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Kinetics of Accumulation of Ag in the Internal Organs and Brain Regions of Mammals at the Long-Term Oral Exposure to Ag Nanoparticles Obtained by Neutron Activation Analysis

A.A. Antsiferova, M.Yu. Kopaeva, V.N. Kochkin, P.K. Kashkarov

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Silver in Human Life

Antsiferova A.A., Kashkarov P.K., Koval'chuk M.V. Effect of Different Forms of Silver on Biological Objects // Nanobiotechnology Reports, 2022, Vol. 17, No. 2, pp. 155–164.

Properties of silver

- Antibacterial
- Antiviral
- Fungicidal

•Silver items were used for medical purposes in ancient Egypt and Mesopotamia. Hindu (Ayurvedic and religious) literature mentions a way of disinfecting water by metallic silver. Thus, up until the 1800s, silver had been used exclusively in its metallic form

•The aseptic nature of silver had become clearer by 1930, and the diversity of its forms had also broadened. Thus, colloidal silver (Argyrol, Protargol), silver nitrate, and silver-arsenic compounds began to be used.

•Silver nanoparticles are widely applied in food, light, cosmetic industries, medicine and pharmaceutics since the beginning of XXI century.

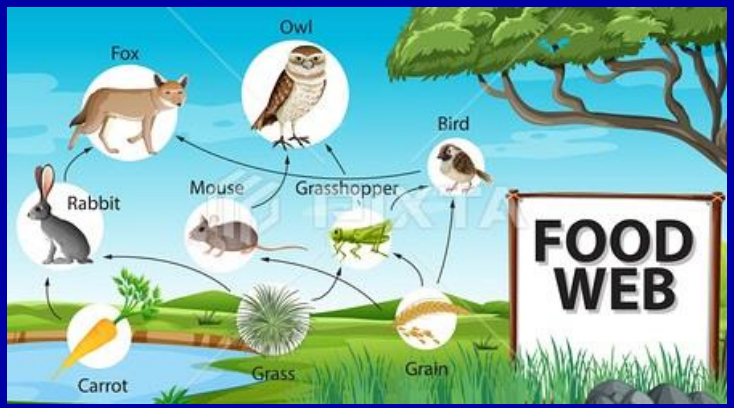
Toxicity of Silver

- Silver can demonstrate toxicity not only to pathogens but to healthy cells as well.
- Several scientific researches has demonstrated influence of silver nanoparticles on behavioral and cognitive functions of mammals.
- American Conference of Governmental Industrial Hygienists has established separate threshold limit values for metallic silver (0.1 mg/m^3) and soluble compounds of silver (0.01 mg/m^3).
- Soluble forms of silver are more biologically active and toxic than insoluble silver. It increases effectiveness and risks in the same time of use of silver nanoparticles with hydrophilic coating in water media.

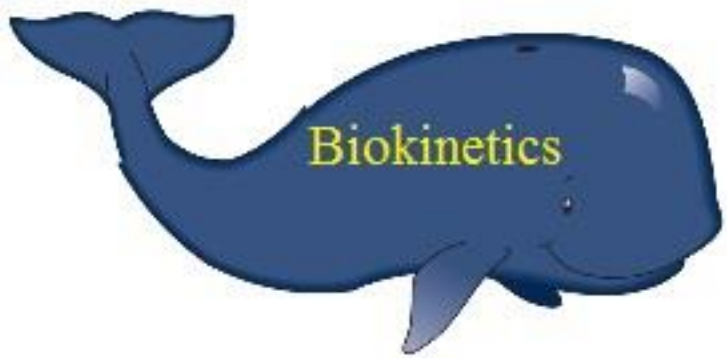
Anna A. Antsiferova, Marina Yu. Kopaeva, Vyacheslav N. Kochkin, Pavel K. Kashkarov, Mikhail V. Kovalchuk. Disturbance in Mammalian Cognition Caused by Accumulationof Silver in Brain // Toxics, 2021, 9, 30.

An Essential Problem

- Silver is not an essential element for mammalian and many other types of organisms.
- Active application of silver in industry leads to essential contamination of ecosphere and biosphere by Ag. The mechanisms and effects of interaction of Ag with living cells are not entirely understood.
- Nanoform brings additional uncertainty into processes of interaction of Ag with living organisms.



