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Assessment of Human Exposure to Lead and Cadmium Through Biological Monitoring

Edited by

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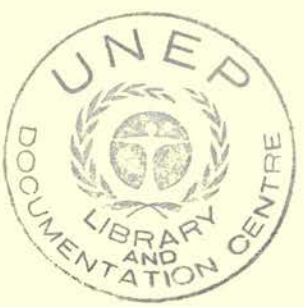

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Contents

Preface

This is the final report of the UNEP/WHO Pilot Project on Assessment of Human Exposure to Pollutants through Biological Monitoring (Metals Component). A companion report covering the Organochlorine Compounds Component of the Pilot Project will be issued separately at a later date.

This report was prepared by the Co-ordinating Institution for the Metals Component at the National Institute of Enviromental Medicine and Karolinska Institute, Department of Environmental Hygiene (Director, Professor Lars Friberg), Stockholm, in collaboration with the network of national institutions which participated in this project component.

The draft report was reviewed and agreed upon at a consultation that was held in Geneva from 14 to 18 December 1981 and at which the Co-ordinating Institution, national institutions participating in the project component, the International Atomic Energy Agency and the Commission of the European Communities were represented. The latter two organizations as well as the United States National Bureau of Standards have greatly contributed to the implementation of the project, which is gratefully acknowledged.

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Abstract

Introduction

This paper describes the development and results of the metals component of the UNEP/WHO Pilot Project on Assessment of Human Exposure to Pollutants through Biological Monitoring. This project was carried out within the framework of the Global Environmental Monitoring System (GEMS). The project was initiated in 1978 following the recommendations from a UNEP/WHO meeting of a Government Expert Group on Health-Related Monitoring, held in Geneva in 1977.

The immediate objectives were: a) to review and agree internationally on selected methods to be used for analysis and evaluation of levels of a limited number of pollutants in selected tissues and body fluids; b) to provide technical advice and arrange training programmes for scientists and technicians; c) to design and implement a programme for rigid quality control in connection with sampling, storage, transport and analysis of tissues and body fluids; and d) to carry out a number of pilot studies on selected segments of the population in a specified number of countries.

The following countries have participated in the project: Belgium, India, Iran (only in the initial phase), Israel, Japan, Mexico, People's Republic of China (from May 1981), Peru, USA and Yugoslavia. In each of the participating countries a national centre was selected for the programme.

Scientific responsibility for the implementation of the project was delegated to a Co-ordinating Institution (CI): the National Institute of Environmental Medicine and the Department of Environmental Hygiene of the Karolinska Institute, Stockholm, Sweden.

Lead and cadmium were measured in samples of venous blood to obtain an indication of recent exposure. Cadmium was also measured in samples of kidney cortex to provide a measure of lifetime accumulation.

About 200 teachers from one urban area in each country constituted the target group for lead and cadmium in blood and about 50 cases of "sudden, unexpected death" in the general population were used for cadmium in kidney cortex.

It was recognized at an early stage in the planning that there was a great need for strict quality control (QC) procedures in the monitoring of trace elements in biological media. A review of published data showed that mean concentrations of lead in blood in the general population of 400–500 μg Pb/l and for cadmium mean values as high as 20–50 μg Cd/l or even higher have been reported. Unfortunately, most published reports lack quality assurance data and a valid international comparison of the exposure to lead and cadmium based on blood levels could therefore not be made. Furthermore, results from several interlaboratory comparisons amplified the need for quality control.

The present project has been divided into two main phases. The first phase has been devoted primarily to training and technical assistance. The second phase has been devoted to the actual monitoring of lead and cadmium, with integrated quality assurance, in the above mentioned tissue and body fluid.

Training phase

It was agreed that no laboratory should start analysis of the monitoring samples until it had achieved satisfactory results in the QC training phase. Criteria for acceptance of data were agreed upon by the participating institutions and the Co-ordinating Institution. CI distributed to the participating laboratories an appropriate number of internal quality control samples (IQC, values known to the laboratories) as well as external quality control samples (ECQ). The results of the analyses were sent to CI for evaluation and the laboratories received feedback in the form of "true" values and whether or not the results met the criteria previously agreed upon.

Quality control samples for analysis of lead in blood consisted of haemolyzed cow's blood containing EDTA and sterilized by gamma-irradiation. The samples were spiked with lead and cadmium. For quality assurance of the analyses of cadmium in kidney cortex, samples of freeze-dried horse kidney cortex were provided. The "true" levels of lead and cadmium in the QC samples were determined with the assistance of reference laboratories. The QC training phase included altogether 12 sets of quality control samples. Each set consisted of 6 blood samples for analysis of lead and cadmium and 4 samples of freeze-dried horse kidney cortex for analysis of cadmium.

It was rare that a laboratory met the criteria for acceptance throughout the training phase. An important component of the project was thus assistance to improve the capability of participating laboratories through training and the provision of equipment. The ability of the participating laboratories to measure lead and cadmium in blood samples and cadmium in kidney cortex improved markedly during the training phase.

It is obvious from the results of the QC analyses that an accepted performance in a limited number of quality control runs was no guarantee for a continuous good performance. Furthermore, correct results reported for the internal quality control samples did not always guarantee accurate results of the external quality control samples. This emphasized the need to include not only internal quality control samples but also external quality control samples in the monitoring phase of the project.

Monitoring phase

The quality control was not focused only on the accuracy of the analytical methods (analytical quality control). It also took into consideration questions relating to sampling and the avoidance of contamination during sampling and storage (preanalytical quality control). In order to avoid contamination through the use of e.g. unsuitable blood collection vials and contaminated anticoagulants, CI provided the participating laboratories with evacuated blood collection tubes (Venoject) with heparin from one and the same batch, after control of the metal content.

During the monitoring phase internal and external quality control samples were analyzed to assess the validity of the obtained results. As a rule five sets of quality control samples were analyzed together with the 200 blood samples and three sets of quality control samples together with the 50 kidney cortex samples.

Calculated regression lines, based on the results of all QC samples analyzed together with the monitoring samples, fell within the acceptance intervals agreed upon, indicating that the obtained average values for lead and cadmium in teachers' blood and cadmium in kidney cortex were valid. In addition, a limited number of duplicate analyses were performed at CI. The obtained values were in good agreement with those obtained at the participating laboratories.

Data from the present programme constitute the only international comparison on a global scale of lead and cadmium concentrations in blood with a rigid quality assurance programme. The results of the quality control analysis, run in parallel with the actual monitoring of the target populations, together with a "preanalytical" quality control programme makes it highly probable that the results are valid and comparable.

The results clearly showed that there is considerable variation in metal exposure between the areas studied. For *lead in blood* the median values thus ranged from about 60 μg Pb/l in Beijing and Tokyo to 220 μg Pb/l in Mexico City. The median values were below 100 μg Pb/l also in Baltimore, Jerusalem, Lima, Stockholm and Zagreb, and between 100 and 200 μg Pb/l in Brussels and the Indian cities Ahmedabad, Bangalore and Calcutta. The 90-percentile values ranged from 89 μg Pb/l in Tokyo to 346 μg Pb/l in Mexico City.

