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Review

ASSESSING RISKS CAUSED BY NICKEL-CONTAINING NANOMATERIALS: HAZARD CHARACTERIZATION *IN VIVO*

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Nanoparticles (NP) of nickel (Ni) and its compounds are promising materials for being used as catalysts in chemical, pharmaceutical and food industry; as construction materials in electronics and optoelectronics, in manufacturing current sources, medications, diagnostic preparations, and pesticides. Annual production volumes for these materials in their nanoform are equal to dozen tons and are expected to growth further. According to data obtained via multiple research nanoforms of Ni and its compounds are toxic to many types of cells; stimulate apoptosis; and can induce malignant transformation in vitro. It indicates that this group of nanomaterials can possibly be hazardous for human health. Risk assessment includes such a necessary stage as quantitative hazard characterization, that is, establishing toxic and maximum no-observed-adverse-effect levels (NOAEL) for a nanomaterial that penetrates into a body via inhalation, through undamaged skin, or the gastrointestinal tract. Experiments in vivo performed on laboratory animals with Ni-containing materials revealed overall toxic effects; toxicity to specific organs (including hepatoxoticity and cardiotoxicity); atherogenic, allergenic, and immune-toxic effects, as well as reproductive toxicity. There are multiple available data indicating that all Ni-containing nanomaterials are genotoxic and mutagenic, though data on their carcinogenic potential are rather scarce. Factors that determine toxicity of Ni and its compounds in nanoform are their ability to penetrate through biological barriers and to release free Ni++ ions in biological media.

The review focuses on analyzing and generalizing data on toxicity signs in vivo and effective toxic doses under various introductions of Ni and its compounds in nanoform into a body over a period starting predominantly from 2011.

Key words: *nickel, nickel oxide, nanoparticles, genotoxicity, allergenic capacity, reproductive toxicity, carcinogenicity, occupational exposure, risk assessment.*

Nanoparticles (NPs) of nickel and its compounds are used as catalysts in chemical, pharmaceutical, and food industry; construction materials production; electronics and optoelectronics; production of current sources, medications, diagnostic preparations, and pesticides. These substances in their nanoforms are produced in dozen tons and production volumes are only expected to grow in future [1].

Obviously, NPs of nickel and its compounds are among nanotechnological products and exposure to them is likely to grow in the nearest future for production workers, consumers of various products and population in general; this unavoidably calls for assessing risks related to this growing exposure [2]. Multiple experimental and epidemiological studies have revealed that metallic nickel and its compounds with common dispersity are carcinogens [3]. Based on this data, Internaitonal Agency for Research on Cancer (IARC) ranked Ni (II) compounds as belonging to

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Group I (carcinogenic for people) whereas metallic Ni was classified as belonging to Group 2B (probably carcinogenic for people). Ni compounds are also active allergens [4, 5].

We generalized data available in scientific literature in works published predominantly from 2011 to 2020 in our previous review and showed that NPs of metallic nickel and its compounds (NiO, $Ni(OH)_2$) as well as nickel nanofibers and nanorods were highly toxic for various cells including cells of bronchopulmonary epithelium, liver, kidneys, intestine, central nervous system and reproductive organs. Cytotoxicity mechanisms include oxidation stress development, functional disorders in cell membranes and mitochondria, expression of nuclear transcription factors, caspases, and proto-oncogenes. Overall, it indicates that Ni-containing nanomaterials are potentially greatly hazardous when they penetrate into the body. However, data of model studies *in vitro* performed on cellular cultures do not allow qualitative assessment of hazards since they don't take into account peculiarities related to how a nanomaterial penetrates through biological barriers as well as its bioaccumulation and biotransformation capabilities.

The aim of this review is to analyze and generalize data on effective toxic doses of Ni-containing nanomaterials under different introduction into laboratory animals' bodies and observed toxic effects as well as possible long-term adverse effects. Attention was primarily paid to data taken from works published in the last decade (starting from 2011); all these works conformed to conventional requirements regarding scientific authenticity and completeness and were found in international reference databases PubMed, WoS and Scopus.

Studies in vivo. Ni NPs are introduced into the body through airways, gastrointestinal tract, and, presumably, skin. When Ni-containing materials are used in medicine (in theranostics) they can possibly penetrate into the body through parenteral (intravenous) introduction. Besides, Ni nanoforms can also be released from implants. Therefore, it seems advisable to consider toxic effects produced by Ni-containing nanomaterials under all these ways of introduction.

Parenteral introduction. A single intravenous introduction of Ni NPs in doses equal to 1, 10 and 20 mg/kg into Sprague Dawley rats caused acute inflammatory damage to the lungs, liver, and spleen as well as acute cardiotoxicity. Histopathologic examination revealed changes in the liver with a growth in its mass depending on a dose as well as changes in the lungs, spleen and some other organs [6].

Marzban A. with colleagues [7] described an experiment involving male rats getting daily intraperitoneal injections of NiO NPs suspension or micronized NiO powder in doses equal to 0, 10, 25 and 50 mg/kg during 8 days. As a result, there was a decrease in recovered glutathione reserves and elevated malonic dialdehyde contents after either Ni-containing form was introduced but NPs introduction additionally led to increased activity of glutathione-S-transferase and catalase. Histopathologic examination revealed necrosis, hyperemia, and sponge-like changes in the brain tissue for both introduced preparations.

