

International project for producing reference values for concentrations of trace elements in human blood and urine — TRACY

by Olof Vesterberg, MD,¹ Lorenzo Alessio, MD,² Dag Brune, PhD,³ Lars Gerhardsson, MD,⁴ Robert Herber, PhD,⁵ George Kazantzis, MD,⁶ Gunnar F Nordberg, MD,⁷ Enrico Sabbioni, MD⁸

VESTERBERG O, ALESSIO L, BRUNE D, GERHARDSSON L, HERBER R, KAZANTIS G, NORDBERG GF, SABBIONI E. International project for producing reference values for concentrations of trace elements in human blood and urine — TRACY. *Scand J Work Environ Health* 1993;19 suppl 1:19–26. In assessing the concentrations of toxic metals, such as cadmium, chromium, and mercury, in human blood and urine samples to determine whether they are abnormal or not, reliable reference values are needed from populations of nonoccupationally exposed subjects. Numerous publications present concentrations claimed to be typical for the study populations, but they can differ by up to an order of magnitude for a particular element. This is the consequence of general problems that are related to the definition of the reference groups, and the sampling and analytical procedures used, and that make it difficult to define typical and unbiased values. An international group of experts now establishes criteria and procedures to evaluate publications containing information on the concentrations of metals in tissues and body fluids for reference populations. These evaluations have been compiled in a data base (TRACY).

Key terms: heavy metals, normal values validation, toxic metals, trace metals.

The TRACY project was initially concerned with typical values of tissue and body fluid concentrations of certain trace elements in healthy populations, elements such as metals known to be connected with toxic effects in humans, especially in relation to occupational exposure (1, 2). Well-known examples include lead, cadmium, and mercury. The reference populations selected should have had no known occupational exposure to the element being studied and preferably should have been in the age range of 20–65 years. The group studied should have also preferably been randomly selected from a larger population. This project was not concerned primarily with

metals such as sodium and potassium, which are seldom regarded as toxic metals in connection with occupational health. Recently, the scope of the TRACY project has been extended to include most essential (eg, selenium and zinc) and toxic elements and populations of all ages (the age range should be specified).

It is well known that different publications sometimes report widely differing concentration mean values and standard deviations for a particular metal 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 groups, and poor quality of the chemical analyses. In the TRACY project it was recognized that certain criteria for the evaluation of publications were necessary; provisional criteria were proposed and published in 1988 (1).

This approach was tested for mercury in an expert evaluation of relevant publications (2). In general, too many papers lacked or gave insufficient information for the complete evaluation of the relevant parameters that influence the concentrations reported (2).

It should be noted that it is only in the last few years that agreement has been reached, mainly among clinical chemists, on how the concept of reference values should be approached (3). A scrutiny of recent publications shows that the principles are not yet generally accepted by scientists studying toxic metals. Contamination of samples with the element

¹ Division of Medical Chemistry, National Institute of Occupational Health, Solna, Sweden.

² Institute of Occupational Health, University of Brescia, Brescia, Italy.

³ Dag Brune Consulting, Oslo, Norway.

⁴ Department of Occupational and Environmental Medicine, University of Lund, Lund, Sweden.

⁵ Coronel Laboratorium, Universiteit van Amsterdam, Amsterdam, The Netherlands.

⁶ Imperial College of Science, Technology and Medicine, Environmental Geochemistry Research, London, Great Britain.

⁷ Department of Environmental Medicine, University of Umeå, Umeå, Sweden.

⁸ Commission of the European Communities, Joint Research Centre, Ispra (Varese), Italy.

Reprint requests to: Professor O Vesterberg, Division of Medical Chemistry, National Institute of Occupational Health, S-171 84 Solna, Sweden.

studied must be avoided as much as possible. Furthermore, the concentrations can be influenced by occupation, life-style, and environmental factors such as diet (eg, fish for mercury) and geographic area of residence. For this reason, the reference values with which this project is concerned differ in principle from those for ordinary clinical analytes, as used in hospital care.

