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Toxic trace element reference levels in blood and urine: influence of gender and lifestyle factors

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Abstract

This study is part of the EURO-TERVIHT project (Trace Element Reference Values in Human Tissues) which aims at establishing reference intervals for trace elements in blood, urine and other human tissues. In this study, reference intervals (0.05–0.95 fractiles) were estimated for lead in blood (105–529 nmol/l for men, 80–340 nmol/l for women), manganese in blood (100–271 nmol/l) and arsenic in urine (36–541 nmol/l for men, 21–475 nmol/l for women). Upper reference limits (0.95 fractile) were established for chromium in urine (13 nmol/l), nickel in urine (52 nmol/l) and cobalt in urine (23 nmol/l for men, 31 nmol/l for women). The reference group was a Danish subpopulation ($n = 189$), age 40–70 years. The influence of gender, age, health status parameters, nutrition and various lifestyle factors was investigated. Urinary arsenic and blood lead levels were found to be higher for men than for women. Arsenic levels also increased with age up to 60 years, and then decreased. Alcohol intake lead to increased arsenic levels in urine as well as blood lead levels. Urinary nickel levels were higher in persons frequently eating porridge and porridge oats. © 1997 Elsevier Science B.V.

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1. Introduction

Environmental and occupational exposure to toxic elements is of growing concern as large quantities of toxic and carcinogenic elements are released to the environment due to human activities. People may be exposed to toxic elements because of their occupation, and the toxic substances may pollute air, soil or water in certain areas and thus constitute a health risk for the population living in that particular area. For many toxic elements, biological monitoring is an efficient tool of monitoring occupational and environmental exposure. However, reliable reference levels are a prerequisite for evaluating biological measurement results within both occupational and environmental medicine. Unfortunately, reference levels cannot be established once and for all. Firstly, reference levels may differ between countries and regions, and they must therefore be established at a national level. Secondly, exposure may change over time due to fluctuations in environmental exposure and change of lifestyle factors. Thus, reference intervals must be established at regular intervals and with respect to the appropriate influence factors (e.g. smoking habits). The EURO-TERVIHT project (Trace Element Reference Values in Human Tissues) aims at establishing and comparing trace element reference values in tissues from inhabitants in the European Community (Sabbioni et al., 1992). At present, trace element values have been reviewed for Italy (Minoia et al., 1990), Belgium (Cornelis et al., 1994), Denmark (Poulsen et al., 1994) and the United Kingdom (Hamilton et al., 1994). A recent review on trace element reference values in blood, serum and urine in the Danish population (Poulsen et al., 1994) showed several shortcomings in some of the previous studies, mainly due to a low number of reference persons or insufficient characterization of the reference group. The aim of the present study, as a part of the EURO-TERVIHT project, is to establish reference intervals for some occupationally important toxic elements in blood and urine, and to investigate the effects of gender, age and some personal lifestyle factors.

2. The reference group

The reference group in this study was a subgroup of the Danish MONICA-10 cohort (MONICA: monitoring of trends and determinants in cardiovascular diseases). Subjects who had attended an earlier study were invited to participate in the study in 1993 (total 4156 subjects, excluding those that have deceased between the two studies). Of these, 2656 attended a medical examination and completed standard questionnaires about former and present health, occupation, family status, health status, nutrition, smoking and drinking habits and the use of medicine. Information on hospital admissions was drawn from public registers. Of the subjects attending MONICA-10, blood and urine samples were obtained from 204 men and women covering equally the age groups 40, 50, 60 and 70 years. The subjects were also asked to complete questionnaires about their environmental and occupational exposure to metal. The reference group was reduced to 189 persons, as 15 persons indicated possible metal exposure. Table 1 shows the type of information obtained from the reference group.

3. Collection of blood and urine samples

3.1. Collection of samples

Teflon catheters (Terumo Europe, Belgium) were used to collect venous blood samples in 5 ml Nalgene cryovials (Nalge Company, New York, USA) containing 250 units of sodium heparin (Sigma Chemical Co., St. Louis, USA). Urine was collected in 100-ml polystyrene flasks with polypropylene lids at Glostrup Hospital (not first void).

Written and oral instructions on blood collection focusing on precautions against contamination were given before the study. Blood samples were taken in a clean room assigned for the purpose. The puncture site was cleaned thoroughly with ethanol. After puncture the steel needle was withdrawn and venous blood drawn

Table 1
Questionnaire data obtained from the reference population

Exposure parameters	Lifestyle factors
Occupational exposure (manufacturing of metals or metal objects	Alcohol drinking
Environmental exposure (traffic, incinerator, power plant)	Tobacco smoking
	Coffee and tea drinking
Other sources (leasure time activities)	Exercise
Nutrition	Health status
Mixed diet/vegetarian	Contact eczema due to white metal
Frequency of fish dinners	Hospital admissions
Food items (frequence of consumption)	Use of medicine (type)
Mineral supplement and vitamins	Use of contraceptives
	Pulse and blood pressure

through a teflon catheter. Blood was collected in 6 vials which were turned slowly five–eight times. Vial no. 5 was used for determination of metals in blood. Blood and urine samples were stored at −50°C until chemical analysis.

