Nickel: a unique allergen – from molecular structure to European legislation

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Nickel is a unique, mysterious and troublesome chemical element. Its molecular structure (unfilled electron shell) determines the high-reactivity and multidirectional biological effects. Some authors classify nickel as trace element, although its biological role in animal and human metabolism remains unclear. Conversely, nickel possesses strong sensitizing potential: as many as 65 million Europeans may be allergic to nickel. In this article, we review chemical and biological properties of nickel, pathomechanism, clinical symptoms and diagnosis of contact allergy to nickel, epidemiology and risk factors. Finally, public health measures and legal regulations of the EU aimed at protecting the population from nickel allergy are discussed, with particular attention devoted to the 'Nickel Directive' 94/27/EC.

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The metal nickel (Ni, atomic number 28, atomic mass: 58.6934 amu, CAS No. 7440–0-20, EINECS No 2311114) is a chemical element, which means it cannot be broken down by chemical means. The nickel atom consists of 28 protons, 28 electrons and 31 neutrons. Nickel is the fifth most common element on Earth, after iron, oxygen, silicon and magnesium [101]. Owing to its structure, nickel possesses unique chemical properties, which make it, among others, a highly bioreactive element. It is an essential microelement for plants, microorganisms and some invertebrates. It is also thought to be an essential microelement for humans; however, its role has not yet been fully elucidated. Conversely, nickel is one of the most frequent sensitizers, causing contact allergy in up to 65 million people in Europe, which poses a major concern for public health. In an attempt to tackle this problem, regulatory actions have been undertaken in the EU, aimed at reducing the frequency of contact allergy to nickel through restrictions on trading consumer products with high nickel content.

The aim of the present article is to give a review of the relationships between the structural, chemical and biological properties of nickel, with their public health implications.

Chemical & biological properties of nickel

Nickel is a transition element, which means that two electrons are missing to completely fill the *d* subshell of its atom. Therefore, nickel has paramagnetic properties and its compounds typically have spectacular colors. Perhaps more important from a biological point of view is that due to the incompletely filled subshell of electrons, nickel is characterized by good catalytic properties and is capable of forming complex compounds. Complex compounds (synonyms: coordination compounds, coordination complexes) are complexes in which an atom of a metal is surrounded by other atoms or groups of atoms, referred to as ligands. The chemical bond between nickel and a ligand is a coordination bond, which means that two electrons are donated by the ligand pair into the incomplete *d* subshell of nickel. An important class of complex compounds are metalloenzymes. Probably the best studied nickel metalloenzymes are the ureases of plants, also present in some microorganisms and invertebrates [1]. Nickel is the essential component of three further bacterial enzymes: hydrogenase (oxidation of hydrogen) [2], methyl coenzyme M reductase (methane biogenesis) [3] and carbon monoxide dehydrogenase (acetate formation) [4]. It

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has been proven that nickel is an essential element for metabolism in plants and other lower organisms: if devoid of nickel, plants and some bacteria cannot complete their life-cycle [1]. Less is known regarding the possible role of nickel in higher animals, including humans. An influence of nickel on erythrocyte formation has been suggested, possibly through influencing vitamin B_{12} metabolism in some way. An animal metalloenzyme with possible involvement of nickel is calcineurin – an important regulatory enzyme in the brain, skeletal muscles and skin [5,6]. Ni^{2+} is a potent activator of calcineurin [7], probably through changing the conformation of its β-chain [8]. Interestingly, it was demonstrated that the calcineurin inhibitor tacrolimus stops allergic inflammation in allergic contact dermatitis (ACD) to nickel [9]. This raises the question of whether nickel ACD is really a good representative of ACD to other haptens in therapeutic trials with calcineurin inhibitors [10]. Taking the above into account, it seems possible that nickel plays a role in human metabolism. Despite the fact that nickel deficiency has not been observed in humans, the UK's Food Standards Agency classifies nickel as a trace element [102]. Examples of nickel-rich foodstuffs (1–10 mg Ni/kg fresh weight) are cocoa and chocolate, liquorice, alfalfa and other legumes, dried beans, peanuts, hazelnuts, almonds, sunflower seeds, oat meal and wheat bran [11,12]. Consumption of these foodstuffs in larger amounts may increase intake of nickel up to 900 µg daily or even more [12].