Many of the problems encountered in measuring trace elements have been described in numerous publications (4–11), but no complete and comprehensive account had been published when the TRACY project was started. Rational procedures for the evaluation of publications on trace metals have been developed by the TRACY group in recent years and discussed at three meetings of the Commission of Toxicology of the International Union of Pure and Applied Chemistry (IUPAC). An account of the rationale of reference values for trace elements and the factors that can influence them has recently been published (10, 12). IUPAC is proposing to publish special guidelines for planning sample collection, analyzing trace elements in biological samples, and publishing the results, which will facilitate the future production of reference values.

In reports on the TRACY project, the abbreviations used for the concentrations of elements in samples from humans and their source (eg, type of body fluid) are those adopted by the International Federation of Clinical Chemistry (IFCC). The concentration of cadmium in blood is thus B-Cd, and the unit is the mole or a fraction of the mole per liter (eg, $\text{mmol} \cdot \text{l}^{-1}$). For purposes of comparability, the units commonly used in the literature will also be given in parentheses.

A list of items and criteria for the evaluation of publications for the TRACY project were agreed upon in 1992 by a group of experts. It was considered that, for each element, a pilot phase would be required to establish the relevant criteria, followed by an independent evaluation by two or more experts, the reaching of a consensus by these experts, and a review by the TRACY group prior to the preparation of a final report for publication (appendix 1). The criteria are listed in appendix 2, which should be useful as a general recommendation. The criteria can vary for the different elements; criteria believed to be very important can be referred to as determinants. It should be noted that, currently, the criteria in the TRACY project are not as stringent as they might well become in a few years, especially once the IUPAC guidelines are implemented.

Certain minimal requirements must be satisfied in respect to the documentation of the analytical procedures, and proper statistical treatment of the data is necessary if a paper is to be included in the TRACY project. Analytical quality ranks very high in the order of importance. In the evaluation of publications it is essential to apply certain fixed criteria in respect to the documentation of the analytical pro-

cedures and the statistical treatment of the data. (See appendix 2.) It has been found that, for each element, only certain of the criteria listed in appendix 2 under "S. Sampling conditions including description of reference group, procedures and protocol for sampling" need to be satisfied. For example, some years ago it was found that B-Cd levels are influenced by tobacco smoking (7), which is therefore an essential criterion (predictor) for evaluating papers on B-Cd. However, this is not true for B-Cr (concentration of chromium in blood), for which, instead, prosthetic material implanted in the subjects studied may influence the level (5). Similarly, fish consumption can significantly increase the concentration of mercury in red blood cells (2). Of critical importance in this respect is how fish-eating habits should be investigated and recorded, for example, whether the species of fish should be recorded as well as the amount consumed over a certain period of time and how long (number of days) this period should be.

Shortage of time does not allow the investigator to collect information on details that (i) cannot be dealt with rationally from a scientific point of view and (ii) have only an insignificant influence on the concentration values. For this reason, it is first necessary to identify which factors are important, and this in turn will depend on the purpose of the study. In publishing, reviewing, and evaluating reports of studies, the aim of each study should therefore be defined. While eating fish will markedly influence mercury concentrations in red blood cells, it will have only a minor effect on levels in serum, plasma, and urine (2, 12). The reverse is true for amalgam tooth fillings, another parameter known to influence levels of mercury in the aforementioned media, so that it may be necessary to collect information on the number of amalgam fillings or amalgam surfaces of each subject.

If certain criteria or factors are more important than others, they may perhaps be given greater weight in the evaluation of publications, but it is difficult to establish logical rules for weighting such factors. However, each publication is graded by each TRACY project evaluator in light of the quality of the sampling, analysis, and statistical treatment, including the presentation of data. A grade given to a paper applies only to its usefulness in TRACY. It is stressed that a paper may be given low grades in TRACY but still be of high quality from other points of view.

Reference individuals or groups for TRACY include "healthy individuals," that is a group that can be matched with the subjects of the study or can be volunteers, students, blood donors, hospital employees, employees not occupationally exposed to the element studied, and the like. Ideally, the group should be a random selection of individuals who should preferably be similar to the study group in respect to age, gender, exposure, and various other parameters — but not in respect to occupational exposure,

since it is what is to be assessed. It is not possible to say which groups are the most suitable for TRACY, but the characteristics of the group should be specified in sufficient detail, and the group should be homogeneous in terms of the specification. A universally accepted definition of "healthy" still seems to be lacking.