The results confirm earlier studies which have indicated that males have higher blood levels than females, also among nonsmokers. There was furthermore a tendency to somewhat higher blood lead values in smokers than in nonsmokers. The differences between the areas studied were, however, not explained by differences in sex distribution or smoking habits.

The reasons for the differences in blood lead levels among the countries are not known and it is an urgent task for future programmes to look into the reasons for such differences. The results of the present study indicate, however, that lead in gasoline may be one important source of exposure to lead.

For *cadmium in blood* the median values among nonsmokers ranged from less than 0.5 μg Cd/l in Jerusalem, Mexico City and Stockholm up to 1.2 μg Cd/l in Tokyo. The highest 90-percentile value was found in Mexico City, 3.2 μg Cd/l.

The values for cadmium in blood were very closely correlated with smoking habits. Smokers thus had considerably higher concentrations than nonsmokers while former smokers were in between. In several countries 90-percentile values of 5–10 μg Cd/l were found among smokers.

Data on *cadmium in kidney cortex* were obtained from all participating countries except Mexico and Peru. Also for cadmium in kidney cortex considerable differences between countries were observed. As could be expected on the basis of earlier data, Tokyo had the highest values with a geometric mean value in the age group 40–59 years of 60–70 mg Cd/kg wet weight. The lowest values were found in Baltimore, Beijing, the Indian cities and Jerusalem, with mean values around 20–25 mg Cd/kg wet weight which is comparable with earlier reported data from Stockholm. As was the case for cadmium in blood there was a tendency towards higher values for smokers than for nonsmokers, but no differences related to sex were found.

1. Introduction

The Global Environmental Monitoring System (GEMS) is a collaborative effort of national institutions and international organizations aimed at the systematic collection of comparable data on the environment throughout the world. One of the main components of GEMS is health-related monitoring which includes programmes for monitoring of air and water quality, and of food contamination. The health-related monitoring component of GEMS was reviewed in 1977 by a Government Expert Group convened by UNEP and WHO. In its report (WHO, 1977a) the Expert Group recommended that the ongoing activities be supplemented by others that could be specifically aimed at the assessment of human exposure to pollutants. In particular, the Group recommended that internationally co-ordinated pilot studies be undertaken for the monitoring of selected metals and organochlorine compounds in human tissues and body fluids (hereinafter referred to as biological monitoring). The Group was aware of the difficulties that would be encountered in obtaining comparable data from laboratories located in different countries and with different degrees of experience and expertise. The Group therefore laid great emphasis on the need to follow rigid quality control procedures as a requisite for the achievement of the objectives of the study.

The *long-term objectives* of the project were:

- to develop and strengthen biological monitoring programmes in Member States, and
- to develop regional and global programmes that will make it possible to accurately assess human exposure to selected toxic pollutants by measuring their concentrations in biological tissues and fluids.

The *immediate objectives* were:

- to review, and agree internationally on, selected methods to be used for the analysis and evaluation of levels of a limited number of pollutants (cadmium, lead, mercury and organochlorine compounds) in selected tissues and body fluids
- to provide technical advice and arrange training programmes for scientists and technicians
- to design and implement a programme for rigid quality control in connexion with sampling, storage, transport and analysis of tissues and body fluids
- to carry out a number of pilot studies on selected segments of the population in a specified number of countries, taking into consideration differences in climate and development, and evaluate and publish these data.

During 1977 and 1978 a consultant for WHO visited several Member States to explore the interest of these countries in participating in the programme and to examine the possibilities of their doing so. Discussions were also held with the Commission of the European Communities (CEC) which was executing the first phase of a biological screening of the population for lead.

Participating countries and institutions were chosen by UNEP/WHO on the basis of

results of the visits, of negotiations with Member States and of advice from the WHO Regional Offices. Criteria for selecting countries included geographical distribution, economic development, environmental problems and participation in other UNEP/WHO health-related monitoring activities. The project was divided into a metals component and an organochlorine compounds component. The following countries have participated in the metals component: Belgium, India, Iran (only in the initial phase), Israel, Japan, Mexico, People's Republic of China* (from May 1981), Peru, USA, and Yugoslavia. In each of the participating countries a national centre was selected.

It was decided that the project would be co-ordinated by WHO headquarters in close collaboration with the Regional Offices. Scientific responsibility for the implementation of the metals component was delegated to the National Institute of Environmental Medicine of Sweden in collaboration with the Department of Environmental Hygiene of the Karolinska Institute, Stockholm, Sweden which was to serve as the Co-ordinating Institution (CI) for the project.

A decision was reached at an early stage to limit the pilot study to the measurements of lead and cadmium in blood and cadmium in kidney cortex obtained from autopsies. At a meeting of the representatives of the participating institutions and the CI held in Stockholm in May 1980 (WHO, 1980a) it was decided to choose teachers (200 from each country) as the target group for the two metals in blood, and cases of "sudden, unexpected death" in the general population for cadmium in kidneys.

It was recognized at an early planning stage that there was a great need for strict quality control procedures in the monitoring of trace elements in biological media. Concentrations which must obviously be grossly in error have often been reported in recent years even from laboratories with adequate facilities and expertise. Results from several interlaboratory comparisons have made the need for quality control even more apparent. A planning meeting on quality control under the project was held in Geneva 26 February–2 March 1979 (WHO, 1979a).

Procedures for sampling, sample handling, analysis and data handling vary among laboratories. The purpose of the pilot project was not to standardize such methods. Indeed, it was recommended that each participating laboratory use methods of its own choice, provided they produced results acceptable according to the quality assurance criteria. However, the CI would provide advice on suitable methods, particularly in cases where the use of new analytical activities was started.

It was recognized that quality control should be planned and implemented before the actual monitoring started. Quality control was considered both as an activity of its own and as a necessary and integral part of the monitoring phase in order to assure a continuous control of sampling and analysis throughout that phase.

An important component of the project was the improvement of the capability of participating laboratories which was achieved through the assistance of consultants and the provision of special training. Whenever possible and required, institutions from developing countries participating in the project received support through the supply of equipment and/or other resources in addition to what the participating institutions themselves could provide.

Within the CEC a programme on biological monitoring of lead in blood had recently started (CEC, 1977). A close collaboration between the UNEP/WHO project and the CEC programme was agreed upon and established through personal contacts between representatives of the CEC and of the CI as well as through formal participation in meetings arranged by either part.

* Hereinafter referred to as China.

2. Background

2.1 The rationale for assessing human exposure to lead and cadmium through biological monitoring

Both cadmium and lead may be found in the working environment, in ambient air, in drinking water, in tobacco smoke and in food. In most countries, certain foods are the major contributors to human exposure to both metals. However, in areas with heavy traffic airborne lead may also be an important source. Smoking may be an additional significant source of cadmium exposure. When exposure through food is low, smoking may contribute to approximately half of the total body burden of cadmium. Within certain industries, occupational exposure to cadmium and/or lead is a dominating factor, but exposure through food and tobacco smoke must not be overlooked as an important contributing factor.