Toxic effects of nickel

Life-threatening toxicity as a result of oral intake of nickel seems rather unlikely. Extrapolation from animal experiments suggests that ingestion of over 250 mg of soluble nickel daily would be necessary in order to produce toxic symptoms in humans [1]. In those chronically exposed to airborne nickel (fumes, aerosols or dusts), there is an increased risk of lung and upper respiratory cancer [13]. A possible mechanism of carcinogenesis is a nickel-induced decrease of activity of cellular enzymes containing iron, and subsequent inhibition of oxidative phosphorylation leading to increased cellular glycolytic activity. Increased glycolysis is one of the fundamental alterations of energy metabolism observed in cancer cells [14]. Chronic exposure to nickel may cause impairment of natural killer lymphocyte function, which is involved, among others, in antiviral immunity [15,16]. The effect of nickel on lymphocytes is probably mediated by oxygen radical intermediates and can be diminished by catalase, glutathione and mannitol [17]. Nickel also induces inhibition of human platelet aggregation, probably due to increased lipid peroxidation, which can be blocked by administration of ascorbic acid [18].

From an ion to an allergen

Nickel ions are too small to be recognized by the immune system. In order to become 'visible', they must bind to a protein first. Only these complexes can initiate an immune response. There are three pathways known to date, by which nickel can activate T cells:

- Nickel can bind to extracellular proteins. The potency of a hapten as a sensitizer is proportional to its protein-binding capacity [19,20]. Nickel ions are highly reactive and can easily form complexes with electron-rich groups of proteins, and can form square planar complexes with histidine and octahedral complexes with oxygenated amino groups. This will result in changes of tertiary structure (spatial conformation) of the protein, which will then be recognized as allergen [21]. When such proteins are taken in by antigen-presenting cells (APCs), they will go through the external pathway and will be presented together with MHC class II molecules, suitable for presentation to the T-cell receptors (TCRs) of $CD4^+$ T lymphocytes [22];
- Nickel may be taken into cells where it binds to intracellular proteins, either as a result of its intrinsic reactivity or through metabolic bioactivation. These proteins are passed through the endosome for degradation and finally the resultant peptides will be expressed together with MHC class I molecules, suitable for presentation to $CD8^+$ T lymphocytes [23];
- A third pathway appears to be metabolism independent, which means that no nickel–protein complex is processed and presented by the APC. Instead, nickel may directly link the MHC molecules of APC with TCR in a process that is similar (although not identical) with the mode of action of superantigens [24,25].

Allergic immune response

When APCs residing in the exposed epidermis encounter nickel or nickel–protein complexes, they process the hapten/allergen in the aforementioned manner and migrate through the lymph vessels to the local lymph nodes. While migrating, the APCs mature, which manifests through increased expression of key surface molecules, including MHC and costimulating molecules, such as CD40, CD80, CD83 and CD86 [22]. During this process, the unique biological properties of nickel become apparent once again: exposure of dendritic cells to nickel results in a stronger upregulation of CD83 and CD86 and greater CXCL8, CCL5, CCL17 and CCL20 expression compared with other contact allergens [26]. This unique potency of nickel may be explained by concomitant triggering of several signal transduction pathways, notably p38 MAPK, ERK and nuclear factor (NF)-κB. In contrast to nickel, a 'model' contact allergen frequently used in sensitization experiments, dinitrochlorobenzene (DNCB) activates p38 MAPK but not ERK, or NF-κB [27–29].