3.2. Quality control of sampling

Flasks and vials were tested for contamination from leachable elements before use by neutron activation analysis (Minoia et al., 1992). To control the risk of contamination from the air during collection of blood samples two vials containing Milli-Q water (Milli-Pore, Molsheim, France) were opened on each sampling occasion at the beginning and corked at the end of the blood sampling procedure. Two unopened vials with Milli-Q water served as blanks. Trace element content of the water was determined by neutron activation analysis.

4. Chemical analysis

In this study blood samples were analyzed for lead and manganese, and the concentration of arsenic, chromium, cobalt and nickel was determined in urine samples. The analytical methods have been described elsewhere (Christensen et al., 1983, 1992; Christensen and Pedersen, 1986; Mürer et al., 1992). All methods were based on electrothermal atomic absorption spectrometry (ETAAS) and Zeeman background correction was used in all cases, except for urinary arsenic. Urinary arsenic was determined by hydride-generation atomic absorption using flow injection (Mürer et al., 1992). In this technique only hydride-generating, i.e. toxicologically relevant, arsenic species are determined. All methods were validated before use by method evaluation (Christensen et al., 1992). Some key performance parameters of the methods used are shown in Table 2.

4.1. Quality control of analysis

Statistical control of the analytical methods were documented by internal and external quality control. Internal quality control was performed by analysing in-house quality control materials together with the samples. Depending on the analytical method internal quality control samples constituted 15–30% of all samples analyzed. Certified reference materials were used if available (e.g. CRM 194, 195, 196 from BCR for lead in blood). Participation in proficiency testing schemes run by Wolfson EQA Laboratory, Birmingham, UK (UK-NEQAS) (lead in blood), Centre de Toxicologie du Québec, Sainte-Foy, Canada (lead in blood, chromium in urine), National Institute of Occupational Health, Denmark (DEQAS) (lead in blood), Deutsche Gesellschaft

Table 2
Performance of methods used for chemical analysis

Measure	Method[a]	LOD[b] (nmol/l)	CV[c] (%)
Lead in blood	ETAAS	70	8% at 500 nmol/l
Manganese in blood	ETAAS	11	7% at 200 nmol/l
Arsenic[d] in urine	HGAAS	40	5% at 200 nmol/l
Chromium in urine	ETAAS	3.8	8% at 100 nmol/l
Cobalt in urine	ETAAS	3.4	8% at 150 nmol/l
Nickel in urine	ETAAS	2	9% at 60 nmol/l

[a] ETAAS, electrothemal atomic absorption spectrometry with Zeeman background correction. HGAAS, hydride generation atomic absorption spectromety.
[b] LOD, detection limit (3σ criteria).
[c] CV, coefficient of variation under 'between run' conditions (%).
[d] Hydride-generating species only.

für Arbeitsmedizin und Umweltmedizin, Erlangen, Germany (DGAU) (lead and manganese in blood; cobalt, chromium and arsenic in urine) served as external quality assurance. Results in the proficiency testing schemes were satisfactory in the period.

5. Statistical analysis

Non-parametric reference limits were preferred because their estimation is robust to the distribution of the measurement results. Reference limits corresponding to the 0.95 and 0.05 fractiles of the distribution were established using the REFVAL computer programme according to the recommendations of the International Federation of Clinical Chemists (Solberg, 1987). IUPAC recommends the use of 95% coverage intervals with a confidence level of 95% (Christensen, 1995; Poulsen et al., 1997), and these intervals were also calculated. No lower reference limit was reported if the 0.05 fractile fell below the detection limit. Univariate statistical analysis of the influence of gender, age, lifestyle factors, etc. was performed using the MINITAB statistical software (MINITAB Inc., Birmingham, UK). A probability level of 0.05 was used as a criteria for significance. When trace element concentrations were found to be logarithmic normal distributed (Anderson–Darling test), the influence of gender, age, diet and lifestyle factors was analyzed after logarithmic transformation by analysis of variance (ANOVA) using the general linear model (GLM) for unbalanced experimental designs. All two-factor interaction terms were included in a preliminary statistical analysis, and excluded in the final analysis if non-significant. For some elements, variable transformation could not be done because of results below the detection limit. In this case, non-parametric statistical tests (Mann–Whitney test, Kruskal–Wallis test) were used to investigate the effect of the influence factors.

Table 3
Definition of influence factors and their levels

Influence factor	Levels of the influence factor
Gender	Men, women
Age	40, 50, 60, 70 years
Smoking habits	Non-smokers Smokers
Alcohol intake[a]	Low intake: 0–0.5 unit per day Moderate intake: 0.51–2 unit per day Drinkers: > 2 units per day

[a] 1 unit is defined as 1 bottle of beer, 1 glass of wine or 1 glass of aquavit.