The fact that accumulation of cadmium and lead in humans may have different sources means that the best way to estimate the total exposure and risks is through biological monitoring. This is possible as we have enough information on metabolism to relate concentrations in certain indicator media and tissues to exposure (dose) and risk of health effects.

Cadmium has a biological half-time in the human body of about 20 years. It is mainly accumulated in kidney cortex and approximately one third of the body burden is found in the kidneys at long-term low-level exposure. Determination of the concentration in kidney cortex can provide a measure of lifetime accumulation of cadmium. At long-term low-level exposure concentrations of cadmium in urine reflect those in the kidneys. Since reasonably good information is available on associations between concentrations of cadmium in kidney cortex and risk of kidney dysfunction, urinary concentrations of cadmium may be used for estimations of risk (Friberg et al., 1974). Monitoring of urinary concentrations of cadmium does not, however, automatically lend itself to evaluation of recent exposure. If exposure in the past has been high, one may well find relatively high urinary levels of cadmium despite current low exposure. Since only about 5% of cadmium taken in through food and water is absorbed, measurement of cadmium concentrations in feces is useful for the evaluation of recent oral exposure. Cadmium concentrations in blood are probably related partly to body burden and partly to more recent exposure. The concentration in blood is a useful indicator of exposure during recent months. In studies of newly employed workers industrially exposed to cadmium (Kjellström, 1977; Lauwerys et al., 1979) it was shown that during the first couple of months of exposure cadmium levels in blood increased and then reached an equilibrium. If exposure can be expected to remain stable, blood levels may be used also for the evaluation of long-term risks (WHO, 1980b). For a detailed discussion of a metabolic model of cadmium reference is made to Kjellström & Nordberg (1978), Camner et al. (1979), Lauwerys et al. (1979) and Friberg et al. (1979).

About 90% of the total body burden of lead is present in the bones and teeth, as a stable fraction, which is not accurately indicated by the blood lead level. In blood 95% of the

lead is bound to the erythrocytes. The level of lead in blood is the best indicator of current exposure. The blood lead level reflects a dynamic equilibrium between exposure, absorption, distribution and elimination of lead (WHO, 1980b).

There is a large number of reports in the literature on "normal" levels of lead and cadmium in blood and of cadmium in kidneys of non-occupationally exposed persons. Selected reports from various countries are reviewed in the following section. Special attention has been paid to whether or not the results have been validated by quality assurance procedures. A computerized literature search for available data on cadmium and lead levels in human blood and kidney was conducted for this project (EPA, 1980). The CEC study on biological screening of the population for lead is presented in a separate section. In section 2.3 results of intra- and interlaboratory comparison studies are reviewed.

2.2 Lead and cadmium in blood and kidneys. A review of data presented in the literature

2.2.1 Lead and cadmium in blood

2.2.1.1 General

A large number of studies conducted in different countries have aimed at evaluating "normal" levels of lead and cadmium in the general population. There are, however, very few international studies in this regard. One such study on lead was carried out in mid 1960s (Goldwater & Hoover, 1967). In addition there is the CEC study on lead (see section 2.2.1.2).

Unfortunately, it is more the exception than the rule that data on quality assurance have been presented as part of the studies. As for the study by Goldwater & Hoover (1967) all analyses were performed with one single method ("a standard dithizone method"), at a recognized laboratory in USA. No quality assurance data were however reported, either for the analyses or the sampling. Data from the study are presented in table 2:1.

Tables 2:2 and 2:3 contain data from selected studies, primarily those carried out during the last 10-year period on lead and cadmium in blood of *adults not excessively exposed* to metals in the working or in the general environment. The populations studied are not defined in detail and may include subgroups such as policemen and taxi drivers. If a detailed comparison of results from different studies is to be made, it is advisable to consult the original publications for information on analytical and sampling procedures.

Published results are only very seldom accompanied by quality assurance data. Therefore, the large variations in levels obtained in the different studies may well be explained by methodological errors rather than by differences in exposure. In cases where extremely high values are reported without any quality control it is obvious that the data cannot be regarded as valid. The situation is much more difficult to evaluate when reported concentrations are within a range which is not completely unrealistic, but where there is no guarantee that a quality assurance programme has been implemented. Unfortunately, most reports fall in this category. The fact that reliable quality assurance data are not presented in a report does not necessarily mean that the data are wrong, but that their accuracy are not known. A valid international comparison of the exposure to lead and cadmium based on blood levels cannot therefore be made.

Table 2:1. Lead concentrations in blood (µg Pb/l) by country and residence, as reported by Goldwater & Hoover (1967)

Country	Urban			Rural		
	Sample size	Median	Range	Sample size	Median	Range
Argentina	*43*	140	50–500	*6*	110	50–150
Chile	*34*	190	50–300	–	–	–
Czechoslovakia	*20*	190	0–500	–	–	–
Egypt	*19*	150	100–500	*9*	110	0–300
England	*30*	210	0–450	–	–	–
Finland	*17*	290	100–500	*29*	210	100–600
Holland	*58*	120	0–450	–	–	–
Israel	*46*	150	50–400	*13*	180	100–250
Italy	*26*	100	50–400	–	–	–
Japan	*32*	210	50–600	*8*	190	100–300
Peru	*32*	60	0–350	–	–	–
Poland	*32*	120	50–250	*44*	120	50–350
Sweden	*14*	90	50–150	*16*	100	50–150
Yugoslavia	*30*	150	50–300	*16*	290	50–600
United States						
California	*27*	190	100–250	*6*	180	100–200
New York City	*105*	170	0–1000	–	–	–
Ohio	*20*	180	100–500	*20*	110	100–350
New Guinea	–	–	–	*38*	210	100–400

2.2.1.2 The CEC study on biological screening of the population for lead

By Council Directive of 29 March 1977 the Member States of the European Community were asked to assess the extent of human exposure to lead in the general environment. This was to be done by measurements of blood lead. The sampling should be carried out on volunteers and include groups of at least 100 persons in urban areas of more than 500,000 inhabitants and, in addition, include where feasible, groups of at least 100 persons from among people exposed to significant pollution. In each Member State and during each of two campaigns the number of analyses to be performed should total at least 50 per million inhabitants. The first progress report gives background information of the programme, an overview of the sampling procedures and the results of the first monitoring campaign (CEC, 1981).

The quality control programme (QCP) consisted of sending blood samples with unknown concentrations to laboratories for analysis. The quality control samples consisted of human blood or cow blood spiked with lead and were prepared at the Regional Toxicology Laboratory, Dudley Road Hospital, Birmingham, U.K. During the first part of the project two quality control studies were implemented (phase 0 and phase 1), and a third more intensive study was mounted during the monitoring period proper (phase 2). During phase 0 nine samples were sent to each of 56 participating laboratories. During phase 1 ten samples were sent in triplicate over three months to 45 participants. During the actual monitoring period (June to August 1979) 30 samples, distributed at weekly intervals, were sent to 41 participants. All of these samples were designated as external quality control samples (EQC).

