

Contact allergy and human biomonitoring – an overview with a focus on metals

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Summary

Humans are widely exposed to chemicals. Today, there is an increased acknowledgement of the importance of measuring human and environmental exposures to man-made or refined chemicals. Different approaches have been applied over time, but during the past 25 years, there has been a general trend towards the use of human biomonitoring. A few studies have used human biomonitoring methodology to track contact allergens together with information on patch test reactivity. Hypothetically, the internal load of reactive chemicals might modify the immune response to haptens and the propensity to sensitize and elicit allergic contact dermatitis or develop tolerance. This review offers a general overview of human biomonitoring, including information about its typical application and methodology. Furthermore, studies that have attempted to perform simultaneous biomonitoring and patch testing are reviewed. It is concluded that all studies conducted until the present have focused on one or two routes of exposure (typically skin and oral exposure, but also skin and airway exposure), whereas no studies have investigated all routes at the same time. Also, there is a need for prospective studies, as all epidemiological studies so far have been cross-sectional.

Key words: biomonitoring; chemical exposure; contact allergy; metal; nickel.

Humans are widely exposed to chemicals. Incidents such as the methyl mercury pollution of the Minamata bay in 1956 (1), leading to the 'Minamata disease', and the high lead concentrations identified in individuals living in urban areas (2) led to an increased acknowledgement of the importance of measuring human and environmental exposures to man-made or refined chemicals. Initially, this control was accomplished by calculations and estimations using data on industry consumptions of a given chemical combined with knowledge of its toxicology and persistence. However, over the past 25 years, there has been a general trend towards the

use of human biomonitoring (HBM). This tool has several advantages over traditional control systems based on 'desk-calculations', as HBM combines all exposure routes and estimates the total internal load of chemicals and metabolites within the body. Accordingly, HBM can be defined as 'the assessment of human exposure to an environmental chemical via the measurement of that chemical, its metabolite(s), or reaction product(s) in human blood, urine, milk, saliva, adipose, or other tissue in individuals taken separately but generally taken together to constitute a population' (3). HBM may offer legislators an enhanced method for making informed decisions concerning regulation of the use of potentially harmful chemicals, as well as a way of monitoring whether existing legislation is adequate.

Contact allergy is very prevalent in both adults and adolescents from Western societies, as indicated by general population studies (4–6). Contact allergy is mainly an environmental disorder that requires cutaneous exposure to low molecular weight chemicals

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in sufficient concentrations to sensitize an individual. A few studies have used HMB methodology to track contact allergens together with information on patch test reactivity. Hypothetically, the internal load of reactive chemicals might modify the immune response to haptens and the propensity to sensitize and elicit allergic contact dermatitis or develop tolerance (7, 8). This review offers a general overview of HBM, including information about its typical application and methodology. For further details, please refer to other review articles (3, 9, 10). Furthermore, we review studies that have attempted to perform simultaneous HBM and patch testing in an effort to build a platform for future studies.

Human Biomonitoring

Methodology

Measurements of absorption, distribution, metabolism and excretion of chemicals are pivotal in HBM study designs. Entry route(s) of the investigated chemical(s) should be identified prior to study initiation, but the behaviour of the chemical(s) in the body and elimination kinetics should also be considered. It is possible to define criteria that are essential to the successful implementation of an HBM study (Table 1):

- (1) The selection of individuals or study groups obviously depends on the purpose of the HBM programme. For large-scale population-wide HBM programmes, the investigator should make sure that the study sample is representative of the overall population. As an individual's occupation can be a significant source of exposure, this should be recorded and taken into account. For smaller studies designed to monitor pollution at specific geographical locations or workplaces, lifestyle confounders such as tobacco smoking should also be considered (11).
- (2) The most frequently sampled biological matrices are blood and urine; however, hair and nails have also been used, and recently the potential use of exhaled breath condensate has been tested (12). Blood is advantageous, because it