The Main Directions of Nanosafety



The Purpose of the Work

- To study the kinetics of accumulation of Ag in internal organs and brain regions of mammalian model organism at long-term oral exposure to Ag nanoparticles.

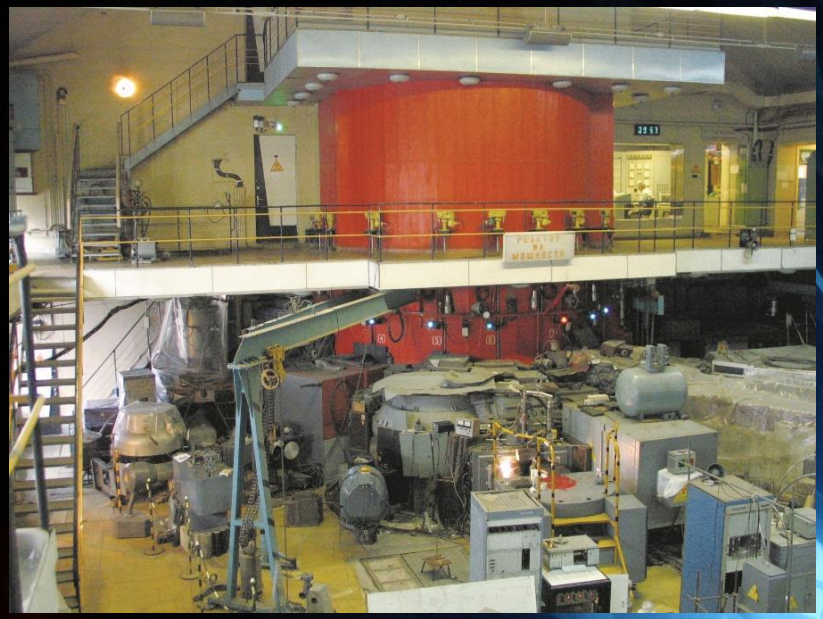
Materials

- Silver nanoparticles coated with polyvinylpyrrolidone performed by dietary supplement Argovit-S (Novosibirsk, Russia).
- Male mice C57Bl/6 since the age of 2 months (initial weight 20-22 g.) (the “Stolbovaya” branch of the Federal Medical Biological Agency of Russia).

Methods of Nanoparticle Characterization

- Dynamic Light Scattering (Malvern Zetasizer, UK)
- Transmission Electron Microscopy (Titan, USA)