Table 4
Characterization of the reference group with respect to age, smoking and intake of alcohol

Influence factor	Men	Women
Age group (years)		
40	43.1 ± 2.4 (22)[a]	42.1 ± 2.2 (25)[a]
50	53.7 ± 3.1 (21)[a]	52.9 ± 2.8 (25)[a]
60	62.5 ± 2.6 (21)[a]	62.5 ± 3.1 (24)[a]
70	73.3 ± 3.1 (26)[a]	72.7 ± 2.8 (25)[a]
Total	58.8 ± 11.8 (90)[a]	57.5 ± 11.8 (99)[a]
Smokers	44	45
Non-smokers	41	48
Alcohol intake		
Low intake	31	39
Moderate intake	36	37
Drinkers	18	17

[a] Average age ± S.D. (number of persons).

5.1. Characterization of the reference group

Table 3 summarizes the levels of the different influence factors tested in the statistical analysis. Table 4 characterizes the reference group after exclusion of 15 persons with possible exposure to metals at work or at home, reducing the group to 189 persons. Of this group, 11 did not give any information on smoking habits or the intake of alcoholic beverages.

Cigarette smoking was the most frequent type of smoking behavior. The average number of cigarettes smoked per day was 17 in the smoking group. Approx. 90% of the cigarettes were with filters. Pipes were smoked by 23 persons (14 women and 9 men). Pipe smokers were asked to

estimate their weekly consumption of tobacco for pipe smoking. The average amount was approx. 9 g/day.

Beer and wine were the most frequently consumed alcoholic beverages, followed by aquavit. Assuming reasonably average values for the volumes and alcohol contents of a bottle of beer (330 ml, 4.6 vol%) a glass of wine (150 ml, 11 vol%) and a glass of aquavit (30 ml, 40 vol%), the average daily alcohol intake for the three groups were estimated at 2, 14 and 44 g/person, respectively.

6. Results

6.1. Estimation of reference intervals

The mean trace element concentration and standard deviation, the range of results and the median and reference limits are reported in Table 5. The reference limits were estimated according to the procedures recommended by IFCC (Solberg, 1987) and IUPAC (Poulsen et al., 1997). The coverage uncertainty associated with the IUPAC coverage interval indicates the uncertainty of the reference limit, e.g. a coverage uncertainty of 0.95 ± 0.043 for the upper blood lead reference limit for women (340 nmol/l) means that the interval 0–340 nmol/l covers on average 95% of the female population, and it covers between 90.7% and 99.3% of the female population with a confidence of 0.95.

The number of metal determinations in urine samples are less than 189 because the limited quantity of urine did not allow determination of all elements. For each element the influence of gender was tested. A significant difference between men and women was found with respect to blood lead and urinary arsenic. With respect to urinary cobalt, women were found to have slightly higher levels than men.

6.2. Factors influencing B-Pb levels

The influence of gender on B-Pb levels was highly significant ($P < 0.001$), but there was no significant effect from the age group (Fig. 1a). The effect of alcohol had borderline significance,

Table 5
Estimates of mean, standard deviation (S.D.), medians and reference intervals for toxic metals in blood and urine. All values in nmol/l. IFCC reference interval, non-parametric, 0.95 fractile is upper limit, 0.05 fractile is lower limit. If the lower limit is below detection limit only the upper limit is presented. IUPAC coverage interval, non-parametric, 0.95 fractile

Matrix and elements[a]	Gender[b]	n	Mean	(±S.D.)	Median	Range	IFCC reference interval	IUPAC coverage interval	Coverage uncertainty
B-Pb	F	99	203	(±90)	190[d]	< 70–50	80–340	< 340	0.95 ± 0.043
B-Pb	M	90	269	(±117)	240[d]	100–580	105–529	< 520	0.95 ± 0.043
B-Mn	F and M	188	165	(±51)	157	74–372	100–271	< 266	0.95 ± 0.032
U-As[c]	F	93	150	(±138)	105[e]	< 20–693	21–475	< 449	0.95 ± 0.044
U-As[c]	M	89	204	(±165)	155[e]	< 20–989	36–541	< 495	0.95 ± 0.045
U-Co	F	97	9.4	(±9.9)	6.7[f]	< 3.4–46.5	< 31	< 31	0.95 ± 0.043
U-Co	M	89	7.0	(±7.2)	3.8[f]	< 3.4–31.5	< 23	< 23	0.95 ± 0.045
U-Cr	F and M	186	5.2	(±4.1)	4.2	< 3.8–25.9	< 13	< 13	0.95 ± 0.032
U-Ni	F and M	118	18	(±18)	15	< 2–97	< 52	< 52	0.95 ± 0.039

[a] B-Pb, lead in blood; B-Mn, manganese in blood; U-As, arsenic in urine; U-Co, cobalt in urine; U-Cr, chromium in urine; U-Ni, nickel in urine.
[b] F, women; M, men.
[c] Hydride-generating species.
[d] Significant difference between men and women, $P = 0.0001$.
[e] Significant difference between men and women, $P = 0.0033$. Mann–Whitney U-test.
[f] Significant difference between men and women, $P = 0.047$, ANOVA on non-transformed data ($P = 0.053$, Mann–Whitney).

