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Review of trace element concentrations in biological specimens according to the TRACY protocol

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Introduction

It is well known that different publications sometimes report widely different concentration mean values and standard deviations for a particular element in blood and urine in a particular population. There are several explanations for such discrepancies, which are related to general problems of sampling, inadequate definition of the reference group, and poor quality of the determinations (Vesterberg et al. 1993).

The goal of the project TRACY (a EUREKA project) is the establishment of reference values. It was recognized that certain criteria for the valuation of publications were necessary; provisional criteria were proposed and published (Vesterberg 1988). The criteria were evaluated and further developed by the Scientific Committee on Toxicology of Metals of the International Commission on Occupational health (ICOH), and the Commission on Toxicology of the International Union of Pure and Applied Chemistry (IUPAC) (Vesterberg et al. 1993). A group of experts with extensive experience in trace elements, occupational or environmental research was formed, and in regular meetings the criteria were discussed, as were the reports on the different elements.

This report describes the TRACY project reviews on the different elements published so far and provides a new review on cadmium in urine.

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Framework

Initially the TRACY project was concerned with typical values of tissue and body fluid concentrations of certain trace element in healthy populations. These elements are known to be connected with toxic effects in humans, especially in relation to occupational exposure (Vesterberg et al. 1998; Brune et al. 1991). The reference populations selected should have had no known occupational exposure to the element being studied and preferably should be between 20 and 65 years of age. Later on, the scope of the project was extended to essential elements and, for some elements, to populations of all ages (Vesterberg et al. 1993). The concept of reference values for blood and urine have been generally adopted in clinical chemistry (Solberg et al. 1989), as the number of factors influencing the reference values is limited. In occupational health, factors such as occupation, lifestyle, geographic area, environment, and diet may influence the element concentration.

It was decided that publications to be used in setting base reference values for occupational health had to give sufficient information regarding: (1) sampling conditions, including description of the reference group, procedures and a protocol for sampling; (2) the presence of analytical and statistical data. A typical example of the detailed criteria used are shown in Table 1.

Elements reviewed

Mercury

Before the TRACY group was formed, in 1991, Brune et al. reviewed 98 publications on concentrations of mercury in blood, blood cells and plasma in individuals not occupationally exposed to mercury. The publication period of the reports was 1976–1990, and the retrieval system used Medline. There were 19 papers that were rejected because of insufficient information, obvious

Table 1 Evaluation of 20 rejected cadmium in urine publications

	Sufficient/relevant information available	
	Yes	No
Sampling conditions		
Age	18	2
Sex	17	3
Country	13	7
Urban/rural	11	9
Food and drinking habits, smoking	10	10
Medication	8	12
Statistically sufficient number of samples	16	4
Sampling technique avoiding contamination	8	12
Storage/preanalytical treatment	10	10
Analytical treatment		
Method	15	5
Tested with reference sample	4	16
Accuracy/precision/sensitivity	5	15
Possible contamination/loss	2	18
Assessment with two methods or participation in cooperative study	4	16
Data treatment (distribution)	16	4
Number of regression studies		4

erroneous sampling or analytical treatment. Parameters influencing the blood Hg concentration, such as fish consumption, medication with Hg-containing drugs and the presence of amalgam fillings, are rarely mentioned in the reports. Because of the shortcomings in the available information, only tentative reference intervals could be established. Tentative pooled reference values for whole blood for non-fish consuming persons were 2 ± 1.8 μg/l (arithmetic mean and standard deviation). For blood cells the value for non-fish consuming persons was 3.8 ± 0.8 μg/kg, and for plasma 1.3 ± 0.8 μg/l. Values for high fish consumers (> 4 times/week) could be up to 20 times higher for whole blood and blood cells. For plasma the values could be three- to fourfold higher for high fish consumers than for non-consumers.

Chromium

Chromium is one of the most striking examples of the necessity of a critical review. Published normal concentrations have varied by a factor of about 1000 during the last few decades (Brune et al. 1993). The retrieval system used was Medline. For chromium in blood/serum/plasma, 53 publications were found to be suitable for reference values, while 34 were unsuitable. For chromium in urine, 41 papers were suitable for establishing reference values, and 17 were unsuitable. Publications were usually rejected because the quality assurance and procedures avoiding contamination were poorly described. The classification categories used were: country, urban, rural; age, gender; alcohol consumption, smoking habits, medication; health status; trace element supplementation; exercise; and iatrogenic exposure from implants. The experimental design must have included the use of siliconized needles for drawing blood, the use of plastic catheters and special precautions to avoid contamination. More than 40 subjects were necessary to establish a distribution.