Instrumental Neutron Activation Analysis

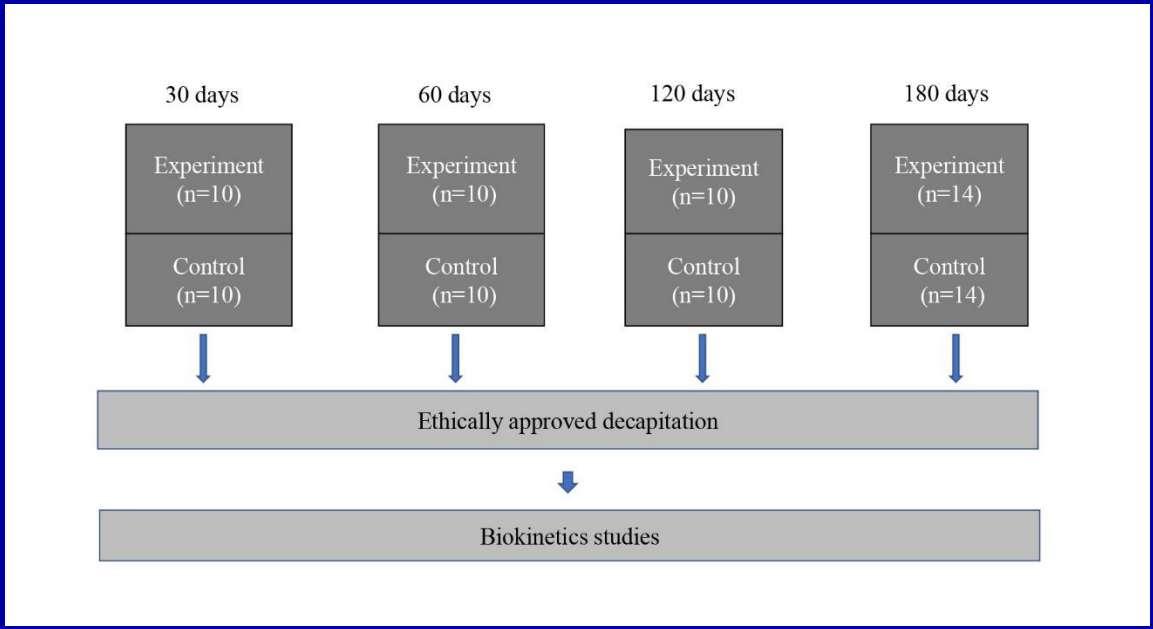


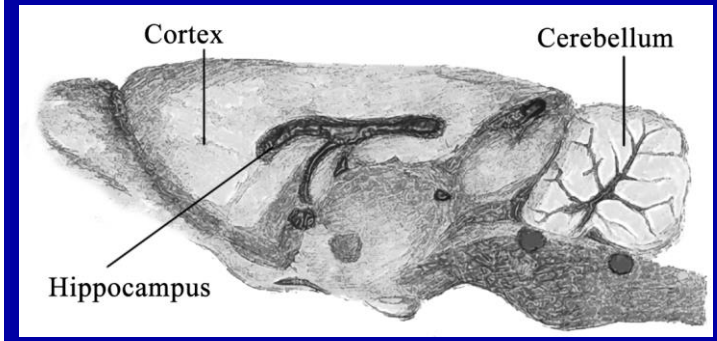
- Instrumental Neutron Activation Analysis was used for elemental analysis of biological tissues.
- It demonstrates high sensitivity (up to 10-11 g) and metrological accuracy (~1-10%). Use of INAA allows to conduct nondestructive macrosample analysis of whole tissues.
- The source of thermal neutrons is a nuclear experimental reactor IR-8 in the National Research Center Kurchatov Institute with neutron flux of no less than 10^{12} $cm^{-2}c^{-1}$

A.A. Antsiferova, Yu.P. Buzulukov, V.A. Demina, V.F. Demin, D.A. Rogatkin, E.N. Petritskaya, L.F. Abaeva, P.K. Kashkarov. Radiotracer Methods and Neutron Activation Analysis for the Investigation of Nanoparticle Biokinetics in Living Organisms // Nanotechnologies in Russia, 2015, V. 10, N. 1–2, pp. 100–108

Scheme of the Experiment

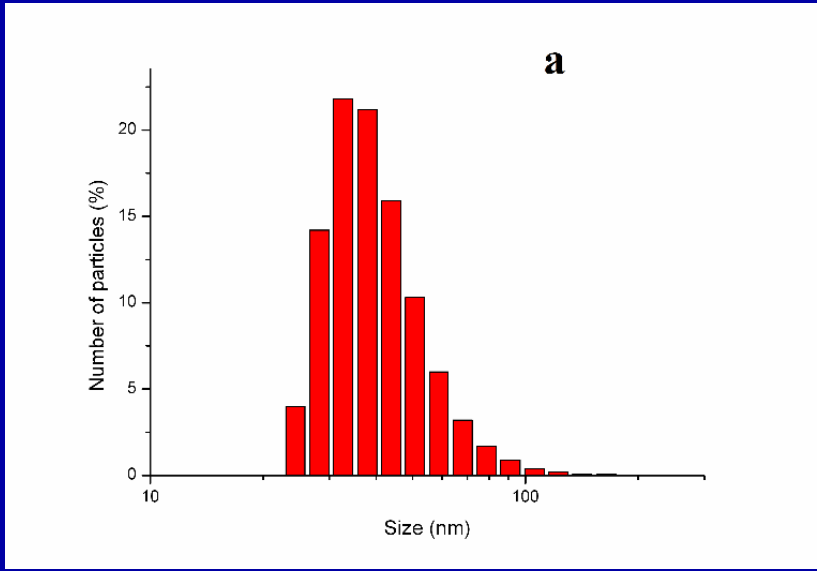
- Everyday oral exposure to 50 μg of Ag nanoparticles per day per animal in ad libitum mode.