In the lymph nodes, APCs present the antigens to T cells. This presentation seems to happen randomly, but if T cells programmed to react specifically to this particular antigen are present, these will be finally found due to a high turnover of T cells in lymph nodes. Once presented with the antigen, the specific T lymphocytes proliferate (clonal expansion) and differentiate into effector cells. Effector cells express receptors that enable them to migrate to sites of inflammation. The trafficking mechanisms that aim the cells to the part of the

body that was exposed to the hapten are not fully understood. It appears that migration to a particular organ is controlled by so-called homing antigens and chemokine receptors that are expressed on the effector cell's surface, similar to an address on an envelope. Such molecules are organ specific; for example, lymphocytes migrating into the skin express on their surface the cutaneous lymphocyte antigen (CLA) and chemokine receptors CXCR3, CCR4 and CCR10 [30].

Effector cells

The allergic response to nickel is a peculiar process that seems to involve both 'type 2' (IL-4, IL-5 and IL-13 secreting) and 'type 1' (IFN-γ secreting) cells, although the latter was not observed in all studies (TABLE 1). Although some authors consider IL-2 as a marker of 'type 1' activation, secretion of IL-2 is not merely restricted to the Th1 subtype, as it is secreted upon the first antigen encounter by naive T cells, before their phenotype (Th1 or Th2, Tc1 or Tc2) is determined [31–34]. It has been demonstrated that in nickel allergy, both 'type 2' and 'type 1' cells are $CD4^+$ T cells [30,35], in contrast to contact allergy to nonmetal allergens, where mostly activation of cytotoxic $CD8^+$ T cells is observed [36,37].

Induction & elicitation of nickel allergy

In some sensitized individuals, an oral dose of as low as 300 µg of nickel is capable of inducing an inflammatory skin reaction (dermatitis). That dose is only a few times higher than the human daily nutritional requirement postulated by using data from animal studies [1], and can be easily found in a normal daily diet [38,39]. As mentioned previously, a nickel-rich diet may increase nickel intake by up to 900 µg daily [12]. The typical route of sensitization and elicitation of allergy to nickel is through the skin [40]. Therefore, the local eliciting doses are of great importance. Most of the local dose–response studies were carried out under occlusion, which means that the allergen applied to the skin is covered, for example by an adhesive dressing. Such application may, to some extent, resemble a situation under a nickel-containing bracelet, watch strap or ear clips. These studies showed that 5% of the sensitized population would react to 0.44 µg nickel applied on 1 cm^2 and 10% would react to 1.04 µg Ni/cm^2. In open application (i.e., solution of a nickel salt is painted once on the skin and is left uncovered), these doses are approximately six-times higher [41].

Table 1. Results of studies on cytokine secretion in response to nickel from peripheral blood mononuclear cells: an overview of published data.

Cytokine	Interpretation	Difference between ACD and controls observed	No difference observed
IFN-γ	'Type 1'	[66,85–87]	[67,88,89]
IL-2	Both*	[67,86,90]	
IL-4	'Type 2'	[85–89]	
IL-5	'Type 2'	[67,85,87,88,90]	
IL-13	'Type 2'	[67,85,87,89]	

*Secretion of IL-2 is not restricted to one lymphocyte subpopulation, as it is secreted upon the first antigen encounter by naive T cells, before their phenotype (Th1 or Th2, Tc1 or Tc2) is determined [31–34].
ACD: Allergic contact dermatitis.

Clinical forms of nickel allergy

The most common clinical form of nickel allergy is ACD, to such an extent that it is frequently understood as a synonym to contact allergy. However, these terms are not synonymous and one must not forget other clinical forms of contact allergy caused by nickel, such as allergic contact stomatitis [42], allergic contact conjunctivitis [43] and systemic nickel allergy [39]. There are also reports of nickel-induced urticaria [44], asthma [45] and rhinitis [46]. Moreover, contact allergy to nickel has been reported, but not proven, to cause rejection of orthopedic and dental implants [47,48] and coronary stents [49].

