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**AIRBORNE NICKEL EXPOSURE:
CAN IT INDUCE NICKEL SENSITIZATION?
A POPULATION BASED STUDY WITH 6-YEAR-OLD CHILDREN**

Dissertation

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Abstract

The present study aimed to evaluate an association between exposure to nickel in ambient air and the health outcome measured in prevalence of nickel sensitization (prevalently T_H1-cell-mediated delayed immune response (Type IV)) in children living close to industrial sources in Germany. Exposure and health outcome were assessed in children at school-beginner age residing in the vicinity of industrial sources of three different hot spots in the State of North Rhine Westphalia (Dortmund Hörde, Duisburg North and Duisburg South), in a rural town without nearby point sources of nickel (Borken), which served as a reference area for the hot spots, and in the comparison areas in other parts of Germany: a city in the State of Bavaria (Augsburg) and a city in the former socialist German Democratic Republic (Halle). The cross-sectional study was conducted between March and June 2000. Exposure assessment comprised modelling of the ambient air quality data and measuring of nickel uptake in tap water (external exposures) and human biomonitoring (HBM), i.e. the measurement of nickel in urine (internal exposure). Health outcome was determined by a questionnaire, a Pharmacia CAP-FEIA system and an epicutaneous patch test (ECT). The influence of exposure variables on nickel sensitization was measured by means of linear and logistic regression analysis. The most striking results were as follows. Children living in the hot spot Dortmund Hörde (increased chromium and nickel levels in ambient air from a steel mill) revealed a peerless prevalence of diverse allergic symptoms and sensitizations, whereas children residing in Duisburg North (a coking plant, sintering plant, steel mill and blast furnace) had elevated current nickel concentrations in urine. Sensitization to nickel in the present study was positively associated with the current internal nickel exposure, and a weak positive significant association between nickel levels in ambient air and in urine was observed. It is concluded that, despite improvements in the general air quality during the last decades, living in the proximity of industrial sources results to some extent in increased internal nickel exposure and in the nickel sensitization.

Keywords:

Sensitization; airborne exposure; ambient air; nickel hypersensitivity; environmental; patch test; children.

1. Introduction

Nickel (Ni), a metal widely distributed in the environment, a constituent of many metal alloys, is one of the most common occupational as well as public contact allergens inducing a prevalently T_H1-cell-mediated delayed immune response (Type IV) (Hengge and Ruzicka, 2006; Janeway, C., 2008; Büdinger and Hertl, 2000), which may lead to allergic contact dermatitis (ACD) (Braun-Falco et al., 2005; Fritsch, P., 2004). In Germany, up to 20 % of women and 6 % of men are sensitized to nickel (Schäfer et al., 2000). According to the studies performed by 22 centres of the German Contact Dermatitis Research Group and filed by the Information Network of Departments of Dermatology (IVDK), the prevalence of nickel sensitizations in 6-15-years-old-children corresponds to 15.9% of all children tested, but in 25.0% of 14-15-years-old-girls, and in only 4.5% of 6-13-years-old-boys (Bratsch and Geier, 1997). Another study (Dotterud and Falk, 1995) carried out in Norway reported a nickel sensitization prevalence of 15 % in 7–12-year-old-children, whereas girls were sensitized almost twice as often as boys.

1.1 Motivation / Objective and Aim of Study

Although nickel is one of the most common contact allergens in humans and the elicitation phase of nickel allergy has been studied rather extensively, the pathways of the sensitization phase, i.e. the occurrence of nickel allergy and especially its sources, have not been sufficiently investigated yet (Artik et al., 2004). One of the reasons for the fragmentary evidence in this field is that an iatrogenic sensitization in human is not permitted. Thus animal models or population based surveillance studies are expected to point toward possible associations. Most research is focused on nickel contact with skin or mucosa as a source of exposure, thus only few studies that deal with inhalative nickel exposure - an aspect that is especially relevant in the environmental medicine - have been carried out until now (Huygens and Goossens, 2001; Karaneva et al., 1999; Nakamura et al., 1999; Schubert, 2000). Some authors argue that a long term oral nickel exposure (in small quantities), e.g. dental inlays, by previously non-sensitized people might prevent hypersensitivity to nickel, inducing thus an immunological tolerance (Artik et al., 2001; Artik et al., 2004; Draeger et al.,

2004; Hensten-Petersen, 1992; Jensen et al., 2002; Kerosuo et al.,1995; Menne, 1996; Mortz et al., 2002; Nowak et al.,2006; Todd and Burrows, 1989 van Hoogstraten et al.,1991; van Hoogstraten et al.,1992; van Hoogstraten et al.,1993; van Hoogstraten et al.,1994).

To date, few studies have focused on airborne nickel exposure to humans and its feasible health effects associated with industrial pollution. Particularly, there has been very little evidence in the literature whether nickel in atmosphere is likely to induce allergic sensitization in humans (Smith-Sivertsen et al., 2002, Smith-Sivertsen et al., 1998); this is what motivates the present study.

The trace amounts of nickel that are present in ambient air are usually not likely to induce significant effects on human health. However, the areas located close to industrial sources, have considerably higher nickel levels in the atmosphere and the influence of nickel there can be of importance.

North Rhine Westphalia (NRW) with the Ruhr District is an important agglomeration area for coal mining, steel production and other heavy industries in Europe. Within the framework of the clean air action plans of the state government of NRW, several large environmental health surveys were started in the 1970s. From 1985 to 1997 nine consecutive cross-sectional studies with children (school beginners) and middle-aged women (55 year old) were undertaken. A summary of the human biomonitoring (HBM) results of these studies has been published recently (Wilhelm et al., 2007a).

As in many parts of the world, ambient air quality has improved during the last decades. However, despite successful programs to improve air quality, ambient air levels of contaminants in the vicinity of certain industrial sources, which are frequently located close to residential areas, can still be increased, and health concerns are raised due to the possible exposure and exposure-related health effects especially of children living adjacent to such sources.

In 2000 the North Rhine Westphalia State Environment Agency identified three “hot spots”. These areas were characterised by increased total suspended particulate (TSP) levels and site specific air pollutants such as benzo[a]pyrene, benzene, cadmium (Cd), lead (Pb), chromium (Cr) and nickel (Ni). The data from the areas outside NRW, namely cities Augsburg in South Germany and Halle in former socialist German Democratic Republic were collected following the study protocol designed by Hot Spot Study NRW regarding study design, questionnaire and allergic testing.

The aim of the whole Hot Spot Study NRW was to evaluate the health outcome of children of school-beginner age living in the vicinity of industrial sources in relation to exposure to different substances relevant to human health that are emitted by these sources. The overview of the Hot Spot Study NRW has been published previously (Wilhelm et al., 2007b). The present manuscript represents the part of the study that is related to airborne nickel exposure as a possible source of nickel sensitization also including the respective results from the studies conducted in Augsburg and Halle. Additionally, prevalences of atopic symptoms have been descriptively evaluated.

1.2 Sources of nickel exposure

Metals and Alloys

The most common cause of nickel hypersensitivity is contact with jewellery items, watches and buttons that contain nickel, as well as the result of sensitization through piercing of ears or other parts of the body (Kerosuo et al., 1996; Morz et al., 2002; Nakada et al., 1990; Todd and Burrows, 1989; van Hoogstraten et al., 1992). Furthermore, nickel allergy could possibly be associated with the frequent inflammatory reactions on stainless steel prostheses, e.g. joint replacements, intraosseous pins (Chang et al., 1995; van Hoogstraten et al., 1992), dental braces (Sunderman, 2004), pacemaker wires or stents in coronary artery disease (CAD). Elevated nickel concentrations in serum were found in haemodialysis and blood transfusion patients in whom devices made of nickel-chrome steel were used (Hopfer et al., 1985).

Foods

The main contribution to nickel uptake in food comes from legumes, where nickel concentration varies between 0.05-0.34 mg/kg (Grandjean, 1984), since some plants accumulate nickel from soils with different intensity. Especially high levels of nickel were assessed in oatmeal (0.3-4.8 mg/kg), cocoa (8.2-12 mg/kg), nuts (0.7-2.3 mg/kg) and soybeans (1.1-7.8 mg/kg) (Nielsen, 1992). It results in a usual daily intake of 90-97 µg nickel in case of mixed food, or 185-196 µg in vegetarians; this quantity can vary up to 900 µg in diets rich in above mentioned food constituents. One to five percents of nickel in food are absorbed and the rest is eliminated by the digestive system (Watzl et al., 1999; Anke, 2004a; Nielsen et al., 1999; Sunderman, 1989; Leitzmann, C., 2002). Similar figures were assessed for Germany, some

Scandinavian countries, U.K. and U.S.A. Release of nickel from stainless steel cooking utensils can cause slight but clinically insignificant increases in the nickel content of foods and beverages (Accominotti, 1998; Flint and Packirisamy, 1997).

Drinking water

Natural sources of nickel in ground water and surface water derive from biological cycles, solubilisation of nickel compounds from soils and rocks, from atmospheric fallout, and especially from industrial processes and waste disposal (Sunderman, 2004). Tap water in Germany contains in average 8 µg Ni/L, hence, a mean of 20 µg Ni/d is consumed and 2% of this quantity is absorbed. (Flint and Packirisamy, 1997; Haber et al., 2000). Drinking water samples occasionally contain much higher nickel concentrations, owing to nickel pollution of the water supply or leaching from nickel-containing pipes or nickel-plated spigots (Sunderman, 1989).

Domestic dust

In domestic dust, a median nickel concentration of 23 µg was assessed in Germany in 1989 (UBA, 2004).

Ambient Air

Nickel concentration in atmosphere in Germany is generally quite low: annual mean levels assessed in 2002 were 2.5-4.6 ng/m³ in urban areas compared with 1.2-1.7 ng/m³ in rural areas (UBA, 2004). The reference concentration of 20 ng/m³ proposed by European Commission in 2003 (EC, 2003) has rarely been exceeded (Beyersmann, 2005). The main sources of nickel in suspended dust are emissions from industrial sources such as coking plants, steel mills and blast furnaces (Beyersmann, 2005). Furthermore, substantial atmospheric emissions of nickel derived from petroleum are released into environment in automotive exhaust fumes. Analyses of nickel in ambient air do not usually specify by nickel forms: it is assumed that nickel is present in atmosphere to greater extent in oxidized form (Sunderman, 2004).

Inhaled nickel mass averages 0.4 µg per day (range: 0.2 to 1.0 g per day) for urban dwellers and 0.2 µg per day (range: 0.1 to 0.4 µg per day) for non-urban dwellers (Bennet, 1984, Sunderman, 1986). Approximately 35 % of inhaled nickel is absorbed from the respiratory tract; the remainder is carried up the tracheobronchial

mucociliary escalator and either swallowed or expectorated (Bennet, 1984; Grandjean, 1984; Sunderman, 1989). As a metal dust component, nickel together with cadmium and zinc is present in high concentrations in tobacco smoke (0.1-1.0 µg in each cigarette and 0.2 to 2.3 µg in secondary and mainstream smoke, respectively) (Exner and Engelhardt, 2002; Ministry of Health, NRW, 1990).

1.3 Biological Effects

Hypersensitivity

The allergic sensitization that is caused by exposure to nickel alloys and nickel compounds can result in allergy that is allergic contact dermatitis (Merk, 2002; Beltrani and Beltrani, 1997). The contact hypersensitivity to nickel is classified according to Coombs and Gell as a delayed type immune response (Type IV). Nearly all nickel compounds release - in different quantities - nickel ions in biological systems. Due to their small size, nickel ions are incomplete antigens (haptens), which need to bind to endogenous peptides (Beyersmann, 2005) that is most probably human serum albumin (HSA), to become full antigens being thus capable to be recognized by the antigen presenting cells (APC) e.g. cutaneous Langerhans cells (LC) of innate immunity. After recognition, APC internalizes Ni-protein-Complex and processes it on into MHC-associated epitope. APC migrates then to the regional lymph node draining the site of exposure where it activates a nickel-specific naive T cell (priming) with the consequent cloning of memory T cells, which end up in the dermis and wait there for potential reexposure. This phase of immune response called sensitization phase has usually an asymptomatic course. In the elicitation phase, a subsequent expose to nickel leads to antigen presentation to memory T cells in the dermis, with the release of T-cell cytokines, such as IFN-gamma and IL-17, it stimulates the keratinocytes of epidermis to release IL-1, IL-6, TNF-alpha, GM-CSF, the chemokines CXCL8, and the interferon inducible chemokines CXCL11 (IP-9), CXCL10 (IP-10), and CXCL9 (Mig; monokine induced by IFN-gamma). The cytokines and chemokines enhance the inflammatory response by inducing the migration of monocytes into the lesion and their maturation into macrophages, and by attracting more T cells (Janeway, 2008; Kimber and Dearman, 1996; Kimber and Dearman, 1998; Kimber and Dearman, 2002).

Nickel hypersensitivity can cause pulmonary asthma, eosinophilic pneumonitis, conjunctivitis, inflammatory reactions around nickel-containing implants and

anaphylactoid reactions after parenteral injection of nickel contaminated medications (Sunderman_1984; Sunderman, 2001).