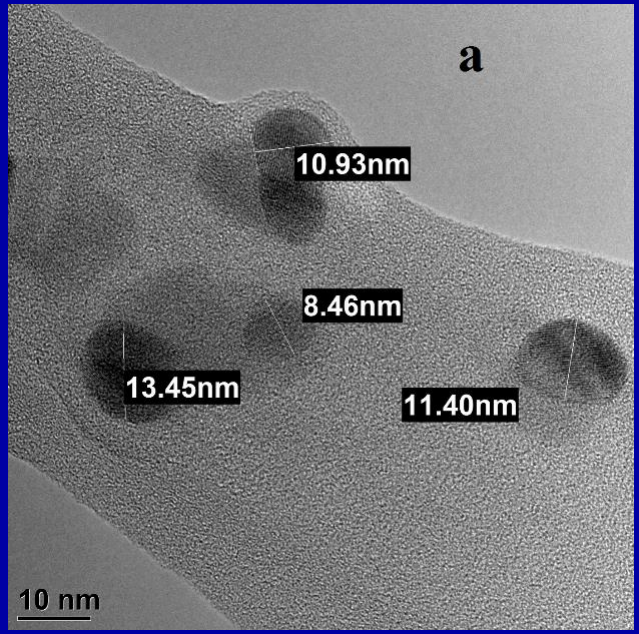




The study was conducted according to the rules of the Ministry of Health of the Russian Federation (№ 267 of 19.06.2013), and approved by the Local Ethics Committee for Biomedical Research of the National Research Center “Kurchatov Institute”(No 01 from 10.02.2017).

Results: Nanoparticles





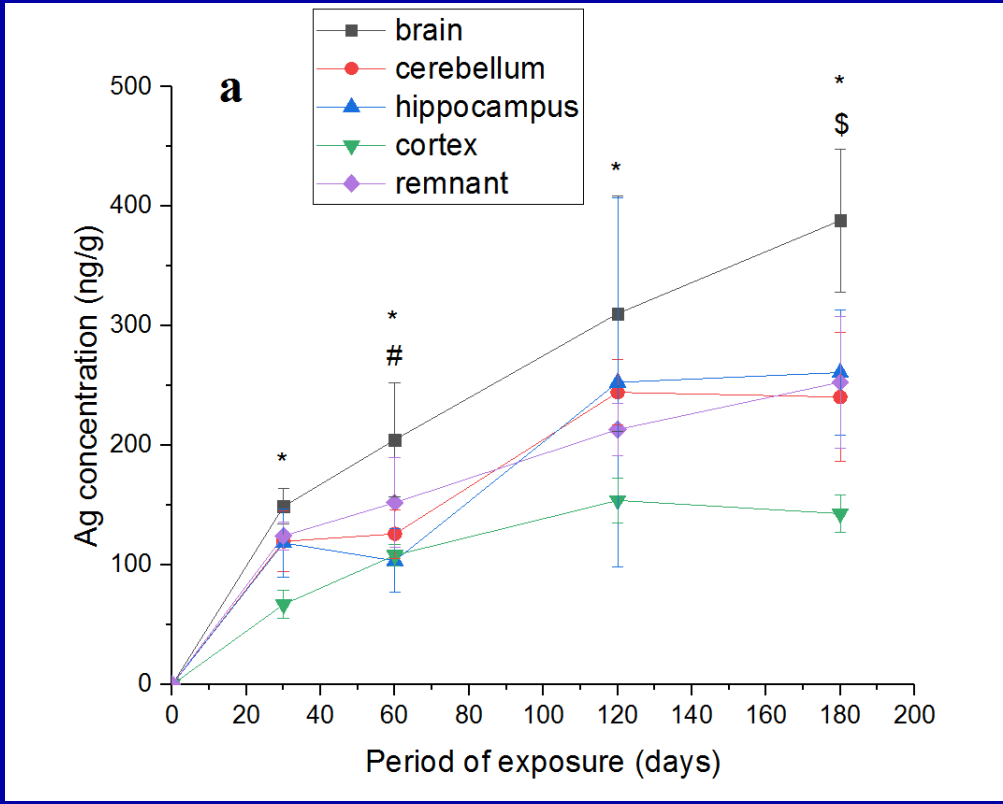
- Average size is size 34±5 nm
- Quasi-spherical form
- The size did not significantly change after storage in the dark at T= +2 °C. It evidences about high stability of the colloidal solution.

Ag Accumulation in Brain Regions

Period of Exposure, days	Mass of silver, ng (M±SD)			
	Hippocampus	Cerebellum	Cortex	Remnant
30	6,4±1,5	16,3±3,4	10,4±3,1	28±2,5
60	5,8±1,5	15±2,4	16,9±1,5	30,3±7,4
120	13,4±8	27,6±3	20,5±6,0	42,9±4,2
180	12,4±2,3	16,6±3,7	31,9±11,3	65,9±14,3

- Masses of Ag in some brain regions are very small quantities.
- INAA allows to detect and measure such small quantities of Ag.