is essentially in equilibrium with different body compartments, making it suitable for testing of chemicals with longer half-lives, for example dioxin and polychlorinated biphenyls (PCBs). Furthermore, it is possible not only to measure chemicals and their metabolites, but also to isolate lymphocytes and measure genotoxic and oxidative stress effects. As blood sampling is an invasive procedure, it may be inconvenient and unsuitable for studies on children. Furthermore, the amount of sampled blood is often limited, which can be a problem if the number of required blood analyses is high. For non-persistent chemicals, blood is unfavourable, as they will usually be present only in very low concentrations, owing to their fast elimination. For volatile compounds and compounds with rapid metabolism, urine is usually the matrix of choice. Polyaromatic hydrocarbons, organophosphate pesticides and phthalates are examples where metabolites are usually measured in urine samples (9, 13). Urine has the advantage of being available in larger quantities, and sampling is non-invasive. As the volume and concentration of urine are dependent on fluid intake, concentrations of a given chemical are typically presented as units per unit of creatinine. Nails and hair are infrequently sampled, but are attractive, because they are easily collected. The inter-individual variation seem to be higher in hair and nails than in blood (9), but there are ongoing efforts to validate analytical procedures with these matrices. Contamination is an issue when these 'external' matrices are used, and should be considered. Hair has been used to monitor cadmium, mercury, nickel, and PCBs (14). Nails have been used to monitor trace metals, including nickel (15). Exhaled breath condensate is also non-invasive, but has yet to be validated. A recent study suggested that the method was unsuitable for monitoring cobalt exposure at the workplace, as the condensate concentration of cobalt was found to correlate poorly with exposure (12). For recent reviews of biological matrices in HBM, see (16, 17).

- (3) In general, three different types of parameter are measured as a part of HBM: (i) internal chemical load; (ii) biochemical effects; and (iii) biological effects. Internal exposure measurements include concentrations of chemicals and their metabolites. Biochemical effect measurements include quantification of the reaction products of reactive substances and biological macromolecules such as haemoglobin and DNA. Biological effect

Table 1. Criteria that are essential to the successful implementation of a human biomonitoring study

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- Availability of suitable test individuals.
 - Identification of a suitable biological matrix.
 - Availability of suitable tests to reliably analyse the sample. The assay should ideally be validated and be a part of a quality control programme.
 - Availability of suitable reference values to interpret results.
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measurements include identification of early non-adverse biological effects, for example alterations of liver enzyme activity. As HBM studies obviously only detect what is measured, biological effect markers have the advantage that they may sometimes reveal effects of unsuspected and untested chemicals. Hence, the inclusion of biological effect measurements, for example patch testing, should always be considered in HBM programmes intended to monitor public health. In general, it is considered that, regardless of study design, all of the above aspects should be validated. The investigator has to consider practical issues regarding sample collection, storage, and shipping. With regard to volatile chemicals, investigators should ensure that they remain stable from sampling to measurement. Containers should preferably prevent adherence of chemicals and should not release any chemicals to the sample. The risk of contamination of samples is an important issue, as the concentration of the monitored chemical is usually very low. Correct interpretation of collected data is also a challenge in HBM. The investigator needs to decide the acceptable internal doses, the possible sources, and whether they are modified by the presence of other foreign chemicals. A key issue within the field of HBM includes the determination of safe internal load concentrations, which is typically a prerogative of national health organizations. The only two countries with established HBM programmes, Germany and the United States, have both set recommendations for acceptable internal loads of a limited range of chemicals. In Germany, the 'Human Biomonitoring Commission', established in 1992, utilizes three different values to interpret the measured levels of a given chemical: (i) the reference value; (ii) HBM I; and (iii) HBM II. The reference value is a purely statistically derived figure representing the 95% percentile within the 95% confidence interval of the distribution of measured concentrations in the reference population (18). As defined, reference values are not linked to any toxicological deliberations or health intervention considerations, but offer an indication of 'what to expect'. HBM I is defined as the value below which detrimental health effects are considered to be unlikely for all categories of the population. HBM II is defined as the value above which adverse health effects are deemed to be possible and immediate action to reduce exposure is to be taken, possibly combined with treatment of affected individuals.

The concentration range between HBM I and HBM II is a zone where health effects cannot be excluded and where action should be taken to confirm the measurements, and if they are confirmed, to reduce exposure. The costs and efforts required to establish HBM I and HBM II values are high, and for these reasons the Human Biomonitoring Commission has only established HBM values for cadmium, mercury, PCBs in blood and urine, and for the sum of the bis(2-ethylhexyl)phthalate metabolites mono(2-ethyl-5-oxo-hexyl)phthalate and mono(2-ethyl-5-hydroxy-hexyl)phthalate in urine. As one may appreciate, this is a very low number, taking into consideration the number of man-made chemicals in circulation and the available detection techniques. In order to achieve urgently needed HBM values, the commission has begun an initiative to derive HBM values with a new approach relying on collation of the currently available values of tolerable daily intake (TDI) and acceptable daily intake as set forward by joint Food and Agriculture Organization of the United Nations/World Health Organization expert meetings (19, 20).