Irritancy, toxicity and carcinogenic effects

Aside from hypersensitivity, the other biological effects of nickel in humans include local irritation followed by inflammation (IPC, 1991) and by persisting inflammation also tissue degeneration as well as carcinogenicity. Nickel is known to be toxic to several organs. In particular, high concentrations of nickel can result in liver damage (Marschall and Oppermann, 2002). About its nephrotoxicity, little has been reported (Baldamus and Friederes, 2002).

Some authors categorize nickel as a first category human carcinogen (Muhle, 2002); in this capacity it is known in the occupational medicine (Breuer and Hanrath, 2002).

Nickel, metallic and alloys, has been classified by the International Agency for Research on Cancer (IARC) in Group 2B i.e. as an agent possibly carcinogenic to humans. For further details see IARC Monographs, 2008.

Nickel possible effects on the upper respiratory tract are mucosairritative, allergen as well as carcinogen (Engelke and Westhofen, 2002; Plinkert and Plinkert, 1993). As to the lung capacity nickel might decrease the forced expiration and the vital capacity (Kilburn et al., 1990; Sobaszec et al., 2000; Osim et al., 1999).

The Californian Air Resources Board (ARB) has identified nickel as a toxic air contaminant (TAC) based on the evidence that nickel presents a public health risk because it is a carcinogen, and because it is capable of inducing irritation and allergic sensitization by large acute exposure as well as asthma and other respiratory ailments by long-term exposure (Californian Air Resources Board, 2004), whereas the studies according to Karas and Bladek, 2004 have deemed monitoring of nickel in air necessary.

Nickel deficiency

Ni is also an important essential component of certain plant and bacterial enzymes, e.g. Urease (Mobley et al., 1995; Dixon_1975; Polacco_1977; Thauer, R.K., 2001). Goats, pigs and rats put on nickel deficiency diet have developed symptoms of growth depression as well as decreased iron absorption followed by hematologic disorders (Anke, 2004b; Anke et al, 1984b). It is not yet clear whether nickel is essential for human organism (Trumbo et al., 2001) and there are no reports

regarding nickel deficiency in humans so far (Sunderman,_2004). However, some foods are being enriched with nickel (Anke et al., 1984a) and many vitamin pills contain up to 6,5 µg per tablet nowadays (Artik et al.,_2004).

Clinical use

In the late nineteenth century, nickel sulphate and nickel bromide were used for therapy of epilepsy and rheumatism in daily dosage of up to 500 mg with a good tolerance (Mastromateo, 1988).

2. Materials and Methods

2.1 Characteristics of study areas and groups

The cross-sectional study consisting of three hot spots and one reference area in NRW was conducted during spring time / early summer 2000 by following a common protocol. All school beginners (2421) in the year 2000 living in the proximity of a coking plant, a steel mill, a sintering plant and a blast furnace in Duisburg North ($n=620$), in the surroundings of metal refiners in Duisburg South ($n=478$), in the vicinity of a steel mill in Dortmund Hörde ($n=390$), in the rural town of Borken ($n=315$) were asked to participate. The study groups Augsburg ($n=369$), and Halle ($n=249$) – both cities without any considerable industrial load – have joined the Hot Spot Study NRW following a common protocol. Finally, 1897 of these children attended the routine examination at the Sanitary Board and 1386 children (response rate 73.1%) took part.

Duisburg (500,000 inhabitants) is an industrialized city situated along the Rhine river in the western part of the Ruhr district. Dortmund (520,000 inhabitants) is an industrialized city in the eastern part of the Ruhr-District. Borken (36,000 inhabitants) is a small county capital in the rural countryside, north of the Ruhr district in North Rhine Westphalia. It has no specific sources of industrial pollution. Borken served as reference area for the hot spots NRW. Augsburg (265,000 inhabitants) is a city in Bavaria, a federal state in South Germany. It is a technological and a high-tech centre of the region. The data from the study group in Augsburg was taken from a cross-sectional study among 5- to 6-year-old preschool children conducted in 1996; the study that had been embedded in the multicentric international study for risk assessment of indoor and outdoor air pollution on allergy and eczema morbidity (MIRIAM). Halle (235,000 inhabitants) is a city in the federal state of Saxony-Anhalt in the eastern part of Germany. After the reunification of Germany its important industrial sources were mostly shut down, hence nowadays only transport as a possible emitting source deserves mention. In times of socialist German Democratic Republic it had important chemical industry, which was mainly shut down short after the reunification of Germany.

Participants living less than 2 years in the study area ($n=208$) were excluded from those parts of the statistical analysis where a long-term exposure was required. Mean

age (years) of the children ($n=1178$) was 6.3 ± 0.4 (range 4.8–9.1) whereas 49.4% of the children were male. The study was approved by the ethical committee of the Ruhr-University Bochum (Register No. 1419 from January 20, 2000). All investigated participants (mothers) gave their informed consent prior to their inclusion in the study. Table 1 gives an overview on the hot spots and the parameters assessed in the cross-sectional study.

Table 1

Sources of exposure and measurements performed in the study.

(TSP=total suspended particulate; HBM=human biomonitoring; PAH=polycyclic aromatic hydrocarbons)

	Borken (Reference)	Dortmund Hörde	Duisburg North	Duisburg South	Augsburg	Halle
Emitting source(s)	--	Steel mill	Coking plant, sintering plant, steel mill, blast furnace	Metal refineries	--	--
Environmental exposure	--	TSP, Cr, Ni	TSP, PAH, benzene	TSP, Cd, Pb	--	--
Ambient air monitoring	Ni in TSP	Ni in TSP	Ni in TSP	--	--	--
HBM	Ni in urine	Ni in urine	Ni in urine [†]	--	--	--
Further nickel monitoring	Ni uptake in drinking water	Ni uptake in drinking water	--	--	--	--
Health outcome	Diagnoses and symptoms of airway diseases and allergic manifestations; skin prick test, CAP-FEIA and epicutaneous patch test					

†: measured in Bochum in 2008 (for further details see below)

2.2 External and internal monitoring of nickel exposure

The external nickel monitoring comprised monitoring of ambient air and tap water, whereas the internal monitoring included sampling of nickel in urine.

Nickel exposure via ambient air was calculated only in subgroups of the study collective in Borken, Dortmund Hörde and Duisburg North by using routinely monitored ambient air quality data of the State Environment Agency of North Rhine Westphalia (www.lanuv.nrw.de) and by means of a Lagrange dispersion model. Thus, a “personal” annual average concentration was obtained and attributed to each dwelling site of the participants within the city. The reference area Borken had only one air quality monitoring station (background level).

For the assessment of nickel concentration in urine, morning urine samples were collected in children in study groups in Borken, Dortmund Hörde and Duisburg North and the nickel level in the urine was measured. The analysis of association between external and internal nickel exposure and also nickel sensitization was performed only for these collectives excluding the study areas Duisburg South, Halle and Augsburg. An analytical procedure was performed by using electrothermal atomic absorption spectrometry (ET-AAS) with the detection limit of 0,3 µg/L, however, no measurements below the detection limit have occurred. Urine samples with creatinine levels >3.0 and <0.2 g/l were excluded from statistical analysis. The nickel content in the drinking water was analysed as a potential confounder. Here, too, the electrothermal atomic absorption spectrometry was used for the analysis (details in DEV, 1992 and also LUA,_2004a). The mothers reported on the average tap water consumption of the children. The measurements of nickel content in drinking water were performed only in study areas Borken and Dortmund Hörde.

The measurement of nickel concentration in urine in the study collectives Dortmund Hörde and Borken was performed routinely during the Hot Spot study in 2000. For the study collective Duisburg North, the measurement was not performed originally, but was assessed later in 2008 using samples, which were frozen in 2000. After the analytical procedure the samples were found reliable and could be considered for further analyses (see appendix for details).

2.3 Health outcome

To assess environmental, hereditary, ethnical and life style factors a standardized questionnaire which included the parental International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaire on asthma, rhinitis and eczema for 5–8-year-old children asked the parents for diseases and symptoms and, furthermore, for socio-economic and environmental factors as potential confounders. Details are given by Sugiri et al., 2006. Clinical observations included dermatological examinations, patch testing, skin prick testing with grass, birch and mugwort pollen, house dust mite, cat, alternaria and food allergens (ALK Scherax, Hamburg, Germany), and determination of specific serum IgE concentrations against grass, birch and mugwort pollen, cat and house dust mite allergens by Pharmacia CAP-FEIA system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The epicutaneous patch test (ECT) (TRUE Test with 24 standard allergens, among them

Ni (II) sulphate, by Pharmacia, Uppsala, Sweden) was applied for 48 hours on the upper back; the readings were performed 72 hours later by dermatologically trained physicians who examined the child's skin and evaluated the diagnosis of allergic eczema (more details are given in Behrendt et al., 2001; Ring, 2005; Schäfer et al., 2000). For statistic analysis only definitely positive (+, ++, +++) and negative (-) test results were considered, whereas questionable results were excluded.

2.4 Statistical analysis

Statistical analysis was performed by using SAS version 8.2 (SAS/STAT Software, Cary, NC: SAS Institute, Inc.). Nickel levels in urine and tap water less than the limit of quantification (LOQ) were set to 2/3 LOQ. For a comparison of groups Mann-Whitney test, Kruskal-Wallis test and χ^2 -test were used. The association between nickel concentrations in urine and nickel concentrations in ambient air was assessed by means of Spearman's correlation coefficient and linear regression. The influence of exposure variables on health outcomes was estimated by means of logistic regression analysis, including potential confounders (such as nationality, gender, eczema in mother, age, piercing, nickel level in drinking water, educational level and residence area) as covariates to control for confounding. Adjusted odds ratios together with their 95% confidence interval were used as measures of association. More details of these methods are available in German from a report released by the North Rhine Westphalia State Environment Agency (LUA, 2004a, b and c).

3. Results

Publikationshinweis

Does airborne nickel exposure induce nickel sensitization? Contact Dermatitis. 2010 Jun;62(6):355-62

Does airborne nickel exposure induce nickel sensitization?

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Background and objective: Nickel is one of the most prevalent causes of contact allergy in the general population. This study focuses on human exposure to airborne nickel and its potential to induce allergic sensitization.

Materials and methods: The study group consisted of 309 children at school-starter age living in the West of Germany in the vicinity of two industrial sources and in a rural town without nearby point sources of nickel. An exposure assessment of nickel in ambient air was available for children in the Ruhr district using routinely monitored ambient air quality data and dispersion modelling. Internal nickel exposure was assessed by nickel concentrations in morning urine samples of the children.

Results: The observed nickel sensitization prevalence rates varied between 12.6% and 30.7%. Statistically significant associations were showed between exposure to nickel in ambient air and urinary nickel concentration as well as between urinary nickel concentration and nickel sensitization. Furthermore, an elevated prevalence of nickel sensitization was associated with exposure to increased nickel concentrations in ambient air.

Conclusion: The observed associations support the assumption that inhaled nickel in ambient air might be a risk factor for nickel sensitization; further studies in larger collectives are necessary.

Key words: ambient air; exposure; nickel; nickel in urine; nickel sensitization. © John Wiley & Sons A/S, 2010.

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Nickel, a metal widely distributed in the environment and as a constituent of many alloys, is one of the most prevalent causes of contact allergy in the general population. Median nickel contact allergy prevalence from studies mainly performed in North America and Western Europe between 1966 and 2007 amounted to 8.6% (range 0.7–27.8%) (1). Nickel allergy is primarily induced by skin exposure to nickel ions. Other routes of nickel exposure in relation to nickel sensitization are considered to be

of lower importance. However, there is increasing evidence that oral intake of nickel via food and drinking water can exacerbate nickel dermatitis in nickel-sensitive individuals (2). Airborne nickel dermatitis (3, 4) and asthma (5) have been reported in a few case studies, mainly from occupationally exposed individuals.

To date, few studies have focused on airborne nickel exposure of humans and its possible health effects associated with industrial pollution.

In particular, there is very little information in the literature as to whether nickel in ambient air might be able to induce allergic sensitization in humans (6).

In recent years, we have performed several studies on the influence of industrial pollution on children's health in North Rhine Westphalia, Germany. This area is important for coal mining, steel production, and other heavy industries. In 2000, two industrial 'hot spots' were identified. These areas were characterized by increased total suspended particulate (TSP) levels in the ambient air and site-specific air pollutants, such as nickel and chromium. In a cross-sectional study, the health outcome of children of school-starter age in relation to this exposure has been evaluated. An overview on the first results has been given elsewhere (7). A striking finding was the high prevalence of nickel sensitization in children living close to a steel mill. Here, we present details on the part of the study that is related to airborne nickel exposure as a possible source of nickel sensitization.

Materials and Methods

Study areas and study groups

The cross-sectional study consisting of two 'hot spots' and one reference area in North Rhine Westphalia was conducted during spring/early summer 2000 by following a common protocol. All school starters (1325) in the year 2000 living in the proximity of a coking plant, a steel mill, a sintering plant, and a blast furnace in Duisburg ($n = 620$); in the vicinity of a steel mill in Dortmund ($n = 390$); and in the rural town of Borcken ($n = 315$) were asked to participate. Of these children, 1079 were attended for routine examination at the Sanitary Board and 696 children (response rate 64.5%) took part in this study. Only 309 children who had a valid result for the nickel sensitization test and had lived >2 years at their actual home address (therefore exposed $>1/3$ of their life to the assessed nickel in ambient air) were included in the analyses, which are as follows.