In addition, internal quality control samples (IQC, the concentrations of which were agreed upon) were used to enable "in-house" assessments of performance at the actual time of the blood lead assay. The total number of EQC and IQC samples distributed to the

Table 2:2. Lead in blood (μg Pb/l) in adults as reported in selected studies from different countries

Area	Population[1]	Mean or median[2]	Quality assurance[3]	Analytical method[4]	Number	Reference
Australia	?	185	–	n.spec.	*202*	Bell (1981)
	U(Sydney)	160	–	n.spec.	*44*	Bisby et al. (1977)
Bangladesh	Small Community	530	–	PIXE	*93*	Khan et al. (1980)
Belgium	M(Belgium)	170	++	AAS	*1678*	Claeys-Thoreau et al. (1980)
	U(Liège)	200	–	AAS	*390*	Sartor & Rondia (1980)
	U(Brussels)	180	++	AAS	*122*	Bruaux et al. (1979)
	U(Brussels, blood donors)	170			*101*	
Denmark	U(Aarhus)	75	–	AAS	*88*	Nygaard et al. (1973)
Finland	U(Helsinki)	85–135	++	AAS	*422*	Nordman (1975)
	R(Pertunmaa)	96–121			*499*	
France	R	278	–	AAS	*95*	Boudene et al. (1975)
	U(Paris)	275–347				
India	U,R(Ahmedabad)	207	–	AAS	*36*	Pandya et al. (1982)
	U(Ahmedabad)	275	–	AAS	*204*	Patel (1980)
	U(Ahmedabad)	400	–	AAS	*60*	Aggarwal et al. (1979)
	U,R(Ahmedabad)	191	–	AAS	*25*	Pandya (1978)
Italy	U(Milan)	240–460	–	AAS	*142*	Secchi & Alessio (1974)
	U(Milan)	240–300	–	Dithiz.	*138*	Zurlo et al. (1970)
Japan	U(Hokkaido)	70	–	AAS	*308*	Saito et al. (1979)
	U(Tokyo, 12–15 yrs)	54–98	–	AAS	*303*	Tsuchiya et al. (1977)
	U(Tokyo, Okinawa)	190	–	AAS	*233*	Mishima (1976)
	U(Tokyo)	180	–	Dithiz.	*2283*	Tsuchiya et al. (1975)
Nepal	R	40	++	AAS	*60*	Piomelli et al. (1980)
Netherlands	U(Amsterdam)	140	–	AAS	*145*	Wibowo et al. (1977)
	U(Arnhem)	110	–	AAS	*222*	Zielhuis et al. (1977)
Papua Guinea	R(children, 7-10 yrs)	50	+	AAS	*100*	Poole et al. (1980)
Sweden	U(Örebro)	56–94	+	AAS	*156*	Andersson et al. (1981)
	(South Sweden)	85–120	–	Dithiz.	*135*	Haeger-Aronsen et al. (1971)
U.K.	U(Birmingham, Renfrew)	190–250	–	AAS	*270*	Beevers et al. (1980)
	U(Birmingham)	290–450	–	AAS	*78*	Khera et al. (1980)
	U(Birmingham)	150–220	–	AAS	*446*	Waldron (1979)
	U(Glasgow)	135	–	AAS	*30*	Moore (1977)
	U(Manchester)	228	–	Dithiz.	*40*	Flindt et al. (1976)
	U(Aberystwyth)	312–393	–	AAS	*626*	Beasley et al. (1973)
	U(London)	290	–	AAS	*50*	Jones et al. (1972)
USA	U(New York)	150	++	AAS	*15*	Piomelli et al. (1980)
	M(Nationwide)	130–180	++	AAS	*2646*	Mahaffey et al. (1979)
	U(San Diego)	270	–	AAS	*86*	Zettner et al. (1977)
	U(Chicago)	200–480	–	AAS	*747*	Creason et al. (1976)
	U(New York)	130	–	AAS	*96*	Mishima (1976)
	U(San Diego, Lancaster)	100–190	–	AAS	*245*	Johnson et al. (1975a)
	U(Houston)	120–280	–	AAS	*216*	Johnson et al. (1975b)
	U(Philadelphia)	130–260	–	n.spec.	*245*	Baloh (1974)
	U(Ann Arbor)	150	–	ASV	*90*	Hecker et al. (1974)
	M(Nationwide)	155–215	–	Dithiz.	*2460*	McLaughlin et al. (1973)
	U(7-city study)	120–200	–	Dithiz.	*1935*	Tepper & Levin (1972)
	U(Chicago, 10–14 yrs)	235	–	AAS	*746*	Blanksma et al. (1969)
Venezuela	R (Indians)	10	–	ASV	*90*	Hecker et al. (1974)
Yugoslavia	U(Trepca)	185	–	AAS/ASV	*45*	Dragović (1980)

[1] U = urban; R = rural; M = mixed

[2] When a range is given, it represents the range of means (arithmetic or geometric) or medians of different subgroups studied

[3] ++ = valid quality assurance data reported; + = probably valid quality assurance but detailed data not reported; – = no valid quality assurance information reported

[4] PIXE = Proton Induced X-ray Emission; AAS = Atomic Absorption Spectrophotometry; ASV = Anodic Stripping Voltammetry; Dithiz. = colorimetric dithizone method

Table 2:3. Cadmium in blood (µg Cd/l) in adults as reported in selected studies from different countries. For further explanation, see footnotes of table 2:2.

Area	Population[1]	Mean or median[2]	Quality assurance[3]	Analytical method[4]	Number	Reference
Belgium	U(Charleroi, Liège)	1.2–1.6	+	AAS	*130*	Lauwerys et al. (1980)
	U(Liège)	4.1	–	AAS	*20*	Lauwerys et al. (1976)
	U	9.5	–	AAS	*24*	Vens & Lauwerys (1972)
Denmark	U(Aarhus)	1.1	–	AAS	*110*	Nygaard et al. (1973)
India	U,R(Ahmedabad)	1.5	–	AAS	*36*	Pandya et al. (1982)
	U(Ahmedabad)	8.1	–	AAS	*204*	Patel (1980)
	Smokers	8.2				
	Nonsmokers	7.9				
	U,R(Ahmedabad)	14	–	AAS	*24*	Pandya (1978)
Japan	U(Tokyo)		–	AAS	*127*	Kjellström (1979)
	Smokers	5.2			*77*	
	Nonsmokers	4.5			*50*	
	U(Hokkaido)	3.4	–	AAS	*308*	Saito et al. (1979)
	U(Tokyo, Okinawa)	38	–	AAS	*238*	Mishima (1976)
Netherlands	U(Arnhem)		–	AAS		Zielhuis et al. (1977)
	Smokers	0.7			*138*	
	Nonsmokers	0.4			*84*	
Sweden	U(Stockholm)	3.8	–	AAS	*39*	Kjellström (1979)
	U(Stockholm)	1.5	–	AAS	*17*	Elinder et al. (1978)
	U(Örebro)		+	AAS	*89*	Ulander & Axelson (1974)
	Smokers	2.0			*45*	
	Nonsmokers	0.5			*44*	
U.K.	U(Renfrew)		–	AAS	*140*	Beevers et al. (1980)
	Smokers	3.3			*53*	
	Nonsmokers	1.8			*87*	
	U(Birmingham)	8–11	–	AAS	*88*	Khera et al. (1980)
USA	U(Houston)	5.5	–	AAS	*216*	Kjellström (1979)
	Smokers	5.2			*77*	
	Nonsmokers	4.5			*50*	
	U(Chicago)		–	AAS		Kowal et al. (1979)
	Smokers	1.3			*67*	
	Nonsmokers	0.8			*228*	
	U(Denver)		–	AAS		Wysowski et al. (1978)
	Smokers	3.0			*26*	
	Nonsmokers	0.4			*79*	
	U(Philadelphia)	3.4–11.1	–	AAS	*27*	Glauser et al. (1976)
	U(New York)	33	–	AAS	*97*	Mishima (1976)
	U(Houston)	4–9	–	AAS	*216*	Johnson et al. (1975b)
	U(Ann Arbor)	17.1	–	ASV	*47*	Hecker et al. (1974)
	U(19 locations)	17.7	–	AAS	*243*	Kubota et al. (1968)
Venezuela	R (Indians)	5.7	–	ASV	*90*	Hecker et al. (1974)
West Germany	Normals (serum)	228	–	Spectrogr.	*29*	von Mertz et al. (1972)

