Biological Effects of Manganese Oxide Nanoparticles after Peroral Intake

N.V. Zaitseva, M.A. Zemlyanova, V.N. Zvezdin* and T.I. Akafieva

Federal State Research Institution "Federal Research Center for Medical and Preventative Technologies of Public Health Risk Management", Perm, 614045, Russia

Abstract: Nanodispersed manganese oxide is a unique substance with a high application potential in nanoelectronics and nanooptics. The scientific literature contains little information about the biological effects and toxic action of this substance after it enters a human body. The biological effects were studied in Wistar rats after intragastric administration of manganese oxide for 30 days. The effects included loss in the body mass, activation of oxidation processes (increased level of lipid hydroperoxides, MDA in the blood serum), decrease in the antioxidant activity (inhibited antioxidant activity in the blood serum), damaged hepatocyte membranes (higher serum AST and ALT levels), and protein synthesizing liver function abnormalities (low albumins, high gamma globulins in the blood serum).

Keywords: Manganese oxide, nanoparticles, hepatotoxity, digestive tract.

1. INTRODUCTION

Today nanomaterials are increasingly coming into contact with people especially the most commonly used metal oxide particles. Because of their physical and chemical properties, metal oxides including manganese oxide powder can pose health and safety threats. This compound is widely used in templates for the production of nanomagnetic, sorbing materials, nanocatalysts, and semi-conducting thermistors [1, 2]. Manufacturing plants involved in such production processes can become the source of nanopowder emission to air, water, and soil. As a result, the local community is exposed to drinking water and agricultural produce contaminated with nanodispersed manganese oxide [3].

Recent research has shown that human exposure to environmental contaminants, including manganese nanoparticles, is recognized as a significant contributing factor for the development of Parkinson's disease [4].

For that reason, it is essential to study the biological effects and toxic action of nanodispersed manganese oxide after peroral intake.

Research shows that metal nanoparticles synthesized by different methods may be used as antibacterial agents [5, 6]; this requires a more thorough analysis of the biological effects of manganese oxide nanoparticles which due to their cytotoxic properties may be also used as bactericidal agents [7].

*Address corresponding to this author at the Federal State Research Institution "Federal Research Center for Medical and Preventative Technologies of Public Health Risk Management", 82 Monastyrskaya St, Perm, 614045, Russia; Tel/Fax: +7 (3422) 371815; E-mail: zvezdin@fcrisk.ru

Accumulated material of the negative effects of manganese oxide nanoparticles in inhalation. As a priority is marked neurotoxic effect [7-10]. One of the pronounced effects associated with the intake of nanoparticles of a number of metals and nonmetals is hepatotoxicity the symptoms of which include changes in enzyme activity, apoptosis activation and fine-structure changes of the hepatocytes and Kupffer cells [11, 12]. Earlier in-house research showed that the one-dose delivery of manganese oxide in aqueous suspension (200 mg/kg) intragastrically administered to mice leads to structural changes in the liver and kidney. Pathomorphology here takes the form of dilation of the venous vessels and proliferation of adenoid tissue [13, 14].

The purpose of this research is to investigate the biological effects of nanodispersed manganese oxide in Wistar rats after intragastrical administration of the aqueous suspension for 30 days.

2. MATERIALS AND METHODS

2.1. Materials

The aqueous suspension of nanodispersed manganese oxide was purchased from the Laboratory of Multiphase Dispersed Systems, Institute of Technical Chemistry at the Ural Research Center of the Russian Academy of Sciences. The investigated substance was synthesized by the template synthesis method according to the procedures presented in these articles [6, 7]. The size of the nanodispersed manganese oxide particles ranged from 24 to 70 nm, and the specific surface area was about 150 m^2/r. The particles had a non-spherical acicular shape.

ISSN: 2223-3806 / E-ISSN: 1927-5951/13

Serum alanine aminotransferase (ALT) and aspartate transaminase (AST) activities were measured by the kits from Human, Germany; albumin and gamma globulin concentrations were measured by the kits from Helena, UK; lipid peroxide concentration and anti-oxidant activity in the serum were measured by the kits from BioChimMak, Russia.