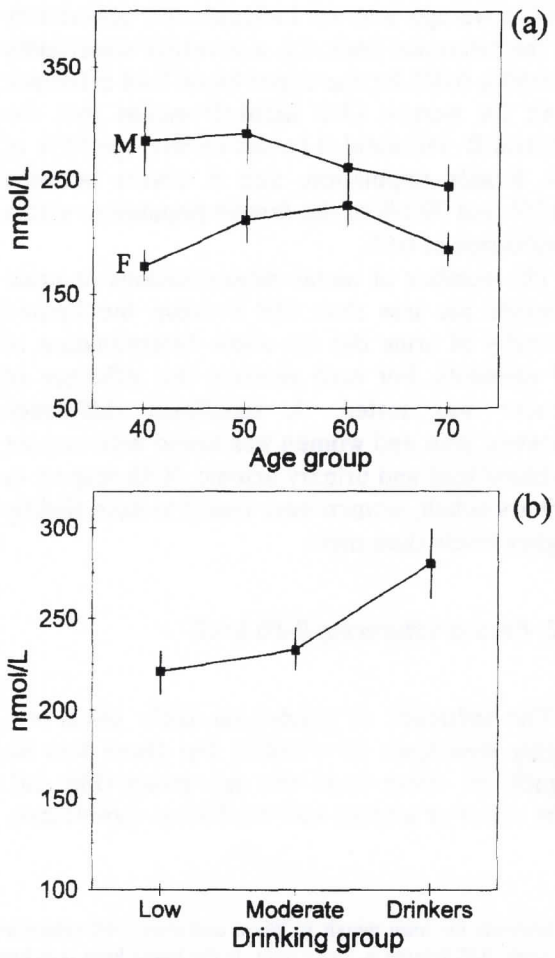

Fig. 1. Influence of gender and age (a) and alcohol (b) on blood lead levels (B-Pb). Mean values ± standard deviation. Gender and alcohol significant at $P < 0.001$ and $P = 0.042$, respectively. Age not significant (ANOVA after logarithmic transformation). M, males; F, females.

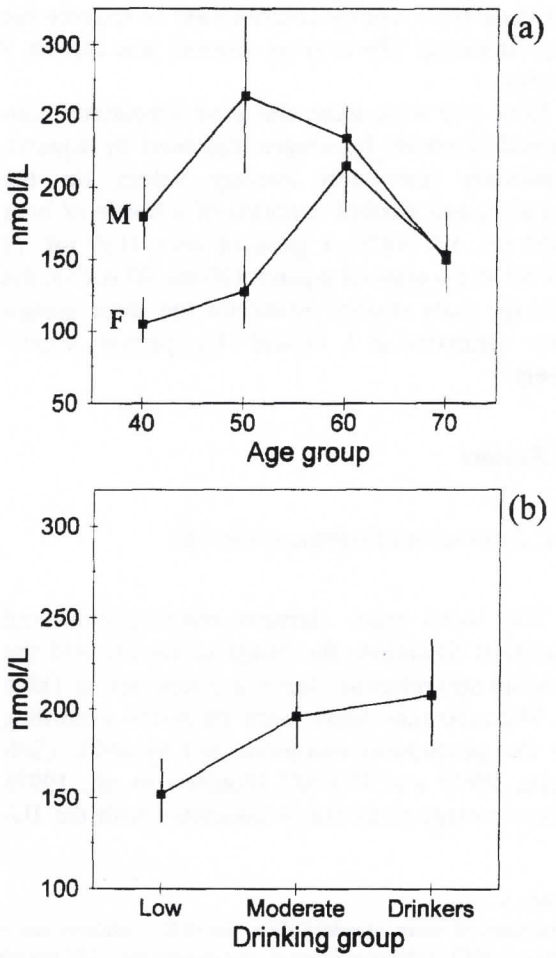

Fig. 2. Influence of gender and age (a) and alcohol (b) on urinary arsenic levels (U-As). Mean values ± standard deviation. Age and gender significant at $P = 0.027$ (interacting terms) and alcohol at $P = 0.016$ (ANOVA after logarithmic transformation). M, males; F, females.

leading to a slight increase in B-Pb levels (Fig. 1b). An effect from smoking on B-Pb levels could not be observed.

6.3. Factors influencing U-As levels

Gender, age and alcohol intake were found to have a significant effect on U-As levels (Fig. 2a,b). Age and gender were interacting terms in the ANOVA, indicating a difference in U-As levels between men and women up to 50 years, but this difference disappears after 50 years (Fig. 2a). Alcohol caused a slight increase in U-As ($P = 0.016$), as illustrated in Fig. 2b. However, alcohol may be a confounder, because it influences on the volume of urine excreted daily, and therefore also the concentration of trace elements. When the statistical analysis was repeated using U-As values corrected for urinary creatinine, the association between U-As and alcohol disappeared (data not shown here), indicating that the confounding effect may explain at least a part of the association.

The effect of selected dietary factors was also investigated. Fish and other seafood products are

known to contain high concentrations of arsenic (Iffland, 1994; Vahter, 1994). The biological half-life of U-As is 5.6 days (Iffland, 1994), and frequent intake of fish meals was therefore defined in this study as more than one fish meal per week. Statistical analysis of the effect of the frequency of fish in the diet on U-As levels did not show any significance (ANOVA).

