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Levels and predictors of urinary nickel concentrations of children in Germany: Results from the German Environmental Survey on children (GerES IV)

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ABSTRACT

Human biomonitoring of nickel has gained interest in environmental medicine due to its wide distribution in the environment and its allergenic potential. There are indications that the prevalence of nickel sensitization in children is increased by nickel exposure and that oral uptake of nickel can exacerbate nickel dermatitis in nickel-sensitive individuals. Urinary nickel measurement is a good indicator of exposure. However, data on nickel levels in urine of children are rare. For the first time, the German Environmental Survey on children (GerES IV) 2003–2006 provided representative data to describe the internal nickel exposure of children aged 3–14 years in Germany. Nickel was measured after enrichment in the organic phase of urine by graphite furnace atomic absorption spectrometry with Zeeman background correction. Nickel levels ($n = 1576$) ranged from <0.5 to $15 \mu\text{g/l}$. Geometric mean was $1.26 \mu\text{g/l}$. Multivariate regression analysis showed that gender, age, socio-economic status, being overweighted, consumption of hazelnut spread, nuts, cereals, chocolate and urinary creatinine were significant predictors for urinary nickel excretion of children who do not smoke. 20.2% of the variance could be explained by these variables. With a contribution of 13.8% the urinary creatinine concentration was the most important predictor. No influence of nickel intake via drinking water and second hand smoke exposure was observed.

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Introduction

Nickel is a metal with no clearly identified biological function in humans. Health effects have been summarized in several reviews (e.g. Barceloux, 1999; ATSDR, 2005). Most concern is raised in occupational settings. Airborne exposure in the workplace to mixed soluble and insoluble nickel compounds occurs via dusts and fumes. The most important adverse health effects due to occupational exposure are skin allergies, lung fibrosis, lung and nasal cancer (review e.g. Zhao et al., 2009). Assessment of carcinogenicity of water-soluble nickel compounds has been provided recently (Goodman et al., 2009). Results from epidemiological, toxicological, and carcinogenic mode-of-action data indicate that soluble nickel compounds are not complete carcinogens, and evidence that they could act as tumour promoters is only limited.

The general population is mainly exposed to soluble nickel compounds as a normal constituent of food or as a contaminant in drinking water. Nickel has gained special interest in

environmental medicine due to its wide distribution in the environment and its allergenic potential. The 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%) (Thyssen et al., 2007). Nickel contact allergy is mainly caused by nickel-containing, and potentially nickel-releasing objects (e.g. costume jewellery, ear piercing). In Germany, data from trend analyses show that the frequency of allergy to nickel in males (1–17 years) ranged between 1.3 and 8.6% per year between 1994 and 2009, while in females (1–17 years) the frequency was between 10.2 and 29.2% with a decreasing trend only between 1994 and 2000 (Schnuch et al., 2011). Oral intake of nickel via food and drinking water can exacerbate nickel dermatitis in nickel-sensitive individuals (Jensen et al., 2006; Krecisz et al., 2011). Results from food duplicate portion measurement revealed an increased nickel dietary intake when compared with recommended intake rates (Wittsiepe et al., 2009). Furthermore, there are indications that airborne nickel exposure increases the prevalence of nickel sensitization in children. In a cross-sectional study performed in 2000, a positive association between nickel in ambient air, urinary nickel content and the prevalence of nickel sensitization in a subgroup of 6-year-old children living near a steel mill was

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found (Wilhelm et al., 2007; Mann et al., 2010). Recently, this observation could be confirmed in a larger study which was performed in 2005 and 2006 (Kasper-Sonnenberg et al., 2011).

For exposure assessment in the general population measurement of nickel in urine is recommended (HBM Commission, 2001; Angerer et al., 2007). This particularly applies to studies with children, since internal exposure assessment is based on non-invasive urine sampling. Thus human biomonitoring can be easily applied in children, even at ages below 6 years. However, data on nickel levels in urine of children are rare. Some human biomonitoring data on nickel in urine are available from studies with children exposed to industrial sources (Wilhelm et al., 2007a,b; Aguilera et al., 2010; Moreno et al., 2010; Kasper-Sonnenberg et al., 2011) or from studies aiming to assess background exposure on a more local scale (Alimonti et al., 2000). The U.S. National Health and Nutrition Examination Survey (NHANES) which provides extensive data sets on human biomonitoring results of representative populations does not include the measurement of nickel (CDC, 2011).