Katsnelson B.A. with colleagues [2, 8] described intraperitoneal introduction of NiO NPs suspension (particles diameter was 17 nm), Mn_3O_4 (18 nm) or their mixture into rats 3 times a week during 6 weeks. Introduction of both NPs resulted in functional and histological disorders in the brain, liver, spleen, and kidneys. Mathematical analysis of data based on Response Surface methodology revealed a variety of combined toxic effects that depended on nature, size, and concentrations of particles.

Male and female mice underwent intraperitoneal injections of NiO NPs in doses equal to 20 or 50 mg/kg of body weight during 14 days and this resulted in male mice having elevated urea contents in blood plasma, an increase in superoxide dismutase (SOD) activity and elevated malonic dialdehyde contents in the liver whereas catalase was less active in the heart and kidneys. Female mice had prevailing signs of decreased total protein and albumin in plasma, lower SOD activity in the lungs and elevated malonic dialdehyde (MDA) contents in the liver [9].

Experiments performed on male Sprague Dawley rats involved intraperitoneal injections of Ni NPs in a dose equal to 10 mg/kg of body weight during 90 days; this led to significant deviations in biochemical and hematological indicators, oxidation stress development, morphological disorders of the kidneys and liver. An extract from Cinnamomum cassia bark (Chinese cassia) in a dose equal to 175–225 mg/kg of body weight that was introduced orally inhibited toxic effects produced by NPs [10].

Introduction into airways. Intratracheal and oropharyngeal instillations of nanomaterials are frequently used in toxicological studies as a model of respiratory exposure. Compared with inhalation introduction, they allow introducing more exact doses of a nanomaterial into each animal although there are certain peculiarities related to distribution of particles with different sizes and chemical structure in the airways given this alternative way of exposure. Both acute and various subacute exposure can be modeled in such experiments.

There was an early study [11] during which NiO NOs sized 8 nm and aggregated into clusters sized 26 nm were introduced into male Wistar rats via single intratracheal administration in a dose equal to 0.33–0.66 mg/kg. Starting from the 3rd day in the experiment, bronchoalveolar lavage (BAL) contained elevated quantities of CINC-1, -2αβ and -3 cytokine-induced neutrophils chemoattractants. The changes persisted up to 6 months after the introduction. This research work seemed to be the first confirmation that Ni NPs were able to induce persistent inflammatory response in the lung even after a single introduction. There was a study similar in its design where elevated contents of CINC-2αβ and CINC-3 in BAL were detected 3 days, 1 week, 3 months, and 6 months after a single intratracheal introduction of nanostructured aggregated NiO with primary NPs sized 8.4 in a dose equal to 3.3 mg/kg into male Wistar rats [12]. In the following experiment, rats got a single intratracheal injection of NiO NOs sized 26 nm in a dose equal to 0.2 mg; after that, researchers observed alveolar macrophages infiltration, long-term increase in MIP-1a contents and transient interleukin (IL) – IL-1α, IL-1β and MCP-1 in lung tissue lysates and BAL within a period of time from 3 days to 6 months after the injection [13]. Therefore, chemokines play an important role in developing lung inflammation caused by Ni-containing NPs.

Spherical NiO NPs and those with irregular shape as well as NiO nanofibers were one-time introduced intratracheally into male F 344 rats in doses equal to 0.67–6.0 mg/kg of body weight; there was translocation into pectoral lymph nodes where the particles were partially dissolved and biologically degraded under exposure to oxidants produced by immune cells. Dissolution, oxidation, and clearance of Ni nanofibers were much faster than for two aforementioned types of NPs [14].

The research work [15] also focused on a relation between clearance of various Ni-containing nanomaterials from the lungs and their dissolubility. NiO NPs were shown to have greater lung toxicity than microparticles (MPs) since they dissolved faster releasing Ni++ ions; the process was reproduced *in vitro* with using artificial alveolar liquid. A peak in responses by inflammation markers in rats' lungs was detected *in vivo* within a period form 1 week to 1 month and to a much lesser extent during the first 3 days; this was in line with a typical period of time required for NPs dissolution *in vitro* that was equal approximately to 1 week. Data available in the work [16] also highlight a role played by dissolubility of Ni-containing nanomaterials and adsorption of surfactant components on their surface in their apparent lung toxicity. In this research [16] effects produced by NiO NPs on mice under pharyngeal aspiration differed depending on a structure of dispersion medium including phosphate-salt buffer, and solution of albumin, non-ion detergent and phospholipids that imitated a structure of surfactant.

NiO NPs were introduced into rats as a suspension through a single intratracheal introduction and induced persistent lung inflammation with inflammatory cell proliferation, alveolar proteinosis and cytokine production 3–28 days after the introduction. *Nlrp3* expres-

sion was elevated together with hyperexpression of caspase 1 (p20) and IL-1β production of which was suppressed due to effects produced by caspase inhibitor on macrophages *ex vivo*. NiO NPs are assumed to induce activation of *Nlrp3*-inflammasome which requires their absorption by cells and production of reactive oxygen species (ROS) [17].