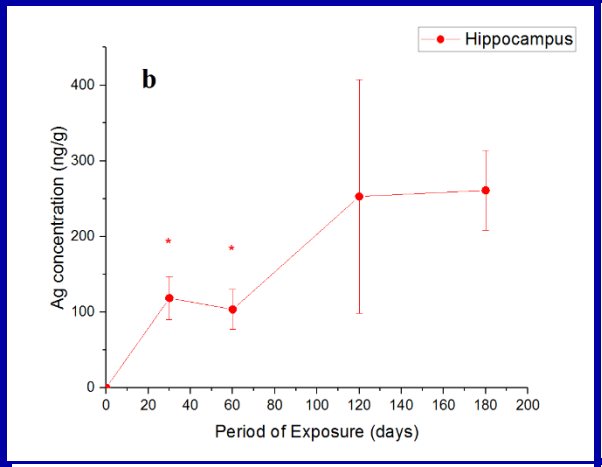
Ag Accumulation in Brain Regions

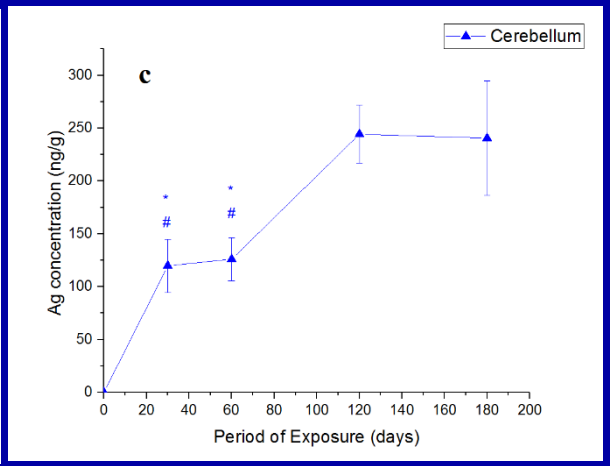


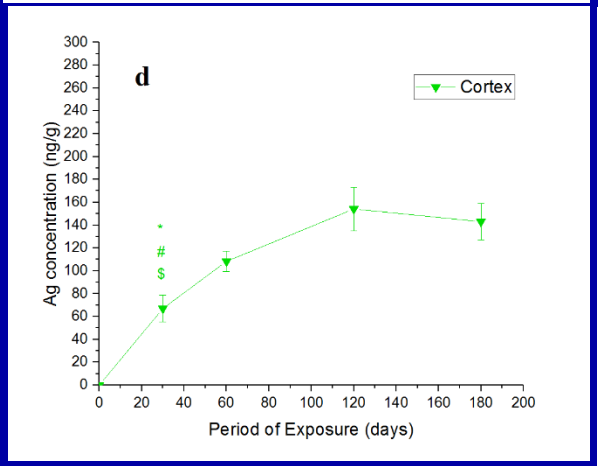
Dependence of the concentration of silver in the brain and its parts on the period of administration of silver nanoparticles (*p < 0,05 – Brain vs Cortex, #p < 0,05 – Brain vs Hippocampus, $ p < 0,05 – Cortex vs Hippocampus in each group). Number of organs per each point n=4-8.

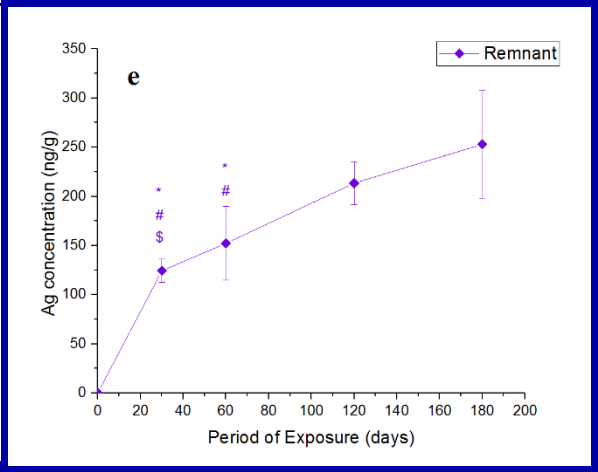
General character of Ag accumulation is similar for brain, hippocampus, cerebellum and remnant. Concentrations of Ag in cortex are lower for all the time points in comparison with other brain regions.