Numerous publications give criteria for analytical reliability, for example, that of Aitio & Jarvisalo (5). To standardize the evaluators' work, the form presented in appendix 3 is used.

Different types of reference values that may be useful are given in appendix 4. The last option listed is particularly useful because it takes care of the uncertainty of the upper limit with a defined degree of confidence (eg, 95%). This approach seems to be particularly suitable for unidirectional intervals (eg, for nonessential elements). More information on tolerance intervals will be presented in the IUPAC guidelines currently being drafted on the calculation and application of the confidence and tolerance intervals of biological reference values [Holst E, Christensen JM, Poulsen OM, IUPAC guideline: intervals for the description of the biological level of a trace metal in a reference population (as yet unpublished) and reference 13].

Most of the publications evaluated to date in the TRACY project do not present enough data or information to allow strict statistical analysis, for example, checking the skewness or type of distribution of the concentration values, so that the standard deviation is neither easy to control nor calculate. (Compare the limits under items 2—4 in appendix 4.) A common shortcoming is that the number of individuals studied is fewer than 40 (1, 4). If this and other important data are lacking in a publication that might be of value for TRACY, it is recommended that the reviewer try to obtain the information via personal contact with the author, in which case a brief description should be given in the evaluation table presented in appendix 2. The foregoing considerations will influence the grading of publications for TRACY. (See table 1.) Publications containing data on fewer than 20 individuals would probably be given one star for statistics unless contact with the author has been successful. If the number of individuals is fewer than 10 and the fit bad, no stars would seem appropriate if the aim is to define an upper reference limit. An approximate median value can be estimated even from only five observations. It is important to be sure that they all belong to the same stratum.

The TRACY project also aims to derive and publish *tentative reference values* after the agreed publications have been evaluated by at least two experts and their reports scrutinized and agreed upon by the TRACY group. In the evaluation of all publications to be used in producing tentative reference values for certain minimal requirements, it is essential to be satisfied with regard to the documentation of the analytical procedures and the statistical treatment of the

Table 1. Proposals for rating (ie, number of stars) publications in respect to sampling conditions. (See appendix 2)

Number of individuals per stratum	Rating ^a
< 10	0
10—20	≤ *
20—40	* but ** if distribution is known
> 40	≥ **

^a Maximum that a publication can receive, provided that the other criteria justify the stars indicated.

data. Provided the quality of the publications is adequate, tentative reference values can be considered, as indicated in items 1—4 of appendix 4.

A tentative reference value for a certain metal is not an absolute value but applies only to the particular questions asked and to certain definitions. The reference values for certain metals may depend on the situation. Ideally, a reference value should relate to a group of individuals characterized by certain defined criteria such as age, gender, and life-style. Such values can be used for purposes of comparison in assessments of the concentrations of a metal, (eg, in blood) in a group considered to be almost identical to the reference group in respect to certain criteria and differing only in the magnitude of the exposure to the metal. If the groups compared differ, for instance, in age, the possibility cannot be excluded that this difference may partly be responsible for any observed discrepancy, unless there is enough reliable published evidence that it can be disregarded. One example is B-Cd, which has a tendency to increase with age. When there does not seem to be enough reliable published information for a certain metal, it may be appropriate to present only preliminary tentative reference values.

The pooling of data described in different publications (ie, meta-analysis) often requires considerably more than 40 individuals per study (1, 2, 10, 14). Information on the relevant criteria for the element must also be available (1, 10, 14).

Tentative reference values and comments on criteria that may influence the values, together with references to key publications, are of interest to occupational health professionals and health care personnel, as well as to many others. To facilitate retrieval, the publication of this information, together with comments made in evaluating the publications, is envisaged; it will also be made accessible in a computerized data base.

Concluding remarks

The aim of the TRACY project is to publish evaluated concentrations of trace elements in human tissues, particularly in blood and urine. It draws the attention of investigators to factors that can influence

the concentrations of such elements and calls on them to publish such information. Scientific journals should modify their instructions to authors along similar lines so as to increase the quality of the data published in the future. TRACY reference values are descriptive of a specified population with a particular life-style and dietary habits, and residence in a certain geographic area in a certain period; they also take into account medication, health status, and occupational exposure. They are fundamental reference points used mainly for (i) the description of measured concentrations of an element (eg, B-Cd) in individuals constituting a certain population and (ii) the classification of the results obtained for the concentration of a certain element such as B-Cd (eg, as "normal" or elevated). A reference value should not, in general, be the same as an action limit if a result of the type indicated in item 2 of appendix 4 is above the limit.