14 days after Ni NPs sized 41 nm and corresponding MPs (350 nm) were introduced in dose equal to 4–20 mg/kg into Sprague Dawley rats via a single intratracheal introduction, inflammatory damage was detected in the lungs, liver, and kidneys, there were hyperplasic changes in the lungs and greater expression of homoxygenase-1 (HO-1) and Nrf2 there without any changes in expression of C-myc oncogene. There were signs confirming that NPs were more cytotoxic than MPs [18].

In the study [19] NiO NPs were one-time introduced intratracheally into mice and then inflammatory reactions were examined in the lungs within 1–28 days after the introduction. There was greater activity of lactate dehydrogenase (LDG), elevated total protein and IL-6 contents and decreased IL-10 contents in BAL. By the 24th hour in the experiment there was a growth in 8-oxo-2-deoxyguanosine (8-oxo-G) and caspase-3 contents in lung tissue and enhancing dose-dependent inflammation in the lower airways. By the 28th day lung tissue fibrosis occurred. Proteome analysis revealed that after 24 hours disorders in the metabolic ways of cell adhesion prevailed in inflammation mechanism whereas on the 28th day prevalence shifted to processes related to tissue glutathione depletion.

Authors of the research [20] introduced NiO NPs into rats through a single intratracheal introduction in doses equal to 0.2 and 1 mg/kg or through inhalation. NiO NPs persisted in the lungs longer than TiO_2, NPs in all tests and biological persistence correlated with histopathologic changes and levels of inflammatory biomarkers in BAL.

Three types of Ni NPs (with non-modified surface, oxide-passivated, and protected with monomolecular carbon layers) were introduced through single intratracheal instillation into common mice and mice with knocked-out micro-RNA (miR)-21 gene; the experiments revealed that expression of *Reck* gene (tumor dissemination inhibitor) that was a direct target for effects produced by miR-21 was inhibited only in wild mice under exposure to the first two types of NPs. These data highlight an important role played by miR-21 in inflammatory response induction *in vivo* and probably carcinogenicity of nickel NPs [21, 22]. There was a comparative study performed on mice who were exposed to 3 types of Ni-containing NPs through a single intratracheal instillation in doses varying from 10 to 100 μg; the exposure resulted in dose-dependent development of acute lung inflammation with elevated contents of neutrophils, CXCL1/KC, total proteins and greater LDG activity in BAL. Time dynamics of the inflammation was as follows: occurrence on the 1st day after introduction, a peak on the 3rd day and a decline after 7 days, although even after 42 days inflammation signs were still more apparent than in the reference group. Oxide-passivated NPs were characterized practically with the same lung toxicity as pure Ni NPs but when NPs were covered with carbon layer, it led to a significant decrease in their toxicity [23].

Macrophage degeneration and necrosis as well as inflammation and proliferation of type II pneumocytes were detected in lungs of Sprague Dawley rats after a single intratracheal instillation of NPs in doses equal to 0, 0.2, 0.67, and 2 mg/kg of body weight [24]. Inter-laboratory studies in 5 laboratories confirmed that the developed procedure was quite reproducible.

A comparison was made between a single and divided (two or four intakes with daily intervals) intratracheal introduction of NiO NPs into rats in a total dose equal to 2 mg/kg of body weight; the experiment revealed that resulting development of lung inflammation with such signs as NPs phagocytosis by alveolar macrophages, their degeneration and necrosis, a response by inflammatory markers in BAL after 3 and days after the last intake were practically the same after either a single or divided introduction of NPs [25].

Sub-acute experimental exposure was modeled in the research [26] where NiO NPs or MPs were introduced intratracheally into male Wistar rats in doses equal to 0.015–0.25 mg/kg twice a week during 6 weeks. Exposed animals had elevated contents of cells in apoptosis in their livers as well as higher expression of IRE-1α, X box protein-1S, pancreatic ER kinase (PERK), eukaryotic initiating factor-2 alpha (eIF-2α), their phosphorylated forms, caspase-3, 9 and 12, glucose-regulated 78 kDa protein and CCAAT-enhancer binding protein; all this indicated there was stress developing in endoplasmatic reticulum.

Histopathologic research by the same authors revealed lung fibrosis and elevated contents of hydroxyproline, collagen-1 and 3 in lung tissues after NiO NPs were introduced into rats in the aforementioned doses. All this was combined with elevated expression of fibrosis factors TGF-1β (transforming growth factor (TGF)), Smad2, Smad4, matrix metalloproteinase (MMP) and their tissue inhibitor of metalloproteinase (TIMP) [27]. Morphological studies revealed dose-dependent widening of alveoli, inflammatory infiltration, and NPs deposition in lung tissues. Exposure to the highest dose resulted in signs of nitrative stress including elevated NO contents and more active total (tNOS) and induced NO-synthase (iNOS); 8-oxo-G was produced in greater volumes, and there were elevated contents of IL-2, TGF-β and IFN-γ starting from a NPs dose equal to 0.06 mg/kg. These effects were authentically more apparent when NPs were introduced than after introduction of MPs in a quantity equivalent as per mass [28]. The central role in immunologic reaction developing in lung tissues as a response to NiO NPs introduction belonged to NF-κB activation and an increase in a relative share of Th2 lymphocytes against Th1 [29].