Diagnosis of nickel allergy

The first step towards diagnosis of nickel allergy is careful history taking of the disease. Typical complaints are itchy skin rash of the skin sites chronically exposed to metal (e.g., watches, bracelets, earrings, ear clips, fashion buttons and rivets). Testing the offending object for nickel release (see later) may be very helpful. For confirmation of contact allergy to nickel, patch test (epicutaneous test) is the method of choice. The principle of the method is very simple: nickel is applied onto the skin in a standardized concentration (either 2.5% or 5% $NiSO_4$ in petrolatum) and time (48 h), and skin reaction is observed on consecutive days. In sensitized individuals, an inflammatory reaction develops in the exposed site – its severity may be graded according to the internationally accepted score introduced by the International Contact Dermatitis Research Group (ICDRG) [50]. As a matter of fact, patch test relies on provoking skin inflammation (dermatitis) under controlled conditions and limited to the patch test site, which is typically less than 1 cm^2 (FIGURE 1).

Patch test remains the diagnostic test of choice for contact allergy and is indispensable in modern dermatology and allergology. The test procedure has undergone very intensive standardization and validation in the last decades, and considerable technological progress has been also made in the production of test substances and accessory equipment. As every clinical test, however, it is not entirely free of certain limitations, such as interobserver variability [51], site-to-site variability [52], and test-to-test variability [53]. Patch test results may be influenced by the time of reading [54], quality of allergens used [55], ultraviolet irradiation [56], topical and oral steroids [57,58], in addition to a host of other factors. In some cases, excessive irritation of the skin makes the interpretation of patch tests difficult or even impossible – a situation referred to as 'angry back' [59] or 'excited skin syndrome' [60]. In addition, the question of the clinical relevance of the results needs to be addressed very carefully. This means that in each

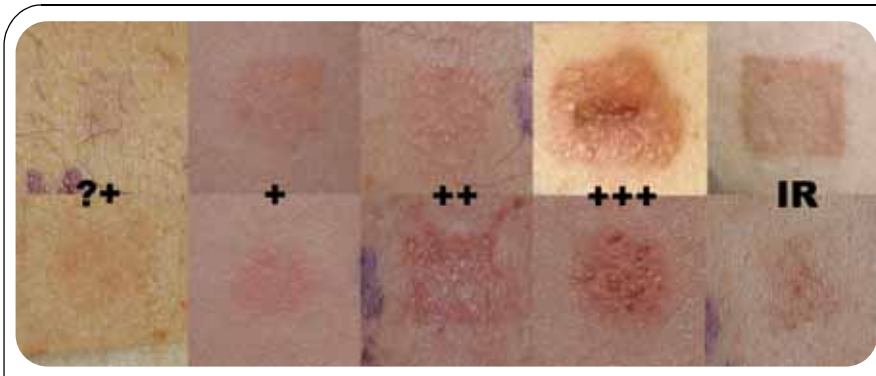

Figure 1. Patch test scoring according to the International Contact Dermatitis Research Group. ?+: Doubtful reaction (faint, nonpalpable erythema); +: Weak reaction (palpable erythema – moderate edema or infiltrate, no or scarce papules, no vesicles); ++: Strong reaction (strong infiltrate, numerous papules, vesicles); +++: Extreme reaction (coalescing vesicles, bullae or ulceration); IR: Irritant reaction. Other symbols used for recording patch test results: – or ø: Negative (no visible change in tested area); NT: Not tested.

case, the answer to the question 'does the positive patch test result really explain the patient's disease?' should be sought for. The proper execution of patch tests and interpretation of the results requires a well-trained and devoted dermatologist or allergist, supported by a reference center in doubtful cases.

Perspectives for laboratory diagnosis

Taking into account the above limitations of patch tests, a reliable *in vitro* test for contact allergy has tantalized researchers for decades. In the past, various *in vitro* tests have been tried in the detection of contact allergy, starting with the macrophage migration inhibition test [61] and the lymphocyte blastic transformation test [62]. A lymphocyte proliferation test (LPT) was also introduced in the 1970s [63] and has been used until today, however, mainly for experimental purposes. Later, the above-mentioned methods were followed by analyses of cytokine and chemokine secretion, surface cell markers and gene expression. Unfortunately, none of these methods has proven sufficient for diagnostic use, mainly due to poor sensitivity and/or specificity. Combinations of two or three different *in vitro* methods or parameters have been proposed to overcome this problem [64,65], however, this approach has not found its way into routine clinical applications either. Recent observations suggest that skewing lymphocytes toward 'type 2' (IL-4- or IL-5-secreting cells) could improve detection of nickel-specific T-cell response in contact allergy [66]. We have shown that combining the ELISpot assay with modified culture conditions may constitute a relevant progress in the detection of contact allergy to nickel *in vitro*. Among the test protocols analyzed, IL-13 ELISpot carried out in cultures supporting the development of 'type 2' lymphocytes was most effective in differentiating people allergic to nickel from those nonallergic (TABLE 1) [67].