In the United States, risk managers are faced with similar discrepancies between what can be measured and the amount of appropriate toxicological study control data. Steps have been taken to utilize tools similar to those of the German Human Biomonitoring Commission, that is, the integration of current toxicological and pharmacological data with current TDI and reference values hitherto used in occupational medicine. The work will result in biomonitoring equivalents (BEs) for each chemical that can be used in a similar way to HBM values (21, 22). BE values have so far been determined for a limited number of chemicals (>10) (23–28). Although the novel approaches in Germany and the United States seem to be less rigorous and expensive, the task of establishing even intermediate HBM and BE values is still monumental. However, it is imperative that it be completed if the full potential of HBM as a risk-managing tool is to be realized.

Obstacles and challenges

Currently, large-scale HBM programmes face important obstacles. First, and most important, there is an economic challenge, as initial costs are high. The estimated cost of the fourth report of the Centers for Disease Control and Prevention (CDC) was \$19.8 million, equivalent to \$8000 per subject evaluated within the programme (13, 29). The cost per test often varies greatly; for example, the CDC

spent \$68 per sample when testing the lead concentration in the blood, but \$1000 per sample when testing a wider panel of chemicals, including dioxins, furans, and dioxin-like PCBs (29). The economic consequences of starting a biomonitoring programme need to be weighed against the potential costs resulting from the harmful health effects of unmonitored environmental pollutants. Besides these financial issues, HMB programmes sometimes face political opposition; a discussion of this falls beyond the scope of this review, but interested readers may consult literature describing the efforts to launch an integrated HBM programme within the EU. Finally, the results from HMB programmes may be difficult to interpret, owing to inadequate understanding of toxicology and biology. There is hence a great need for better understanding of the consequences of broad chemical exposure in humans and the resulting biological alterations, including contact allergy.

Contact Allergens and Routes of Exposure

Consumers and workers are exposed to chemicals in their daily lives (30–36). Depending on the setting, exposure may be via air or food, or through the mucosa or skin. In the case of skin exposure, an individual may develop contact allergy, a delayed-type hypersensitivity event. Upon repeated skin exposure, allergic contact dermatitis may be elicited; this is an eczematous disease that is persistent if allergen exposure is not reduced or removed. Historically, haptens resulting in contact allergy and allergic contact dermatitis mainly include metals, fragrances, and preservatives, but drugs, adhesives, hair dyes, local anaesthetics and other substances have also contributed to morbidity. Recent epidemiological trends for contact allergy were thoroughly reviewed in 2010 (37). Briefly, contact allergy is observed in 15–20% of the general population (4–6). On the basis of the accumulation of data from general population studies, nickel allergy as a proportion of contact allergy to at least one allergen increased significantly over the period 1966–2007, to about 60–70% (6). Thus, nickel is by far the most important contact allergen. Contact-sensitized individuals may develop systemic allergic dermatitis if they are systemically exposed to contact allergens in high concentrations. Also, it has been demonstrated that individuals may become immunologically tolerant if they are orally exposed to haptens before cutaneous sensitization (38) or managed with hyposensitization therapy (either orally or subcutaneously) (39, 40). Oral tolerance and systemic contact dermatitis reactions have been extensively reviewed recently, and will not be discussed further (41, 42). However, exposure routes

should be considered by readers of this review, as none of the presented studies has performed systematic investigations to determine routes of exposure, although techniques are available (Table 2). Thus, individuals may be extensively exposed to, for example, metals, fragrances and other substances through air (e.g. pollution, cigarette smoke, or working in refineries or perfumeries), food (nickel is present in beans, nuts, and chocolate, and cinnamyl alcohol, cinnamal, eugenol and α -amyl cinnamal are present in some foods) (43), water (44), and skin (45), depending on their jobs, personal habits, and geographical location. Studies have suggested that cigarette smoking (cigarette smoke contains nickel), for example, may increase the prevalence of patch test reactivity to nickel and other contact allergens (46, 47). Airway exposure to haptens other than nickel may possibly also result in contact sensitization, but there is very little available information on this topic. Elberling et al. suggested an association (but did not specify in which direction) between the skin and the airways in fragrance-allergic but not nickel-allergic individuals (48). In an experimental model, Schnuch et al. provoked skin disease but not respiratory disease when provoking fragrance-allergic individuals with fragrance inhalations (49). Also, hapten exposure at different stages of life may alter the immune system. To our knowledge, no studies have yet investigated an association between hapten exposure in pregnancy and the propensity of the offspring to develop contact allergy. However, it has been suggested that hapten exposure in pregnancy may alter the immune system and increase the risk of atopic disease (8, 50). It is evident that the route of exposure may have an effect on the immune system, resulting in increased or decreased susceptibility to contact allergens. At present, very little is known about the effects of simultaneous exposure to chemicals through different routes. Thus, our knowledge is limited to insights from separate systems.