For the first time, the German Environmental Survey (GerES IV) 2003–2006 provided representative data to describe the internal nickel exposure of children aged 3–14 years on a nationwide scale. Based on the survey the German Human Biomonitoring Commission has recently set a reference value for nickel in children's urine of 4.5 µg/l (Schulz et al., 2009, 2011), while for adults a value of 3.0 µg/l was derived from literature data (Wilhelm et al., 2004).

An overview of human biomonitoring results from GerES IV has been given in a report by Becker et al. (2008, 2009). Here we analyze for the first time urinary nickel levels in children from Germany in detail and evaluate relevant predictors.

Methods

Study design and study population

The German Environmental Survey on children (GerES IV) was carried out from 2003 to 2006 by the German Federal Environment Agency (UBA). 1790 children (907 girls and 883 boys) aged 3–14 years living in 150 study locations (communities or urban districts), respectively, took part in GerES IV (more details in Becker et al., 2008, 2009; Kolossa-Gehring et al., 2012; Schulz et al., 2012). These participants were a sub-sample randomly selected from participants of the German Health Interview and Examination Survey for Children and Adolescents (KiGGS), which was carried out by the Robert Koch Institute (RKI), Germany (Kurth et al., 2008). The KiGGS study sample included 17,641 0- to 17-year-old children. The Ethics Committee of the Charité University Hospital of the Humboldt University of Berlin, and the Federal and Province Commissioners for Data Protection had approved the project.

Questionnaire data

In GerES IV, several standardized interviewer guided questionnaires were used (Becker et al., 2008). Additionally, information on GerES IV participants collected in the KiGGS study could also be included. Thus information such as child's drinking water consumption, exposure relevant behaviour (e.g. wearing body jewellery, such as earrings), food consumption, socio-demographic characteristics, socio-economic status (SES) and health related behaviour and risks (e.g. exposure to tobacco smoke, overweight) were taken for the present evaluation.

Sampling

Urine

Morning urine (total quantity excreted) was collected as follows. From children aged 3–4 years who no longer wore diapers at night

(from girls up to 6 years of age if required or after consultation with their parents), in decontaminated (cleaning in dishwasher, rinsing with 1.3% nitric acid and double-distilled water) 750 ml toilet seat inserts (Tyco Healthcare Neustadt/Donau, Germany) and from children aged 5 years and above (girls aged 7 and above, if required) in 1 l decontaminated polyethylene wide-neck square vessels (Kautex, Bonn-Holzlar, Germany). No morning urine specimens were taken from children still wearing diapers at night because collection of a standardized urine specimen largely free from external contamination was impossible under such conditions. All parents were provided with the respective information sheet on correct sampling. All urine samples were transferred at the examination centre into 15 ml tubes (Sarsted, Nümbrecht, Germany) for the analysis of nickel and were stored at –20 °C until analysis. The tubes had been tested for nickel contamination. No contamination was found. Before analysis at our laboratory, the urine samples were acidified (1% nitric acid, vol/vol). The urine samples were coded and analyzed in a randomized sequence to avoid observer bias.

Tap water

Drinking water was taken in the households from the tap normally used by the participants to draw water for cooking and drinking. Two kinds of samples were collected: random tap water (1 l sample collected without letting the water run first) and stagnation water (water standing in the water pipe at least 4 h during the night and taken without letting it run first; first draw samples, 0.5 l). Samples were collected in decontaminated 0.5 and 1 l square vessels (Kautex, Bonn-Holzlar, Germany). The samples were stored at room temperature (as cool as possible) until analysis. Before analysis at AnBUS e.V. Fürth, Germany, the tap water samples were acidified (0.6% nitric acid, vol/vol).

Chemical analysis

Nickel in urine

Determination of nickel in the organic phase of urine was performed by graphite furnace atomic absorption spectrometry with Zeeman background correction (Perkin Elmer SIMAA 6000 or 6100 spectrometer) according to "Analyses of Hazardous Substances in Biological Materials, Part II" (Angerer and Schaller, 2008). Four ml of acidified urine were extracted with 500 µl of MHA-HMCD solution (13.6 g/l hexamethylenammonium-hexamethylene-dithiocarbamate in xylene/isobutyl-methylketone (3 + 7), enrichment factor 8). Calibration was performed with 4 samples of spiked natural urine in the range of 0–10 µg/l nickel. For internal quality control, standard reference material was used (Clin Check® – Control, urine control for trace elements, Level I, Recipe, Munich, Germany). The limit of quantification (LOQ) was 0.5 µg/l. Quality control was maintained through successful participation at the German External Quality Assessment Scheme performed by the Institute for Occupational, Environmental and Social Medicine, Friedrich-Alexander-University Erlangen-Nuremberg.