2.2. Experimental Animals

Fifty mature Wistar male rats were obtained for this study from the federal state Biomedical Technology Research Center of the Russian Academy of Medical Sciences. The body weight of the rats averaged 220±15 g. All rats were maintained in polypropylene cages of standard dimensions in a ventilated room at a constant temperature of 23.0±2.0 ºC and humidity of 60.0±5.0 %; the rats were housed in groups of 2 rats per cage. The rats were fed a semisynthetic diet with the nutritional and biological values in accordance with the recommended norm of physiological requirements. Access to food was not limited. All procedures and studies were approved by the Animal Care Ethical Committee at the Federal Scientific Center for Medical and Preventive Health Risk Management Technologies.

2.3. Experimental Procedure

The animals were divided into 5 groups (10 animals each). The experimental rats in groups 1, 2, 3 and 4 received manganese oxide in aqueous suspension administered intragastrically for 30 days at doses of 234.0 mg/kg, 46.8 mg/kg, 9.4 mg/kg and 4.7 mg/kg which corresponds to the lethal doses of 1/10. 1/50. 1/250. 1/500 LD_{50}. The control group 5 kept in the same environmental conditions was administered distilled water. The volume of water and experimental substance administered to the animals did not exceed 1.0 ml.

The following parameters were studied during the experiment: the survival rate, the body weight before the experiment (base level), on the 10^{th}, 20^{th}, and 30^{th} days of the experiment. Blood sampling was obtained from the tail veins in the rats of all the groups before the experiment and on the 30^{th} day of the experiment to assess serum values.

2.4. Determining the Values of Serum Biochemistry

The values of serum biochemistry including ALT and AST were measured with the kits following the guidelines for the automatic biochemical analyzer (Konelab20. Thermo Scientific, Finland). The albumin and gamma globulin concentrations were measured following the guidelines for the automated electrophoresis analyzer (SAS -1 plus, SAS-2, Helena, UK).The lipid peroxide concentrations and antioxidant activity were measured following the guidelines for the robotic absorbance microplate reader Infinite F50. Tecan, Australia)

2.5. Determining the Values of Plasma Biochemistry

The plasma MDA levels were measured in accordance with the methodological procedure. Tris Buffer in the amount of 1.7 ml was added to 300 mcl of serum in a centrifuge tube; 0.5 ml of 34% trichloroacetic acid (TCA) was used to precipitate proteins. Then the precipitation was purified in a centrifuge tube for 10 min at 300 rpm – 20 min. The supernatant fluid was later transferred into tubes, 2 ml at a time, and mixed with 1 ml of 0.8% TCA aqueous suspension, the generated samples were placed into the boiling water bath for 10 min. The samples containing Tris-Buffer-HCl instead of the supernatant fluid were used as control samples. After the samples turned pink, they were cooled down to room temperature, and the measurements of the optical density (OD) were taken at 532 nm in a tray, 10 mm ray path length, against the control sample using a spectrophotometer PE-5300 (Ekros, Russia). MDA concentration was determined by using a molar extinction coefficient according to the formula OD*156=mcM/ml.

2.6. Data Analysis

The data was analyzed with Statistica 6.0 data analysis software. The results were analyzed with parametric statistical methods. Student's t-test was used to ensure the accuracy of results. For the results, the P values less than or equal to 0.05 were considered statistically significant.

3. RESULTS

3.1. Nanodispersed Manganese Oxide Effects on the Body Weight Change in the Experimental Animals

The analysis of the body weight change in the experimental animals determined a 7.7% decrease in the body weight in group 1 by the 10^{th} day. The base weight was re-gained by the 30^{th} day. The body weight decrease in the animals of this group during the course of the experiment was significantly different from the

Table 1: Nanodispersed Manganese Oxide Effects on the Body Weight Change in the Experimental Animals

Group	Average body weight in the group, g (M±m)				Average body weight in the group / this indicator ratio before the substance administration, %		
	Before the experiment	10th day	20th day	30th day	10th day	20th day	30th day
№ 1	194.4±5.2	179.4±8.5*^	184.4±9.9*^	193.6±21^	-7.7	-5.2	-0.5
№ 2	196.3±6.2	195±9.5	205.6±11*	221.7±18.4*	+0.7	+4.7	+12.9
№ 3	190±9.2	210±10.3*^	220±7.1*^	235±16.3*	+10	+15.7	+23.6
№ 4	194.5±8.6	198.2±5.2	209.4±11.2*	217.5±9.5*	+1.9	+7.7	+11.9
№ 5	198.8±10.2	198.8±9.9	208.8±11.1*	233±19.9*	+0	+4.6	+17.2

*p<0.05 as compared to the indicator values before the experiment (Base Level).
^p<0.05 as compared to the control values (group № 5).

control group values. In other groups, the body weight in the experimental animals increased (Table **1**).