HBM and Contact Allergy

Exposure to metals (e.g. nickel, cobalt, and chromium) may pose a threat to an individual's health, as metals can be allergenic, toxic and/or carcinogenic above a certain level. Also, certain hair dye chemicals, for example *p*-phenylenediamine, have been associated with cancer risk (76). In metal refineries, foundries, metal reprocessing plants and the welding industry, exposed workers are at particular risk of morbidity, and for these reasons numerous HBM studies have been performed in such workers. When systemically absorbed, most metals are fairly quickly excreted, with half-lives between 20 and 100 hr (77), and urine is often the medium of choice

Table 2. Patch test studies in which simultaneous human biomonitoring has been conducted

Author	Controls, n	Allergics, n	Matrix				Conclusion
			Urine	Blood/ serum	Nails	Hair	
Menné (51)	20	71	–	+	+	+	Nickel-allergic women had significantly higher levels of nickel in toenails, hair and plasma than controls
Menné (52)	0	4	+	–	–	–	Urine nickel levels were elevated in 4 women with vesicular hand eczema prior to eruption of vesicles when compared to periods without vesicles
Menné (53)	13	0	+	–	–	–	Ingestion of 5.6 mg of nickel resulted in elevated urine nickel levels in the following days
Menné (54)	0	11	+	+	–	–	Serum nickel levels rose following Antabuse intake, but showed a tendency to decline during the treatment period. The results suggest that Antabuse is able to reduce nickel deposits in humans
Spruit (55)	–	–	+	+	–	+	No difference in urine nickel level was found between allergic and non-allergic individuals
Andreassi (56)	10	15	+	+	–	–	Similar serum and urine nickel levels were observed following ingestion of 10 mg of nickel sulfate in controls and nickel-sensitized women without atopy
Boscolo (57)	20	20	+	+	–	–	Controls and nickel allergic individuals showed similar levels of urine and serum nickel and serum chromium
Di Gioacchino (58)	6	12	+	+	–	–	Serum and urine nickel levels increased significantly after nickel ingestion, with no differences between allergic individuals and controls
Katz (59)	38	7	–	–	–	+	Hair nickel content was dependent on sex but not on nickel allergy status
De Jongh (60)	0	1	+	+	–	–	A limited degree of correlation was identified between the nickel levels in plasma and urine and clinical activity of dermatitis
Hindsén (61)	32	20	+	+	–	–	Urine nickel levels were significantly higher in atopic than in non-atopic individuals, and also decreased with age
Santucci (62)	0	11	+	–	–	–	Subjects were given a gradually increasing daily oral dose of nickel sulfate in water over 49–152 days. The ratio of nickel serum level to oral intake showed that, as the doses administered increased, the absorption of nickel decreased, perhaps because of intestinal adaptation
Santucci (63)	0	8	+	+	–	–	In some nickel-sensitive subjects, prolonged oral exposure to nickel sulfate in water reduces the intestinal adsorption of nickel and activates its renal excretion
Christensen (64)	16	0	+	+	–	–	Healthy volunteers without nickel hypersensitivity were given 5.6 mg of nickel orally. The nickel level in serum increased in all subjects, and in most cases in urine, but not decisively in sweat

Table 2. Continued

Author	Controls, n	Allergics, n	Matrix				Conclusion
			Urine	Blood/ serum	Nails	Hair	
Christensen (65)	8	0	+	+	—	—	The peak level of nickel in blood was reached 2.5 hr after nickel ingestion, and the maximal urinary excretion of nickel occurred during the first 8 hr after ingestion. Great individual variations in nickel levels in blood and nickel excretion in urine were observed
Christensen (66)	30	35	+	+	—	—	Similar levels of nickel were found in urine, but not in serum, of nickel-allergic individuals with hand eczema and healthy controls
Nielsen (67)	20	20	+	+	—	—	A stable nickel isotope, nickel-61, was given in drinking water to allergic and non-allergic individuals. No differences in serum and urine nickel levels were detected
Jensen (68)	20	40	+	+	—	—	Dose dependency was found for nickel levels in both serum and urine after oral nickel exposure, but no differences were identified between controls and nickel-allergic individuals
Gawkrødger (69)	0	1	+	+	—	—	Urinary nickel levels decreased together with clinical symptoms in a nickel-allergic woman who commenced a low-nickel diet
Bonde (70)	12	12	+	—	—	—	The nickel levels in intercellular fluid from blisters and urine in non-atopic women with nickel allergy and vesicular hand eczema were similar to those of matched controls without nickel allergy
Smith-Sivertsen (71, 72)	1236, 531, 657	0	+	—	—	—	Nickel water levels were higher in Nikel, Russia than in Sør-Varanger, Norway, and urine nickel levels were much higher in Russian women; these findings suggest that Russian women may have become tolerant to nickel following oral exposure to nickel from contaminated water and foods
Wilhelm & Mann (73, 74)	1000	0	+	—	—	—	A positive correlation was identified between nickel allergy, urine nickel levels and nickel levels in ambient air from Dortmund, Hörde, an area with heavy industrial activities
Schwieger (75)	163	Not stated	+	—	—	—	Nickel urine levels were not associated with nickel dermatitis