Nickel in tap water

Nickel in tap water was measured by AnBUS e.V. Fürth, Germany, according to standards of the German Institute of Standardization (DIN No. 38406 E29) by ICP-MS (for more information see Schulz et al., 2008). Limit of quantification (LOQ) was 0.5 µg/l. AnBUS e.V. is an accredited testing laboratory and took part successfully in external quality control schemes for nickel in drinking water.

Cotinine

Exposure to second hand smoke was assessed by analysis of cotinine in urine (details in Conrad et al., 2010) by the Institute of

Medical Microbiology, Virology and Hygiene, University Hospital of Rostock as well as by parental or self report.

Creatinine

Urinary creatinine was measured according to the Jaffé method (Larsen, 1972) by the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Friedrich-Alexander-University Erlangen-Nuremberg.

Statistical analyses

The procedures for the descriptive statistics have been described in detail by Becker et al. (2008). Briefly, GerES IV-specific case weights were applied for the descriptive calculations. The nickel levels were described for all children and selected sub-groups. Selection of sub-groups was based on significant predictors of nickel levels calculated by multivariate linear regression analysis. Additionally, active and passive smoking, and nickel consumption via drinking water were included. Bivariate analyses were performed to compare the sub-groups. After log transformation the nickel levels were approximately normally distributed and were used to test for differences between geometric means of sub-groups by one way ANOVA and Student's *t*-test. Values below LOQ were set to 1/2 LOQ for calculations.

To investigate predictors of urinary nickel levels multivariate linear regression analysis was used. The log-transformed concentration of nickel in urine was used as the dependent variable to achieve normally distributed residuals. To facilitate interpretation, each resulting regression coefficient *b* was transformed by exponentiation ($\exp(b)$). For a binary predictor, the transformed coefficient gives the ratio – or relative change – of the estimated geometric mean concentration in the exposed group as compared to the estimated geometric mean in the reference group. It is therefore called geometric means ratio (MR) and its interpretation is analogous to a risk ratio or odds ratio.

Multiple regression analyses were only performed with the data of nonsmokers, as only very few children in GerES IV smoked and as active smoking was strongly associated with age. In addition, the children's urinary creatinine concentrations had to be within 0.3 and 3 g/l and the data necessary for the regression analyses had to be complete.

The covariates comprised gender, age, body-mass-index (BMI), urinary creatinine, socio-economic status (SES), migration status, residential area, environmental tobacco smoke exposure, nickel levels in household drinking water, nickel intake via household drinking water, consumption of several food items (such as nuts, chocolate, cake, cookies, fish, milk products, meat, and tea),

potential contact to nickel via piercing and wearing of metal-containing jewellery. The relevant predictors were identified by a stepwise approach. To reach the final model, we used backward elimination with a threshold of $p < 0.05$ for retaining a variable in the model. We tested the models for collinearity to make sure that predictors (such as food items) were not too highly correlated. Case weights were not considered. Data analyses were performed using SPSS 17 (SPSS Inc.).

Results

Response

When evaluating the response rate it has to be taken into account that in GerES IV only those children of the respective age group were examined who had also participated in KiGGS. For all KiGGS participants selected also for GerES IV, the participation rate achieved in GerES IV was 77.3%. If referred to the KiGGS response rate of the subjects aged 3–14 years (67.7%), the response rate of GerES IV was 52.6%, so that altogether, the response rate achieved in GerES IV was lower. For further details see Schulz et al. (2012).

Urinary nickel concentrations

Nickel was detected in most (91%) of the 1567 urine samples available from GerES IV. Concentrations ranged from below 0.5 µg/l to 15 µg/l. The geometric mean and the 95th percentile concentrations were 1.26 and 4.50 µg/l respectively (Table 1). Urinary nickel levels for the sub-groups defined by gender, age and SES are also summarized in Table 1. Geometric means did not differ between boys and girls (1.27 and 1.26 µg/l). For the different age groups, geometric means were highest in the children aged 3–5 years (1.40 µg/l), values decreased to 1.34 (6–8 years) and 1.14 µg/l (9–11 years), and then increased again (1.23 µg/l) in children aged 12–14 years. Nickel levels were lower in children with higher SES compared to children with low or medium SES (1.11 µg/l and 1.33 µg/l respectively).