3.2. Nanodispersed Manganese Oxide Effects on the Oxidation and Anti-Oxidant Processes

Assessment of the oxidation and anti-oxidation processes in the experimental animals determined that in groups 1-3 the level of lipid peroxides was 1.5 – 2 times higher ($p<0.05$) and the plasma MDA levels were 1.7 – 2 times higher ($p<0.05$) as compared to the base line and the control group. A significant reduction in the AOS levels as compared to the base level and the control values was registered in groups 1 – 3 ($p<0.05$). Nevertheless, the maximum indicator reduction (by 5.1 times as compared to the control group, and by 5.5 times as compared to the base level) was registered in group 1. The minimum AOS level reduction (by 2.4 times as compared to the control group, and by 2.5 times as compared to the base level) was registered in group 3. See Figure **1**, Table **2**.

3.3. Nanodispersed Manganese Dioxide Effects on the Liver Cells Membranes

A significantly higher plasma AST level was registered in group 1 as compared to the base and control levels ($p<0.05$). A higher serum ALT level was

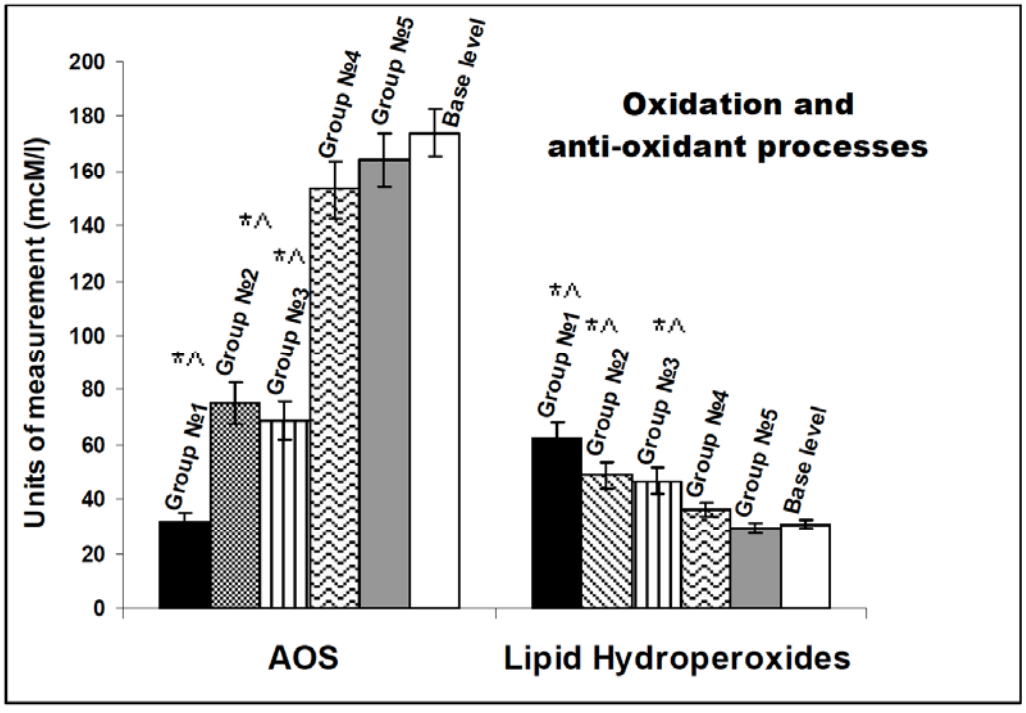

Figure 1: Nanodispersed manganese oxide induced changes in serum on the 30th day of the experiment. Levels of AOS and Lipid Hydroperoxides, *p<0.05 as compared to the indicator values before the experiment (Base Level), ^p<0.05 as compared to the control values (group № 5) controls.