—, not given. The list may not be exhaustive.

in HBM projects. However, when inhaled, nickel and chromium may accumulate in pulmonary tissue and be retained for years (78). We have not been able to identify any studies, besides investigations on metal allergy, that have investigated whether patch test reactivity to chemicals is associated with an increased load of these chemicals in biological matrices. For these reasons, we present an overview of information gathered from metal studies (Table 2).

Metal penetration though intact skin

Here, skin penetration of metal ions is presented, as it has been intensively studied. Before metal ions can elicit a cutaneous immune response, they must gain access to the viable epidermis. Exogenous (e.g. dose, size, counterions, polarity, valence, and pH) and endogenous (e.g. age of the skin, anatomical site, and oxidation and reduction) factors are important, and should be accounted for when investigating metal absorption (79–81). Little is known about how aging affects penetration, apart from incomplete barrier function in infants and young children, which tends to increase the penetration of chemicals (82). However, no studies have been performed on metals. Permeability depends on anatomical location (scrotum > forehead > postauricular > abdomen > forearm > leg > back) (82). The differences are explained by the thickness of the stratum corneum and by intercellular lipid composition and shunt density (82). Nickel chloride is more rapidly absorbed than nickel sulfate when experiments are performed with occlusion (83–85). Without occlusion, permeation of nickel chloride is reduced by 90% and permeation of nickel sulfate is hardly detectable (82, 85). The counterion (sulfate or chloride) plays a role in the passive diffusion of nickel through the stratum corneum (82, 85). Nickel diffuses slowly (time lag of 50 hr) through the stratum corneum, and passage is believed to be mainly intercellular (82). Once nickel has passed through the stratum corneum, diffusion rates increase (82). Nickel is mostly accumulated in the stratum corneum, where 90% is bound, and only minor amounts are found in the lower layers, independently of the counterion (83, 86, 87). Little is known about cobalt absorption and penetration. Volunteers who were cutaneously exposed to cobalt had higher concentrations of urinary cobalt than controls (88, 89). Linnainmaa and Kiilunen found an increase in the urinary cobalt concentration 4–6 hr after skin exposure to cobalt powders in 5 subjects (89). One *in vitro* study confirmed cobalt penetration, and showed that cobalt remained in the skin after penetration (90). Also, the permeation of both nickel and cobalt ions may be closely associated with the oxidizing capacity of sweat (79, 91). Only trivalent

and hexavalent chromium are sufficiently stable to act as haptens. Hexavalent chromium may easily pass through the epidermis, whereas trivalent chromium tends to form stable complexes within the epidermis (92, 93). Once hexavalent chromium has entered the skin, it is reduced to trivalent chromium, which then binds to proteins. Filon et al. found that 99% of chromium accumulated in the skin when performing *in vitro* skin permeation experiments (94).

Metal penetration through damaged skin

It has been shown that cobalt and nickel powders penetrate damaged skin more easily than intact skin (94–98). Two clinical studies have investigated the association between damaged skin and chromium penetration. One study showed that workers exposed to cement without the addition of ferrous sulfate had significantly higher concentrations of urinary chromium than workers exposed to cement with the addition of ferrous sulfate (99). Another study showed that the average urinary chromium concentration was approximately six times higher in non-smoking workers with hand eczema than in non-smoking workers with no skin disease (45.5 versus 7.6 µg/l) (100). Studies have also shown that nickel-allergic and cobalt-allergic subjects with irritated skin react to lower concentrations of nickel and cobalt than controls with intact skin (101–103).