Table 2 summarizes nickel levels in urine by overweight, intake of food groups naturally high in nickel and intake of drinking water (stagnation sample). Geometric mean of children who were overweight (gender- and age-specific 90th BMI percentile according to Kromeyer-Hauschild et al. (2001) was lower compared to those who were not overweight (1.14 and 1.29 µg/l; $p < 0.05$). Intake of nickel-rich food (nuts, hazelnut spread, cereals, and chocolate) was reflected by higher geometric mean values. As shown in

Table 1

Nickel levels in urine of 3–14-year-old children in Germany by gender, age and socio-economic status (SES).

	N	% ≥ LOQ	P10	P50	P90	P95	MAX	AM	GM	95% CI GM
All	1567	91	0.51	1.30	3.15	4.50	15.0	1.68	1.26	1.21–1.31
Gender										
Boys	814	92	0.54	1.30	3.10	4.00	9.10	1.62	1.27	1.21–1.34
Girls	753	91	0.51	1.30	3.40	5.10	15.0	1.75	1.26	1.19–1.34
Age groups ($p < 0.01$)										
3–5 years	311	92	0.57	1.50	3.50	5.07	15.0	1.90	1.40	1.28–1.53
6–8 years	390	90	<0.5	1.40	3.87	4.90	13.0	1.82	1.34	1.23–1.46
9–11 years	402	90	0.50	1.20	2.90	3.78	9.00	1.47	1.14	1.06–1.23
12–14 years	464	93	0.52	1.20	3.10	3.97	11.0	1.61	1.23	1.15–1.32
SES ($p < 0.001$)										
Low	364	95	0.58	1.36	3.40	4.59	15.0	1.73	1.33	1.23–1.43
Medium	723	92	0.51	1.40	3.50	5.00	12.0	1.80	1.33	1.25–1.41
High	463	88	<0.5	1.20	2.70	3.45	11.0	1.46	1.11	1.03–1.19

Data from the German Environmental Survey IV 2003/2006 (Becker et al., 2008).

Notes: N = number of samples; % ≥ LOQ = percentage of values at or exceeding LOQ; P10, P50, P90, P95 = percentiles; MAX = maximum; AM = arithmetic mean; GM = geometric mean; 95% CI GM = 95% confidence interval of GM; LOQ = limit of quantification; LOQ = 0.5 µg/l; for calculation of AM, GM, and 95% CI GM levels below LOQ were replaced with half of the LOQ; p = p value of Student's *t*-test or one way ANOVA with log transformed nickel levels.

Table 2
Nickel levels in urine of 3–14-year-old children in Germany by body weight, intake of food groups naturally high in nickel and intake of drinking water (stagnation sample).

	N	% $\geq$ LOQ	P10	P50	P90	P95	Max	AM	GM	95% CI GM
<i>BMI/Overweight ($p < 0.05$)</i>										
Overweight ^a	233	88	<0.5	1.30	2.70	3.30	15	1.51	1.14	1.04–1.26
Not overweight	1328	92	0.53	1.30	3.30	4.60	13	1.72	1.29	1.23–1.34
<i>Consumption of nuts ($p < 0.001$)</i>										
$\geq$ One portion per month	474	95	0.59	1.40	3.80	4.90	13	1.85	1.40	1.31–1.50
<One portion per month	1027	89	<0.5	1.20	3.00	4.00	15	1.60	1.20	1.14–1.25
<i>Consumption of hazelnut spread ($p < 0.001$)</i>										
$\geq$ One teaspoon per day	284	96	0.70	1.50	3.70	4.90	9.90	1.90	1.51	1.39–1.63
<One teaspoon per day	1224	90	0.50	1.20	3.10	4.30	15	1.63	1.21	1.16–1.26
<i>Consumption of cereals ($p < 0.01$)</i>										
$\geq$ Once a week	1105	76	0.54	1.30	3.20	4.50	15	1.72	1.31	1.24–1.38
<Once a week	410	88	<0.5	1.20	3.10	4.70	12	1.58	1.15	1.06–1.24
<i>Consumption of chocolate ($p < 0.001$)</i>										
$\geq$ 25 g per day	372	93	0.57	1.50	3.90	4.90	12	1.87	1.41	1.30–1.52
<25 g per day	1139	91	0.51	1.20	3.10	4.10	15	1.62	1.22	1.17–1.27
<i>Nickel intake via drinking water^b ($p < 0.05$)^c</i>										
$\leq$ 0.5 μ g per day	599	92	0.52	1.30	3.20	4.70	11	1.69	1.28	1.20–1.36
>0.5–2 μ g per day	443	93	0.57	1.30	3.40	4.40	12	1.80	1.36	1.26–1.45
>2 μ g per day	512	89	<0.5	1.20	3.00	4.10	15	1.57	1.17	1.09–1.25