6.4. Factors influencing U-Co levels

Smoking or alcohol did not have any effect on U-Co levels. Statistical analysis of U-Co data indicated that women may have slightly higher U-Co levels compared to men (Table 5). A possible reason for this observation may be differences in iron uptake as cobalt is similar to iron and share the same absorptive pathways in the duodenal mucosa and the small intestine (Thunus and Lejeune, 1994). Therefore, it could be expected that post-menopausal women (having a low iron uptake and presumably also a low cobalt uptake) have lower U-Co levels than pre-menopausal women. In Fig. 3 the U-Co levels (medians and 0.75 fractiles) are compared for two age groups of men and women, namely the group below 50 years (i.e. presumably pre-menopause for women) and the group above 50 years (i.e. presumably post-menopause). The trends in U-Co levels confirm the suggested change in Co uptake at the menopause. As it can be seen, the tendency is downward from the age group of 40 to the age group of 50, 60 and 70 for women, while the opposite tendency is observed for men. The difference between men and women is significant ($P = 0.018$, Mann–Whitney) for the age group of 40, but not for the higher age group.

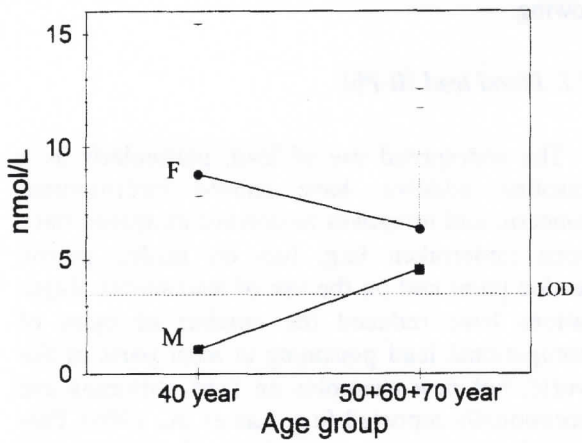

Fig. 3. Influence of gender and age on urinary cobalt levels (U-Co). Medians and 0.75 fractiles. M, males; F, females; LOD, limit of detection.

6.5. Factors influencing U-Ni

Some types of cereals contain relatively high concentrations of nickel (Schaller et al., 1994; National Food Agency of Denmark, 1995). It could be shown that frequent intake of porridge or porridge oats lead to significantly higher U-Ni levels (Mann–Whitney, $P = 0.024$), see Fig. 4. Frequent intake was here defined as one meal per week or more (biological half-life of U-Ni: 17–39 h according to Schaller et al., 1994).

6.6. Factors influencing B-Mn

Blood manganese levels are regulated by the liver by excretion in the bile (Chiswell and John-

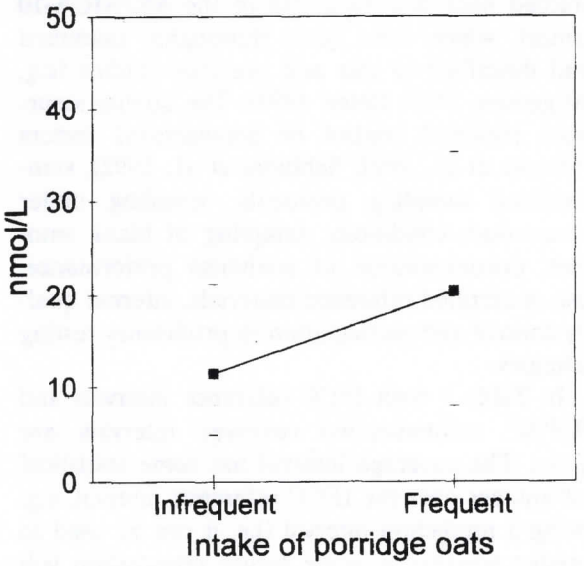

Fig. 4. Influence of frequency of meals with porridge or porridge oats on urinary nickel levels (U-Ni). Medians and 0.25 and 0.75 fractiles. Frequent: more than two meals per month; infrequent: less than two meals per month. The difference is significant at $P = 0.024$ (Mann–Whitney test).

son, 1994). Thus, the effect of paracetamol (acetaminophen) intake was investigated, but no significant difference in B-Mn levels was found between individuals taking paracetamol at least once a week (average intake approximately 2.5 g of paracetamol per week) and individuals who have not taken this medicine for the last 5 years. Other factors (gender, age, alcohol, smoking) had no effect on the B-Mn levels.

6.7. Factors influencing U-Cr

Urinary chromium levels were not significantly influenced by gender, age, alcohol or smoking.

7. Discussion

Biological reference limits are indispensable tools in evaluating environmental or occupational exposure of groups or individuals at risk. As many factors influence on the biomonitoring results, characterization of the reference population and strict quality assurance during sampling and chemical analysis are extremely important (Minoia et al., 1990, 1992; Alessio, 1993; Vesterberg et al., 1993; Christensen, 1995). The reference group studied here is a subgroup of the MONICA-10 cohort, which have been thoroughly examined and described in this and previous studies (e.g. Jørgensen, 1987; Osler, 1993). The quality assurance included control of preanalytical factors (Minoia et al., 1992; Sabbioni et al., 1992), standardized sampling protocols, sampling under clean-room conditions, sampling of blank samples, documentation of analytical performance, use of certified reference materials, internal quality control and participation in proficiency testing schemes.