Implant surgery and metal ion release into biological matrices

Metal implants in contact with biological fluids may corrode and release metals that can activate the immune system. In the 1960s and 1970s, the early prostheses used for total hip arthroplasty were metal-on-metal. Their use was gradually abandoned (and replaced by the use of metal-on-polyethylene prostheses), as they resulted in the release of cobalt and chromium into the blood, hair, and urine, as well as metal sensitization and prosthesis loosening (104–106). Recent studies have shown that patients with metal-on-metal prostheses may still have elevated serum and urine concentrations of cobalt and chromium (107–109). Large particulate wear debris particles (more than 150 nm to 10 µm) are released to the surrounding tissue, and may become systemic (110). Hallab et al. found a linear correlation between serum metal ion levels and lymphocyte reactivity, suggesting that *in vivo* metal release is directly associated with metal contact allergy (111). Taken together, the experience from implant surgery has taught us that elevated metal concentrations in serum and urine may initially result in local delayed-type hypersensitivity reactions around

the prostheses and also in more distant reactions, for example the skin, where patients may react with positive patch test reactions to the metals in question. Orthodontic metal exposure may also result in increased metal levels in serum and urine, but this topic will not be discussed further in this review.

Nickel in biological matrices

Nails. Attempts have been made to correlate the internal nickel load with nickel allergy. Menné et al. showed that nickel concentrations were significantly higher in fingernails than in toenails in 71 nickel-allergic patients and 20 controls (51). Nickel-allergic women had significantly higher levels of nickel in toenails, hair and plasma than controls, whereas no significant difference was found in fingernails between the two groups (51). Another study showed that the nickel concentration was significantly elevated in fingernails from subjects who were heavily (employees in the electronic nickel-plating industry) or moderately (assemblers of electrical switches, relays, and locks) exposed to occupational nickel as compared with controls (112). The authors suggested that nail nickel analysis could be performed in subjects who were suspected of having occupational nickel exposure, and defined occupational nickel exposure by a cut-off value of 8 µg nickel/g (112).

Urine, serum, and hair. Menné and Thorboe showed that the urine nickel concentration was elevated in 4 women with vesicular hand eczema prior to eruption of vesicles when compared to periods without vesicles (52). Later, Menné et al. studied nickel concentrations in urine from 13 patients with psoriasis or leg ulcers (53). Each subject ingested 5.6 mg of nickel, resulting in elevated urine nickel concentrations in the following days. Spruit et al. measured the nickel content of plasma, urine and hair in individuals with and without nickel allergy (55). Two distinct groups were enrolled in their study: non-occupationally exposed patients, and 89 exposed workers from a factory. No difference in the urine nickel concentration was found between allergic and non-allergic individuals (55), a finding that has been replicated by others (56, 58, 67). However, the authors showed that the nickel content in hair was significantly higher in women than in men, and that it was 10 times higher in occupationally exposed individuals than in patients, perhaps as an indication of higher nickel exposure. Katz et al. collected hair samples from 7 nickel-allergic and 38 non-allergic individuals, and showed that the hair nickel content was dependent on sex rather than on nickel allergy status (59). De Jongh et al.

followed a very nickel-sensitive female patient for two prolonged periods (43 and 43 days, respectively) (60). The authors found a limited degree of correlation between the nickel concentrations in plasma and urine and the clinical activity of her dermatitis. Hindsén et al. investigated urine nickel concentrations in nickel-allergic patients with and without atopy after oral nickel administration (61). Apparently, urine nickel concentrations were significantly higher in atopic than in non-atopic individuals, and also decreased with age. In a larger study, Boscolo et al. could not confirm this finding, as they found comparable levels of nickel in the urine and serum in atopic and non-atopic individuals (57). Santucci et al. suggested that prolonged oral exposure to nickel sulfate in water might reduce the intestinal adsorption of nickel and activate renal nickel excretion (63). In a small 3-day study including 8 healthy controls, Christensen and Lageson showed that serum and urine nickel concentrations varied greatly, but suggested that urine seemed to be most reliable as a biological matrix (65). Contradicting this finding, Christensen et al. showed that the nickel concentration in serum increased in healthy volunteers without nickel hypersensitivity following oral intake of 5.6 mg of nickel sulfate, but that the nickel concentration did not increase in the urine and sweat of all subjects (64). A Danish study found similar levels of nickel in the urine of 35 nickel-allergic individuals with hand eczema and 30 healthy controls (66). However, the authors showed that the level of nickel in serum was significantly lower in nickel-allergic individuals than in controls. This was explained by a significantly lower intake of nickel-rich food items by nickel-allergic individuals. Jensen et al. investigated the cutaneous response in nickel-allergic and non-allergic individuals who were orally exposed to nickel sulfate hexahydrate (68). Dose dependency was found for the nickel content of both serum and urine after oral nickel exposure, but no differences were identified between controls and nickel-allergic individuals. Gawkrödger et al. found that urinary nickel levels decreased together with clinical symptoms in a nickel-allergic woman who commenced a low-nickel diet (69). Menné et al. found an increase in urinary nickel in nickel-allergic patients who were orally exposed to tetraethylthiuram disulfide as an indication of decreasing nickel deposits in the body (54). One study showed that the nickel concentrations in intercellular fluid from blisters in non-atopic women with nickel allergy and vesicular hand eczema were similar to those of matched controls without nickel allergy (70). Finally, only one study has, to our knowledge, directly investigated to what degree the load of metal contact allergens within the blood and urine may affect the