Reference values

Seven publications were selected for chromium reference values in serum. Arithmetic means from these publications ranged from 0.05 to 0.15 μg/l. A total of 11 publications were selected for chromium in urine. Five expressed the values in μg/l. The arithmetic means were 0.1–0.5 μg/l. Six expressed the values in μg/g creatinine. The arithmetic mean values were 0.1–0.5 μg/g creatinine.

Nickel

Reference values for nickel concentrations in blood, serum, plasma, and urine were evaluated by Templeton et al. (1994). The retrieval systems used were Medline, Toxline, Chemical Abstracts, and Current Contents. Eight nickel in serum/plasma studies out of 39 were suitable for reference values. Six nickel in urine studies out of 36 were suitable for reference values, and four out of 15 nickel in blood studies were suitable. Few methods have used external quality assurance and determination by a second, independent method. Because of the limited number of suitable studies on nickel, studies with 15–39 subjects were included while noting the inadequacies of the studies. Publications were rejected usually because they lacked information about the analytical methods, reported suspiciously high values, or furnished little information on the source of the specimens. Parameters that might influence the nickel concentration in body fluids are, beside occupation, age (little information), sex, environment, food and water intake, alcohol intake (little information), smoking, health status, medication, trace element supplementation, and hobbies. The experimental design must have included the use of siliconized needles for drawing blood, the use of plastic catheters and special precautions to avoid contamination.

Reference values

Arithmetic means for nickel in serum/plasma were 0.14–0.63 μg/l, for nickel in urine 0.9–3.2 μg/l (four studies), and 1.5–1.6 μg/g creatinine (two studies). For nickel in blood the values were < 0.05–3.8 μg/l.

Selenium

As may be expected, the purpose of the review of this essential element was different from the toxic elements.

Reference values were reviewed not for occupationally exposed groups, but for the general population by Alfthan and Neve (1996). The retrieval systems used were Medline and Current Content, selenium in serum and plasma were chosen because these were the most frequently published values. From the period 1983–1993, 36 papers were selected out of 291 for reference values. Contamination is a minor problem, and sample handling for serum/plasma is easy. Reasons for rejection were inhomogeneous population (often not specified) and number of subjects (too small). Important parameters for the selenium in plasma/serum concentrations were living location, time, age, diet. Minor influence is expected from gender, liver and kidney dysfunction, acute infection, supplements, and medication (e.g., oral contraceptives, corticosteriods).

Reference values

Due to the large geographical variation in selenium intake, one universal reference range cannot be given. A tentative value for omnivorous adults, 0.5–2.5 μmol/l, is given.

Lead

Lead in whole blood has been the main indicator medium used for biological monitoring (Gerhardsson et al. 1996). Papers on lead in blood were reviewed by using the Medline retrieval system. More than 1000 papers from the period 1980–1994 were reviewed for their suitability for reference values. From these 1000, ten were selected as examples of the criteria required for lead in blood reference values. Contamination is a well-known problem in the determination of lead in blood and the not-mentioning of a contamination-free procedure was reason to reject a paper. A number of papers did not mention the use of a reference sample and/or participation in an intercomparison program and had to be rejected as well. Parameters influencing the lead in blood concentration are the degree of urbanization, year of sampling, age, gender, ethnic origin, diet including consumption of water and beverages, health status, smoking, occupational exposure also from household members, and hobbies. As distributions of lead in blood concentrations generally are skewed, information about this distribution had to be given.

Reference values

The conclusion of the review was that, mainly due to the influence of the year of sampling (a decreasing tendency is shown, probably caused by the diminished emission of leaded gasoline), and the place of sampling, it is not possible to give international reference values for lead in blood.