Ambient air nickel exposure

Using routinely monitored ambient air quality data of the State Environment Agency of North Rhine Westphalia (www.lanuv.nrw.de) and by means of Lagrange dispersion modelling, an individual annual average concentration was obtained and attributed to each dwelling site of the participants within the city. The reference area of Borcken had only one air quality monitoring station and this provided a single background exposure level for the children living in this area.

Nickel concentration in urine

For assessment of internal nickel exposure, morning urine samples were collected. The acidified samples were adjusted to pH 3 and subsequently mixed with 1 ml of an aqueous solution containing 2% ammonium pyrrolidinedithiocarbamate (APDTC; p.a. grade, Merck, Darmstadt, Germany). The formed APDTC complexes were then extracted with 1 ml 4-methyl-2-pentanone (MIBK; p.a. grade, Merck, Darmstadt, Germany) for 1 min. After centrifugation (30 min, 4000 g, 4°C), the organic layer was separated and used for nickel analysis by electro-thermal atomic absorption spectrometry (ET-AAS) using a Perkin-Elmer ET-AAS instrument model 4100 ZL. The limit of quantification (LOQ) of the analytical procedure was 0.3 µg/l. No samples below LOQ were observed. Urine samples with creatinine levels >3.0 and <0.2 g/l were excluded from statistical analysis.

Nickel from drinking water

The nickel content in the drinking water was analysed as a potential confounder. Here, too, the ET-AAS was used for the analysis (for details see Ref. 8). The mothers reported on the average tap water consumption of their children. The measurements of nickel content in drinking water were performed only in the study areas of Borcken and Dortmund. The LOQ of the analytical procedure was 2.5 µg/l.

Nickel sensitization test and potential confounders

To assess environmental, hereditary, ethnic and life style factors as potential confounders, a standardized questionnaire was used. Details are given by Sugiri et al. (9). Clinical observations included dermatological examinations and patch testing. The TRUE test[®] with 24 baseline allergens, among them nickel(II) sulfate (Pharmacia, Uppsala, Sweden) was applied for 2D on the upper back; the readings were performed on D2 and D3 by dermatologically trained physicians who examined the child's skin. Patch tests were performed as recommended by the International Contact Dermatitis Research Group (10). For the final analysis, only definitive positive and negative results were considered, whereas irritant and questionable results were excluded. More details are given by Ring (11).

Statistical analysis

Statistical analysis was performed using SAS version 8.2 (SAS/STAT Software, SAS Institute, Inc., Cary, NC, USA). Nickel levels in tap water less than LOQ were set to two-thirds of LOQ.

For a comparison of groups, Mann–Whitney, Kruskal–Wallis, and χ^2 tests were used. The association between nickel concentrations in urine and nickel concentrations in ambient air was assessed by means of Spearman's correlation coefficient and linear regression. The influence of exposure variables on health outcomes was estimated by means of logistic regression analysis, including potential confounders (such as nationality, sex, age, piercing, nickel uptake by drinking water, parents' educational level, passive smoking, and residential area) as covariates to control for confounding. Adjusted odds ratios (OR) together with their 95% confidence interval were used as measures of association.

Results

Characteristics of the study group

The characteristics of the study group specified by study area and in total are summarized in Table 1. The collective of Dortmund stands out by its strikingly high-prevalence rate of nickel sensitization of 31%. The percentage of children that had ear piercing was relatively high and was strongly related to being female. Further stratification by sex showed a significant association between sex and ear piercing, as 88.5% of pierced children were girls ($P < 0.001$) with 42.6% of children having pierced ears in total. The socio-economic and socio-demographic background of the children in Duisburg differed strongly from the two other areas as only 20% of their parents had more than 10 years of school education in contrast to 34% in Borken and Dortmund and 60% were of non-German nationality (predominantly Turkish nationality) compared with 100% and 81% German nationality in Borken and Dortmund, respectively. Furthermore, the children in Duisburg less frequently had an atopic predisposition (prevalence: 28%) than those in the two other areas (prevalence: 54%) where prevalence of eczema, allergy,

hay fever or asthma in the child's parents ever diagnosed by a physician (lifetime prevalence) was used as an indicator of the child's predisposition. Environmental tobacco smoke exposure was lowest in Borken.

External and internal nickel exposures and their associations

The differences in ambient air quality data specified by area are listed in Table 2. As showed, the children residing in Dortmund had higher values of nickel concentration in ambient air compared with the reference area Borken. The correspondent values in the study area Duisburg were even higher (Mann–Whitney test: $P < 0.001$) than in Dortmund. Because the daily uptake of nickel in tap water has been assessed only in Borken and Dortmund the further analysis of this parameter was restricted to these two areas. As shown in Table 3, the observed differences of distribution parameters between both areas were not significant (Mann–Whitney test: $P = 0.289$). All nickel levels in urine were above the LOQ. The urinary nickel distribution for the three study groups of Borken, Dortmund, and Duisburg is shown in Table 4. Corresponding to the ambient air nickel exposure concentrations, the children living in Duisburg had the highest median nickel concentration in morning urine. The highest urinary nickel concentrations were observed in children living in Dortmund, whereas the median urinary nickel concentration in Dortmund was nearly identical to the median level of the children from the reference area Borken. The comparison of distribution of urinary nickel exposure for the three areas by means of Kruskal–Wallis test showed significant differences ($P < 0.001$), whereas the difference between Dortmund and Borken was not significant (Mann–Whitney test: $P = 0.195$). A weak but significant correlation

Table 1. Characteristics, atopic diseases and symptoms, and allergic test results of the study group of children from Borken, Dortmund and Duisburg, Germany

	Borken ^a	Dortmund ^a	Duisburg ^a	Total ^a	P-value ^b
Age, mean (years)	6.3 (105)	6.2 (101)	6.5 (103)	6.3 (309)	<0.001
Sex (male)	45.7 (105)	42.6 (101)	52.4 (103)	46.9 (309)	0.353
Nationality, German	100.0 (104)	81.2 (101)	40.2 (102)	73.9 (307)	<0.001
Parents' education ^c	33.0 (103)	35.4 (96)	19.8 (96)	29.5 (295)	0.037
Ear piercing	39.1 (105)	41.8 (98)	47.1 (102)	42.6 (305)	0.498
Predisposition ^d	54.3 (105)	53.0 (100)	27.7 (101)	45.1 (306)	<0.001
Passive smoking	19.4 (103)	45.5 (101)	48.0 (100)	37.5 (304)	<0.001
Current allergic eczema ^e	5.8 (104)	8.1 (99)	4.0 (101)	5.9 (304)	0.465
Patch test Ni(II)SO ₄	13.3 (105)	30.7 (101)	12.6 (103)	18.8 (309)	<0.001

^aPercentage and sample size in parentheses.

^bTest of differences between areas: χ^2 -test and Kruskal–Wallis test, respectively.

^cSchool years >10.

^dFather or mother with allergy or eczema or asthma or hay fever.

^ePoint prevalence, day of investigation.

Table 2. Ambient air exposure to nickel in TSP (ng/m³) of children from Borken, Dortmund and Duisburg, Germany

Study area	Borken ^a	Dortmund	Duisburg	Total
<i>n</i>	105	99	99	303
Range	2.5	5.0–16.5	5.5–24.8	2.5–24.8
P25	–	5.0	11.8	2.5
Median	–	5.0	14.9	5.0
P75	–	7.2	18.8	11.8

TSP, total suspended particulate; *n*, sample size; Px, xth percentile.
^aBorken had only one ambient air measuring station (background level).

Table 3. Nickel uptake in drinking water (µg/d) in children from Borken and Dortmund, Germany

Study area	Borken	Dortmund	Total
<i>n</i>	80	92	172
Range	0.00–26.00	0.00–48.10	0.00–48.10
P25	0.52	0.53	0.53
Median	0.90	1.16	1.01
P75	2.08	2.58	2.39

n, sample size; Px, xth percentile.

Table 4. Nickel in the morning urine (µg/l) of children from Borken, Dortmund and Duisburg, Germany

Study area	Borken	Dortmund	Duisburg	Total
<i>n</i>	78	73	59	210
Range	0.30–15.07	0.39–26.02	0.67–16.00	0.30–26.02
P25	1.53	1.66	3.00	1.83
Median	2.61	2.67	4.60	3.07
P75	4.07	5.79	6.75	5.70

n, sample size; Px, xth percentile.

($r = 0.256$, 95% confidence limits: 0.137–0.375, $P < 0.001$) between nickel concentration in ambient air and urine was observed using Pearson correlation of the log-transformed values (Fig. 1). Because Borken had only one air quality monitoring station the same correlation analysis was repeated without this area. Here too, a weak but significant correlation ($r = 0.195$, 95% confidence limits: 0.029–0.361, $P < 0.025$) between urinary and ambient air nickel levels was found.

Association between nickel exposure and nickel sensitization

The comparison of the nickel concentrations in TSP of ambient air between sensitized and non-sensitized children is shown in Fig. 2a and it shows an association of nickel sensitization prevalence with the exposure to nickel in ambient air for the Duisburg area (Mann–Whitney test: $P = 0.094$). A similar association in Dortmund was not observed probably because the nickel concentrations were higher in ambient air registered in

Duisburg than that in Dortmund and so the differences (sensitized/non-sensitized) in Duisburg were more pronounced. Children's urinary nickel concentrations separated by study area and patch-test results are shown in Fig. 2b. Overall, nickel levels of sensitized children were higher in urine than that of non-sensitized children (Mann–Whitney test: $P < 0.001$). Children who had an urinary nickel exposure or an ambient air nickel exposure below the median of the respective exposure showed a higher prevalence of nickel sensitization than those with both levels below median (χ^2 -test: $P = 0.109$).

Logistic regression analysis

Besides nickel in ambient air and internal nickel exposure, the following factors are assumed to affect the prevalence of nickel allergy in children and, therefore, are considered as potential confounders for the association between the external and, respectively, internal exposures of nickel and nickel sensitization: sex, social status, nationality, ear piercing, passive smoking, family predisposition, and nickel uptake in tap water. The social status was assessed by classifying the school grade achieved by the parents. Additionally, the children's area of residence was introduced as an independent determinant in the regression model to cover unknown risk factors specific for the respective area. Borken was used as the reference. Nickel uptake in tap water on the day of examination was only available for Dortmund and Borken. In a subgroup analysis, the daily nickel uptake in tap water showed no influence on the nickel sensitization in the study group with tap measurements available and, therefore, was not considered anymore as a potential confounder. Adjusted OR estimated by logistic regression analysis (Table 5) show the associations of ambient air and internal nickel exposure and other potentially influencing factors with the nickel sensitization prevalence within the study group. Additionally, the raw OR of each influencing factor are also shown in Table 5. The internal nickel exposure assessed by nickel in urine turned out to be an important and significant risk factor for nickel sensitization (Table 5, Model 2). The prevalence of nickel sensitization was about twice as high as in children with nickel urine concentrations above the 75th sample percentile compared with children with concentrations below the 25th percentile. Furthermore, the external nickel exposure assessed by the nickel concentration in TSP of ambient air as well showed a significant influence ($P = 0.062$) on the nickel sensitization prevalence (Table 5, Model 1). As estimated by the regression model, the prevalence of nickel sensitization was about four times as high as in children with an exposure to nickel in ambient air

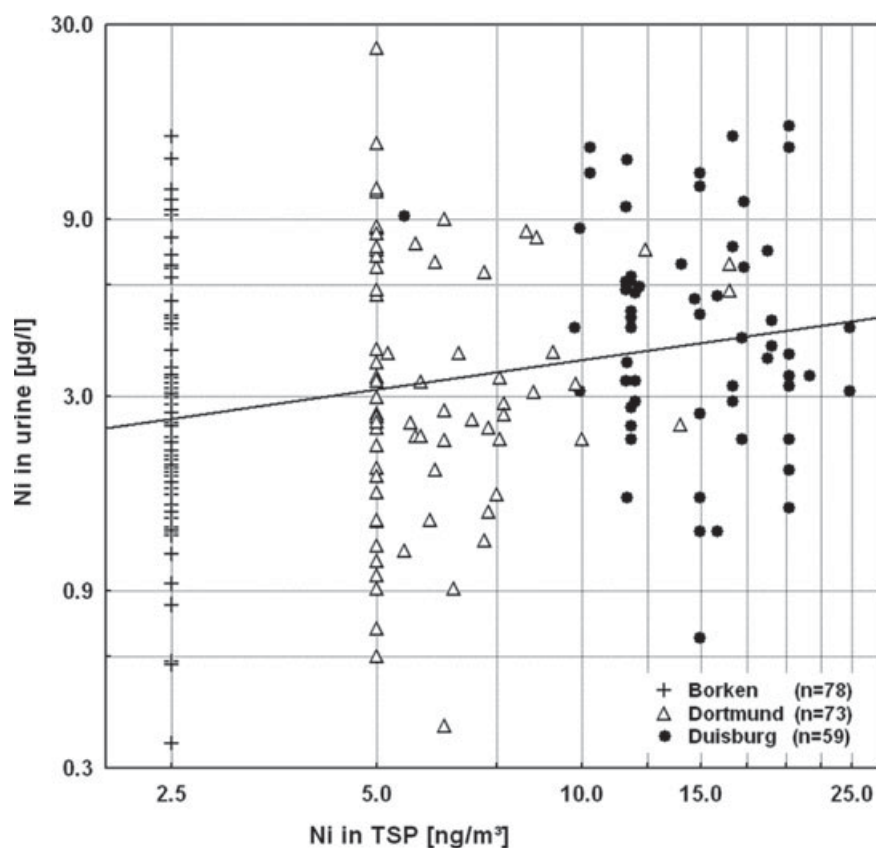


Fig. 1. Association between exposure to nickel in total suspended particulate in ambient air and urinary nickel of children from Borken, Dortmund and Duisburg, Germany, with linear regression in double logarithmic scale. TSP; total suspended particulate.

above a concentration level of 12 ng/m^3 compared with children with concentrations below 2.5 ng/m^3 . Obviously by comparing the raw and adjusted OR, the study area was a strong confounder for the influence of nickel in ambient air on nickel sensitization. Furthermore, the lower prevalence level in Duisburg in comparison with Borken remained unexplained in regression modelling. Higher social status (parents' education level) was a further risk factor of nickel sensitization, but with weak significance. All the other factors considered in the regression analysis were of no significant influence. By comparing the pseudo R^2 of the two regression models, the nickel concentration in urine turned out to have a higher explanatory value than nickel in ambient air.