Table 2: Nanodispersed Manganese Oxide Effects on the Oxidation and Anti-Oxidant Processes in Animals on the 30th Day of the Experiment

Group, №	Indicator (M±m)		
	AOS (mcM/l)	Lipid Hydroperoxides (mcM/l)	MDA (mcM/ml)
1	31.95±3.35*^	62.21±5.50*^	3.244±0.29*^
2	75.36±6.94*^	48.88±6.07*^	2.746±0.29*^
3	68.97±4.73*^	46.71±5.67*^	3.35±0.52*^
4	153.21±5.13	36.52±5.24	1.82±0.42
5	164.26±8.29	29.30±2.28	1.60±0.12
Base Level	174.1±10.13	30.76±2.64	1.62±0.195

*p<0.05 as compared to the indicator values before the experiment (Base Level).
^p<0.05 as compared to the control values (group № 5).

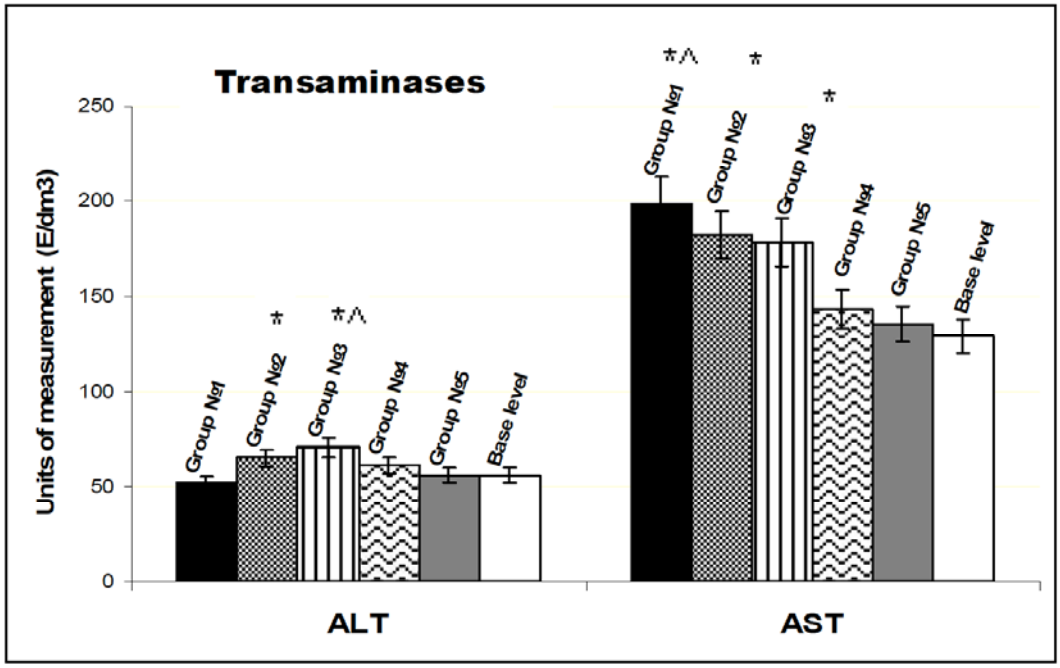

Figure 2: Nanodispersed manganese oxide induced changes in serum on the 30th day of the experiment. Levels of ALT and AST, *p<0.05 as compared to the indicator values before the experiment (Base Level), ^p<0.05 as compared to the control values (group № 5) controls.

Table 3: Nanodispersed Manganese Oxide Effects on the Liver Cells Membrane on the 30th Day of the Experiment

Group, №	Indicator (M±m)			
	ALT (E/dm^3)	AST (E/dm^3)	Albumin (%)	Gamma-globulin (%)
1	51.83±4.12	198.83±19.2*^	39.48±1.21*^	17±2.1*^
2	65.14±10.16*	182.28±23.16*	43.56±0.78	12.76±1.29*
3	70.66±15.51*^	178.33±27.52*	43.1±1.18	12.43±2.06*
4	61.31±11.21	143.12±25.67	44.6±0.39	12.97±1.19*
5	56.00±7.56	135.33±26.39	45.1±1.15	11.32±1.12
Base Level	55.73±8.31	128.9±17.1	45.43±1.03	10.78±0.92

*p<0.05 as compared to the indicator values before the experiment (Base Level).
^p<0.05 as compared to the control values (group № 5).