Acknowledgments

In addition to the co-authors of this paper, who have participated in four meetings of the TRACY group, the following persons have made valuable contributions to the project: Antero Aitio, Finland; George Alfthan, Finland; Pietro Apostoli, Italy; Mario Braga, Italy; Jean Neve, Belgium; F William Sunderman, Jr, the United States; and Doug Templeton, Canada.

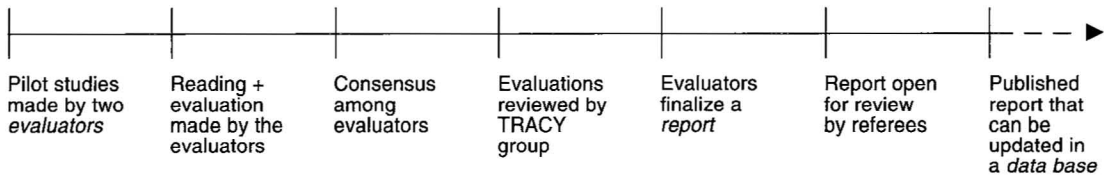
We are grateful for the cooperation of the International Commission on Occupational Health (ICOH), the International Atomic Energy Agency (IAEA), the International Union of Pure and Applied Chemistry (IUPAC), and the World Health Organization (WHO). EUROENVIRON is an umbrella organization for improved environmental quality in Europe. The TRACY project has been recognized as such a project and is supported by EUREKA. Thanks are due to Mr H Vesterberg for his computer assistance.

References

1. Vesterberg O, Nordberg G, Brune D. Database for trace element concentrations in biological samples. *Fresenius Z Anal Chem* 1988;332:556—60.
2. Brune D, Nordberg G, Vesterberg O, Gerhardsson L, Wester PO. A review of normal concentration of mercury in human blood. *Sci Total Environ* 1991;100:235—82.
3. Solberg HE, Grasbeck R. Reference values. *Adv Clin Chem* 1989;27:1—79.
4. Notten WR, Herber RFM, Hunter WJ, Monster AC, Zielhuis RL. Health surveillance of individual workers exposed to chemical agents. *Int Arch Occup Environ Health* 1988; suppl:161p.
5. Aitio A, Jarvisalo J. Collection, processing and storage of specimens for biological monitoring of occupational exposure to toxic chemicals. *Pure Appl Chem* 1984;56:549—66.
6. Versieck J, Cornelis R. Trace elements in human plasma or serum. Boca Raton, FL: CRC Press, 1989.
7. Clarkson TW, Friberg L, Nordberg GF, Sager PR, ed. *Biological monitoring of toxic metals*, New York, London: Plenum Press, 1988.
8. Aitio A, Aro A, Jarvisalo J, Vainio H. Trace elements in health and disease. Cambridge (England): The Royal Society of Chemistry, 1991.
9. Schaller KH, Angerer J, Lehnert G. Internal and external quality control in the toxicological analysis of blood and urine samples in the Federal Republic of Germany. *Int Arch Occup Environ Health* 1991;62:537—42.
10. Vesterberg O, Nordberg G, Gerhardsson L, Brune D. A database for easy retrieval of normal concentration ranges of toxic metals in blood and urine. In: Merian E, Haerdi W, ed. *Metal compounds in environment and life*. Northwood, UK: Science and Technology Letters, 1992;4:63—70.
11. Minoia C, Pietra R, Sabbioni E, Ronchi A, Gatti A, et al. Trace element reference values in tissues from inhabitants of the European Community: III. the control of preanalytical factors in the biomonitoring of trace elements in biological fluids. *Sci Total Environ* 1991;120:63—79.
12. Nordberg G, Brune D, Gerhardsson L, Grandjean P, Vesterberg O, Wester PO. The ICOH and IUPAC international programme for establishing reference values of metals. *Sci Total Environ* 1992;120:17—21.
13. Holst E, Molin Christensen J. Intervals for the description of the biological level of a trace metal in a reference population. *Statistician* 1992;41:233—42.
14. Duca P. Statistical aspects of the estimation of reference limits. *Sci Total Environ* 1992;120:155—71.