Data from the German Environmental Survey IV 2003/2006 (Becker et al., 2008).

Notes: N = number of samples; % $\geq$ LOQ = percentage of values at or exceeding LOQ; P10, P50, P90, P95 = percentiles; MAX = maximum; AM = arithmetic mean; GM = geometric mean; 95% CI GM = 95% confidence interval of GM; LOQ = limit of quantification; LOQ = 0.5 μ g/l; for calculation of AM, GM, and 95% CI GM levels below LOQ were replaced with half of the LOQ; p = p value of one way ANOVA or Student's t -test with log transformed nickel levels.

^a Overweight is defined as a BMI above the 90th age- and gender-specific percentile of the German reference system (Kromeyer-Hauschild et al., 2001).

^b Intake data are based on the nickel levels in drinking water which was taken after a stagnation time period of at least 4 h.

^c p for linear trend not significant.

Table 2, nickel intake >2 μ g per day via drinking (stagnation sample) resulted in a lower urinary excretion compared to a lower intake rate. However, nickel intake via drinking water was not a significant predictor for nickel levels in urine as tested by multiple linear regression analysis.

Concerning tobacco smoke exposure results were somewhat inconsistent. 65 children were smokers (according to their own or their parent's answers in the questionnaires or their cotinine levels). Most of these children (68%) were low-frequency smokers (non-daily smoking or smoking <6 cigarettes daily; data not shown). Geometric mean of urinary nickel of smokers was significantly lower compared to non-smokers (1.17 and 1.27 μ g/l; $p < 0.001$). Based on tobacco exposure assessment by urinary cotinine levels (<2 μ g/l, $n = 775$ versus ≥ 2 μ g/l, $n = 727$; smokers excluded) a significantly higher geometric mean ($p < 0.05$) was found for levels ≥ 2 μ g/l compared to levels <2 μ g cotinine/l (1.33 and 1.22 μ g nickel/l urine). The number of smokers in the household was not significantly associated with urinary nickel levels (smokers excluded). Children with second hand smoke exposure at home ($n = 365$; parental report; smokers excluded) had a higher geometric mean urinary nickel level compared to those without second hand smoke exposure ($n = 1137$; 1.40 and 1.23 μ g/l; $p < 0.01$). However, tobacco smoke exposure tested on 3 different assessments (cotinine levels, number of smokers in household, environmental tobacco smoke exposure at home) was not a significant predictor for nickel levels in urine as tested by multiple linear regression analysis.

Among the description of nickel values samples with urinary creatinine levels below 0.3 and above 3.0 g creatinine/l were not excluded from further analysis. Among the 1567 children of GerES IV for whom a nickel and a creatinine level of a morning urine sample was available, only 9 children had a creatinine level below 0.3 g/l and 6 above 3.0 g/l. Urinary creatinine levels are given in Table 3.

Predictors of nickel levels in urine

Table 4 shows the results of the multiple linear regression analysis. The final model included gender, age, SES, being overweighted,

consumption of nuts, hazelnut spread, cereals, chocolate and creatinine levels. 20.2% of the variance could be explained by these variables. With a contribution of 13.8% the urinary creatinine concentration was the most important predictor. All other predictors had a low contribution to the explained variance.