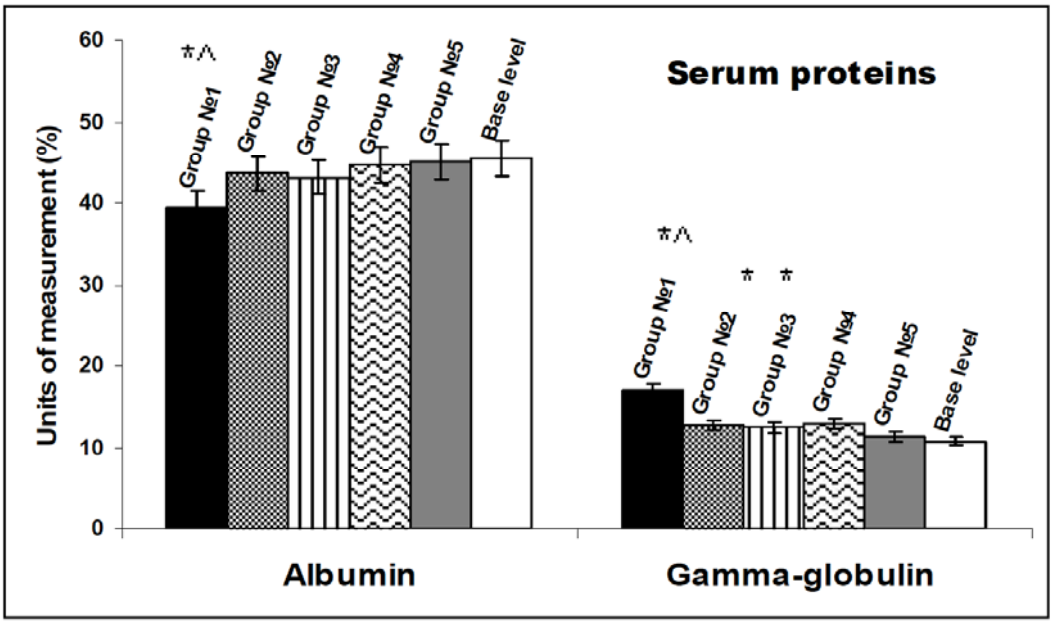

Figure 3: Nanodispersed manganese oxide induced changes in serum on the 30th day of the experiment. Levels of Albumin and Gamma-globulin, *p<0.05 as compared to the indicator values before the experiment (Base Level), ^p<0.05 as compared to the control values (group № 5) controls.

registered in group 3 as compared to the base and control levels (p<0.05). See Figure **2**, Table **3**.

Imbalance in serum protein levels points to a change in the protein synthesizing liver function. A significant decrease in serum albumin concentrations (p<0.05) was registered in groups 1. A higher serum gamma globulin level (a 1.6 time increase as compared to the base level, and a 1.5 time increase as compared to the control level) was registered in group 1 (Figure **3**, Table **3**).

4. DISCUSSION

These registered effects give evidence to a possible negative impact that the studied nanodispersed manganese oxide suspension has on the experimental animals after intragastric administration for 30 days at the doses of 234.0 mg/kg, 46.8 mg/kg, 9.4 mg/kg. After administration of the suspension at a dose of 4.7 mg/kg for 30 days, no significant change was registered in the studied indicators as compared to the base and control levels.

Compilation of the test data allows us to draw conclusions in regards to a possible toxic action of the studied particles. Activation of lipid peroxidation following a direct cytotoxic impact by nanodispersed manganese oxide (typical of metals and their compounds) could serve as a possible trigger [8]. This effect develops on the system level of the lipid hydroperoxide and MDA (its product) levels build-up which results in inhibited antioxidant processes. In the course of our study, we determined that hepatocytes are the target cells for the tested compound after peroral intake. The reason for this, first of all, is the intake method – peroral – which is connected with the functional activity of the liver cells involved in detoxification processes. It is necessary to take into account a high penetration power of the metal nanoparticles [9] which can intensify the contact of the particles with the target cells. Hepatocyte damage intensifies transaminase inflow in the general circulation and results in reduced protein synthesizing liver function which is reflected in a higher AST and ALT activity, reduced albumin concentrations, and increased gamma globulin concentrations in the blood serum [10].