laboratories was in excess of thirty thousand. Measures were also taken to avoid contamination by using only trained operators to collect blood samples and conducting "preanalytical" QC exercises of the blood container tubes. Some of the samples were sent for confirmation to a second laboratory and up to 15% were submitted to the CEC reference laboratory in Ispra, Italy, for analysis.

Details of QC procedures used have been reported (WHO, 1979a; Yeoman, 1981). For acceptance a laboratory needed to obtain 80% of its results in any series of test within the

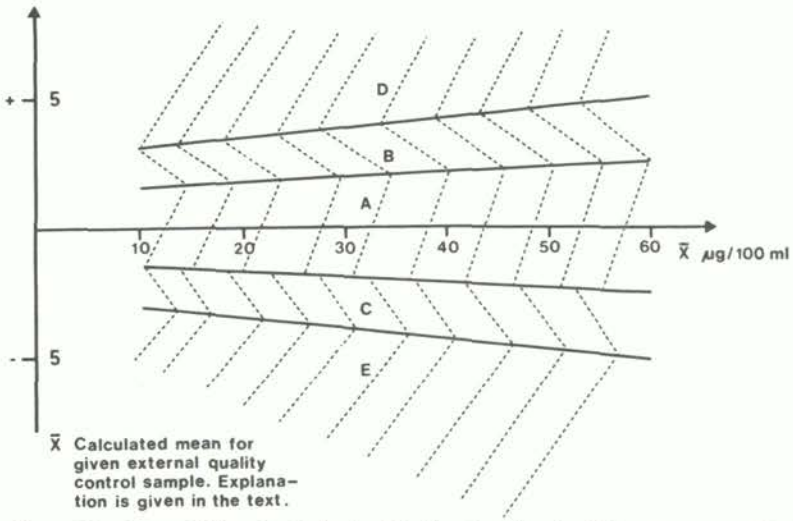


Figure 2:1. Acceptability criteria for lead in blood within the CEC programme. Modified from: Yeoman (1981) (For explanation, see text).

intervals A, B and C in figure 2:1. If two thirds of these results lie outside segment A, the proportion of results within B and C must not be biased to a higher proportion than 2:1 between these two segments. The limiting values for segment A are 100±15 µg Pb/l to 600±25 µg Pb/l, and for segments B and C 100±30 µg Pb/l to 600±50 µg Pb/l. It is appreciated that the criteria may not be tight enough due to the acceptability of distribution of results in those segments outside segment A. The effect of a bias towards segment B or segment C can have far-reaching effects on the apparent distribution of blood lead levels in a population during a monitoring exercise.

Initially 56 laboratories participated in the QCP. Assessment of performance was made in each Member State by "Competent National Authorities". Of the 33 laboratories which were finally retained for the campaign by the National Authorities 11 (33%) obtained more than 90% of results within the acceptable criteria, a total of 16 laboratories (48.5%) obtained more than 80% of acceptable results, and a total of 22 laboratories (66.6%) attained more than 70% of results within the acceptance band. No other results of the QCP have been reported.

The results obtained during the first CEC study are summarized in table 2:4 (Berlin, 1982). Some selected results for locations comparable to those monitored in the UNEP/WHO project are given in table 2:5. It can be seen that the median values as a rule were above 100 µg Pb/l. In very few cases for men the median exceeds 200 µg Pb/l. In general, the median levels for women are between 20 and 50 µg Pb/l lower than for men, and the number of women with high blood lead levels are substantially lower than for men.

Table 2:4. CEC biological screening of the population for lead; overall results (µg Pb/l). From: Berlin (1982)

Total number of subjects	Median (community)	Number of subjects with >300 µg Pb/l	Number of subjects with >350 µg Pb/l
17,609	130	367 (2%)	184 (1%)

Table 2:5. CEC biological screening of the population for lead. Blood lead (μg Pb/l) of non-exposed adults in selected cities (inner urban areas with more than 500,000 inhabitants) From: Berlin (1982)

Area	MEN No. of subjects	MEN Percentile 50	MEN Percentile 90	WOMEN No. of subjects	WOMEN Percentile 50	WOMEN Percentile 90
Belgium						
Brussels	*75*	190	250	*47*	160	210
Denmark						
Copenhagen	*35*	118	180	*36*	89	130
Federal Rep. Germany						
Hamburg	*43*	120	209	*42*	110	188
	22	120	189	*42*	115	169
Hannover	*60*	125	179	*42*	90	129
	44	100	159	*39*	80	120
France						
Bordeaux	*22*	150	280	*39*	120	230
Lille	*38*	120	260	*60*	110	210
Lyon	*96*	150	240	*110*	110	190
Marseille	*165*	160	270	*104*	100	160
Nantes	*20*	155	255	*69*	90	200
Nice	*40*	140	240	*57*	100	150
Paris	*350*	170	270	*424*	120	190
Toulouse	*51*	130	210	*54*	100	160
Ireland						
Dublin	*25*	160	200	*25*	125	199
Italy						
Bologne	*68*	210	350	*32*	110	208
Milan	*122*	180	280	*277*	130	190
Naples	*98*	210	280	*100*	150	208
Rome	*241*	200	280	*180*	150	219
Torino	*84*	200	380	*112*	150	230
Luxembourg						
Luxembourg	*59*	150	210	*52*	115	160
Netherlands						
Amsterdam	*50*	150	280	*50*	100	130
United Kingdom						
Birmingham-Sparkbrook	*46*	170	234	*51*	120	180
Birmingham-Handsworth	*55*	160	245	*44*	115	186
Leeds	*55*	170	260	*45*	130	210
Liverpool	*43*	150	250	*57*	120	200
London-Islington	*39*	140	201	*48*	100	144
London-Lambeth	*95*	150	200	*105*	100	150
Manchester	*46*	190	274	*54*	150	216
Sheffield	*52*	160	230	*48*	120	172