Cadmium

For cadmium, three studies were performed. The first one was a meta-analysis of cadmium in blood values in the general population (Alessio et al. 1994). Cadmium in blood is used for biological monitoring and indicates recent exposure. Since this study was planned before the TRACY guidelines were performed, not all phases of the TRACY evaluation procedure were fulfilled. The retrieval systems were Medline and Toxline for papers published between 1976 and 1991. A total of 18 studies were dedicated to cadmium in blood reference values, from which four were used for the meta-analysis. Contamination is a well-known problem in the determination of cadmium in blood and the not-mentioning of a contamination-free procedure was reason to reject a paper. A number of papers did not mention the use of a reference sample and/or participation in an intercomparison program and had to be rejected as well. The main variables influencing the cadmium in blood concentration are smoking, diet, and country; a moderate variable is indirect occupational exposure; and minor variables are age, sex, residence, hobbies, and medication. For the meta-analysis it was assumed that the distribution of the original value was a log-normal density function. Only papers reporting the geometric mean and the geometric standard deviation, and that were accepted for reference values were considered in the analysis.

Results of the meta-analysis

For the variables "smoking" and "area", there was enough information to subdivide the sample according to these variables only. For nonsmokers, a geometric mean (GM) of 0.56 μg/l with a geometric standard deviation (GSD) of 1.75 was calculated for a general case list, while the for a Japanese case list a GM of 3.42 μg/l and a GSD of 1.50 were calculated. For smokers these figures for the general case list were: GM of 1.50 μg/l and GSD of 1.97, and for the Japanese case list a GM of 4.19 μg/l and a GSD of 1.48. It was concluded that results obtained by pooled analysis from Italian and English studies confirmed the major role of smoking and geographic area. Age, smoking and geographic area could predict 50% of the total variability. It was stated that further aspects of heterogeneity should be studied.

In a second review on cadmium in blood, the heterogeneity causing differences was studied (Herber et al. 1997). The retrieval systems were Medline, POLTOX, and OSH-ROM Silver Platter. About 800 references for the period 1983–1992 were obtained. There were 67 papers selected for further study, and finally four studies selected for reference values. The analytical problems found in the studies were the same as in the first cadmium in blood study. A stratification according to smoking habits, occupation, sex, age, medication, hobby, and country, area, urban/rural was performed.

Table 2 Reference values from a Belgian environmental study on cadmium pollution (Cadmibel, Sartor et al. 1992)

Area	Males		Females	
	n	U-Cd GM and range	*n*	U-Cd GM and range
Liège[a]	194	0.75 (0.08–3.42)	249	0.64 (0.06–4.91)
Charleroi[a,b]	106	0.83 (0.09–3.63)	165	0.65 (0.12–7.96)
Liège-Engis[a,c]	66	0.96 (0.20–3.39)	93	0.89 (0.17–4.43)
Hechtel-Eksel[d]	151	1.01 (0.08–3.79)	197	0.89 (0.16–4.04)
N-Kempen[d,e]	86	1.12 (0.22–3.81)	216	1.09 (0.24–5.31)

U-Cd, urinary cadmium; *n*, number of individuals; GM geometric means, units µg/l (recalculated from nmol/l)
[a] Urban area
[b] Industrialized area without Cd emissions
[c] Area with previous Zn and Cd milling
[d] Rural area
[e] Area with current Cd milling

Reference values

Reference values were given for nonsmokers only, because a smokers' group is heterogeneous per definition, and the value will be simply dependent on the number of cigarettes smoked. It was stated that it is difficult to give general cadmium in blood values. The easiest stratum to construct is that of nonsmoking white collar workers in the age group of 19–65 years. Reference values are < 0.8 µg/l (geometric mean). In countries with a higher oral intake, as in Japan, values are higher. Also, values for blue collar workers are higher, and it can be concluded that in this case there is still a small exposure.

A third review, not published earlier, concerns cadmium in urine. Literature searches were performed using Medline, POLTOX, and OSH-ROM Silver Platter, CD-ROM's for the period 1983–1994. After a first screening by title alone, 541 abstracts seemed useful; after a second screening of abstracts, three remained. Stratification was performed according to occupation, sex, age (it was reported that cadmium in urine increases with age (Ewers et al. 1985) medication, hobbies (some hobbies have the risk of exposure to Cd, e.g., welding, pottery), country, area, urban/rural. The body burden, expressed as Cd in kidney cortex, differs considerably between different countries (WHO 1992). Thus, it can be expected that Cd in urine depends on the country as well. Urine sampling avoiding contamination has to be mentioned: Urine