Therefore, the biological effects of the aqueous suspension of nanodispersed manganese oxide after daily intragastric administration to Wistar rats for 30 days include reduced body weight (at a dose of 234.0 mg/kg), activation of oxidation processes connected to higher serum lipid hydroperoxides and plasma MDA (at a dose of 9.4 mg/kg), a lower antioxidant activity connected to lower serum AOS level (at a dose of 9.4 mg/kg), liver cell membrane damage connected to higher serum AST (at a dose of 234.0 mg/kg) and ALT levels (at a dose of 9.4 mg/kg), a liver protein synthesizing function disorder connected to lower albumin concentrations (at a dose of 9.4 mg/kg), and higher serum gamma globulin concentrations (at a dose of 234.0 mg/kg).

Nanodispersed manganese oxide did not cause the above effects at a dose of 4.7 mg/kg.

CONFLICT OF INTEREST

No conflict of interest took place during the experiment and preparation of the publication.

FINANCIAL DISCLOSURE

The research was conducted within the Federal Scientific Research Program called “Hygienic Reasoning for Public Health Risk Elimination in Russia” (2011-2015).

ACKNOWLEDGEMENTS

We are thankful to the staff of the Laboratory of Multiphase Dispersed Systems, Institute of Technical Chemistry at the Ural Research Center of the Russian Academy of Sciences for providing the aqueous suspension of nanodispersed manganese oxide

We would like to thank R.R. Makhmudov, Associate Professor, Candidate of Chemical and Physical Sciences (Perm State national Research University) for providing consulting services during the experiments with the animals.

ABBREVIATIONS

AOS = anti-oxidant activity

ALT = alanine aminotransferase

AST = aspartate transaminase

LD = lethal dose

MDA = malondialdehyde

REFERENCES

[1] Amatucci GG, Nathalie P. Fluoride based electrode materials for advanced energy storage devices. J Fluorine Chem 2007; 128: 243-62. http://dx.doi.org/10.1016/j.jfluchem.2006.11.016

[2] Ming H, Shujuan Z, Bingcai P, Weiming Z, Lu Lv, Quanxing Z. Heavy metal removal from water/wastewater by nanosized metal oxides: A review. J Hazardous Mater 2012; 211: 317-31. http://dx.doi.org/10.1016/j.jhazmat.2011.10.016

[3] Zaytseva NV, May IV, Kleyn SV. K voprosu ustanovleniya i dokazatelstva vreda zdorovyu naseleniya pri vyyavlenii nepriyemlemogo riska, obuslovlennogo faktorami sredy obitaniya [To the question of establishing and evidence of harm to public health in identifying unacceptable risk due to environmental factors]. Analiz Riska Zdorovyu 2013; 2: 14-27. Available from: http://fcrisk.ru/sites/default/files/upload/page/747/health-risk-analysis-2013-02.pdf#page=14 (Russian).

[4] Caudle WM, Guillot TS, Lazo CR, Miller GW. Industrial toxicants and Parkinson's disease. Neurotoxicology 2012; 33: 78-88. http://dx.doi.org/10.1016/j.neuro.2012.01.010

[5] Borah D, Deka P, Bhattacharjee P, Changmai A, Yadav RNS. Ocimum sanctum mediated silver nano particles showed better antimicrobial activities compared to citrate stabilized silver nano particles against multidrug resistant bacteria. J Pharm Res 2013; 7: 478-82. http://dx.doi.org/10.1016/j.jopr.2013.06.018

[6] Borah D, Buragohain P, Saikia A, Yadav RNS. Synthesis and Evaluation of Antimicrobial Silver Nanoparticles on Multidrug-Resistant Bacterial Isolates from Urine Samples of Diabetic Patients and Infected Human Soft Tissues. BioNanoScience 2012; 2: 322-28. http://dx.doi.org/DOI 10.1007/s12668-012-0053-6