In Table 5 both IFCC reference intervals and IUPAC recommended coverage intervals are given. The coverage interval has some statistical advantages over the IFCC reference interval, e.g. being a prediction interval (i.e. it can be used to predict whether a single future observation will be covered by the interval with a defined probability) (Poulsen et al., 1997). In this study there is good accordance between the limits calculated by the two methods (Table 5), which indicates that the number of samples are sufficient to estimate reliable reference limits.

Table 6
Comparison of mean values obtained in this study with other studies

Element	This study	Other Danish studies	Studies in other other countries
B-Pb (mmol/l)	234 ($n = 189$)	240 ($n = 200$)[a]	761 ($n = 959$)[b]
B-Mn (nmol/l)	165 ($n = 188$)	No data[c] —	160 ($n = 88$)[b]
U-As (nmol/l)	177 ($n = 182$)	No data[c] —	224 ($n = 540$)[b]
U-Co (nmol/l)	8.2 ($n = 186$)	16 ($n = 51$)[d]	9.7 ($n = 468$)[b]
U-Cr (nmol/l)	5.2 ($n = 186$)	4.4 ($n = 10$)[e]	12 ($n = 879$)[b]
U-Ni (nmol/l)	18 ($n = 116$)	14 ($n = 10$)[e]	15 ($n = 878$)[b]

[a] Grandjean et al., 1992.
[b] Minoia et al., 1990.
[c] No data available.
[d] Raffn et al., 1988.
[e] Christensen and Pedersen, 1986.

In Table 6 the mean values of toxic trace element concentrations in this study are compared with the mean values obtained in other Danish studies (Christensen and Pedersen, 1986; Christensen et al., 1991; Grandjean et al., 1992) and with the values presented by Minoia et al. (1990) for an Italian reference population. The results for each metal are discussed in the following.

7.1. Blood lead (B-Pb)

The widespread use of lead, particularly as a gasoline additive, have caused international concern, and initiatives to prevent its spread have been undertaken (e.g. ban on leaded petrol, leaded paint and on the use of lead shots). Regulations have reduced the number of cases of occupational lead poisoning in most parts of the world, but new examples on lead poisoning are occasionally reported (e.g. Lax et al., 1996). Biological monitoring campaigns of the general populations have been run in many countries at regular intervals since the beginning of the 1980s

(Ducoffre et al., 1990; Minoia et al., 1990; Berode et al., 1991; Christensen and Kristiansen, 1994; Yang et al., 1996). Usually a downward time trend in B-Pb levels is observed, but with considerable differences between countries (Christensen and Kristiansen, 1994). Although the downward trend may feel reassuring, the blood lead concentration for onset of adverse effects has been adjusted even more downwards. Currently, lead is believed to be able to induce subtle neurological changes in children down to a B-Pb level of approx. 500 nmol/l (approx. 100 μg/l) (Needleman and Gatsonis, 1990; Mushak, 1992; Leviton et al., 1993). In adults, B-Pb levels of the same magnitude are believed to be associated with increased blood pressure, coronary heart disease and cardiovascular disease (Møller and Kristensen, 1992; Hertz-Picciotto and Croft, 1993; Schwartz, 1995), although this view is questioned (Dolenc et al., 1993; Staessen et al., 1995).

In this study the upper 0.95 reference limit was estimated to 529 nmol Pb/l for men and 340 nmol/l for women (Table 5). In the recent study of Grandjean et al. (1992) parametric reference limits for B-Pb were estimated to 60 nmol/l (lower 0.025 fractile) and 580 nmol/l (upper 0.975 fractile), which are in reasonable accordance with the reference limits established in this study. The mean value in this study (234 nmol/l for both genders) was significantly lower than the mean value observed by Minioa et al. (761 nmol/l). This large difference can probably be ascribed to the large geographical variations and time trends observed for lead in blood reference levels caused by variability in environmental exposure (Christensen and Kristiansen, 1994).

From many studies it is known that B-Pb levels depend on gender, age, health status, nutrition, smoking and drinking habits and other lifestyle factors (e.g. Ducoffre et al., 1990; Berode et al., 1991; Grandjean et al., 1992; Schuhmacher et al., 1993). The dependence on gender and alcohol intake was confirmed (Table 5 and Fig. 1), while neither the effect of smoking nor age turned out to be significant in this study. The reason that age is not a significant factor may be because the reference group in this study is relatively old (starting at 40 years) compared to other studies. Regarding smoking, the reference group may have been too small since the influence of smoking on B-Pb levels is weak (e.g. Schuhmacher et al., 1993).

Thus, separate B-Pb reference limits should be stipulated for men and women. Heavy drinkers may have elevated levels (15–20% increase) compared to abstainers or persons with low alcohol intake. Medians: 240 (male) and 190 (female) nmol/l; 0.05 fractiles: 105 (male) and 80 (female) nmol/l; 0.95 fractiles: 529 (male) and 340 (female) nmol/l.