Discussion

This study shows a statistically significant association between nickel sensitization and internal nickel exposure in the investigated study group of 6-year-old children, as urinary nickel values of sensitized children were significantly higher than that of non-sensitized children. At a weaker significance level, the association between the mean annual exposure to nickel in ambient air and the nickel sensitization

also was obvious in multivariate logistic regression analysis ($P = 0.062$). Furthermore, the assumption that inhaled nickel in ambient air was a risk factor of nickel sensitization is supported by the significant association between urinary nickel concentration and exposure to nickel in ambient air. The otherwise potentially influencing factors such as sex, age, nationality, familial predisposition, environmental tobacco smoke or nickel exposure through skin or through drinking water did not show noticeable associations with the prevalence of nickel sensitization in the study group. The only exception was a higher parental school education which resulted in increased nickel sensitization prevalence. This result of an influence of nickel exposure in ambient air on nickel sensitization in children must be appraised in view of the large variability of nickel sensitization prevalence within the study areas. The sensitization prevalence in the industrialized area of Dortmund was more than twice as high as in the rural area of Borken, but the prevalence in the other industrialized area of Duisburg was even less than in Borken. In the statistical modelling, the environmental nickel exposure was not able to explain the large differences of sensitization prevalence between study areas.

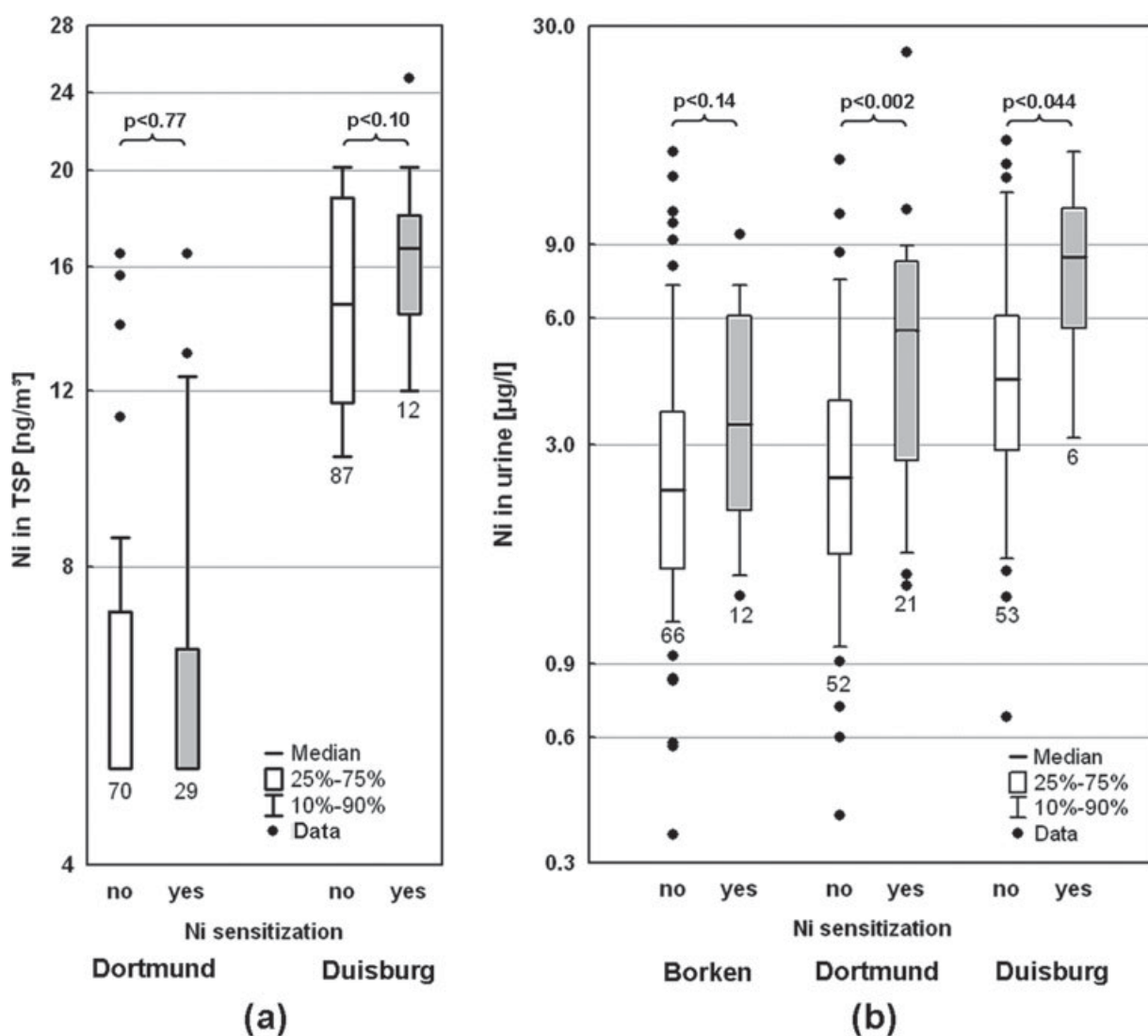


Fig. 2. (a) Exposure to nickel in TSP (total suspended particulate) in ambient air and (b) urinary nickel levels of nickel sensitized and non-sensitized children from Dortmund and Duisburg, Germany; distributions represented by box–whisker plots, Mann-Whitney test for group comparison.

Recent epidemiological studies have showed that the differences in air pollution are associated with allergic symptoms and diseases in the general population (12). These findings are consistent with previous observations showing that the metal composition of ambient particulate matter influences the severity of allergic airway diseases in humans and mice (13, 14). In our study, we observed remarkably high prevalences of allergic symptoms, diseases, and sensitizations in the children from the study area in Dortmund, which was situated in close vicinity to a steel mill (7).

Our findings could have the following potential explanation. Although cutaneous nickel exposure occurs from time to time, airborne exposure is constantly present; hence, even the exposure of the low concentrations of nickel in ambient air may be sufficient to induce sensitization. Furthermore, the

mucosa of the respiratory tract of children is often inflamed or irritated. Recent studies have shown that the metal composition of ambient particulate matter influences the severity of allergic airway diseases in humans and mice (12, 13). Inflammation or irritation causes the creation of an acid milieu that facilitates penetration of particles into the tissue. Nickel present in the inhaled air may be oxidized by oxidative species usually present in inflamed tissue. Nickel in oxidation states III and IV, as previously described by Artik et al. (14), is more potent in inducing nickel sensitization, as it probably causes the enhancement of production of major histocompatibility complex (MHC) and co-stimulatory molecules responsible for priming and cloning of T-cells. The clinical evidence that allergy occurs more readily in inflamed tissue supports this hypothesis.

Table 5. Association between nickel sensitization determined by patch test and exposure to nickel in ambient air and urine, respectively, for children from Borken, Dortmund and Duisburg, Germany, estimated by raw OR and OR adjusted for potential confounders using logistic regression (sample size: 193)

Influencing factors	Model 1				Model 2		
	OR _{raw}	OR _{adj}	95% CI	P	OR _{adj}	95% CI	P
Ni in TSP (9.3 ng/m ³) ^a	0.99	4.23	0.92–19.43	0.062	–	–	–
Ni in urine (3.9 µg/l) ^a	1.80	–	–	–	2.07	1.34–3.19	0.001
Sex (male)	0.71	0.70	0.27–1.80	0.451	0.69	0.26–1.82	0.450
Age (years)	1.17	1.76	0.52–5.89	0.359	2.15	0.59–7.79	0.243
Nationality, German	1.78	1.77	0.48–6.59	0.391	1.64	0.41–6.56	0.480
Parents' education ^b	2.03	2.27	0.99–5.17	0.050	1.84	0.81–4.19	0.141
Predisposition ^c	1.23	0.91	0.40–2.05	0.818	0.91	0.40–2.08	0.818
Ear piercing	1.93	1.13	0.43–2.95	0.804	0.87	0.32–2.36	0.781
Passive smoking	0.84	0.85	0.35–2.03	0.705	0.63	0.25–1.61	0.335
Duisburg	0.76	0.15	0.01–1.82	0.133	0.68	0.16–2.85	0.591
Dortmund	2.35	1.48	0.49–4.53	0.486	2.56	1.00–6.55	0.048
Borken (reference)	1.00	1.00	–	–	1.00	–	–
Pseudo R ²	–	–	8.37%	–	–	13.16%	–

TSP, total suspended particulate in ambient air; OR_{raw}, raw odds ratios; OR_{adj}, adjusted odds ratios; 95% CI, 95 percent confidence interval; P, P-value.

^aFor comparison of continuous influencing factors a rounded quartile range (P₂₅–P₇₅) was used as a unit step.

^bSchool years >10.

^cFather or mother with allergy or eczema or asthma or hay fever.

In contradiction to our results, Smith-Sivertsen et al. (6) conjectured an immuno-tolerance effect in an adult population from the Russian town of Nikel with nickel refineries and extremely elevated nickel concentrations in ambient air. Living in this environment was likely to reduce nickel hypersensitivity, i.e. it contributed to the development of immunological tolerance in non-sensitized people prior to exposure, although, no correlation between nickel in ambient air and nickel in urine was found in this investigation. Several studies argued that a long-term oral nickel exposure, (e.g. dental braces), by previously non-sensitized people might prevent nickel allergy by inducing immunological tolerance (15–18). A possible conclusion of the seemingly contradiction between our results and the above-mentioned in the literature might be that immunological reactions to long-term oral and inhaled nickel uptake follow different pathways and, therefore, that long-term airway exposure in early age could lead to allergy rather than tolerance.

The following limitations that could have influenced the outcome of our study deserve mention. Individual exposure to nickel in ambient air was assessed by estimated annual nickel concentrations in total suspended particles at the children's home addresses. Individual activity profiles were not taken into account. Therefore, the applied exposure estimates were likely to contain large errors which could have biased the estimation of the association between external nickel exposure and the prevalence of nickel sensitization. Internal nickel exposure was assessed by individual urinary nickel concentrations measured in morning urine samples

and collected only once during the investigation phase. Nevertheless, these measurements were used as long-term internal exposure. The patch-test readings in the study groups were performed 2D and 3D after the patch application. Hence, false positive results due to skin irritation were expected to be rare. Only one irritant reaction was excluded in the study group. Furthermore, other possible causes that can provoke false positive patch-test results were not considered in the study.

In conclusion, this study suggests that the elevated nickel concentrations in ambient air might be able to certain extent to induce nickel sensitization in children. Therefore, a similarly designed study with larger collectives and in other areas with known elevated nickel concentrations in ambient air is under way to check the findings of this study.

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3.1 Characteristics of study samples and prevalences of health parameters

The characteristics of the study populations, prevalence of atopic diseases and symptoms as well as allergic sensitizations divided by study area and in total are summarized in Table 2.

Table 2

Study groups' characteristics, atopic diseases and symptoms, allergic tests.

Percentages observed. Number of samples is indicated in parentheses.