Nickel detection

Once a person has been diagnosed with contact allergy to nickel and the clinical relevance of the finding has been confirmed, the question of allergen avoidance comes to the foreground. The avoidance of nickel is particularly difficult due to its broad presence in everyday life. In the modern world, it is hard to imagine an environment without nickel. Fortunately, patients do not need to avoid all nickel-containing objects – it is sufficient to avoid nickel-releasing objects instead. A helpful aid in identifying such objects is the dimethylglyoxime test: in the presence of free Ni^{2+} ions, dimethylglyoxime turns red. The detection level of the test is 10 ppm, whereas most nickel-allergic individuals will only develop the symptoms of skin inflammation when exposed to higher concentrations of nickel [68].

Epidemiology of nickel allergy

Nickel is one of the most common causes of allergy. In the general population, the frequency of nickel allergy is estimated at approximately 17% adults and 8% children [69,70]. Women are affected more frequently: in a German population study, 20.4% women and 5.8% men were diagnosed with contact allergy to nickel [71]. Assuming that these figures are representative for the European population, the number of nickel-allergic people in the EU (including Bulgaria and Romania) could be roughly estimated at 65 million, including 51 million women and 14 million men (for calculation of these figures, population of the EU27 = 488.5 million [103] and sex ratio of 105.5:100 [104] were used). It should be stressed, however, that not all people with a contact allergy to nickel will develop any disease symptoms.

Risk factors

Among known risk factors for nickel allergy, the female gender and body piercing seem most relevant [72,73]. It has been long discussed whether female sex is an intrinsic factor or is it related to the more frequent use of nickel-containing objects, such as jewelry and fashion items [74]. In Denmark, after introducing a ban on earrings with high nickel content, the risk of nickel allergy in girls wearing earrings dropped by 64% [75], which favors the view that sex differences in the prevalence of sensitization to nickel reflect differences in exposure. The legal restrictions of nickel-containing consumer goods will be discussed later in this article. Cigarette smoking was also suggested as a risk factor for nickel allergy [76]. The presence of atopy was proposed by various authors either as a risk factor or a protecting factor; finally, it seems that there is no relationship between both phenomena [77]. Some authors highlight the protective role of oral contact with nickel (dental braces) against a later development of contact allergy to nickel, however, this protection seems not to take place in those who were previously pierced with nickel-containing objects [73]. Until now, no evidence was found for the role of genetic factors in contact allergy to nickel [78].

Legal actions regarding nickel

As mentioned previously, up to 65 million European citizens may be allergic to nickel – a substance abundantly present in daily life. Appreciating this as a serious burden to public health, the European Parliament and European Council issued on 30 June 1994 the Directive 94/27/EC nicknamed as the 'Nickel Directive' [79]. In fact, the Nickel Directive is an amendment to the Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to the restrictions on the marketing and use of certain dangerous substances and preparations [80]. The amendment 94/27/EC added nickel to the list of hazardous substances and imposed restrictions on the marketing and use of nickel in the EU (BOX 1). The regulations came fully into force in July 2001.