7.2. *Blood manganese (B-Mn)*

Manganese is vital in human nutrition with an estimated daily requirement of 2.5–5 mg/day. However, excessive doses of manganese may cause chronical poisoning characterized by central nervous system manifestations, including psychiatric disturbances and neurologic disorders (Greenhouse, 1982; Yamada et al., 1986; Wennberg, 1994). The demonstration in a number of studies of 'subclinical' adverse neurological effects in workers exposed to low air concentrations of manganese (approx. 1 mg/m^3 or less) has caused concern about the consequences of low level long-term exposure to this element (Roels et al., 1987; Wennberg et al., 1991; Mergler et al., 1994; Lucchini et al., 1995). Thus, the addition of methylcyclopentadienyl manganese tricarbonyl (MMT) to gasoline in some countries is causing some concern with respect to the risk of environmental pollution (Lytle et al., 1995) and occupational exposure (Buchet et al., 1993). In addition, the World Health Organization considers reducing the limit value for manganese in ambient air (World Health Organization, 1996). Egyed and Wood (1996) concluded, that the addition of MMT to gasoline in Canada is unlikely to represent a significant health risk to the general population.

As manganese is excreted in the bile, enhanced B-Mn levels have been observed in patients with liver failure (Mehta and Reilly, 1990; Krieger et al., 1995). In a recent study (Krieger et al., 1995), subclinical to moderate encephalopathy was observed in 10 patients with liver failure. The patients B-Mn levels ranged from 271 nmol/l to

2024 nmol/l (mean: 900 nmol/l) with the control group having a mean of 217 nmol/l. However, in studies of occupational manganese exposure, subclinical neurological symptoms have been detected in groups with a much more moderately increased B-Mn level (70–100 nmol/l increase over the control group) (Mergler et al., 1994; Lucchini et al., 1995).

The mean B-Mn level observed in this study (165 nmol/l) is similar to the mean level, 160 nmol/l, observed for an Italian reference population in the study by Minoia et al. (1990) (Table 6). The results in the present study indicate that reference limits of B-Mn do not depend on gender or age (in the range 40–70 years), on smoking or drinking habits or on taking paracetamol. Median: 157 nmol/l; 0.05 fractile: 100 nmol/l; 0.95 fractile: 271 nmol/l.

7.3. Urinary arsenic (U-As)

Essentiality of arsenic to humans is yet to be established. Occupational and environmental exposure may lead to skin lesions (hyper- and hypopigmentation, papular keratosis and ulcerative zones) and cancer in various organs (Iffland, 1994). The various chemical species of arsenic differ very much in toxicity, the inorganic form of arsenic being the most toxic (Iffland, 1994; Le et al., 1994). In humans, most of the inorganic arsenic is detoxified by methylation and excreted in the urine as monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA). Both inorganic arsenic and MMA and DMA are hydride-generating species.

Seafood is known to contain relatively large quantities of arsenic, and therefore frequent intake of seafood increases the total amount of arsenic excreted daily (Iffland, 1994; Vahter, 1994). However, seafood arsenic are non-toxic organic compounds (mainly arsenobetaine) which are not, or only to a minor extent, metabolized in humans (Vahter, 1994; Christensen, 1995). These organo–arsenic compounds are relatively stable, and they are not hydride-generating. It should be kept in mind, however, that ingestion of seaweed (with arsenosugars as the major arsenic species) lead to excretion of hydride-generating arsenic species in urine (Le et al., 1994). Also mussels may contain organoarsenic compounds that can be metabolized to hydride-generating species in humans (Buchet et al., 1994).

In this study a hydride-generating method for arsenic speciation was used (Mürer et al., 1992). The U-As levels determined in this study probably reflect exposure to arsenic from other sources than seafood, as neither seaweed nor mussels are common in a normal Danish diet. The finding that a diet rich in fish did not lead to increased U-As levels confirms this assertion.

The influence of gender and age on U-As levels (Fig. 2a) has not been demonstrated before as far as we know. Men have markedly higher U-As levels than women at 40 and 50 years, but this difference disappears with increasing age. It should be noted that the period from 60 to 70 years is the normal retirement period in Denmark, and changes in lifestyle (food and drinking habits) could be a part of the explanation of the changes with age. The onset of the menopause may also add to the explanation of the change of U-As level in women older than 50 years (Fig. 2a). However, at present the causal link between age and U-As levels is purely speculative.

Alcohol intake led to a slight increase in U-As levels (Fig. 2b), but the effect is probably due to a confounding effect of alcohol on the volume of excreted urine.

The conclusion is that reference limits for U-As levels should be stipulated separately for men and women. In interpreting reference values one should be aware that heavy drinkers may have elevated U-As levels (20–30% increase) compared to alcohol abstainers or persons with low alcohol intake. The effect of age is complicated (see Fig. 2a), but at least in women U-As levels seems to increase at increasing age (Fig. 2a). Median: 155 (male) and 105 (female) nmol/l; 0.05 fractiles: 36 (male) and 21 (female) nmol/l; 0.95 fractiles: 541 (male) and 475 (female) nmol/l.