[7] Oszlánczi G, Horváth E, Szabó A, Horváth E. Subacute exposure of rats by metal oxide nanoparticles through the airways: general toxicity and neuro-functional effects. Acta Biol Szegediensis 2010; 54: 165-70. Available from: http://doktori.bibl.u-szeged.hu/769/1/og_phd_thesis.pdf

[8] Sárközi L, Horváth E, Kónya Z, Kiricsi I. Subacute intratracheal exposure of rats to manganese nanoparticles: behavioral, electrophysiological, and general toxicological effects. Inhalation Toxicol 2009; 21: 83-91. http://informahealthcare.com/doi/abs/10.1080/08958370902939406

[9] Elder A, Gelein R, Silva V. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 2006; 114: 1172-78. http://dx.doi.org/10.1289/ehp.9030

[10] Hussain SM, Javorina AK, Schrand AM, Duhart HM. The interaction of manganese nanoparticles with PC-12 cells induces dopamine depletion. Toxicol Sci 2006; 92: 456-63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16714391.

[11] Tseng MT, Lu X, Duan X, Hardas SS, Sultana R, Wu P, *et al*. Alteration of hepatic structure and oxidative stress induced by intravenous nanoceria. Toxicol Appl Pharmacol 2012; 260: 173-82. http://dx.doi.org/10.1016/j.taap.2012.02.008

[12] Chen Q, Xue Y, Sun J. Kupffer cell-mediated hepatic injury induced by silica nanoparticles *in vitro* and *in vivo*. Int J Nanomed 2013; 8: 1129-40. http://dx.doi.org/10.2147/IJN.S42242

[13] Zaytseva NV, Zemlyanova MA, Zvezdin VN, Sayenko YV, Tarantin AV, Makhmudov RR, *et al*. Toksikologo-gigiyenicheskaya otsenka bezopasnosti nano- i mikrodispersnogo oksida margantsa (III,IV) [Toxicological and hygienic safety assessment of nano-and microfine manganese oxide (III, IV)] Voprosy pitaniya 2013; 5: 13-19. Available from: http://www.fesmu.ru/elib/Article.aspx?id=271722 (Russian).

[14] Zaytseva NV, Zemlyanova MA, Zvezdin VN, Lebedinskaya OV, Melekhin SV, Sayenko YV, *et al*. Morfologicheskiye osobennosti tkaney vnutrennikh organov i sistem pri vozdeystvii nanodispersnogo oksida margantsa (III, IV) [Morphological properties of tissues of the internal organs and systems under the influence of nanosized oxide of manganese (III, IV)] Vestnik RAMN 2013; 470: 18-23. Available from: http://elibrary.ru/item.asp?id=18834547 (Russian).

[15] Tsuda M, Arai H, Sakurai YJ. Improved cyclability of Na-birnessite partially substituted by cobalt. Power Sources 2002; 110: 52-56. http://dx.doi.org/10.1016/S0378-7753(02)00217-3

[16] Meynen V, Cool P, Vansant EF. Verified syntheses of mesoporous materials. Microporous Mesoporous Materials 2009; 125: 170-23. http://dx.doi.org/10.1016/j.micromeso.2009.03.046

[17] Jeng HA, Swanson J. Toxicity of metal oxide nanoparticles in mammalian cells. J Environ Sci Health Part A 2006; 41: 2699-11. http://dx.doi.org/10.1080/10934520600966177

[18] Elder A, Vidyasagar S, DeLouise L. Physicochemical factors that affect metal and metal oxide nanoparticle passage across epithelial barriers. Wiley Interdisciplinary Reviews: Nanomed Nanobiotechnol 2009; 1: 1434-50. http://dx.doi.org/10.1002/wnan.44

[19] Tseng MT, Lu X, Duan X, Hardas SS, Sultana R, Wu P, *et al*. Alteration of hepatic structure and oxidative stress induced by intravenous nanoceria. Toxicol Appl Pharmacol 2012; 260: 173-82. http://dx.doi.org/10.1016/j.taap.2012.02.008

Received on 16-07-2013 Accepted on 10-09-2013 Published on 11-11-2013

DOI: http://dx.doi.org/10.6000/1927-5951.2013.03.04.3