Ag Accumulation in Brain Regions



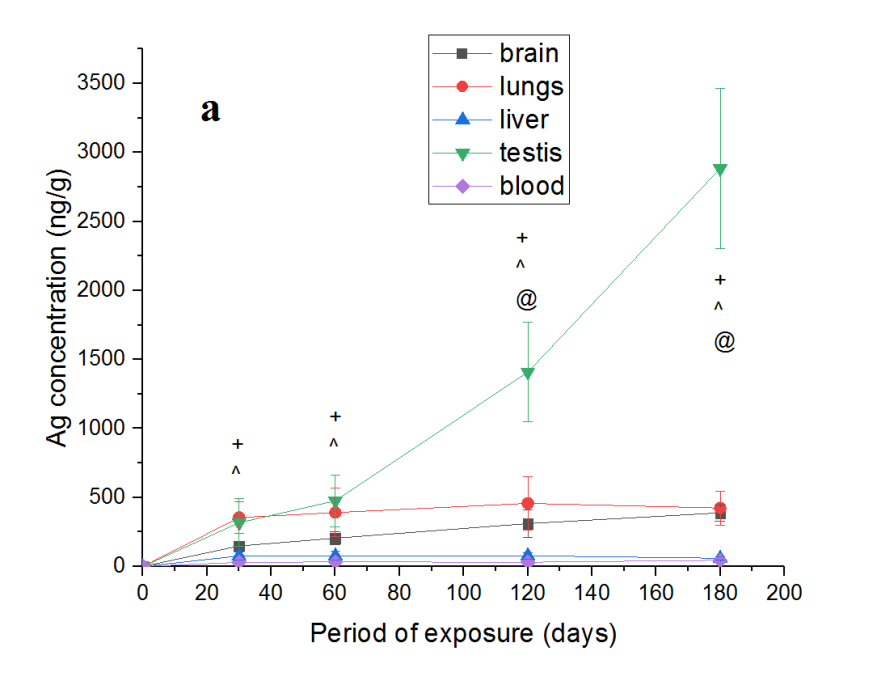


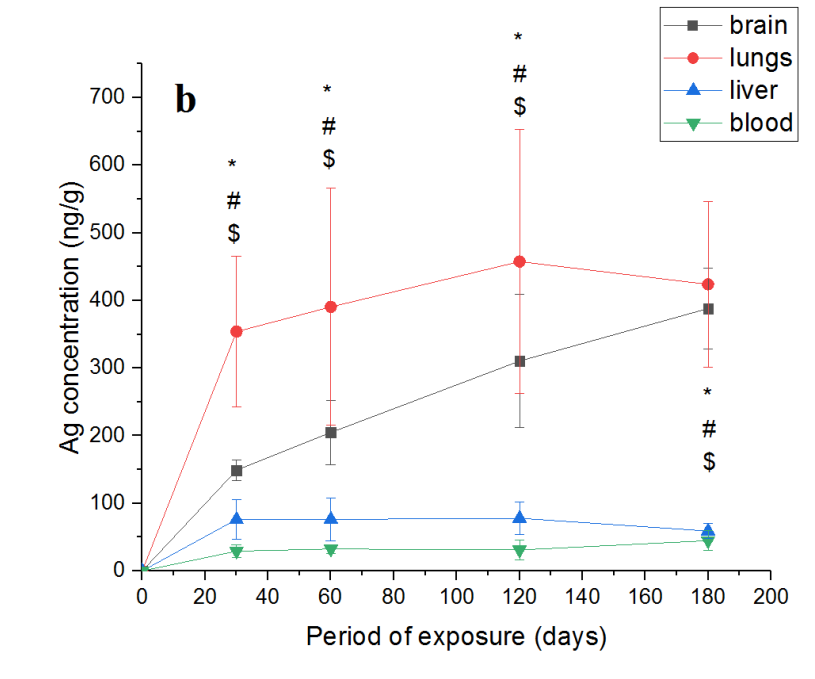




The points to the left denoted by * are statistically different from the 180 day point; the points to the left denoted by # are statistically different from the 120 day point; the points to the left denoted by $ are statistically different from the 60 day point. Number of organs per each point n=4-8.