According to data available in the research by Yu S. [30], sub-acute intratracheal introduction of NiO NPs into male Wistar rats led to changes in the liver including growing mass of the organ, cellular edema, biliary ducts closing, and multinuclear cells occurrence. NO synthase became more active and NO contents in the liver grew under exposure to a dose of NiO equal to 0.25 mg/kg. Besides, there were growing concentrations of hydroxyl radical, lipid peroxides, catalase, glutathione peroxidase, and SOD. Therefore, intratracheal introduction of NiO NPs results in systemic effects including damage to the liver.

Ni NPs toxicity for male and female mice was compared under two oropharyngeal instillation regimes: as a single dose (an acute experiment) or 6 intakes during 3 weeks. Acute exposure resulted in elevated contents of CXCL1 and IL-6 and higher neutrophils quantity in BAL in male mice; there was also more intense STAT3 phosphorylation in lung tissues. Sub-acute exposure to Ni NPs caused greater monocytes quantities in lavage among male mice and there was also CXCL1 and CCL2 induction but STAT1 phosphorylation under such exposure was detected only in female mice. Male mice also tended to have greater IL-6 expression in the liver under acute exposure and greater CCL2 expression under sub-acute exposure than female mice. Differences in the lung responses to Ni NPs introduction were to a greater extent determined by animals' sex than introduction regime [31].

Zhang Q. and colleagues [32] performed experiments that involved introducing NiO NPs into male Wistar rats in doses varying from 0.015 to 0.25 mg/kg intratracheally two times a week during 9 weeks. Elevated accumulation of type I and III collagens was detected in the liver combined with elevated expression of TGF-β1, phosphorylated Smad2, Smad3, α-actin, MMP9, TIMP1, and a decrease in E-cadherin and Smad7 which indicated fibrosis was developing. The results were similar to those obtained by the same authors in experiments *in vitro* on liver cells culture and this indicates there is probably systemic NPs translocation after their introduction into the airways.

Inhalation exposure. Inhalation exposure to nanomaterials is modeled by placing an animal into an aerosol chamber or by putting on a specially designed helmet on its head. Such experiments are more suitable for reproducing occupational exposure than direct introduction into the airways and they are also

less traumatic. But still, there are certain problems related to them caused by a necessity to take respirable dose into account (that is, a dose that produces effects on the lower airways) as opposed to "inhaled one", or a dose of a nanomaterial that occurs direct in inhaled air. Effects that occur due to these two exposure types were described by Mizuguchi Y. and colleagues [33] who performed experiments involving NiO NPs introduction into male Wistar rats either through intratracheal instillation or sub-acute 4-week inhalation. Examinations were performed with a wide range of NPs doses. It was shown that both procedures could yield comparable and reproducible results as per polymorphonuclear leukocytes contents in BAL if a dose of a nanomaterial was measured as per surface area and not as per a mass or mass concentration.

As per data provided by Horie M. and colleagues [34] a response by oxidation stress markers in the lungs including HO-1, 8-isoprostaglandin-F2α, thioreodoxin, myeloperoxidase and iNOS developed more intensely at the first stage in inflammatory response under intratracheal introduction of NiO NPs as opposed to inhalation; however, later difference between these two introduction ways disappeared.

There were also experiments that involved comparing effects produced on rats by 4-week exposure to NiO NPs, Multi-Walled Carbon Nanotubes (MWCNTs) or fullerene in doses equal to 0.13–0.37 mg/m^3; it was revealed that NiO NPs caused the gravest and the most adverse changes in animals' lungs regarding contents of phospholipids and SP-D (surfactant-specific protein D). MWCNTs also produced certain effects, though less apparent; there were no any toxicity signs detected under exposure to fullerene [35]. Also, micron-sized NiO particles as well as TiO_2 particles turned out to have low lung toxicity for rats according to expression of MMP-1, TIMP, and type 1 collagen. These data indicate that inhalation toxicity of Ni-containing particles obviously grows when they are in nanoform.

The longest inhalation exposure (10 months) of rats to NiO NPs was accomplished by Sutunkova M.P. and others [36]. Nanomaterial concentration in aerosol amounted to 0.23 ± ± 0.01 mg/m^3. BAL examination revealed changes in cytological and some biochemical properties with paradoxically feebly apparent histopathologic picture of lung tissues and comparatively feebly apparent NPs accumulation in the lungs. But at the same time there were signs of systemic toxicity including damage to the liver and kidneys, allergic effects, transient stimulation of erythropoiesis and NiO NPs penetration into the brain through the olfactory pathway. Genotoxicity became obvious due to DNA fragmentation in nuclear cells in blood (RAPD test) with a tendency to grow as exposure became longer. It was also established that a majority of these adverse effects could be inhibited with some bioprotectors introduced orally into animals including vitamins C and E, fish oil, glycine, monosodium glutamate, etc. There are reviews focusing on issues related to using nutrients (antioxidants, polyunsaturated fatty acids, or amino acids) as biological protectors to eliminate toxic effects produced by nanomaterials including Ni-containing NPs [2, 37].