Discussion

Other studies

Only few studies have assessed urinary levels of nickel in children (Table 5). Previously, results from several studies on nickel levels found in urine of 6-year-old-children (school beginners) living in different areas of North Rhine-Westphalia, Germany have been reported (Wilhelm et al., 2007a,b; Kasper-Sonnenberg et al., 2011). Nickel levels in urine were slightly higher than those measured in the GerES IV study. It has to be considered that these previous studies were performed with children living in industrialized cities, and in the vicinity to industrial sources, including nickel-emitting steel mills, but also a reference group was included (Wilhelm et al., 2007b; Kasper-Sonnenberg et al., 2011). However, in contrast to the population representative GerES IV results, these data were not population representative. Nickel exposure in other countries, like Australia (Callan et al., 2011), Spain (Aguilera et al., 2010) and Italy (Alimonti et al., 2000) was slightly higher than that observed in GerES IV. In contrast, a high internal exposure with all nickel levels exceeding the reference value of 4.5 μ g/l (RV₉₅) (Schulz et al.,

Table 3
Urinary creatinine concentrations (g/l) grouped by age for children in Germany.

Age group	N	Range	AM	95% CI AM
All children	1567	0.19–3.65	1.16	1.13–1.18
3–5 years	311	0.19–1.86	0.83	0.79–0.86
6–8 years	390	0.24–2.40	1.08	1.02–1.10
9–11 years	402	0.19–2.79	1.15	1.11–1.19
12–14 years	464	0.23–3.65	1.46	1.41–1.51

Data from the German Environmental Survey IV 2003/2006.

Notes: N = number of samples; AM = arithmetic mean; 95% CI AM = 95% confidence interval of AM.

Table 4

Multiple regression analysis of predictors of urinary nickel concentration in 3–14-year-old children (non smokers only) in Germany.

	N	MR	95% CI MR	p	β	β^*r^*100
<i>Gender</i>						
Girls	697	1.09	1.02–1.18	0.017	0.06	0.2%
Boys	708	Reference				
<i>Age</i>						
3–5 years	297	1.77	1.57–1.99	<0.001	0.30	2.1%
6–8 years	377	1.35	1.22–1.50	<0.001	0.17	0.5%
9–11 years	384	1.09	0.99–1.21	0.090	0.05	–0.4%
12–14 years	347	Reference				
<i>Socio-economic status</i>						
Low/medium	986	1.12	1.04–1.22	0.005	0.07	0.6%
High	419	Reference				
<i>Overweight^a</i>						
No	1203	1.16	1.05–1.29	0.004	0.07	0.4%
Yes	202	Reference				
<i>Consumption of nuts</i>						
≥One portion per month	424	1.16	1.08–1.26	<0.001	0.09	0.7%
<One portion per month	981	Reference				
<i>Consumption of hazelnut spread</i>						
≥One teaspoon per day	261	1.20	1.09–1.31	<0.001	0.09	1.0%
<One teaspoon per day	1144	Reference				
<i>Consumption of cereals</i>						
≥Once a week	1034	1.12	1.03–1.21	0.008	0.06	0.5%
<Once a week	371	Reference				
<i>Consumption of chocolate</i>						
≥25 g per day	344	1.11	1.02–1.21	0.016	0.06	0.6%
<25 g per day	1061	Reference				
Creatinine in urine (log)	1405	2.17	1.98–2.39	<0.001	0.43	13.8%

Data from the German Environmental Survey IV 2003/2006.

Notes: N = number of samples (total n in model = 1405; unweighted data); MR = geometric means ratios; 95% CI MR = 95% confidence interval of MR; β = standardized regression coefficient, p = p-value; r = bivariate correlation coefficient of dependent variable and predictor; β^*r^*100 = contribution of predictor to explained variance.^a Overweight is defined as a BMI above the 90th age- and gender-specific percentile of the German reference system (Kromeyer-Hauschild et al., 2001); samples only included if creatinine = 0.3–3.0 µg/l; final R = 0.449, R² = 0.202, R² corrected = 0.195.

2009, 2011) was observed in children living in a mine tailing zone in Southern Mexico (Moreno et al., 2010).

Some of the above mentioned studies provided data on predictors of urinary nickel levels. In the hot spot study (Wilhelm et al., 2007b), 35.3% of the variance could be explained by gender, nationality, body weight, creatinine in urine, nickel in tap water (stagnation samples), parental education and place of residence (Eberwein et al., 2004). In the North Rhine-Westfalian study performed in 2005–2006, the results of the linear regression analysis showed that 21% of urinary nickel excretion could be explained by gender, nationality, parental education, body mass index, nickel in ambient air, and urinary creatinine concentration (Kasper-Sonnenberg et al., 2011). The only significant predictor of urinary nickel concentration in the study of Callan et al. (2011) was creatinine. Thus similar to the present GerES IV evaluation, the urinary creatinine concentration was by far the most important predictor for nickel levels in urine. Using regression analysis of creatinine

adjusted nickel levels, 8% of the variance could be explained in the Spanish study (Aguilera et al., 2010). Place of residence, season, food intake such as vegetables, fish, and canned food during the week previous to sampling of urine showed a significant association to urinary nickel.