immunological response to nickel. Boscolo et al. suggested that the urine nickel concentration was correlated with 'memory' CD4⁺/CD45RO⁺ lymphocytes in non-allergic women, and with CD4⁺/CD45RO⁺ and CD3⁺/CD25⁺ T cells in nickel-allergic women (57). Furthermore, nickel-allergic subjects showed a statistically significant correlation between serum nickel concentration and CD5⁺/CD19⁺ B lymphocytes and serum interleukin-13 levels. Nielsen et al. debated in what way nickel may influence immune reactivity, as nickel binds in higher amounts to the cell membranes of T lymphocytes from nickel-allergic individuals than to the membranes of those from non-allergics (67). The findings from human studies were supported in guinea pigs, as sensitized animals had higher amounts of nickel in the dermis and lower urinary nickel levels than to controls (67). These findings indicate that nickel concentrations may affect the immune response.

Large-scale epidemiological studies. Numerous large epidemiological studies have investigated the content of trace metals in biological matrices, but very few have conducted simultaneous patch testing. It has been established that elevated nickel concentrations may occur following inhalation (smoking or from occupational exposure) (113), skin deposition (typically in workers) (45), and oral intake of food and water (individuals and general populations) (71). In an interesting study on the possible association between nickel allergy and air pollution, Smith-Sivertsen et al. found a similar prevalence of nickel allergy in 1236 adults from the general population in Sør-Varanger near the Russian border, an area with industrial nickel pollution, and in 531 adults from Tromsø, a reference city with little nickel pollution (71). No statistical association was found between nickel allergy and nickel urine concentrations among adults from Sør-Varanger. However, in further studies, the authors showed that the prevalence of nickel allergy was 7.2% in 657 Russian women from Nikel, as compared with 27.5% in Norwegian women from Sør-Varanger, despite a similar prevalence of ear piercing (72). As nickel water concentrations were higher in Nikel than in Sør-Varanger, and urine nickel concentrations were much higher in Russian women, their findings suggest that Russian women may have become tolerant to nickel following oral exposure to nickel from contaminated water and foods (72). A recent German study made an attempt to correlate metal patch test reactivity with the metal content of ambient air samples and urine samples in 6-year-old children (73). They found a positive correlation between nickel allergy, urine nickel concentration and nickel levels in ambient air from Dortmund, Hörde, an area with heavy industrial activity.

However, the authors also identified much higher prevalences of contact allergy to chemicals from panels 1 and 2 of the TRUETM test in this area, although this was not discussed. In further papers on nickel patch test data, the same research group suggested that elevated nickel levels in ambient air samples and urine samples, respectively, were associated with nickel allergy, because of the high prevalence (30.7%) of nickel patch test reactivity in children from Dortmund as compared with the nickel allergy prevalence in Borken (12.6%) (74). However, the nickel content of drinking water was comparable in the two areas. The study had several weaknesses, and it is our opinion that the results need to be repeated. Finally, one recent German study investigated nickel levels in urine among 163 female dermatological patients aged 18–46 years (75). The authors showed that nickel urine levels were not associated with nickel dermatitis.