Cardiotoxic and atherogenic effects produced by Ni-containing NPs under inhalation exposure are especially interesting. In particular, it was shown that when C57BL/6 mice underwent 5-hour inhalation exposure to NPs of $Ni(OH)_2$ with their diameter being up to 40 nm in a dose equal to 100–900 μg/m^3, vasoconstriction went down in the exposed animals' carotid due to effects by phenylephrine and vasorelaxation decreased due to effects by acetylcholine. Thus, even relatively short-term inhalation exposure to Ni-containing NPs results in changes in endothelium of great vessels that are located far from a place where a nanomaterial penetrates into the body [38]. Later it was shown that inhalation exposure of mice to NPs of metallic Ni under similar conditions led to a growth in quantity of endothelial progenitor cells (EPCs) in bone marrow and blood flow and it indicated there was damage to vascular endothelium. Tubes formation and chemotaxis *ex vivo* of EPCs taken from mice exposed to Ni NPs were disturbed substantially. There was also a decrease in a

number of receptors for mRNA on EPCs that were responsible for mobilization and tissue fixation due to exposure to NPs [39]. Kang G.S. with colleagues [40] noted in their research work that inhalation exposure to NPs of $Ni(OH)_2$ in a dose equal 79 μg Ni/m^3 5 days a week during a period of time starting from 1 week and up to 5 months resulted in graver vascular atherosclerosis in sensitive mice with knocked-out gene of lipoprotein E (ApoE-/-).

Oral introduction. There are relatively few research works that focus on examining effects produced by orally introduced nickel-containing nanomaterials since such an exposure scenario is not considered as a priority one in most studies. Dumala N. and others accomplished an experiment [41] where acute oral toxicity of Ni NPs exceeded 2,000 mg/kg of body weight and it meant the substance was classified as belonging to the 5th hazard category according to Organization on Economic Cooperation and Development Guidelines Test No. 420. There were no lethal cases detected during 14 days after acute oral introduction of Ni NPs sized approximately 16 nm into female Wistar rats in doses varying from 5 to 2,000 mg/kg of body weight. Rats which were exposed to Ni NPs in a dose equal to 2,000 mg/kg became languid and irritated, they consumed food in slightly smaller quantities, and their body weight and relative masses of organs decreased insignificantly. According to Comet Assay data, exposure to the highest dose led to DNA damage in the liver and kidneys after 24 hours. Similar results were yielded in a micronucleus test. Use of this model to examine NiO NPs toxicity revealed an authentic decrease in a number of erythrocytes and AchE inhibition in rats' brains under exposure to high doses of NPs. Transaminases became more active in the liver and blood serum and less active in the kidneys. Exposure to NPs in high doses also involved disorders of enzyme balance in antioxidant protection [42].

Sub-acute oral toxicity and biological distribution of NiO NPs sized 13 nm was examined in a 28-day experiment performed on Wistar rats [43]. There were histopathologic changes in some organs, growing transaminases activity in the liver and kidney homogenates, less active SOD and more active catalase. Reserves of recovered glutathione depleted and there was a decrease in malonic dialdehyde contents indicating there was oxidative stress developing. The liver was the primary place where Ni accumulated; the kidneys followed. Ni was excreted mostly with feces and to a very small extent with urine.

Sub-acute oral toxicity of Ni NPs was examined in experiments performed on male and female rats exposed to doses varying from 5 to 45 mg/kg of body weight during 10 weeks (research design conformed to Organization on Economic Cooperation and Development Guidelines No. 415). The experiments revealed ultra-structural changes in the ovaries and testicles, oxidation stress development and expression of proteins that were associated with apoptosis [44, 45]. It was mentioned in the same works that exogenous ascorbic acid introduced with food protected animals from adverse effects produced by Ni NPs.

NiO NPs sized approximately 50 nm were introduced intragastrically into male Wistar rats during 7 or 14 days in doses from 1 to 4 mg/kg of the body weight. This resulted in a significant growth in a number of chromosome aberrations, micronuclei, and damage to DNA. Flow cytometry procedure revealed apoptosis, reactive oxygen species (ROS) generation and dysfunction of mitochondria membrane potential. There was antioxidant enzymes imbalance and histological changes in the liver. Immunoblotting revealed interaction between apoptosis stimulating factor (p53) and mitogen-activated protein kinase (MAPK)-signal pathway with activation of MAPAPK-2 (phosphorylation substrate for p38 MAP kinase), caspases 3 and 8, cytochrome C being released from mitochondria, expression of Bcl-associated X-protein (Bax) and inhibition of intracellular apoptosis regulator (Bcl-2) [46].

Antagonism between toxic effects as per some integral and biochemical parameters was revealed in rats after single combined oral exposure to 0.5 or 1 g of NiO NPs and Co_3O_4 NPs. Each nanomaterial was more toxic for rats when introduced separately than in combination with another [47].