Ag Accumulation in Internal Organs

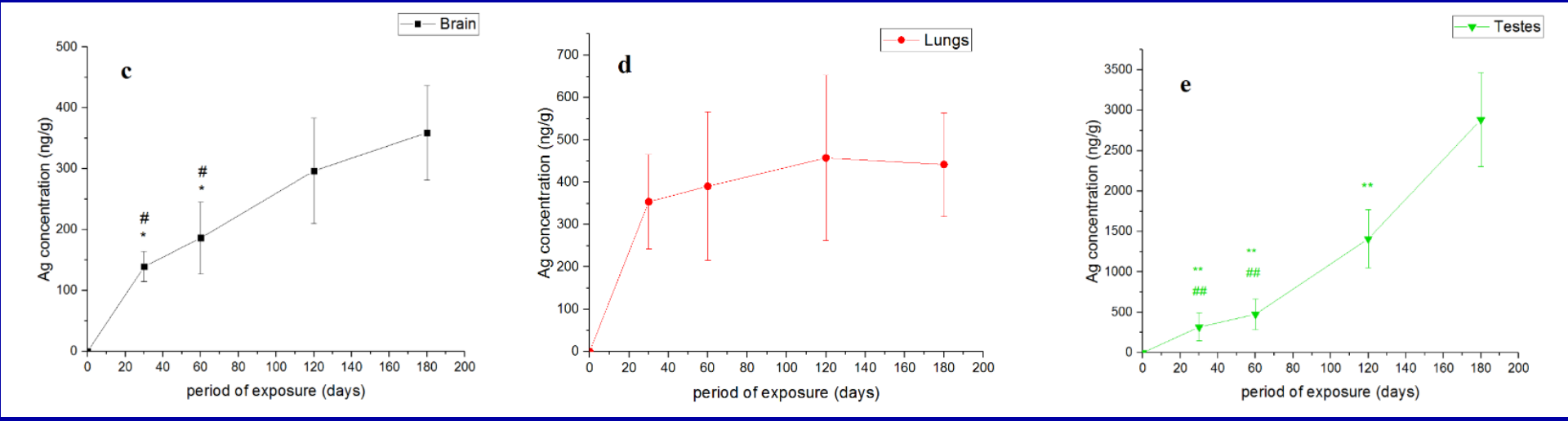




Dependence of the concentration of silver in the internal organs on the period of Ag nanoparticle administration in 2 scales: (a) — all organs (+p < 0,05 – Testis vs Liver, ^p < 0,05 – Tests vs Blood, @ p < 0,05 – Testis vs Brain in each group); (b) — excluding testis (*p < 0,05 – Brain vs Blood, #p < 0,05 – Lungs vs Liver, $ p < 0,05 – Lungs vs Blood in each group). Number of organs per each point n=6-8.

Extremal accumulation of Ag was observed in testes, rather significant accumulation of Ag was found in liver and brain.