Factor	Borken	Do-H	Du-N	Du-S	Augsbg	Halle	Total	p-value ^a
Age, mean (years)	6.3 (178)	6.2 (193)	6.4 (246)	6.4 (226)	6.4 (198)	6.2 (137)	6.34 (1168)	0.0001
Gender (male)	45.5 (178)	44.56 (193)	50.4 (246)	49.1 (226)	52.5 (198)	55.5 (137)	49.4 (1178)	0.3270
Nationality, German	98.9 (177)	86.0 (193)	48.6 (245)	72.1 (226)	73.1 (197)	94.9 (137)	76.3 (1175)	0.0001
Parents' education, school years >10 years	35.4 (175)	36.4 (184)	11.9 (235)	25.8 (217)	28.1 (185)	53.7 (136)	29.9 (1132)	0.0001
Ear piercing	36.8 (155)	37.7 (162)	49.7 (185)	39.2 (194)	44.1 (170)	27.4 (135)	39.8 (1001)	0.0024
Predisposition [§]	53.4 (178)	55.8 (190)	28.9 (243)	48.0 (225)	51.3 (195)	48.2 (135)	46.7 (1166)	0.0001
Allergy ^b	14.4 (174)	15.8 (190)	10.3 (243)	10.3 (223)	8.4 (191)	6.7 (135)	11.1 (1156)	0.0601
Asthma ^b	4.0 (174)	9.2 (184)	1.7 (239)	1.8 (224)	5.2 (172)	4.0 (124)	4.1 (1117)	0.0016
Wheezing ^c	8.4 (178)	18.2 (192)	6.9 (246)	7.1 (225)	6.7 (195)	8.2 (135)	9.1 (1171)	0.0003
Hay fever ^b	2.9 (172)	8.0 (178)	4.9 (243)	2.2 (226)	4.2 (167)	4.1 (122)	4.4 (1118)	0.0923
Hay fever min. 1 year ^d	1.2 (173)	3.2 (188)	0.4 (243)	1.3 (225)	0.0 (172)	0.8 (122)	1.2 (1123)	0.0704
Sneezing attacks ^c	6.2 (177)	12.2 (189)	7.0 (244)	8.0 (223)	6.8 (192)	11.5 (131)	8.4 (1156)	0.1845
Running nose and red eyes ^c	1.8 (165)	5.5 (181)	2.5 (239)	2.4 (213)	7.8 (180)	1.6 (126)	3.6 (1104)	0.0079
Allergic eczema ^b	13.8 (174)	16.4 (189)	6.2 (242)	15.3 (223)	18.6 (173)	24.6 (126)	14.8 (1127)	0.0001
Current allergic eczema ^e	6.5 (154)	7.9 (165)	3.8 (183)	3.6 (195)	5.2 (173)	8.2 (134)	5.7 (1004)	0.2946
Itching skin rash ^c	4.0	4.7	5.8	2.2	3.6	8.9	4.7	0.0831

Factor	Borken	Do-H	Du-N	Du-S	Augsbg	Halle	Total	p-value ^a
	(175)	(190)	(242)	(224)	(193)	(135)	(1159)	
RAST ^f	21.4 (117)	31.5 (130)	19.2 (141)	19.9 (166)	41.8 (103)	20.2 (84)	25.1 (741)	0.0001
Prick test ^f	25.0 (136)	26.5 (147)	18.8 (170)	18.2 (181)	28.4 (148)	22.6 (124)	23.0 (906)	0.1689
Patch test Ni(II) SO ₄	13.3 (105)	30.7 (101)	12.6 (103)	5.0 (100)	11.7 (77)	38.1 (42)	16.7 (528)	0.0001

Do-H: Dortmund Hörde, Du-N: Duisburg North, Du-S: Duisburg South, Augsbg: Augsburg

^a: Test of differences between areas: Chi-square test and Kruskal-Wallis test, respectively

^b: In father or mother: allergy or eczema or asthma or hay fever.

^g: Ever diagnosed by a physician (life time prevalence).

^c: Within last year.

^d: Ever lasting the whole year long.

^e: Point prevalence.

^f: At least one test positive

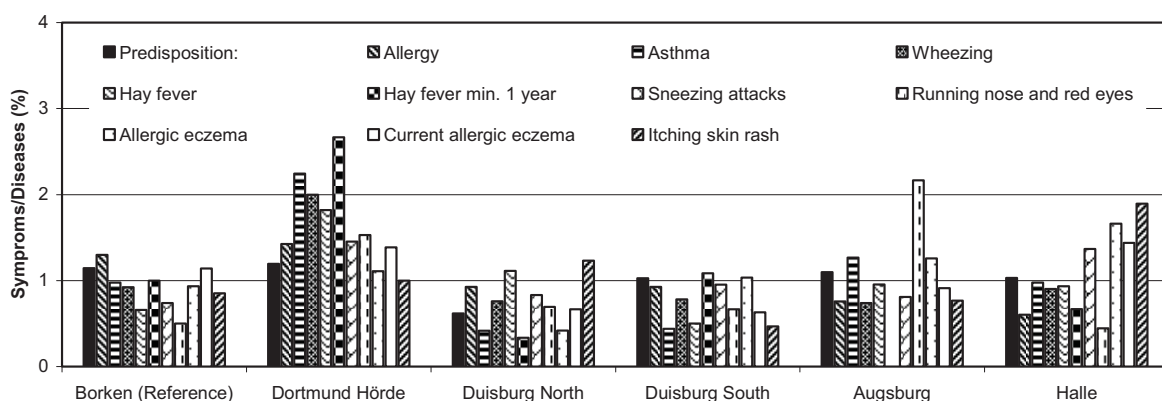


Figure 1

Atopic diseases and symptoms, which were assessed by questionnaire as a quotient of relative frequency of appearance in the respective area compared to the relative total frequency of appearance of symptoms.

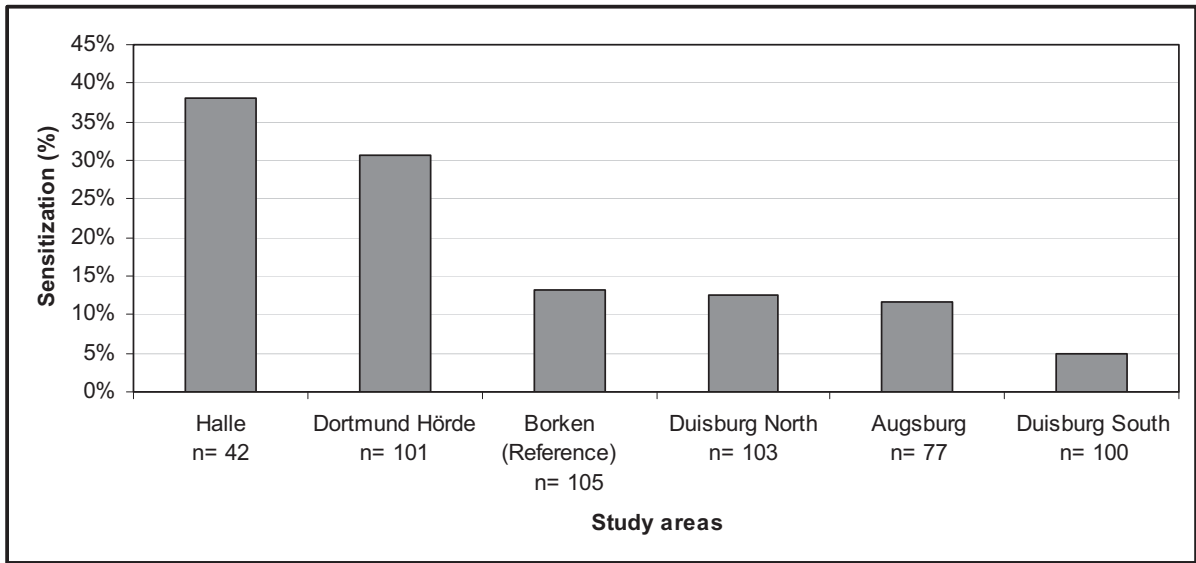


Figure 2

Prevalence of the Nickel sensitization assessed by skin patch test with Ni (II) sulphate in the study areas in percentage of total study sample.

Out of all study centres the collective of Dortmund Hörde allots much attention due to its strikingly peerless prevalences of almost all the assessed parameters as demonstrated in Table 2 and Figure 1.

The percentage of children that had ear piercing is relatively high as listed in Table 2. This factor is strongly related to female gender, since further stratification by gender revealed a significant association between gender and ear piercing, as 87.4 % of pierced children were girls ($\chi^2_1= 373.4$; $p<0.001$) with 39.8 % of children ear pierced in total.

The socio-economic and socio-demographic background of the children in Duisburg-North differed strongly from the two other areas as only 11.9 % of their parents had more than 10 years of school education in contrast to Halle (53.7%) and around 30% in the other investigated areas. In Duisburg-North, 48.6% were of non-German origin (predominantly Turkish) compared to much lower percentages in all other areas (Table 2). Furthermore, the children in Duisburg-North less frequently had an allergic predisposition (prevalence: 28 %) than the children in all other areas (prevalence: mostly around 50%) where prevalence of eczema, allergy, hay fever or asthma in the child's parents ever diagnosed by a physician (life-time prevalence) was used as an indicator of the child's predisposition. A life time prevalence of allergy defined in the

questionnaire as allergy ever diagnosed by a physician, was higher in Dortmund and Borken than in the other samples (Table 2).

Furthermore, as shown in Table 2 and additionally graphically illustrated in Figure 2, considerable variations in prevalence of nickel sensitization measured by patch test were observed in different study areas within Germany. Interestingly, nickel sensitization rate in Halle was about sevenfold higher than in the hot spot Duisburg South and the nickel sensitization level in Duisburg North was 2,5-fold higher than in the southern part of the same city.

3.2 Description of external and internal nickel exposures and their associations

Nickel levels in ambient air and morning urine were measured only in the study areas Borken, Dortmund Hörde and Duisburg North, therefore, the analysis of the correlation between the external and internal exposure, and the prevalence of nickel sensitization is restricted to these collectives. There are 178, 184 and 208 valid ambient air quality data, respectively. One hundred and four, 101 and 72 valid urine samples, respectively, were evaluated. There were all together 275 valid samples of both measurements. Nickel uptake with drinking water was assessed only for study areas Borken and Dortmund Hörde with 105 and 130 valid samples, respectively.

Nickel in ambient air

The differences in ambient air quality data specified by area are listed in Table 3. As demonstrated, the investigated children residing in Dortmund Hörde had higher mean and median values of nickel concentration in ambient air compared with the reference area Borken. The correspondent values in the study area Duisburg North were even higher.

Table 3

Nickel in TSP [ng/m³] in Borken, Dortmund Hörde and Duisburg North

Study area	Borken †	Dortmund Hörde	Duisburg North	Total
N	178	184	208	570
AM [†] / SD	2.50 [†] / 0.00	6.29 [†] / 2.41	14.91 [†] / 3.94	8,25 ± 5,94
CI _{95%} Range of AM	--	5.94 – 6.64	14.37 – 15.45	7,76-8,74
Range	2.50 – 2.50	5.00 – 16.50	5.50 – 26.38	2,50-26,38
P ₅	2.50	5.00	9.94	2,50
P ₁₀	2.50	5.00	10.31	2,50
P ₂₅	2.50	5.00	11.66	2,50
Median	2.50	5.00	14.67	5,05
P ₇₅	2.50	6.55	17.32	11,84
P ₉₀	2.50	9.00	20.18	17,27
P ₉₅	2.50	12.40	20.18	20,18

†:Borken had only one ambient air measuring station (background level). TSP = total suspended particulate;

AM = arithmetic mean; SD = standard deviation; CI = confidence interval; P₅ = 5th percentile

Given the fact that the reference area Borken had only one air quality monitoring station (background level) and therefore, the same level of nickel in ambient air was attributed to every child residing there, this area was not considered by the comparison of distribution between the groups. The comparison of distribution of airborne nickel exposure in Dortmund Hörde and Duisburg North by means of Mann-Whitney Test showed that the exposure levels in Dortmund Hörde were different from those in Duisburg North ($p < 0.001$).

Nickel uptake in drinking water

Because the daily uptake of nickel in tap water has been assessed only in Borken and Dortmund Hörde the further analysis of this parameter is restricted to these two areas. As demonstrated in the Table 4, the observed differences of distribution parameters between both areas were not significant (Mann-Whitney test: $p=0.289$).

Table 4

Nickel uptake in drinking water [$\mu\text{g}/\text{d}$] in children in Borken and Dortmund Hörde

Study area	Borken	Dortmund Hörde	Total
N	105	130	235
AM \pm SD	1.94 \pm 3.99	2.11 \pm 4.51	2.03 \pm 4.28
CI _{95%} Range of AM	1.17 -2.71	1.32 – 2.89	1.48 – 2.58
Range	0.00 – 31.20	0.00 – 48.1	0.00 – 48.1
P ₅	0.21	0.21	0.21
P ₁₀	0.21	0.21	0.21
P ₂₅	0.53	0.53	0.53
Median	0.94	1.16	1.16
P ₇₅	2.08	2.46	2.28
P ₉₀	3.00	4.14	3.86
P ₉₅	5.40	5.07	5.07

AM = arithmetic mean; SD = standard deviation; CI = confidence interval; P₅ = 5th percentile.

Nickel in urine

The urinary nickel distribution for study populations of Borken, Dortmund Hörde and Duisburg North is shown in Table 5. As demonstrated, the children living in Dortmund Hörde had higher means and medians of nickel concentration in morning urine at the day of sampling compared with the reference area Borken, whereas the correspondent values in the study area Duisburg North were even higher.