Long-term effects produced by toxicity of Ni-containing nanomaterials. ***Carcinogenicity of Ni-containing NPs in vivo.*** Mechanisms of carcinogenesis induced by metals have not been studied enough. As it can been seen from all aforementioned data NPs of Ni and its compounds induce oxidative stress and inflammatory reactions and inhibit apoptosis factors, that is, they act as non-genetic pathologic factors that can be considered carcinogenic. Besides, experiments accomplished in model systems *in vitro* revealed that Ni and NiO NPs were highly genotoxic and mutagenic as well as able to induce malignant transformations of certain cell lines. Given that, it seems vital to examine carcinogenicity of various Ni-containing materials *in vivo*. However, there are hardly any research works on the subject available at the moment. There was a single study accomplished by Hansen T. and others [48] where authors observed development of rhabdomyosarcoma in rats with implants that contained Ni NPs in their spinal columns. Epigenetic mechanisms can play a significant role in carcinogenesis that is possibly induced by Ni compounds [3, 49]. It is assumed that absorption of Ni NPs by cells is a crucial component that determines their carcinogenicity [50]. Still it is widely known that absorption is greatly influenced by surface charge of a particle and it should be taken into account when planning experiments *in vivo*.

Therefore, additional studies are required to establish carcinogenicity of Ni NPs and NPs of other Ni-containing nanomaterials when they are into the body through natural ways

Immunotoxicity and allergenic capacity. Allergenic properties of Ni NPs are to a great extent determined by their immunotoxicity and they can become apparent both in a direct contact (when applied on skin) [51] and in indirect ones through the airways and gastrointestinal tract [4]. Single intratracheal introduction of NiO NPs into female Wistar rats in doses varying from 50 to 200 cm^2 recalculated as per particle surface resulted in growing inflammation markers in humoral and cellular fractions of BAL. Quantity of eosinophils didn't correlate with total IgE and anaphylatoxines but alveolar macrophage lysis and extracellular LDG activity positively correlated with eotaxin release. A conclusion was that NPs accumulation in phagolysosomes of immune cells induced their lysis that was accompanied with eotaxin production and eosinophilia. Allergenic capacities of NiO NPs were comparable to those of Ni salt as well as ovalbumin [52].

According to data provided by Glista-Baker E.E. with colleagues [53], transcription factor *Tbx21* (T-bet) has a significant role in preventing the immune response from switching from antigen Th1 type to Th2 type and, consequently, it is necessary for preventing development of allergic reactions such as bronchial asthma. A study was performed on mice with knocked-out gene *Tbx21* and with T-bet-/- genotype in comparison with wild animals. Animals were exposed to nanomaterials through oropharyngeal aspiration; the following histopathologic research revealed that cell metaplasia in alveoli mucosa was authentically higher in T-bet-/- mice on the 21st day after the experiment than in mice from the reference group. This effect was less apparent after exposure to Multi Walled Carbon Nanotubes (MWCNTs). Chronic alveolitis developed in T-bet-/- mice on the 21st day after the exposure to Ni NPs, but not to MWCNTs. Higher expression of MUC5AC and MUC5B was also observed in the test group against the reference one under exposure to Ni NPs. T-bet-/- mice had elevated levels of IL-13, CCL2 and elevated quantity of eosinophils in BAL (bronchoalveolar lavage) already 1 day after the exposure to Ni NPs. When T-bet-/- mice were treated with monoclonal antibodies to CCL2, this resulted in higher metaplasia in mucosa and MUC5AC expression. These data confirm a significant role that belongs to T-bet in protection from allergenic effects produced by Ni NPs.

Roach K.A. and others performed a study [54] where mice were twice exposed to NiO NPs (42 nm in diameter) or MPs (181 nm) through pharyngeal aspiration, on the 1st and 19th day in the experiment, in a dose equal to 3–40 μg and were simultaneously parenterally sensitized with ovalbumin. Exposure to NiO in doses that were comparable in particle surface resulted in changes in total IgE, cytokine con-

tents in blood and the lungs. When an introduced surface was low, the immune response developed predominantly as per Th2-pathway, but in case it was high, there was a switch to Th1 type. There was also a growth in lung eosinophilia under exposure to high doses of NPs.

Possibility that the immune response could switch to Th2 type was also mentioned in the work [29] where intratracheal introduction of NiO NPs into male Wistar rats led to greater expression of GATA-3 and T-bet against elevated levels of cytokines TNF-α, IL-2, IL-10 and neutorphil chemoattractants CINC-1, CINC-2αβ and CINC-3.

The research work [55] revealed there was a possibility of enhanced mice sensitization with Ni NPs combined with lipopolysaccharide when introduced subcutaneously. Silver NPs produced the same effects but that was not the case with Ag+ ions, NPs of gold and amorphous SiO_2.