Appendix 1

Proposed phases for evaluating publications in the TRACY project for producing reference values for trace elements in samples from humans



In cooperation with the International Commission on Occupational Health and the International Union of Pure and Applied Chemistry, the TRACY group chooses at least two *evaluators* for each element. Both evaluators perform a *pilot study* to gain experience and to find the *relevant criteria* for the element studied. The TRACY group then confirms the criteria and the evaluators' tasks. The publications are located through computerized literature search. The evaluators read and evaluate the publications independently. Then they meet to form *consensus documents* for the evaluations, which should contain proposals for *tentative reference values* for the element studied, if possible derived by meta-analysis. The documents are then reviewed by the TRACY group. Then the evaluators prepare *one common evaluation report*, which is made available for review by journal referees and other experts. If the published reports are present in a *data base*, they can easily be upgraded over time to take account of relevant comments and also the subsequent evaluations for additional publications.

Appendix 2

Form for the evaluation of each publication in the TRACY project¹

Author (s):

Journal:

Sufficient information present — yes/no²

S. Sampling conditions including description of reference group, procedures and protocol for sampling

- Number of samples or individuals³
- Statistical selection criteria, for example, random selection and participation rate

¹ Used as of May 1992. The importance of the different items and criteria varies among the elements.

² Yes = Y; no = N in relation to the relevant criteria, which can be different for each element. Only *original papers* approved by a *board of editors* are evaluated. Certain criteria can be more important for a particular element, for example, smoking for cadmium. It is desirable that a consensus be reached by the TRACY group on which criteria are relevant and important for each element. In the document for each element each evaluator should specify which criteria have been used. If the sampling or analysis is given a rating of zero stars, the publication is unacceptable for TRACY. It should also be stated whether only publications in English have been evaluated. See also footnotes 3 and 4.

³ More than nine individuals are required to obtain an approximate mean concentration value. Provided that the concentration values are not widely scattered over a large range, a "trimmed mean" can be derived by first deleting the two highest and lowest values. See also footnote 4. If the distribution is narrow, five individuals may be the minimum required for one cell, for example, an age range, and five times the number of factors or criteria gives the minimum total number of individuals required to derive a reasonable estimate of the mean or median value for each category. Before a calculated standard deviation (SD) for a particular distribution of values is used, it is first necessary to determine the type of distribution, for example, symmetrical and normal (Gaussian) or skewed. The number of observations (individuals) required for such a test is often more than 40, and it increases as the range of measured values becomes wider and the distribution becomes more scattered (Solberg HE, Grasbeck R. Reference values. Adv Clin Chem 1989;27:1—79 & Duca P. Statistical aspects of the estimation of reference limits. Sci Total Environ 1992;120:155—71). For a rough estimate, fewer observations may be enough (Solberg HE, Grasbeck R. Reference values. Adv Clin Chem 1989;27:1—79). If the distribution is skewed but can be normalized by logarithmic transformation, the geometric mean (GM) and geometric standard deviation (GSD) should preferably be calculated and reported in the tables of the TRACY evaluations. The transformations may sometimes reveal nonhomogeneity, for example, bimodality. See also footnote 5.

- Age of individuals
- Gender (if females, whether pregnant or not, and use of oral contraceptives)
- Ethnic origin
- Country
- Urban/rural
- Living near contaminated area, eg, traffic, main combustion plant or incinerator
- Food, including drinking-water source and alcohol habits
- Tobacco use
- Health status (eg, for mercury, an estimate of the amount of amalgam in teeth), how health status is documented and whether certain individuals are excluded due to, for example, obesity, diabetes or high blood pressure
- Medication and supplementation with minerals and vitamins, prosthesis
- Occupation
- Hobbies, for example, shooting, especially indoors, may increase lead concentrations
- Sample location in tissue
- Sampling technique to avoid contamination, for example, use of appropriate needles,³ collection vials, including stoppers (brand and type should be specified), and pipette tips. It should be stated whether items are used uncleaned; if cleaned, the cleaning procedure should be described. The anticoagulants used should be specified
- Storage of samples, for example, temperature and time
- Other relevant comments

Rating⁵ for S: ...