2.2.2 Cadmium in kidney cortex

In several studies cadmium concentrations in kidney cortex, obtained at autopsies, have been analyzed in order to estimate the accumulation of cadmium in the general population. Some of these studies have been reviewed and discussed by Friberg et al. (1974). A general increase in cadmium levels with age was found up to about 50 years after which the concentrations levelled off and decreased. The highest values at age 50 (means

60–120 mg Cd/kg wet weight) were observed in Japanese studies (Ishizaki et al., 1970; Kitamura et al., 1970; Tsuchiya et al., 1972). In Europe and the USA mean values between 15 and 50 mg Cd/kg were reported (Schroeder et al., 1967; Anke & Schneider, 1971; Piscator & Lind, 1972; Hammer et al., 1973). Valid quality assurance was often lacking, but the risk of gross analytical errors is not the same as in the analyses of cadmium in blood because of much higher concentrations of cadmium in the kidneys.

In one internationally co-ordinated study (compiled by Kjellström, 1979; based on data from Elinder et al., 1976; Tsuchiya & Iwao, 1978; and Kowal et al., 1979) with quality control, cadmium concentrations from cases of accidental or "sudden death" showed geometric means at age 50 in Tokyo, Dallas and Stockholm of 65, 20 and 15 mg Cd/kg wet weight, respectively. Another large study also with quality control (Gross et al., 1976) confirmed the U.S. data. A Finnish study (Vuori et al., 1979) showed similar results, 25 mg Cd/kg wet weight.

2.3 Intra- and interlaboratory comparison studies on the analysis of lead and cadmium

To evaluate the accuracy of trace metal analysis in biological materials, a number of intra- and interlaboratory comparison studies have been carried out in the past by different laboratories and organizations. As the results of such studies may be of importance in interpreting the validity of reported data, some of these are summarized below.

The American Industrial Hygiene Association sponsored interlaboratory comparisons to test the accuracy of blood lead analysis (Keppler et al., 1970). In this study samples of spiked blood (200–2910 μg Pb/l) were sent to ten different laboratories to be analyzed by methods routinely used by these laboratories. The overall variation in results was large for both the high and the very low concentrations. Results of up to 4300 μg Pb/l were reported for normal blood and down to 0 μg Pb/l for blood spiked with 1050 μg Pb/l. There was also great variation between results obtained from the same laboratory with time. The problem was not associated with specific analytical techniques.

Donovan et al. (1971) have reported on lead analysis at laboratories in Pennsylvania, USA. In response to an enquiry to 267 different laboratories, 20 laboratories agreed to participate in an intercomparison programme for analysis of lead in urine. Spiked urine (136 μg Pb/l urine) was sent to each laboratory to be analyzed by the method normally used at the laboratory. Reported results ranged from being 10 times lower to 20 times higher than the "true" value. When notified of their inaccuracy, some of the laboratories sought technical assistance. Seven laboratories with relatively good results on lead analysis in urine were later provided with blood samples spiked with 155 μg Pb/l blood. The results reported ranged between 10 and 150 μg Pb/l blood.

In 1972, the reference laboratory of the European Intercomparison Programme (Berlin et al., 1973) sent an aqueous solution of lead nitrate (100 μg Pb/l) and three samples of blood (two from persons occupationally exposed to lead and one from an unexposed person) to 22 different laboratories. Methods used for analyses included atomic absorption spectrophotometry, dithizone extraction and colorimetry, polarography and emission spectrophotometry. For the aqueous solution, results varied from 51 to 180 μg Pb/l, but 70% of the results did not deviate more than 10% from the "true" values. Blood lead levels were reported to range from 140 to 860 μg Pb/l (median: 500 μg Pb/l), 210 to 1170 μg Pb/l (median: 660 μg Pb/l) and 120 to 740 μg Pb/l (median: 410 μg Pb/l) for the three samples, respectively.

Large variations were reported also in "experienced" laboratories by Browne et al. (1974). Samples of heparinized blood (45 in 1973 and 48 in 1974) from lead workers were sent to three different laboratories for analysis by atomic absorption spectrophotometry or anodic stripping voltammetry. Each laboratory had been reported to handle more than 2,000 blood samples per year for lead analysis. The mean difference between results reported by the participating laboratories was as high as 290 μg Pb/l blood in 1973 and 440 μg Pb/l blood in 1974.

In another study (Lerner, 1975) one blood sample obtained from a single person was divided into 35 separate samples and sent to a well recognized laboratory (the Kettering Laboratory) over a period of nine months together with other samples. A considerable variation in lead levels was found with a mean of 191.3 μg Pb/l blood and a standard deviation of 57.2 μg Pb/l. The values ranged from 120 to 420 μg Pb/l blood.

Lauwerys et al. (1975) evaluated the results from 66 European laboratories participating in an interlaboratory comparison programme for the analysis of cadmium, lead and mercury in water, blood and urine. The analytical methods used were atomic absorption spectrophotometry (flame and flameless), anodic stripping voltammetry, colorimetry and neutron activation analysis. There were large variations in results for all the metals and all the media analyzed. As an example three samples of lead in blood showed median values, interlaboratory coefficients of variation (CV) and ranges as follows: median 128 μg Pb/l, CV 52.2%, range 27–490 μg Pb/l; median 227 μg Pb/l, CV 42.9%, range 103–873 μg Pb/l; median 233 μg Pb/l, CV 77.5%, range 10–1150 μg Pb/l. Corresponding results for cadmium in the same blood samples were: median 7 μg Cd/l, CV 168%, range 1–92 μg Cd/l; median 9 μg Cd/l, CV 116%, range 0–73 μg Cd/l; median 10 μg Cd/l, CV 143%, range 0–110 μg Cd/l. The variability of the results, according to the authors, could not be attributed to either the different analytical methods used or to the difference in experience in trace metal analysis at the laboratories.

Paulev et al. (1978) sent blood, urine and aqueous solutions spiked with lead and cadmium to five laboratories, three in Scandinavia and one each in Britain and Canada. Recovery of the added lead in the blood samples (470 μg Pb/l) ranged between 250 and 470 μg Pb/l. The results for two blood samples spiked with cadmium (20 and 67 μg Cd/l) ranged for three laboratories between 10 and 21 μg Cd/l and 40 and 62 μg Cd/l, respectively.

Maher et al. (1979) sent pooled blood from lead workers to 24 laboratories on nine occasions during 1974 and 1977. They tested different methods but found no significant bias that could be attributed to the method of analysis as such. In six different rounds, the coefficient of variation (CV) varied between 1.7% for a sample with a mean value of 575 μg Pb/l and 10.7% for a sample with a mean value of 250 μg Pb/l. The CV did not improve significantly with time. It was concluded that with available methodologies (atomic absorption spectrophotometry, flameless and Delves Cup, dithizone extraction, colorimetry, anodic stripping voltammetry and spectroscopy) the optimum coefficient of variation between laboratories would be about 7%.