Creatinine adjustment

For multiple regression analysis the nickel levels were included on a weight/volume basis (µg/l) instead of using adjustment for urinary creatinine. Urinary creatinine was added as a separate independent variable. This approach is in line with the recommendation given by Barr et al. (2005) and by the German Human Biomonitoring Commission (HBM, 2005). Urinary creatinine levels are known to vary for example by age, sex and BMI. In the present nickel study, creatinine levels in urine of the 3–14 years old children (all 1527 children, no values excluded) steadily increased with increasing age

Table 5

Nickel concentrations in urine (µg/l; unless otherwise stated) of children reported in the literature.

Group, age	N	P50	P95	Year of sampling	Area, remarks	Reference
3–14 years	1567	1.3	4.5	2003–06	Representative survey for children in Germany	Present study
School beginners	104	1.9	4.6	1991	Industrialized cities, NRW ^a	Wilhelm et al. (2007a)
School beginners	124	2.7	10.2	2000	Vicinity to industrial sources, NRW ^a	Wilhelm et al. (2007b)
	141	2.5	8.2	2000	Reference area, NRW ^a	
School beginners	594	2.9	7.6	2005–2006	Vicinity to steel mills, NRW ^a	Kasper-Sonnenberg et al. (2011)
1–12 years	39	3.0	<LOD–9.0 ^b	2009	Australia	Callan et al. (2011)
6–10 years	131	0.2–1.6 ^c	1.6 ^c	n.a.	Urban area of Rome, Italy	Alimonti et al. (2000)
5–17 years	224	1.3	10.7	2003–04	Highly polluted area, Spain	Aguilera et al. (2010)
	194	1.9	9.0		Urban area Andalusia, Spain	
6–11 years	50	75.4 ^d	120	2003–04	Mine tailing zone, Mexico	Moreno et al. (2010)

Notes: N = number of samples; P50, P95 = percentiles, unless otherwise stated; see below; n.a. not available; LOD = limit of detection.

^a North Rhine-Westfalia, Germany.^b Range of all data.^c Range P5–P95 and P95 both in µg/g creatinine.^d Mean.

group 3–5, 6–8, 9–11, and 12–14 years (Table 3). Since creatinine is produced from muscle metabolism, this increase mainly reflects the increase in muscle mass during children's growth. For a better comparison with other studies in which nickel levels in urine are only provided after creatinine adjustment we here additionally give the corresponding values for the whole study group (1567 children, age 3–14 years): range 0.11–40.7, P 50 1.19, P 95 4.24, AM 1.65, and GM 1.20 $\mu\text{g/g}$ creatinine. Creatinine adjusted nickel levels (GM) decreased with increasing age group as follows: 3–5 years 1.83 $\mu\text{g/g}$ creatinine, 6–8 years 1.35 $\mu\text{g/g}$ creatinine, 9–11 years 1.08 $\mu\text{g/g}$ creatinine, and 12–14 years 0.91 $\mu\text{g/g}$ creatinine.

Influence of tobacco smoke exposure on urinary nickel levels

Nickel is present in cigarettes and levels may vary according to factors such as geographical region and fertilizer treatment. Nickel levels in the range of about 1–5 mg/kg have been reported for cigarettes and tobacco (Stojanovic et al., 2004; Vania et al., 2011). Some studies with adults indicate that smoking increases urinary nickel (Alfridi et al., 2010; Stojanovic et al., 2004; Talio et al., 2010) while others could not confirm this observation (Torjussen et al., 2003). Results from GerES IV did not supply clear evidence for an influence of tobacco smoke exposure on urinary nickel. However, the number of smokers was low and most of these children were low-frequency smokers. Furthermore, second-hand smoke exposure was not a significant predictor for nickel levels in urine of nonsmokers as tested by multiple linear regression analysis.