A. Analytical (A1) and statistical data (A2) treatment⁶

- Method and performance of the chemical analysis
- Determination (detection) limit⁷
- Reference material used⁸
- Bias, imprecision, sensitivity
- Possible contamination or loss
- Participation in interlaboratory comparison or external quality-control programs
- Assessment by two or more different methods
- Data treatment and presentation, that is, frequency distribution of concentration values, number of individuals and statistics²
- Other relevant comments

Rating⁵ for: A1 ... A2 ...

⁴ Stainless steel needles are generally satisfactory, except for nickel and chromium (Minora C, Sabbioni E, et al. Trace element reference values in tissues from inhabitants of the European Community: III. the control of preanalytical factors in the biomonitoring of trace elements in biological fluids. *Sci Total Environ* 1991;120:63—79). Siliconized needles may be free from contamination with chromium, nickel and manganese, but some types are only siliconized on the outside (D Templeton, personal communication). Glass vials cannot be used for storing samples to be analyzed for aluminum because of the risk of metal contamination. More information can be found in the guidelines of the International Union of Pure and Applied Chemistry.

⁵ At a TRACY expert meeting in May 1992, it was decided that the grades used previously (Brune D, Nordberg G, Vesterberg O, Gerhardsson L, Wester PO. A review of normal concentration of mercury in human blood. *Sci Total Environ* 1991;100:235—82) should be replaced by *stars* as indicators of the usefulness of publications for TRACY, so that excellent = 3, good quality = 2, acceptable = 1, and unsuitable for TRACY = 0. It is stressed that the rating does not apply to a publication evaluated from other points of view, that is, a publication can be excellent by other criteria even if few stars are obtained in TRACY.

⁶ A1 and A2 can be rated separately but, if desirable, they can be combined as A.

⁷ The detection limit should preferably be less than one-tenth of the mean average for the population and must always be significantly lower than the lowest value found so that the type of distribution can be determined and the mean and standard deviation calculated. The median can be calculated and presented if the detection limit is higher than the lowest value of the concentration or if these values are widely scattered over a large range. Many of the evaluated publications report analytical methods that have high detection limits and thus justify the calculation of the median. Such publications can be useful for TRACY even if the number of individuals is less than 40 and the rating was only one star, especially for elements with only a few publications suitable for TRACY.

⁸ The reference material used should preferably have concentrations in the same range as the measured values; if they are much higher, their significance is reduced.

Appendix 3

Form used for evaluating publications for TRACY

Overview of evaluations of publications for TRACY by:..... date:.....

Sufficient information in each publication Yes = Y / No = N		Table for collecting evaluation parameters for many publications									
		REFERENCE									
SAMPLING = S	Year										
	Country, district										
	Urban/ Rural										
	Sufficient number of subjects										
	Gender										
	Ages										
	Ethnic origin										
	Health Status										
	Living near contaminated area										
	Food + water										
	Alcohol										
	Smoking										
	Medication/supplementation										
	Occupation										
	Hobbies										
	Contamination control										
	Storage of samples										
	Other relevant details										
ANALYSIS = A											
	Method:described/published										
	Detection limit										
	Precision within batch										
	Precision between batch										
	Reference material										
	Recovery, contamination/loss										
	Two or more methods										
	Interlaboratory comparison										
DATA TREATMENT											
	Data presentation										
	Distribution type examined										
	Mean / median										
	Standard deviation (SD)										
	Reference limit(s)										
	Other relevant details										
	Rating S/A										

The numbers in the boxes refer to information to be found after the tables

Appendix 4

Different types of reference values

1. Interval, for example, mean $\pm$ 2 standard deviations, encompassing 95% of a normal population or one which has been normalized.
2. A limit that is the mean $\pm$ 2 standard deviations.
3. A limit that is the 95th percentile of the distribution, independent of its type.
4. A limit that is the upper limit of the confidence interval for the limits under items 2 or 3 (ie, the tolerance interval) (1, 2).

References

1. Holst E, Molin Christensen J. Intervals for the description of the biological level of a trace metal in a reference population. *Statistician* 1992;41:233—42.
2. Duca P. Statistical aspects of the estimation of reference limits. *Sci Total Environ* 1992;120:155—71.