Reproductive toxicity. Ni NPs (90 nm in diameter) were daily introduced into female rats through a gavage in a dose equal to 3–45 mg/kg during 14 days. As a result, there was mitochondria swelling in ovary tissue, mitochondrial cristae disappeared, and endoplasmatic reticulum grew in size. There was an authentic decrease in SOD and catalase activity and a growth in contents of ROS, malonic dialdehyde, and NO. There also was an authentic growth in expression of mRNA, caspase-3, 8 and 9, Fas, cytochrome C, Bax and Bid with a simultaneous decrease in Bcl-2 expression. Effects produced by Ni MPs were weaker as per several parameters than those produced by NPs [44]. The authors conducted the next research where they orally exposed male rats to Ni NPs (90 nm in diameter) in doses equal to 15–45 mg/kg of body weight every day during 10 weeks prior to coupling and estimated a number of fertilized female rates after it. There was lower SOD and catalase activity as well as lower contents of gonad-stimulating hormone GSH in testicles of the exposed animals with simultaneous elevated contents of NO, malonic dialdehyde, and ROS. Expression of caspases 3, 8 and 9 grew but there was a decline in expression of Bcl-2-associated X protein (Bax) and apoptosis-induced factor (AIF). The aforementioned effects could be partially inhibited by ascorbic acid introduced into animals in high doses and this indicates they were pro-oxidant in their essence [45].

Ni NPs sized 90 nm were one-time introduced through a gavage into male ICR mice in doses varying from 5 to 45 mg/kg of body weight; 30 days after the introduction there was apoptosis of cells in spermatophore tubes, a decrease in testicle mass index, lower activity of marker tissue enzymes in them and lower sperm mobility [56].

Sub-acute intratracheal introduction of NiO NPs into male Sprage Dawley rats in a dose equal to approximately 1 mg once every three days during 3 months resulted in a decrease in total number of sperm cells, number of live cells, and a growth in a number of morphologically abnormal sperm cells. Female rats who coupled with exposed male rats had greater number of dead fetuses. Ni concentration which correlated with lower spermatogenesis parameters grew in male rats' blood and sperm [57].

Table 1 contains a list of the most significant genetic and molecular markers showing toxicity of Ni-containing NPs according to data provided by research *in vivo*.

Table 2 provides data on experimental estimates for maximum non-effective dose (NOAEL) for Ni-containing NPs as per data available in several sources. In most cases authors failed to establish NOAEL; therefore, its estimates are given "from above", that is, according to detected toxic effects.

Toxicity in clinical observations. There are very few clinical or epidemiologic observations regarding adverse effects produced by NPs that contain Ni and its compounds despite occupational exposure to them is quite probable [2]. NPs of heavy metals including Ni were detected through retrospective analysis in bodies of patients who died from Hodgkin lymphoma; given that, they were considered a factor that might cause this neoplasm [58]. Another research revealed that workers who had contacts with Ni nanopowder at their workplaces had irritated throat and stuffy nose, red skin on their face and other skin reactions

Table 1

The most significant biomarkers of toxic effects produced by Ni-containing nanomaterials *in vivo*

No.	Biomarker	Abbreviation	Place of detection	Source
1	Interleukins	IL-1α, IL-1β, IL-2, IL-6, IL-8, INF-γ, TNF–α	Lungs BAL	[17, 28, 31] [13]
2	Matrix metalloproteinase	MMP 2, 9	Lungs Liver	[27] [32]
3	Matrix metalloproteinase inhibitors	TIMP 1,3	The same	[32]
4	Micro-RNA 210	miR210	Lungs	[21, 22]
5	8-oxo-2-deoxyguanosine	8-oxo-G	The same	[19]
6	Glutathione	GSH	Liver	[7]
7	Superoxide dismutase	SOD	Blood plasma, brains	[9]
8	Catalase	Cat	The same	[9]
9	Malonic dialdehyde	MDA	The same	[9]
10	Chemokines	MIP-1a, MCP-1, CINC-1, CINC-2αβ, CINC-3	Lungs, BAL	[11, 13]
11	Caspases 1, 3, 8, 9, 12	-	Lungs, liver, ovaries	[19, 26, 44, 46]
12	Heme oxygenase-1	HO-1	Lungs	[18]
13	Lactate dehydrogenase	LDH	Lungs	[19]
14	Nuclear transcription factor erythroid-2	NRF-2	Lungs	[18]
15	Nuclear transcription factor T-bet	Tbx21	Lungs	[29]

Note: LDH – lactate dehydrogenase; IL – interleukin; INF – interferon.

Table 2

Estimates of maximum non-effective doses (NOAEL) for NPs of Ni and its compounds as per data available in literature