General Remarks

Very few studies have combined HBM and patch testing. As stated, such studies have been limited to nickel allergy. This is a shame, as patch testing is easy to perform in general populations and workers, and other metals and chemicals are relatively easy to collect and analyse. On the basis of the accumulation of data from the presented studies and former reviews on contact allergy, one may generate some overall conclusions, as shown in Fig. 1, whereas an in-depth immunological understanding is still to be fully achieved. Although the mechanisms are expected to work for all haptens, this may be an incorrect assumption. Chemical exposure in humans has been observed to occur through several routes; the most important are the skin, the oral cavity, and the gastrointestinal and respiratory systems. Skin exposure to chemicals and metals is by far the most important cause of contact allergy and allergic contact dermatitis. In cases of very high cutaneous contact allergen levels, contact allergens may be found within the body. Whether subjects with filaggrin deficiency have higher internal nickel levels than wild-type individuals has not yet been investigated, but is possible (114). It has been convincingly shown in epidemiological and immunological studies that oral hapten exposure prior to hapten skin exposure may result in immunological tolerance and affect the prevalence of contact allergy (41). Also, hyposensitization therapy may increase the threshold level and, to some degree, result in immunological tolerance. It is clear that high experimental nickel doses given orally result in nickel dermatitis, as well as in elevated nickel concentrations in the blood and urine. Similarly, depletion of nickel results in decreasing levels of nickel in these fluids. In our opinion, there are not yet any studies that have convincingly

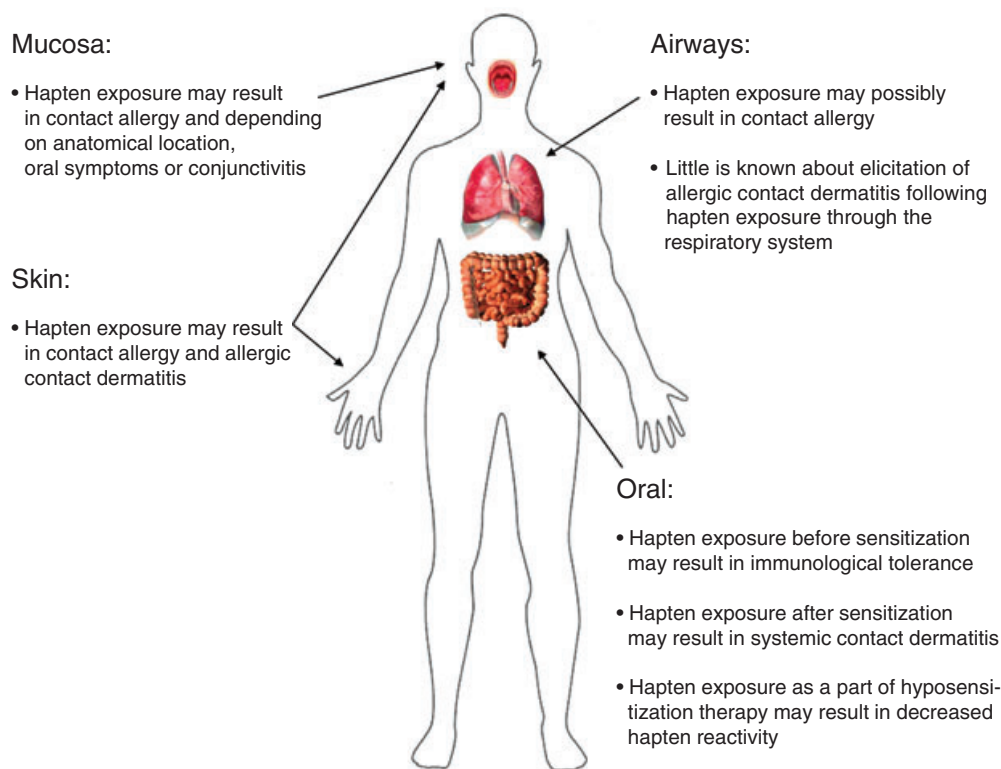


Fig. 1. An overview of the effects of haptens in relation to route of exposure. No published study has yet taken all of these variables sufficiently into account.

determined whether airway exposure to chemicals and metals may result in decreased or increased contact sensitivity, but the studies conducted so far suggest that it results in contact allergy. Animal and human studies have been conducted to support a link between the skin and the airways, as primary sensitization or hapten exposure may occur on the skin and subsequently result in respiratory tract hyperreactivity, including asthma, following airway hapten exposure (115, 116). All studies conducted so far have focused on one or two routes of exposure (typically skin and oral exposure, but also skin and

airway exposure), whereas no studies have investigated all routes at the same time. This clearly needs to be done to determine the overall effects of hapten exposure. Also, there is a need for prospective studies, as all epidemiological studies conducted so far have been cross-sectional. HBM in combination with patch testing is an important area that should be a priority in the future. It is possible that, by using HBM, we can find new ways to better protect citizens against contact allergy and dermatitis, as systemic exposure may alter the immune response to cutaneous allergens.

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