Table 5Nickel in the morning urine [$\mu\text{g/L}$] of children from Borken, Dortmund Hörde and Duisburg North

Study area	Borken	Dortmund Hörde	Duisburg North	Total
N	104	101	72	277
AM \pm SD	3.42 \pm 2.278	4.08 \pm 3.76	5.50 \pm 3.64	4,20 \pm 3,47
CI _{95%} Range of AM	2.88 – 3.96	3.34 – 4.82	4.64 – 6.35	3,79-4,61
Range	0.30 – 15.07	0.39 – 26.02	0.67 -16.00	0,30-26,02
P ₅	0.80	0.91	1.40	0,83
P ₁₀	1.13	1.08	1.70	1,23
P ₂₅	1.53	1.66	3.00	1,83
Median	2.61	2.67	4.60	3,07
P ₇₅	4.07	5.79	6.75	5,70
P ₉₀	7.71	8.22	12.00	8,48
P ₉₅	9.25	10.64	14.00	12,00

AM = arithmetic mean; SD = standard deviation; CI = confidence interval; P₅ = 5th percentile.

Corresponding to the ambient air Ni exposure concentrations, the children living in Duisburg-North had the highest median Ni concentration in morning urine, whereas the median urinary Ni concentration in Dortmund was nearly identical to the median level of the children from the reference area Borken. The comparison of distribution of urinary Ni exposure for the three areas by means of Kruskal-Wallis Test showed significant differences ($p < 0,001$) whereas the difference between Dortmund and Borken was not significant (Mann-Whitney test: $p = 0.195$).

Associations between external and internal nickel exposures

To assess the association between nickel concentrations in ambient air and in morning urine, the correlation analysis of the united data of Borken, Dortmund Hörde and Duisburg North ($n=275$) was performed. A weak but highly significant correlation ($r_s = 0.257$, $p < 0.001$) between nickel concentration in ambient air and in urine was observed. Because the reference area Borken had only one air quality monitoring station the same correlation analysis was repeated without this area. There as well we could observe a weak but highly significant correlation ($r_s = 0.221$, $p < 0.004$) between urinary and ambient air nickel levels. The results of both correlations shown in Table 6 did not reveal large differences in the compared parameters.

Table 6

Spearman's rank correlation between nickel concentration in ambient air and in urine in children of Borken, Dortmund Hörde and Duisburg North: Analysis with and without Borken.

Study area considered in correlation	Spearman's correlation (r_s)	Level of significance (p)	Number of samples (N)
Borken, Dortmund Hörde, Duisburg North	0.257	0.001	275
Dortmund Hörde, Duisburg North	0.221	0.004	171

The scatter plot shown in Figure 3 demonstrates single data for the association between urinary and airborne nickel concentration in double logarithmic scale separated by study groups.

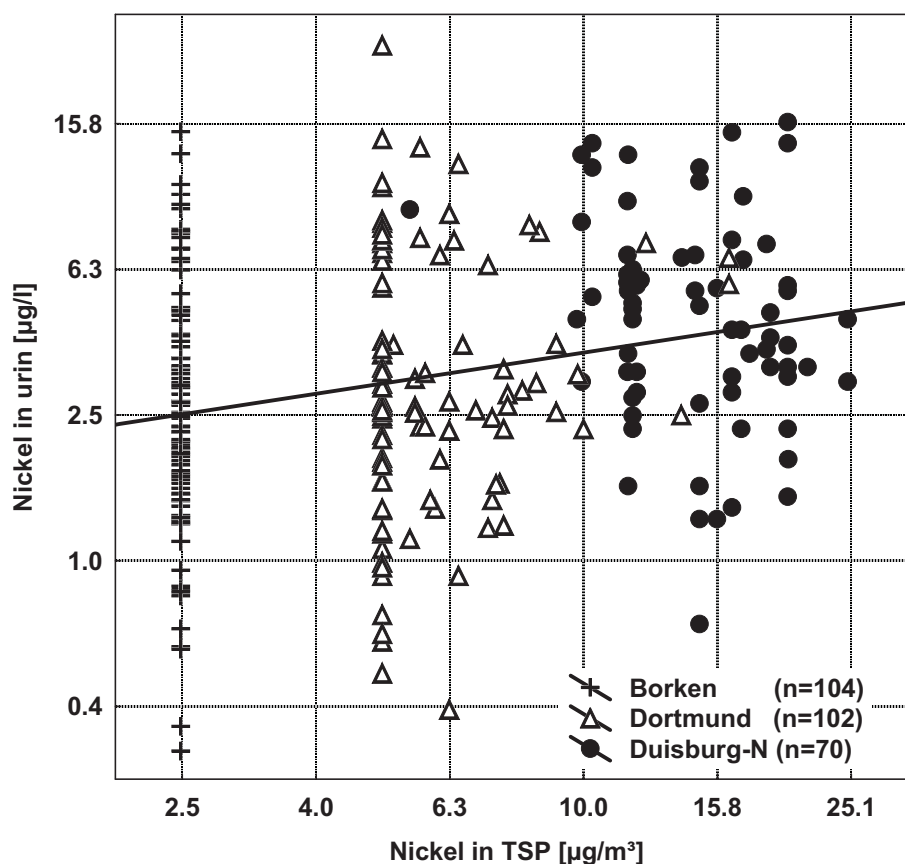


Figure 3

Association between nickel concentrations in ambient air and urine in children of Borken, Dortmund Hörde and Duisburg North with linear regression in double logarithmic scale. (TSP= total suspended particulate).

Associations between nickel in ambient air and nickel sensitization

The comparison of the nickel levels in TSP of sensitized and non-sensitized children was performed only for the study areas Duisburg North and Dortmund Hörde. The study area Borken had only one measuring station, therefore the children living in this area were all attributed the same concentration of nickel in TSP (2.50 ng/m^3). The results shown on the box-and-whisker diagram (Figure 4) demonstrate for the study area Duisburg North a positive association ($p < 0.10$), of nickel sensitization status of children with the nickel level in TSP. A respective association in Dortmund Hörde could not be observed probably because the nickel concentrations in ambient air registered in Duisburg North were higher than in Dortmund Hörde and so the differences (sensitized / not sensitized) in Duisburg North were stronger expressed.

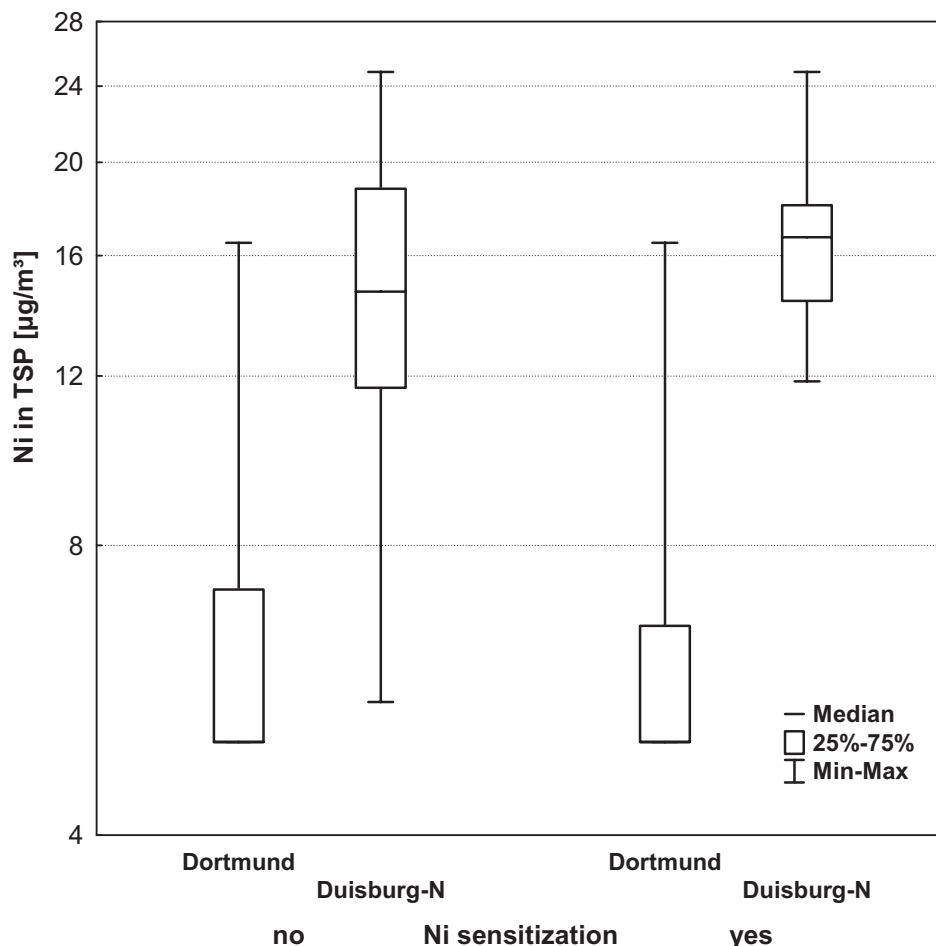


Figure 4

Nickel levels in TSP of sensitized and non-sensitized children in Dortmund Hörde and Duisburg North; distributions represented by box-whisker plots..(TSP= total suspended particulate; 25% = 25th – 75th percentile range).

Association between nickel level in urine and nickel sensitization

The box-and-whisker plots of childrens' urinary nickel levels separated by study area by function of their epicutaneous patch test results are displayed on Figure 5.

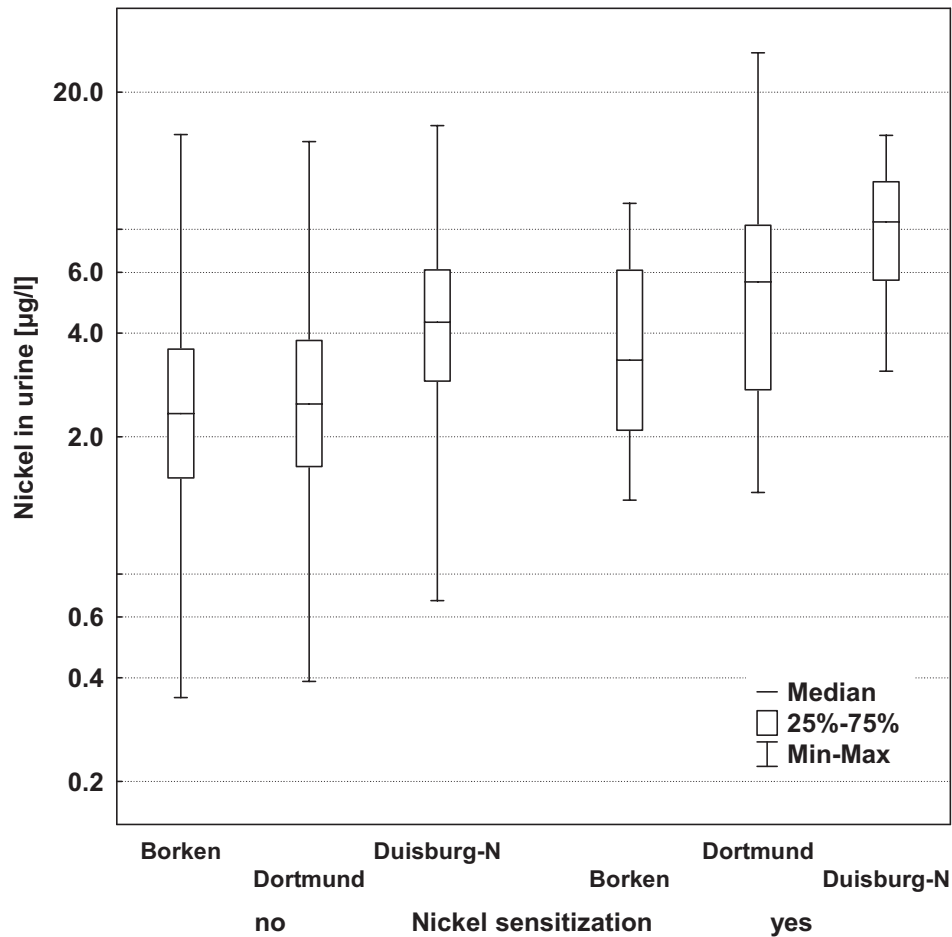


Figure 5

Urinary nickel levels of sensitized and non-sensitized children in Borken, Dortmund Hörde and Duisburg North; distributions represented by box-whisker plots..

The same distribution as in Figure 5 but not separated by the area is displayed in Figure 6. A comparison of both groups using Mann-Whitney test showed with $p < 0,001$ that both groups were significantly different and the sensitized children had higher nickel levels in urine than the non-sensitized.

