gain	↓
Spleen							
2 – 6 mg/kg	M/F	Spleen weight	↑	6 mg/kg	M/F	Spleen weight	↑
6 mg/kg	M/F	Spleen cell number	↑	6 mg/kg	M/F	Spleen cell number	↑
6 mg/kg	M/F	T cells spleen	↑	6 mg/kg	M/F	T cells spleen	↑
6 mg/kg	M/F	B cells spleen	↑	6 mg/kg	M/F	B cells spleen	↑
6 mg/kg	M/F	NK cells spleen	(↑)	6 mg/kg	M/F	NK cells spleen	(↑)
2 – 6 mg/kg	M/F	NK cell activity	↓↓↓	6 mg/kg	M/F	NK cell activity	↓↓↓
2 – 6 mg/kg	M/F	IL-10 production	↓	6 mg/kg	M/F	IL-10 production	↓
2 – 6 mg/kg	M/F	IFNγ production	↓	6 mg/kg	M/F	IFNγ production	-
Blood							
6 mg/kg	M/F	AST	↑	6 mg/kg	M/F	AST	↑
6 mg/kg	M/F	PMN blood values	↑	6 mg/kg	M/F	PMN blood values	↑
2 – 6 mg/kg	M/F	MCV – RBC	↓	6 mg/kg	M/F	MCV – RBC	(↓)
2 – 6 mg/kg	M/F	MCH – RBC	↓	6 mg/kg	M/F	MCH – RBC	(↓)
6 mg/kg	M/F	Hemoglobin	↓	6 mg/kg	M/F	Hemoglobin	-
6 mg/kg	M/F	IgG	-	6 mg/kg	M/F	IgG	-
6 mg/kg	M/F	IgM	↑	6 mg/kg	M/F	IgM	↑
6 mg/kg	M/F	IgE	↑	6 mg/kg	M/F	IgE	↑

Summary of histopathology in IV 28 days toxicity study Ag-NP

- Histopathology
  - Ag-NP (20 nm and 100 nm) present in various organs (spleen, liver, kidney, adrenal glands, mesenteric and popliteal LN, intestinal tract)
  - No tissue response to Ag-NP (liver)
  - Lung, some granulomas present both in control and Ag-NP treated
    - Granulomas containing Ag-NP (Ag-NP animals), control negative
    - Granulomas due to injection technique (repeated IV injection)

Overall conclusion of IV 28 days toxicity study Ag-NP

- At highest dose administered main effect on spleen including effects on functionality of spleen cell populations (immunotoxicity)

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