In context of the media discussion coming up occasionally about the nickel content in Euro coins and its sensitizing potential, it is worth mentioning that the Nickel Directive does not apply to Euro coins, as these are not considered as objects coming into prolonged contact with the skin. The possibility of nickel release and sensitizing potential of Euro coins was raised immediately after introducing the currency into circulation [81]. The high release of nickel ions is attributed to the fact that 1-Euro and 2-Euro coins are each made of two alloys. In the presence of human sweat (which acts as an electrolyte) the bimetallic structure produces a galvanic potential of 30–40 mV that enhances corrosion of the coin, thus the release of nickel ions [82]. Conversely, it has been demonstrated that even an artificially prolonged, 2-day contact of the hands with the Euro coin only rarely causes symptoms in nickel-sensitive people, possibly due to the thick horny layer preventing penetration of nickel ions into the skin of the hands [83]. Nevertheless, some EU Member States, decided to introduce a nickel-free alloy called 'Nordic gold' in their currency systems, and the European Council encourages such initiatives [84].

As mentioned before, in Denmark, where restrictions similar to the 'Nickel Directive' are in force since 1992, a 64% decrease of nickel allergy was achieved among girls wearing earrings [75]. This is an excellent success story of how appropriate legal regulations can effectively improve public health.

Expert commentary

Nickel is a highly reactive element and interferes (both in positive and negative ways) with a range of metabolic processes of living organisms. Nickel sensitization is a major public health concern due to its high prevalence and the wide presence of nickel in daily life. The clinical diagnosis of nickel allergy (history taking, medical assessment and patch testing) is relatively simple and reliable. However, such diagnosis requires several visits at the doctor's office. A simple screening test is not yet available. In such circumstances, increasing public awareness of the problem and undertaking regulatory actions seem the only available effective way of decreasing the prevalence of nickel allergy to date.

Five-year view

The most important issue for the next 5 years is to develop a reliable *in vitro* test to determine nickel allergy. Having such a test within this 5-year time frame appears quite possible, however, most probably, it will be based on cell cultures. This means that execution of such a test would be restricted

Box 1. Restrictions on nickel imposed by the 'Nickel Directive' 94/27/EC: Nickel CAS No 7440–0-20 EINECS No 2311114 and its compounds.

May not be used:

- In postassemblies that are inserted into pierced ears and other pierced parts of the human body during epithelization of the wound caused by piercing, whether subsequently removed or not, unless such postassemblies are homogeneous and the concentration of nickel – expressed as mass of nickel to total mass – is less than 0.05%
- In products intended to come into direct and prolonged contact with the skin (If the rate of nickel release from the parts of these products coming into direct and prolonged contact with the skin is greater than 0.5 µg/cm^2/week), such as:
  - Earrings
  - Necklaces, bracelets and chains, anklets, finger rings
  - Wrist-watch cases, watch straps and tighteners
  - Rivet buttons, tighteners, rivets, zippers and metal marks, when these are used in garments
- In products such as those listed above, where these have a non-nickel coating unless such coating is sufficient to ensure that the rate of nickel release from those parts of such products coming into direct and prolonged contact with the skin will not exceed 0.5 µg/cm^2/week for a period of at least 2 years of normal use of the product

Furthermore, products that are the subject of the above points may not be placed on the market unless they conform to the requirements set out in those points

From [79].

to specialized laboratories. The chance for having a simple screening *in vitro* test for nickel allergy seems rather low at this point. On the public health side of the problem, more evidence can be expected to be presented for the effectiveness of the Nickel Directive within the next 5 years throughout EU countries. Such data could demonstrate whether the regulations need adjustment or reinforcement in some aspects, or in some countries. Research of possible alternatives to nickel, especially in consumer products, should be encouraged and supported through sufficient funding and regulatory actions.

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Key issues

- Nickel is a highly reactive transition metal and is the fifth most abundant element on Earth.
- Nickel is a microelement and central component of metalloenzymes necessary for the growth of plants, microorganisms and some invertebrates. It is also considered a microelement to humans; however, symptoms of nickel deficiency have never been described.
- Toxic effects of nickel on humans are very rare, whereas hypersensitivity to nickel (manifest in most cases as allergic contact dermatitis) is a very important burden to public health in Europe.
- Recent experience shows that reduction of the frequency of contact sensitization to nickel can be achieved through regulatory means protecting consumers from excessive exposure to this metal.

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