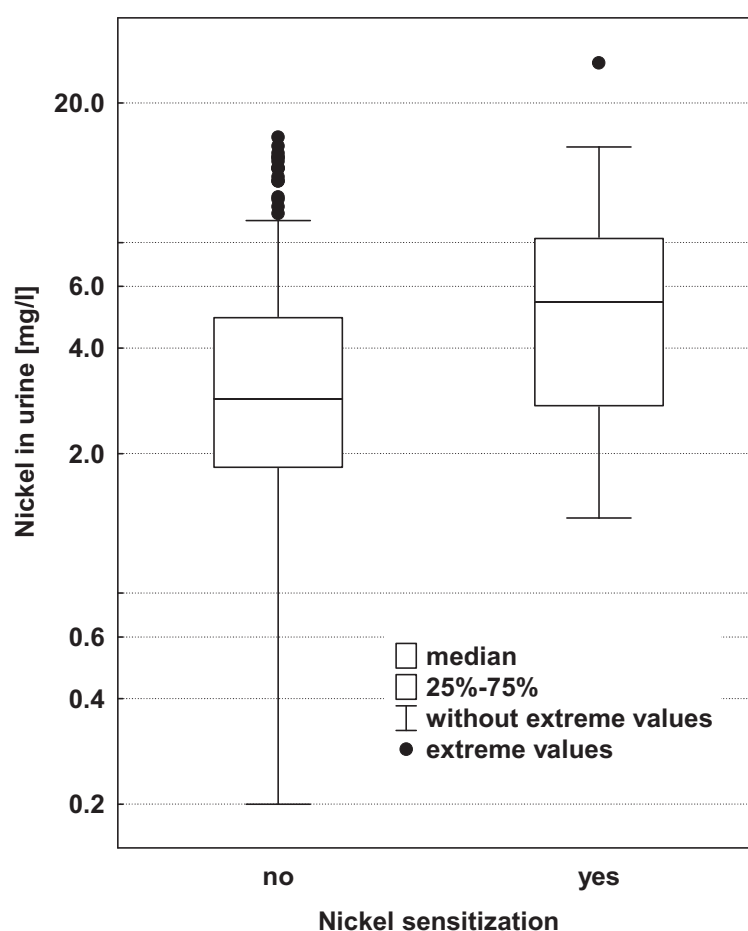


Figure 6

Urinary nickel levels of sensitized and non-sensitized children in the study area; distributions represented by box-whisker plots.

Associations between external, internal nickel exposures and nickel sensitization

Out of 275 children that had data of nickel in TSP and in urine 200 children had also valid epicutaneous patch test (ECT) results. In order to see the performance of the prevalence of nickel sensitization by function of external or internal exposure, or the nickel sensitization by function of the both rates within one descriptive scheme a contingency table was built. The rates of the external and internal exposure were divided into two groups each. Medians (4.5 for the ambient air and 3.0 for the morning urine) were used as cut points.

As shown in Figure 7 children who had levels higher than median of either external or internal exposure, or both showed higher rates of prevalence of nickel sensitization than those with the levels below median (χ^2 -test: p=0.109).

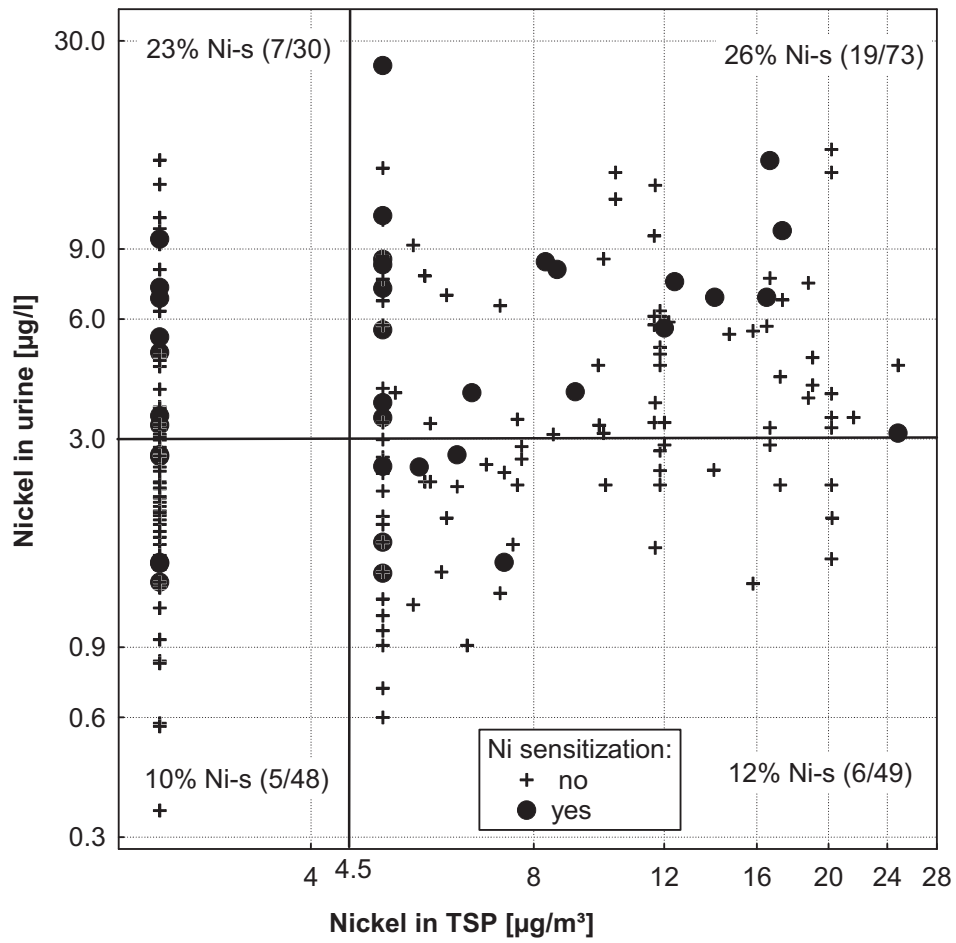


Figure 7

Correlation between nickel concentrations in ambient air and urine not separated by the area in children of Borken, Dortmund Hörde and Duisburg North (n=200). In addition, nickel sensitization status is indicated for respective groups. Cut points: medians. (TSP= total suspended particulate).

3.3 Logistic regression analysis

Besides Ni in ambient air and the internal Ni exposure, the following factors are assumed to affect the prevalence of Ni allergy in children and, therefore, are considered as potential confounders for the association between the external and, respectively, internal exposure of Ni and Ni sensitization: gender, social status, nationality, ear piercing, passive smoking, family predisposition and Ni uptake in tap water. The social status was assessed by classifying the school grade achieved by the parents. Additionally, the children's area of residence was introduced as independent determinant in the regression model to cover unknown risk factors specific for the respective area. Borken was used as reference. Ni uptake in tap water on the day of examination was only available for Dortmund and Borken. In a subgroup analysis, the daily Ni uptake in tap water showed no influence on the Ni sensitization in the study group with tap measurements available and, therefore, was not considered anymore as a potential confounder. Adjusted odds ratios estimated by logistic regression analysis (Table 7) demonstrate the associations of ambient air and internal Ni exposure and other potentially influencing factors with the Ni sensitization prevalence within the study group. Additionally, the raw odds ratios of each influencing factor are also shown in Table 7. The internal Ni exposure assessed by Ni in urine turned out to be an important and significant risk factor for Ni sensitization (Table 7, Model 2). The prevalence of Ni sensitization was about twice as high in children with Ni urine concentrations above the 75th sample percentile compared to children with concentrations below the 25th percentile. Furthermore, the external Ni exposure assessed by the Ni concentration in TSP of ambient air as well showed a significant influence ($p = 0.062$) on the Ni sensitization prevalence (Table 7, Model 1). As estimated by the regression model, the prevalence of Ni sensitization was about four times as high in children with an exposure to Ni in ambient air above a concentration level of 12 ng/m³ compared to children with concentrations below 2.5 ng/m³. Obviously by comparing the raw and adjusted odds ratios, study area was a strong confounder for the influence of Ni in ambient air on the Ni sensitization. Furthermore, the lower prevalence level in Duisburg in comparison to Borken remained unexplained in regression modelling. Higher social status (parents' education level) was a further risk factor of Ni sensitization, but with weak significance. All the other factors considered in the regression analysis were of no

significant influence. By comparing the pseudo R² of the two regression models, the Ni concentration in urine turned out to have a higher explanatory value than Ni in ambient air.

Table 7

Association between nickel sensitization determined by patch test and exposure to Ni in ambient air and urine, respectively, for children from Borcken, Dortmund and Duisburg, Germany, estimated by raw odds ratios and odds ratios adjusted for potential confounders using logistic regression. Sample size: 193.

Influencing factors	OR _{raw}	OR _{adj}	Model 1		Model 2		
			95%CI	p	OR _{adj}	95%CI	p
Ni in TSP [9.3 ng/m ³] ¹	0.99	4.23	0.92-19.43	0.062	--	--	--
Ni in urine [3.9 µg/l] ¹	1.80	--	--	--	2.07	1.34-3.19	0.001
Gender (male)	0.71	0.70	0.27-1.80	0.451	0.69	0.26-1.82	0.450
Age [years]	1.17	1.76	0.52-5.89	0.359	2.15	0.59-7.79	0.243
Nationality, German	1.78	1.77	0.48-6.59	0.391	1.64	0.41-6.56	0.480
Parents' education ²	2.03	2.27	0.99-5.17	0.050	1.84	0.81-4.19	0.141
Predisposition ³	1.23	9.91	0.40-2.05	0.818	0.91	0.40-2.08	0.818
Ear piercing	1.93	1.13	0.43-2.95	0.804	0.87	0.32-2.36	0.781
Passive smoking	0.84	0.85	0.35-2.03	0.705	0.63	0.25-1.61	0.335
Duisburg	0.76	0.15	0.01-1.82	0.133	0.68	0.16-2.85	0.591
Dortmund	2.35	1.48	0.49-4.53	0.486	2.56	1.00-6.55	0.048
Borcken (reference)	1.00	1.00	--	--	1.00	--	--
Pseudo R ²	--		8.37 %			13.16 %	

¹ For comparison of continuous influencing factors a rounded quartile range (P₂₅ – P₇₅) was used as a unit step.

² School years >10 years

³ Father or mother with allergy or eczema or asthma or hay fever

TSP : total suspended particulate in ambient air; OR_{raw} : raw odds ratio; OR_{adj} : adjusted odds ratio; 95%CI : 95 percent confidence interval; p : p-value

4. Discussion

In the present study, the human biomonitoring of nickel has been assessed only in the hot spots Dortmund Hörde, Duisburg North and the reference area Borken, hence the correlation analysis of the results of external and internal nickel exposure and nickel sensitization was performed only for these study centres, whereas the prevalence of allergic symptoms, diseases and sensitizations were additionally evaluated in Duisburg South, Halle and Augsburg.

Our study demonstrated a statistically significant association between Ni sensitization and internal Ni exposure in the investigated study group of 6-years-old children, since urinary Ni values of sensitized children were significantly higher than those of non-sensitized. At a weaker significance level, the association between the mean annual exposure to Ni in ambient air and the Ni sensitization also was obvious in multivariate logistic regression analysis ($p = 0.062$). Furthermore, the assumption that inhaled Ni in ambient air was a risk factor of Ni sensitization is supported by the significant association between urinary Ni concentration and exposure of Ni in ambient air. The otherwise potentially influencing factors like gender, age, nationality, familial predisposition or Ni exposure through skin or through drinking water did not show noticeable associations with the prevalence of Ni sensitization in the study group. The only exception presented a higher parental school education which resulted in increased Ni sensitization prevalence. This result of an influence of Ni exposure in ambient air on Ni sensitization in children must be appraised in view of the large variability of Ni sensitization prevalence within the study areas. The sensitization prevalence in the industrialized area in Dortmund was more than twice as high as in the rural area of Borken, but the prevalence in the other industrialized area in Duisburg North was even less than in Borken. In the statistical modelling, the environmental Ni exposure was not able to explain the large differences of sensitization prevalence between study areas.

Recent epidemiological studies have demonstrated that differences in air pollution are associated with allergic symptoms and diseases in the general population (Brauer et al., 2007). These findings are consistent with previous observations showing that the metal composition of ambient particulate matter influences the severity of allergic airway diseases in humans and mice (Gavett et al., 2003;

Schaumann et al., 2004). In our study, remarkably different prevalences of allergic symptoms, diseases and sensitizations were observed in different study areas throughout Germany, a rather unexpected fact for young study collectives. Particularly striking were peerlessly high prevalences in Dortmund-Hörde, (Table 2 and Figure 1), the hot spot situated closely to a steel mill and characterized by increased Ni and Cr levels in ambient air (109).

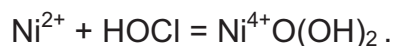
Further, we could reveal considerable variability in prevalence of sensitization to nickel between the investigated areas, as, for instance, nickel sensitization in Halle was about sevenfold higher than in the hot spot Duisburg South and the nickel sensitization level in Duisburg North is 2,5-fold higher than in the southern part of the same city. No factors that could explain these discrepancies have been detected yet. Possible explanations for this finding are given by the following four hypotheses.

H_I: While cutaneous nickel exposure occurs from time to time, airborne exposure is constantly present; hence, smaller concentrations of nickel could be threshold to induce sensitization (Menne, 1994).