7.4. Urinary chromium (U-Cr)

The essentiality of chromium to humans is debated (Herold and Fitzgerald, 1994). Chromium (VI) containing compounds are toxic to humans

as they may cause cancer (IARC, 1990; Aitio and Tomatis, 1991), skin allergy (Basketter et al., 1993; Menné et al., 1994), or possibly renal injury (Petersen et al., 1994). Chromium (VI) compounds have many important uses, and potential occupational exposure is common in the metal industry (e.g. welders, electroplaters), in wood, leather and textile treatment, in dye manufacturing, etc. (IARC, 1990).

Urinary chromium (U-Cr) cannot be used to differentiate between trivalent and hexavalent chromium. In evaluation of occupational exposure of welders both Knudsen et al. (1992) and Stridsklev et al. (1993) demonstrated good correlation between chromium (VI) in air and chromium in post-shift urine samples, while Lukanova et al. (1996) found only a weak correlation between air-chromium (VI) and urinary chromium (post-shift) from electroplaters. As indicated by these studies, chromium is excreted rapidly via the urine. However, if the daily dietary intake is less than 760 nmol (40 μg), the urinary excretion will be constant (Herold and Fitzgerald, 1994). The short biological half-life and the influence of dietary intake on chromium must be considered when planning biological monitoring activities.

The reference values of U-Cr obtained in this study were independent on gender, age (in the range 40–70 years), and on smoking and drinking habits. The mean value observed in this study (5.2 nmol/l) is somewhat less than the mean value observed by Minoia et al. (12 nmol/l, see Table 6). Until further information is available on geographical differences and time trends, the difference remains unexplained. Median: 4.2 nmol/l; 0.95 fractile: 13 nmol/l.

7.5. Urinary cobalt (U-Co)

Cobalt is essential to humans in the form of vitamin B_{12}. Gastrointestinal absorption of vitamin B_{12} is 70% under normal conditions. Due to its use in alloys and pigments potential occupational exposure is important in many industries. The health risks associated with occupational exposure are allergic dermatitis, rhinitis, asthma, coughing and irritation of the airways (Raffn et al., 1988; Lauwerys and Lison, 1994; Midtgård and Binderup, 1994).

The absorption of ingested inorganic cobalt is approx. 25% (Thunus and Lejeune, 1994). In a study by Christensen et al. (1993) on ingested inorganic cobalt, women were found to have significantly higher U-Co levels compared to men. This finding is partly confirmed by the results presented here (Table 3, Fig. 3). In the present study the difference between men and women (all age groups) with respect to U-Co levels had only borderline significance (Table 3). The difference between men and women seems to be largest in the lowest age group (here 40 years) and disappears with increasing age (Fig. 3). We suggest that this observation could be associated with a change in iron uptake in women before and after the menopause.

In both the study of Christensen et al. (1993) and of Lison et al. (1994) it was observed that urinary cobalt was a good indicator of exposure only when cobalt was soluble or semi-soluble. This must be taken into account in interpreting biomonitoring results.

In conclusion, gender must be considered when evaluating U-Co results. For women, U-Co levels may decline after the menopause. Median: 3.8 (male) and 6.7 (female) nmol/l; 0.95 fractiles: 23 (male) and 31 (female) nmol/l.

7.6. Urinary nickel (U-Ni)

Nickel is probably not essential to humans. Occupational exposure to nickel increases the risk of cancer in the nose and in the lung (IARC, 1990; International Committee on Nickel Carcinogenesis in Man, 1990; Aitio and Tomatis, 1991; Aitio, 1995). The carcinogenicity of nickel seems to be associated with both soluble and insoluble forms of nickel (International Committee on Nickel Carcinogenesis in Man, 1990; Aitio, 1995). A good correlation usually exists between U-Ni levels and nickel in air with regard to soluble nickel compounds, while the correlation is less pronounced for slightly soluble nickel (Aitio, 1995). Soluble nickel is excreted rapidly in the

urine. Thus, U-Ni levels reflects exposure over the working day. Slightly soluble forms of nickel is excreted less rapidly (Aitio, 1995).

Skin contact to nickel may induce sensitization to nickel and eczema in persons already sensitized. Approx. 10% of the females in Denmark are sensitized to nickel (Menné et al., 1994). It is believed that many have been sensitized from ear piercing and contact to jewellery made from nickel-containing alloys (Santucci et al., 1989).

With regard to intake, food is the most important source of non-occupational exposure. Cocoa, beans and cereals are particularly rich in nickel. In a recent study on foodstuffs on the Danish market, two kinds of morning cereals (main constituent porridge oats) were found to contain (medians) 421 and 381 ng Ni per g fresh wt., respectively. Meat, vegetables, fruits contained in general less than 50 ng Ni per g fresh wt. (National Food Agency of Denmark, 1995). The present study demonstrates that porridge oats contributes significantly to U-Ni levels (Fig. 4).

In conclusion, in evaluating U-Ni results one must take into account frequent intake of foodstuffs with high nickel concentrations. Eating porridge oats had a significant influence on the U-Ni levels (Fig. 4). Moreover, other studies have demonstrated that time of sampling and the solubility of the relevant nickel compounds are important in evaluating occupational exposure using U-Ni measurements. Other factors had no significant effect in this study. Summary: median, 18.2 nmol/l; 0.95 fractile, 52 nmol/l.

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