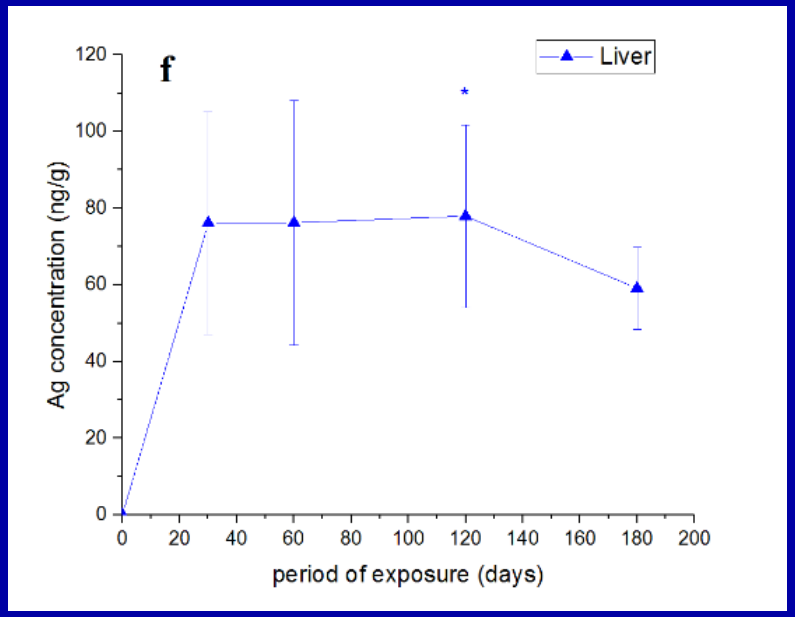
Ag Accumulation in Internal Organs

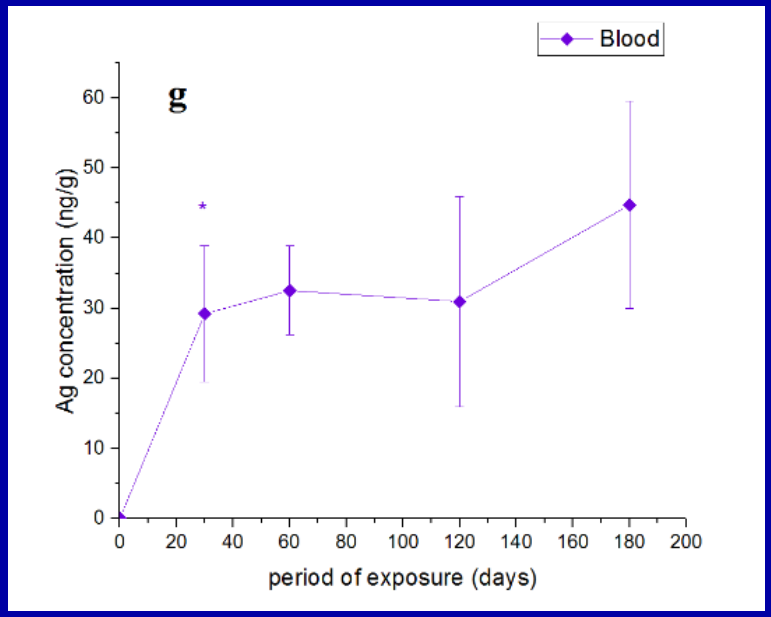


- Dependence of the concentration of silver in the internal organs on the period of Ag nanoparticle administration. The points to the left denoted by * are statistically different from the 180 day point; the points to the left denoted by # are statistically different from the 120 day point; the points to the left denoted by $ are statistically different from the 60 day point). Number of organs per each point n=6-8.

- An extremally high accumulation of Ag was found in testes, rather significant accumulation of Ag was observed in lungs and brain.
- Saturation in brain was achieved 120 days after the beginning of exposure to Ag nanoparticles, apparently, saturation in lungs was reached earlier the observed time points, saturation in testes was not achieved during the observed period.

Ag Accumulation in Internal Organs





- Dependence of the concentration of silver in the internal organs on the period of Ag nanoparticle administration. The points to the left denoted by * are statistically different from the 180 day point; the points to the left denoted by # are statistically different from the 120 day point; the points to the left denoted by $ are statistically different from the 60 day point). Number of organs per each point n=6-8.

- The effect of elimination of Ag from liver since 120 days of exposure in the conditions of constant exposure to Ag nanoparticles was observed. Apparently, saturation in blood was reached earlier than the observed time points.

Conclusions

- General character of Ag accumulation is similar for brain, hippocampus, cerebellum and remnant. Concentrations of Ag in cortex are lower for all the time points in comparison with the other brain regions. Saturation in cortex occurs earlier than in other regions. It may evidence of some selectivity of Ag nanoparticle action.
- An extremally high accumulation of Ag was found in testes, rather significant accumulation of Ag was observed in lungs and brain after long-term everyday oral exposure to PVP-coated Ag NPs into mice organisms. The results evidence about the potential risk for reproductive, respiratory and nerve systems of an organism at the long-term application of Ag nanoparticles. Also an unforeseen influence on other systems of an organism cannot be excluded due to the concept of neurovisceral integration.
- The effect of elimination of Ag from the liver (since 120 days of exposure) in the conditions of constant exposure to Ag nanoparticles was observed. It may point to the possible adaptation of an organism to the xenobiotic.

Antsiferova, A.A.; Kopaeva, M.Y.; Kochkin, V.N.; Kashkarov, P.K. Kinetics of Silver Accumulation in Tissues of Laboratory Mice after Long-Term Oral Administration of Silver Nanoparticles. Nanomaterials 2021, 11, 3204.

"Solely the dose determines that a thing is not a poison“
Paracelsus