H_{II}: Mucosa of respiratory tract of children is often inflamed or irritated, usually with an asymptomatic course. Inflammation or irritation cause, however, the creation of acid milieu that facilitates penetration of particles in the tissue. That can make the occurrence of nickel sensitization easy by transforming nickel, which is present in the inhaled air, into higher oxidation states by oxidative species like HOCl and H₂O₂ usually present in the inflamed tissue. This synthesis occurs according to Fenton reaction:



or:



Nickel in oxidation states III and IV as it was previously described by Artik et al., 1999 and Artik et al., 2004 is more potent to induce nickel sensitization, since it probably causes the enhancement of production of MHC- and co-stimulatory molecules responsible for priming and cloning of T cells (sensitization phase of delayed-type immune response). It is notorious that the side effects of gold-based antirheumatic medicine are caused not through the ingested gold(I), but through its reactive metabolite gold(III) (Goebel et al., 1995; Griem et al., 1996; Griem et al., 1998; Schuhmann et al., 1990). By analogy to gold, Artik et al. asked whether the higher

oxidation states of nickel would be more potent than Ni^{2+} to induce de novo sensitization to this heavy metal. In their study, they succeeded to demonstrate in the mouse ear swelling test (MEST), a standard screening test for contact allergens, that mice could be sensitized easier, when nickel was applied at oxidation states III or IV (to be precise, the Ni^{3+} - and Ni^{4+} -bearing reaction products that occur as a result of oxidation of Ni^{2+}) (Artik et al., 1999 and Artik et al., 2004). The clinical evidence that allergy occurs easier in inflamed tissue supports this hypothesis. Hence, further investigations are needed to confirm or refute these presumptions.

H_{III}: Additionally, oral nickel uptake may affect nickel sensitization less than an uptake via respiratory tract probably because the oral uptake is less efficient, since the intestinal absorption of nickel is depressed by metals such as zinc and iron or other food constituents, which compete with nickel for transport process (Sundermann, 2004; Foulkes and McMullen, 1986).

H_{IV}: Lacy et al. (1996) corroborated the experimental data showing a difference between different tissues in terms of nickel uptake. According to their studies, there is much more uptake of nickel by keratinocytes than by fibroblasts. That lets us suggest that nickel uptake by mucosa cells of respiratory tract (upper as well as lower) could be more efficient than by keratinocytes; therefore, certain concentrations of nickel in the inhaled air could be sufficient to induce nickel sensitization.

According to numerous literature a long term oral uptake of nickel (in small quantities) does not seem to induce nickel sensitization but is most likely to protect from it, thus, inducing immunological tolerance (Menne, 1996; van Hoogstraten et al., 1991; van Hoogstraten et al., 1992; van Hoogstraten et al., 1993; van Hoogstraten et al., 1994; Artik et al., 2001; Artik et al., 2004; Hensten-Petersen, 1992; Jensen et al., 2002; Kerosuo et al., 1995; Mortz et al., 2002; Todd and Burrows, 1989). Living in the environment of nickel refineries in Russian town of Nikel with extremely elevated nickel concentrations in ambient air is likely to reduce nickel hypersensitivity, i. e. contributes to development of immunologic tolerance in non-sensitized people prior to exposure, according to results of a population based study conducted by Smith-Sivertsen et al., 2002 with adult population, although no correlation between nickel in ambient air and nickel in urine was found in this investigation (Smith-Sivertsen et al., 1998, Smith-Sivertsen et al., 1997). Another study suggested that trace amounts of nickel present in the general environment do not induce nickel sensitization (Menne, 1994). In contrast, our data based on children imply that high nickel concentrations in

the environment do not protect against nickel sensitization but are most prone to accelerate it (see also Wilhelm et al., 2007b). A possible conclusion of the seemingly contradiction between our results and the above mentioned in the literature might be that immunological reactions to long-term oral and inhalative Ni uptake follow different pathways and, therefore, that long-term airway exposure in early age could lead to allergy rather than tolerance.

Study limitations

Following points could have modified the outcome of our study and therefore deserve mention. Individual exposure of Ni in ambient air was assessed by estimated annual Ni concentrations in total suspended particles at the children's home addresses. Individual activity profiles were not taken into account. Therefore, the applied exposure estimates were likely to contain large errors which could have biased the estimation of the association between external nickel exposure and the prevalence of Ni sensitization. Internal Ni exposure was assessed by individual urinary Ni concentrations measured in morning urine samples and collected only once during the investigation phase. Nevertheless, these measurements were used as long-term internal exposure. The patch test readings in the study groups were performed 48 and 72 hours after the patch application. Hence, falsely positive results due to skin irritation were expected to be rare. Actually, only one irritant reaction was excluded in the study group. Furthermore, other possible allergic causes that can provoke falsely positive patch test results were not considered in the study. (details in German are given in the report on the whole Hot Spot Study NRW (LUA, 2004a).

5. Conclusions

The present study clearly shows that despite improved air quality in the last decades 'hot spot' emissions still occur, and children who live in the vicinity of industrial sources are to certain extent at risk of increased internal contaminant exposure (the present study focused on nickel) and elevated health effects when compared with the children from a reference area and comparison cities in other parts of Germany (see also LUA, 2004a; b; c; Wilhelm et al, 2007a). Elevated nickel concentrations in ambient air seem to some extent to have an impact on development of nickel

sensitization in children. Therefore, a similarly designed follow up study with larger study samples in the other areas with known elevated Ni levels in ambient air should be undertaken to verify the findings of the present study. Additionally, our study suggests that a peerless prevalence rate of almost all assessed allergic ailments and sensitizations in children in Dortmund Hörde compared with other study collectives that could not be explained by present study needs further investigation.

6. Perspective

A further cross-sectional study with children has been conducted in 2005-2006 at four different hot spots with increased nickel level in ambient air aiming to investigate if elevated airborne nickel exposure has an influence on the manifestation of asthma, allergies, atopic disease and lung function disorders in children. Results of this study have recently been published (Monika Kasper-Sonnenberg, Dorothee Sugiri, Sabine Wurzler, Ulrich Ranft, Heinrich Dickel, Jürgen Wittsiepe, Jürgen Hölzer, Friederike Lemma, Georg Eberwein, Peter Altmeyer, Martin Kraft, Ursula Krämer, Michael Wilhelm: Prevalence of nickel sensitization and urinary nickel content of children are increased by nickel in ambient air. *Environmental Research* 111 (2011) 266–273).

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Appendix

Assessment of urinary nickel concentration in the study collective Duisburg North in 2008

The measurement of nickel concentration in urine in the study collectives Dortmund Hörde and Borken was performed routinely during the Hot Spot study in 2000. For the study collective Duisburg North, the measurement was not performed originally, but assessed later in 2008 using samples, which were frozen in 2000. In the few samples available in which nickel concentration was measured in 2000 it was measured again in the laboratory of the IUF in Düsseldorf and in the laboratory of the Department of Hygiene, Social and Environmental Medicine, Ruhr University Bochum. The results of all three measurements were compared (Table 7). The comparison has shown that the measurements performed in IUF and in the University of Bochum were comparable with the original measurements done in 2000 [and therefore reliable]. Further comparison of the results from the laboratories in IUF and Bochum demonstrated on the scatter plot (Figure 8) has shown that the results from both laboratories were comparable.

Table 7

Study group Duisburg North: comparison of measurements of urinary-nickel levels assessed originally during Hot Spot study in 2000 in the laboratory of IUF in Düsseldorf with measurements of preserved urine samples that were performed in 2008 in the laboratory of IUF in Düsseldorf and the laboratory of the University of Bochum.

Sample number	Urinary-nickel concentration, [$\mu\text{g/L}$]		
	Original measurement Düsseldorf 2000	Bochum 2008	Düsseldorf 2008
#01	10.00	9.00	8.29
#02	2.17	2.60	2.03
#03	3.38	3.20	2.73
#04	2.43	2.30	1.63
#05	3.50	2.40	2.49
#06	1.10	1.30	0.87

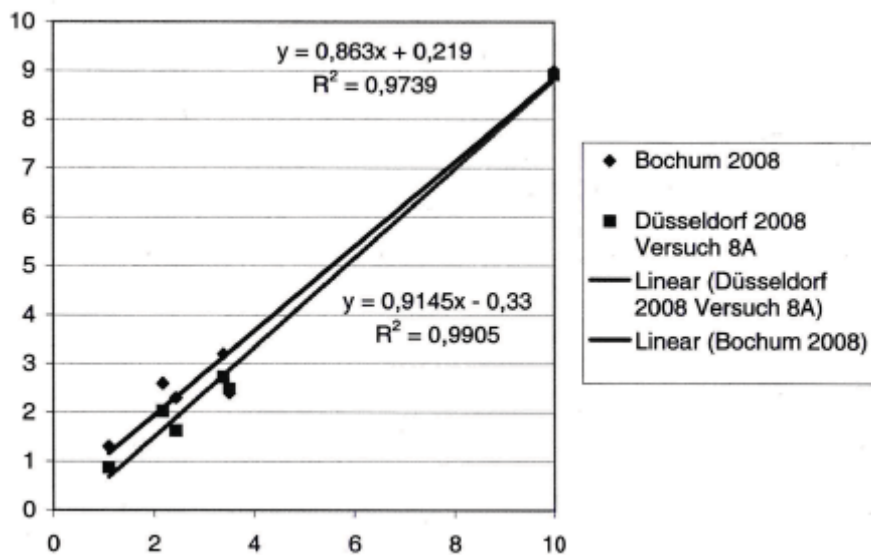


Figure 8

Study group Duisburg North: comparison of measurements of nickel in defrozen urine samples performed in Bochum and Düsseldorf in 2008.

Zusammenfassung

Nickel Exposition in der Luft: kann sie eine Nickelsensibilisierung hervorrufen? Eine populationsbezogene Studie über T_H1-zellvermittelte Immunantwort mit Kindern im Einschulungsalter

Die vorliegende Studie hatte den Zweck, den Zusammenhang zwischen der Nickelbelastung in Außenluft und der Ausprägung der gesundheitsrelevanten Faktoren – gemessen u.a. in Prävalenz der Nickelsensibilisierung, vorwiegend T_H1-zellvermittelte Immunreaktion - bei sechsjährigen Kindern, die in unmittelbarer Nähe der Industriegebiete in der Bundesrepublik Deutschland wohnen, zu evaluieren. Belastung und Gesundheitseffekte wurden bei Kindern in Schulanfängeralter ermittelt. Der Wohnsitz der Studienteilnehmer war in der Nähe der Industriequellen der drei unterschiedlichen „Hot Spots“ in NRW (Dortmund-Hörde, Duisburg-Nord und Duisburg-Süd), in einem ländlichen Gebiet ohne unmittelbarer industrieller Belastung (Stadt Borken in Westfalen), das als Referenzareal für die „Hot Spot“ dienen sollte, sowie in den Vergleichsarealen in anderen Gebieten Deutschlands: einer Stadt in Bayern (Augsburg) und einer Stadt in den neuen Bundesländern (Halle). Die Querschnittstudie wurde im Zeitraum zwischen März und Juni 2000 durchgeführt. Die Erfassung der Belastung beinhaltete Modellierung von Außenluftdaten und Messung der Nickelaufnahme mit Trinkwasser als Außenbelastung sowie die Human Biomonitoring (HBM), i.e. Erfassung der Nickelwerte in Morgenurin als innere Belastung. Gesundheitseffekte wurden mittels eines Fragebogens, des CAP-FeIA-Systems sowie des Epikutantests (ECT) erfasst. Der Einfluss der Belastungsfaktoren auf Nickelsensibilisierung wurde mittels linearer und logistischer Regressionsanalyse gemessen. Die wichtigen Ergebnisse waren wie folgt: die beobachtete Nickelsensibilisierungsprävalenz variierte in den Studienzentren zwischen 5.0% (Dortmund-Hörde) und 38.1% (Halle). Kinder, die im „Hot Spot“ Dortmund-Hörde wohnten (v.a. erhöhte Chrom- und Nickelwerte in der Außenluft aus Stahlwerk), zeigten erhöhte Prävalenzen verschiedener Allergiesymptome und Sensibilisierungen, während Kinder, deren Wohnsitz in Duisburg-Nord lag (Kokerei, Stahlwerk, Hochofen und Sinteranlage), u.a. erhöhte akute Nickelkonzentrationen in Urin aufwiesen. In dieser Studie wurde ein positiver signifikanter Zusammenhang

zwischen Nickelsensibilisierung und der akuten inneren Nickelbelastung gemessen in Nickelkonzentration im Morgenurin beobachtet. Des Weiteren, wurde ein tendenzieller, jedoch signifikanter Zusammenhang zwischen Nickelwerten in der Außenluft und Nickelkonzentration in Urin festgestellt. Zusammenfassend lässt sich festhalten, dass erhöhte Nickelbelastung in der Außenluft zu einem gewissen Grad zur Entstehung der Nickelsensibilisierung beitragen kann. Weitere Studien sind notwendig, um Hinweise dieser Studie in größeren Kollektiven in anderen Arealen mit bekannter erhöhter Nickelbelastung in Außenluft zu verifizieren.