Boone et al. (1979) compared the results from 113 laboratories participating in the "Blood Lead Proficiency Testing Program" conducted by the Center for Disease Control, USA, with the results from isotope dilution mass spectroscopy at the U.S. National Bureau of Standards. The blood for the interlaboratory comparison was obtained from cattle orally fed with lead nitrate, and the concentrations ranged from 130 to 1020 μg Pb/l blood. Twelve separate samples were dispatched to each laboratory for analysis. It was concluded that most methods overestimated the lead concentration when the actual concentration was low (less than 400 μg Pb/l blood) and underestimated it when the actual concentration was high (more than 500 μg Pb/l blood). The overall coefficient of variation

ranged from 29 to 73% at NBS values of 124 μg Pb/l blood and from 9 to 37% at an NBS value of 1020 μg Pb/l.

The reviewed studies show that the accuracy and precision of trace metal analysis in biological specimens, especially lead and cadmium in blood and urine, in general appear to be unsatisfactory. This may also hold for laboratories that have gained considerable experience by analyzing a large number of biological samples over many years. Of the analytical methods currently available for analyzing trace metals in biological materials, no single method has been found to be distinctly superior to the others.

3. Quality control

3.1 Introduction

It was recognized at an early stage of the project that there was a great need for quality control (QC) to enable comparison of data and that quality control procedures should be planned and implemented before the actual monitoring of lead and cadmium was started. Quality control was considered to be both an activity of its own and a necessary and integral part of the subsequent monitoring operation. Although it was foreseen that common instructions for sampling and sample handling would be needed, it was not the intent to standardize analytical procedures. At the planning meeting on QC in Geneva 1979 it was decided that each laboratory would be permitted to use procedures of its own choice, as long as they produced acceptable results (WHO, 1979a).

QC was not to be concerned only with the accuracy of the analytical methods (analytical quality control) but also took into consideration questions relating to sampling and avoidance of contamination during sampling and storage (preanalytical quality control). It was agreed that a rather pragmatic approach should be taken and requirements would be kept within reasonable limits since the project was of a pilot nature, and since the data resulting from the project would not be used within regulatory or other legal processes.

The QC programme has involved the preparation of blood and kidney quality control samples, some of which were spiked with known concentrations of lead and cadmium. The samples were tested with respect to stability and homogeneity. Procedures for sterilization and long-range transport were worked out. "True" values were obtained with the assistance of reference laboratories. A detailed procedure was worked out for the evaluation of results in order to get criteria for acceptance and rejection of the results. Follow-up meetings on QC were held with representatives of the participating laboratories in Mexico City, 10–15 December 1979 (WHO, 1979b), Zagreb, 20–22 February 1980 (WHO, 1980c) and Stockholm, 27–30 May 1980 (WHO, 1980a).

3.2 Analytical quality control procedures

The analytical quality control has by and large followed the original design throughout the project. The CI distributed an appropriate number of quality control samples to the participating laboratories. Each quality control set included both internal quality control samples (IQC samples, concentrations of metals known to the laboratories) and external quality control samples (EQC samples, concentrations not known to the laboratories). The use of IQCs followed the pattern worked out within the CEC programme. The operational procedure is shown in figure 3:1.

When the project started IQC samples for the analysis of lead in blood were provided by the CEC and were used during the major part of the quality control programme. They contained about 70 and 370 μg Pb/l blood, respectively (median values from several

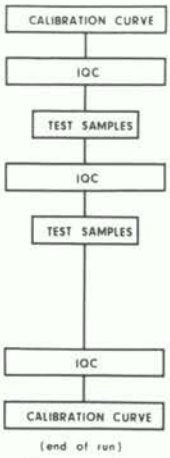


Figure 3:1. Flow sheet to illustrate use of internal quality control samples (IQC) in analytical programme.
From: WHO (1979a).

laboratories within the CEC programme). Similar samples for the analysis of cadmium in blood were prepared at the CI. They contained 2.3 and 10.7 μg Cd/l blood, respectively. These samples were also spiked with lead and contained 94 and 394 μg Pb/l blood, respectively. For the analysis of cadmium in kidney cortex, samples of freeze-dried horse kidney cortex containing about 220 mg Cd/kg dry weight were provided as IQC samples.

For the external quality control participating laboratories received sets of EQC samples to be analyzed together with the IQC samples. The results were sent to CI for evaluation. The laboratories received a feed-back, usually via telex, in the form of "true" values (reference values), acceptance intervals, and information on whether or not the results met the criteria for acceptance previously agreed upon (see section 3.5).

On average twelve sets of quality control samples were distributed during the QC training phase. The first two sets (QC 1 and 2) consisting of 14 and 11 blood samples, respectively, were obtained from the CEC programme. From QC 3 onwards each QC set consisted of six blood samples prepared at the CI for analysis of cadmium and lead and from QC 4 each set also included four kidney cortex samples for cadmium analysis.

It was agreed that analysis of blood and kidneys from the target populations should not start until results from the training phase were considered satisfactory according to CI. Quality control during the monitoring phase was based on five QC sets to be analyzed together with about 200 blood samples and three QC sets to be analyzed together with about 50 kidney cortex samples. It was recommended that the analysis of collected blood samples should start with one QC set and continue with one set every 50 samples. For kidney cortex samples the QC sets were to be analyzed before and after the autopsy samples as well as after about 25 autopsy samples. As a rule the QC samples were not analyzed on a blind basis, i.e. the analysts knew that they were QC samples although the concentrations were unknown.

3.3 Preanalytical quality control

There are many possibilities to contaminate biological samples through use of e.g. unsuitable blood collecting vials and contaminated anticoagulants (Zief & Mitchell, 1976;

Nackowski et al., 1977; Nise & Vesterberg, 1978). Furthermore, contamination may originate from the skin if not properly cleaned, or from contaminated cleaning solutions (Bratzel & Reed, 1974). Studies on commercially available blood collection tubes have shown that diluted nitric acid as well as blood may extract lead and cadmium from certain vials and syringes in quantities which would invalidate any measurement of these metals in blood within the normal range of concentration (Nise & Vesterberg, 1978).

To avoid contamination as much as possible, evacuated blood collection tubes (Venoject, Terumo Corp., Tokyo, Japan) with heparin from the same batch were provided by the CI after control of the metal content in a suitable number of tubes from the batch. Eight tubes were randomly selected from a box of 100 heparinized blood collection tubes and ten grams of 0.01 M nitric acid added to each tube. After 11 days of storage at room temperature the content of lead and cadmium was analyzed by AAS (ETA). Some tubes were turned over so that the acid came into contact with the rubber caps during storage. The results showed a mean cadmium concentration of 0.06 μg Cd/l in the acid solution, and a lead concentration of less than 0.8 μg Pb/l (both close to the detection limit of the analysis) in all tubes tested. No contamination from the rubber caps was noticed. A similar study on 20 Venoject tubes containing EDTA as anticoagulant showed a mean cadmium content of 0.14 μg Cd/l and a mean lead content of 0.85 μg Pb/l. It was decided to use tubes with heparin since they seemed to contain less metals compared to the tubes with EDTA. If in the future EDTA tubes with low metal content will be available, the use of such tubes may have certain advantages (see Appendix 1).