Table 3 Overview of the TRACY studies

Element	Matrix	Main parameters	Remarks	Reference values in g	Reference values in mol
Hg	B	Fish consumption, medication, amalgam fillings	Tentative values, values for non-fish consumers	2.0 ± 1.8 µg/l (AM ± SD)	10 ± 9.0 nmol/l (AM ± SD)
	B cells	Fish consumption, medication, amalgam fillings	Tentative values, values for non-fish consumers	3.8 ± 0.8 µg/Kg (AM ± SD)	
	P	Fish consumption, medication, amalgam fillings	Tentative values, values for non-fish consumers	1.3 ± 0.8 µg/l (AM ± SD)	6.5 ± 4.0 nmol/l (AM ± SD)
Cr	S/P	Trace element supplementation, implants		0.05–0.15 µg/l (AM)	1.0–2.9 nmol/l (AM)
	U	Trace element supplementation, implants		0.1–0.5 µg/l (AM) 0.1–0.5 µg/g creat (AM)	1.9–9.6 nmol/l (AM) 0.22–1.1 µmol/mol creat (AM)
Ni	S/P	Trace element supplementation, medication, hobbies		0.14–0.63 µg/l (AM)	2.4–11 nmol/l (AM)
	B	Trace element supplementation, medication, hobbies		<0.05–3.8 µg/l (AM)	<0.85–65 nmol/l (AM)
	U	Trace element supplementation, medication, hobbies		0.9–3.2 µg/l (AM) 1.5–1.6 µg/g creat (AM)	15–55 nmol/l (AM) 2.9–3.1 µmol/mol creat (AM)
Se	S	Living location, time, age diet	Tentative values	39–197 µg/l (AM)	0.5–2.5 µmol/l (AM)
Pb	B	Degree of urbanization, year of sampling, hobbies, water and beverage consumption, smoking	No values possible		
Cd	B	Smoking, living location, diet, occupation	Occupation: not clearly exposed to Cd values for non smokers	<0.8 µg/l (GM) for white collar workers in Europe	<7 nmol/l (GM) for white collar workers in Europe
	U	Age, place of living, smoking		Females: 0.64–1.09 µg/l (GM) Males: 0.75–1.12 µg/l (GM)	Females: 5.7–9.7 nmol/l (GM) Males: 6.7–10.0 nmol/l (GM)

Abbreviations: S serum; P plasma; B blood; U urine; AM arithmetic mean; GM geometric mean; SD standard deviation samples must be taken with normal precautions regarding contamination. A proof of normality of the distribution by statistical testing had to be given. If the distribution was not Gaussian, adequate transformation had to be done (preferably the geometric mean had to be calculated). A common reason for rejection was a poor description of the analytical factors regarding checking for contamination, missing method description, and lack of quality control. A number of studies gave no information about the food and drinking habits, and about the medication. Some studies gave for the item "Method" a suitable literature reference.

Reference values

All three suitable studies were performed by the same groups and were conducted in four Belgian districts, i.e., the Cadmibel study (Staessen et al. 1991, 1992; Sartor et al. 1992). The first study (Staessen et al. 1991) was performed to investigate a possible effect of environmental exposure to cadmium on calcium metabolism. The age group was 20–80 years old, with 965 men and 1022 women in four Belgian districts. The food and drinking habits were mentioned, medication, current and past occupation were asked for, and a statistically sufficient number was present. The method was mentioned in a separate paper (Lauwerys et al. 1990) and adequate quality control was performed by using (certified) reference material, participation to intercomparison programs and testing for contamination.

Table 2 shows the results of these studies. The table shows that the urban Cd in urine values are lower than the rural values, and that Cd-polluted areas are responsible for higher urinary Cd values than in polluted non areas. According to the authors, age was the most important factor that influenced Cd in urine, followed by place of residence, and current smoking. These three parameters accounted for nearly 50% of the explained variance.

Conclusions

For the elements Hg, Cr, Ni, Se, and Cd (tentative) reference values were given for various matrices (Table 3). For Pb no reference values could be given. The most important issue in all evaluations was the small number of papers suitable to extract reference values. Generally, the lack of analytical quality and unsuitable groups were the reasons to reject papers.

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