Drinking water and food

Absorption rate of nickel from drinking water is much higher compared to food (Sunderman et al., 1989; Nielsen et al., 1999). Therefore one may expect that even a low concentration of nickel in drinking water and/or a low consumption rate of drinking water could be reflected in urinary nickel excretion. In contrast to the study from North Rhine-Westfalia (Eberwein et al., 2004), here we could not confirm an influence of nickel intake via drinking water on internal nickel exposure. However nickel levels in drinking water (stagnation sample) were higher in the hot spot study (stagnation sample: median 5.7 $\mu\text{g/l}$ in 147 samples from residents living in industrialized city and 7.3 $\mu\text{g/l}$ in 119 samples taken in reference group; corresponding values for random samples: most levels below 2.5 $\mu\text{g/l}$; Eberwein et al., 2004) compared to GerES IV (stagnation sample 3.9 and random sample 2.5 $\mu\text{g/l}$; Schulz et al., 2008). When using creatinine-adjusted concentrations as dependent variable, Callan et al. (2011) could explain 26% of variance of urinary nickel excretion by distance to the port and drinking water levels.

Food is a main source of nickel exposure for the general population. In their duplicate study Wittsiepe et al. (2009) identified from food and nutrition tables (Souci et al., 2000) the following nickel-rich foodstuffs: cereals, rice, oats, pulse, cocoa containing products, seeds, nuts, and black tea. According to EFSA (2005) nickel concentration is highest in cocoa, soya beans, oatmeal, hazelnuts, almonds and legumes. In the present study we confirm the previous finding concerning the influence of nickel-rich nutrition on urinary nickel concentrations (Kasper-Sonnenberg et al., 2011). Consumption of nuts, hazelnut spread, cereals, and chocolate were significant predictors of urinary nickel levels in GerES IV (Table 4). Kasper-Sonnenberg et al. (2011) showed by the multivariate regression analysis an 8% increase in urinary nickel concentration if nickel-rich foodstuff consumption (using a score system 0–6 scores) increased by one unit of the consumption score.

Though food is the main source for nickel exposure, food intake was not a major predicting factor on urinary nickel. Several aspects have to be considered. Nickel is mainly excreted via feces and not

via urine (Patriarca et al., 1997). Nickel levels in food show great variation and assessment of nickel intake by questionnaire or even by dietary records is limited (Wittsiepe et al., 2009). Plant tissue contains more nickel than animal tissues (Sharma, 2007). Nickel content of food is influenced by the nickel concentration in soil. Thus the concentration of nickel varies with geographic location (Sharma, 2007). Furthermore, transition of nickel from kettles or other household utensils made from stainless steel into the food may increase the nickel content of food (Wittsiepe et al., 2009). Aguilera et al. (2010) showed that urinary concentrations of nickel increased with the intake of canned food. In areas with high fish consumption, fresh fish intake increase urinary nickel (Aguilera et al., 2010). In our analysis we did not consider geographic variation. As shown by Wittsiepe et al. (2009), influence of regional differences on dietary nickel intake in an area of Germany could only be detected by using the time consuming duplicate portion measurements which is not applicable among a survey as described here.

Limitations of the study

One may argue that a single morning urine measurement has the potential to misclassify exposure. The majority of absorbed nickel is rapidly excreted in the urine. There are striking differences in absorption rate depending on route of exposure: 27% from drinking water, only 0.7% from food (Sunderman et al., 1989). After administration of nickel in the drinking water an average elimination half-life of 28 h has been reported (Sunderman et al., 1989). In a study with 4 healthy adults who were given isotope ^{62}Ni , 65% (range 51–82%) of the absorbed dose was excreted in urine over 5 days (Patriarca et al., 1997). Longer half-lives have been observed in exposed workers: in a highly exposed flame sprayer 650 days (Schaller et al., 2007) and in stainless steel welders 59.5–95.5 h depending on the exposure period (Colli et al., 2005). However, the present study revealed in line with a further observation that presumably continuous intake of food items known to be rich in nickel was related to nickel levels in urine. Therefore, one may assume that under steady state conditions, estimation of nickel by a single urine sample seems to be suitable to detect long-term nickel exposure.

Conclusion

We provide for the first time urinary nickel concentrations for children on a nationwide level. Creatinine in urine was the strongest determinant of urinary nickel levels. Intake of nickel-rich food is reflected in urinary nickel levels. From other studies it can be assumed that exposure to industrial sources may increase urinary nickel excretion. The data presented here can be used to interpret human biomonitoring measurement.

Conflict of interest

All authors declare they have no competing financial interests.

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