Nanomaterial (composure and particle size)	Experimental model	Examined parameters	Estimate for NOAEL, meas. units	Source
Intratracheal and inhalation introduction				
NiO 8–26 nm	Wistar rats	CINC-1, CINC-2αβ, CINC-3 in BAL	< 0.33 mg/kg	[11]
NiO 8.4 nm	Wistar rats	CINC-1, CINC-2αβ, CINC-3 in BAL	< 3.3 mg/kg	[12]
NiO 26 nm	Rats	Cytokines in BAL	< 0,2 mg/kg	[14]
Ni 41 nm	Sprague Dawley rats	Histopathology, HO-1, NRF-2 (Lungs)	< 4 mg/kg	[18]
NiO	Rats	Histopathology of internal organs	< 0.2 mg/kg	[20]
Ni	Mice	Neutrophils, LDH, CXCL1/KC in BAL	< 0.4 mg/kg	[23]
NiO	Sprague Dawley rats	Histopathology, (Lungs)	< 2 mg/kg	[24]
NiO	Rats	Histopathology, (Lungs)	< 2 mg/kg	[25]
NiO	Rats	Apoptosis index (Liver)	0.015 mg/kg	[26]
NiO	Male Wistar rats	Histopathology, (Liver)	< 0.25 mg/kg	[30]
Ni 20 nm	Mice, males and females	Macrophages, LDH in BAL	4 mg/kg (females)	[31]
NiO 10–20 nm	Male Wistar rats	Polymorphonuclear leukocytes in BAL	200 cm^2 per 1 animal	[33]
NiO 15–35 nm	Male Fischer 344 rats	HO-1 (Lungs)	< 0.2 mg/kg	[34]
NiO 23 nm	Female rats **	Neutrophils and macrophages in BAL, histopathology of liver, kidneys, and spleen	< 1 mg/m^3	[36]
NiO 54 nm	Male Wistar rats	Surfactant components in BAL	< 0.2 mg/m^3	[35]
$Ni(OH)_2$	Male C57Bl/6J mice	Pharmacologically stimulated vasoconstriction and vasorelaxation	< 0.15 mg/m^3	[38]
Ni	ApoE(-/-) mice	Vascular atherosclerosis	< 0.08 mg/m^3	[40]
Oral introduction				
NiO 20 nm	Female Wistar rats ***	Genotoxicity (bone marrow), Enzyme activity (blood serum, liver, and kidneys)	125 mg/kg	[41, 42]
NiO 13 nm	Wistar rats, males and females	Hematologic parameters, histopathology (liver)	< 50 mg/kg	[43]
Ni 90 nm	Male Sprague Dawley rats	Glutathione (gonads)	< 5 mg/kg	[45]
NiO 50 nm	Male Wistar rats	Apoptosis (bone marrow)	< 1 mg/kg	[46]
NiO 50 nm	Male Wistar rats ***	Integral, biochemical, and hematologic parameters	< 1 g/kg	[47]

Note: * means abbreviations are given in Table 1; ** means sub-chronic experiment (10 months); *** means acute experiment (a single introduction); LDH – lactate dehydrogenase.

in case they were not properly protected [59]. There was a clinical case when a worker who dealt with metal arc welding involving inhalation exposure to Ni NPs died due to respiratory distress-syndrome. Autopsy revealed Ni NPs sized 25 nm in lung macrophages, elevated Ni contents in biological media and tubular necrosis [60].

Conclusions. Therefore, literature analysis reveals that NPs of metallic Ni and its compounds (NiO, $Ni(OH)_2$) as well as nickel nanofibers and nanorods produce local and systemic toxic effects when introduced into the body both parenterally and naturally, though the airways and gastrointestinal tract. Systemic effects occur due to these NPs being able to move into organs that are located far from the place where they penetrated the body, either with blood or lymph flow.

Toxicity of both Ni NPs and Ni-compounds with traditional dispersity is to a certain extent related to their ability to penetrate through membranes and biological barriers, dissolve and degrade biologically in the body. Other things being equal, better soluble NPs (NiO) turn out to be more toxic than poorer soluble NPs of metal Ni or Ni MPs. On the other hand, in some cases Ni-containing NPs turn out to be even more toxic than soluble nickel salts due to their greater ability to penetrate into cells through membranes. It further confirms an assumption that substances in their nanoform can be substantially different from their micro-dispersed analogues regarding their impacts on biological systems, and hazards caused by nanomaterials are to be described profoundly in each specific case [61].

Ni-containing nanomaterials are known to produce various adverse effects including overall toxic ones, toxic effects on specific organs (hepatotoxic and cardiotoxic included), atherogenic, fibrogenic, allergenic, and immunotoxic ones; they also have reproductive toxicity. But at the same time, data obtained via experiments *in vitro* and indicating that Ni-containing nanomaterials are genotoxic, mutagenic, and have transforming capabilities, have found practically no confirmations in chronic experiments *in vivo* that focused on establishing whether these materials were carcinogenic. Given that, carcinogenicity of Ni and its compounds in their nanoform remains rather disputable.

Experimental estimates of maximum non-effective doses and concentrations of Ni-containing nanomaterials show that such doses are within a range being lower than 0.2 mg/kg of body weight or 1 mg/m^3 when they are introduced into the airways regardless of particle size and structure of a nanomaterial. At present there are no reliable estimates of maximum non-effective or toxic doses for multiple (subacute and chronic) oral exposures to these nanomaterials.

A necessary stage in risk assessment procedure is to determine exposure to examined adverse factors at workplaces, due to environmental factors, and consumer products. Unfortunately, at present such data on NPs of Ni and its compounds are practically unavailable. The only exceptions are rare reports on detecting Ni-containing NPs in working area air at metallurgic enterprises. So, it is practically impossible to assess risks caused by Ni NPs including those caused by their occurrence in food products and water, as residual quantities of nickel catalysts or due to the environmental contamination with these nanomaterials.

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