It was recommended that before collecting blood, the skin should be carefully washed and then cleaned with disposable napkins, saturated with 70% isopropyl alcohol (Medi-Swab, Pharmax Limited, Bexley, U.K.), provided by CI after check for metal content. Written instructions for the sampling of blood were worked out (WHO, 1980a) and a demonstration was made at CI during the meeting in Stockholm in May 1980. The participating institutions were requested to prepare a protocol with information on the personnel collecting the samples, procedures for collection, transport and storage of samples and any additional procedure.

After collection of the blood samples, the blood from each tube was transferred to three 5 ml tubes of polypropylene (washed with diluted nitric acid and deionized water) provided by the CI. They were deep-frozen as soon as possible. One of the three tubes was stored to allow duplicate analysis at the CI or a reference laboratory. Blood not used for the initial analysis was stored to make possible reanalysis at the laboratory if necessary.

The risk of a significant contamination was considered less pronounced for kidney cortex samples owing to the higher concentrations of cadmium in the kidneys than in blood. To avoid contamination to the greatest possible extent and to get comparable samples, the laboratories received a film showing procedures for collection of kidney cortex samples at autopsies. The film was produced by WHO/IAEA in relation to the project "WHO/IAEA Joint Research Programme on Trace Elements in Cardiovascular Diseases (Autopsy Studies)" (Masironi & Parr, 1979). The kidneys were to be opened longitudinally (with e.g. stainless steel knives) and a slice containing the cortex and medulla was to be isolated. From this slice pyelic fat should be eliminated and a portion of the cortex carefully isolated and collected, then put in a suitable container of polypropylene or polyethylene (previously washed with diluted nitric acid and deionized water) and deep-frozen as soon as possible. After collection the samples were kept deep-frozen until analysis. Some of the collected material was kept in storage to enable reanalysis and/or duplicate analysis at a reference laboratory. A description of the procedures used for the kidney cortex collection was prepared by each laboratory and sent to CI.

3.4 Quality control samples

3.4.1 General

Blood samples for quality control purposes have been used within the CEC programme (section 2.2.1.2). The samples consisted of human or bovine blood with EDTA as anticoagulant, hemolyzed by ultrasonication and sterilized by gamma irradiation (Yeoman & Berlin, 1979). Some of the samples were spiked with lead nitrate. The stability of lead in the blood samples was studied in a series of experiment and found satisfactory. Some of the CEC samples were used during an early phase (QC 1 and QC 2) within the UNEP/WHO project.

The QC samples in the UNEP/WHO project had to meet certain criteria. The number of samples of various concentrations of both lead and cadmium had to be large enough to make possible several quality control runs at the participating laboratories. Samples had to be stable over long periods of time and withstand transport from the CI to the participating laboratories in different parts of the world. Transport could last more than 24 hours and the outside temperatures might reach 30—40°C. The QC samples had to be sterilized to facilitate custom clearance. To get enough blood with a low background level it was considered advantageous to use animal blood. The possibility of using freeze-dried blood spiked with the metals was considered, since such samples would be less sensitive to temperature variations than liquid blood. Preliminary studies both at the CI and within CEC, however, indicated problems in the reconstitution of the blood. Furthermore, freeze-dried blood samples would require distribution of deionized water or buffer solutions, free of metals, to the laboratories in order to minimize the possibilities of contamination at the reconstitution step. It was therefore decided to use the blood as such without freeze-drying.

Some information on the preparation of blood samples and the storage and handling of such samples was available from the literature and for lead from the experience within the CEC programme. By and large it was felt necessary, however, to make a thorough study at the CI on the influence of different environmental factors. This was felt particularly important for cadmium in blood as virtually no information existed which could be used directly for this project.

For analysis of cadmium in kidney cortex the NBS standard reference material bovine liver was used in an initial phase of the project. It has a matrix similar to that of kidneys, but the main disadvantage is that the concentration of cadmium is much lower than normally found in kidney cortex. Furthermore, bovine liver is available only with one concentration of cadmium whereas for quality control purposes several samples with different concentrations of cadmium were required.

Horses accumulate cadmium in the kidney cortex to an even greater extent than humans (Piscator, 1976). As cadmium concentrations in kidney cortex increase with age, it was possible to obtain kidney cortex samples with varying concentrations of cadmium by selecting kidneys from horses of different ages at slaughter houses. The kidneys were homogenized and freeze-dried in a way similar to that of the NBS bovine liver.

The studies on which the preparation of QC samples were based are reported in Appendix 1. The procedures eventually adopted are reviewed in the following section for both blood and kidney cortex.

3.4.2 Procedures used for preparation of quality control samples

3.4.2.1 Blood

Type of blood: Cow blood collected at slaughter house.

Anticoagulant: EDTA (dipotassium salt), 1.5 mg EDTA/ml, added to the beaker before collection of blood.

Hemolysis: Ultrasonication (MSE Ultrasonic Disintegrator, 150 W). Aliquots of 50 ml blood, cooled with ice, were ultrasonicated for 10 minutes at an amplitude of 8 μm and a frequency of 20 kHz/sec. Less than 1% of the original red cells remained after ultrasonication as evaluated in a Bürker cell counting chamber. The blood was centrifuged at 5000 rpm for 20 minutes to remove cell debris.

Spiking: Cadmium nitrate and lead nitrate in deionized water were added to the blood. The volume of the added standard solutions was always less than 2% of the total blood volume.

Dispensing: During the first phase of the project blood was dispensed in 1.5 ml blood centrifuge tubes of polypropylene with press-on-caps washed with diluted nitric acid and deionized water. To avoid the risk of leakage from the press-on-caps when tubes were exposed to variations in temperature, 5 ml polypropylene tubes with screw-on-caps, acid washed, were used during the main part of the project.

Sterilization: After dispensing the samples were sterilized by gamma irradiation (total dose of 2.5 Mrad).

Storage and transport to participating laboratories: Samples were stored deep-frozen. For the transport to participating laboratories they were packed with ice in neopolyene containers. With four cooling blocks (each containing about 600 g of a water solution of CMC) and the containers kept at a room temperature of about +20°C, the inside temperature remained below +5°C for 48 hours and below +10°C for 72 hours. All samples were sent air freight and laboratories were informed in advance of the exact arrival time. It was requested that parcels be stored in freezer at the airports during transit whenever possible.

3.4.2.2 Horse kidney cortex

Material: Quality control samples for cadmium in kidney cortex were prepared from horse kidneys. The kidneys were obtained from a slaughter house (Kalmar county slaughter house, Kalmar, Sweden) where they had been deep-frozen immediately after slaughter. The kidneys were thawed just enough to make possible their cutting in 2–3 mm slices. The cortex was separated from the medulla and cut into 5 mm pieces. These were deep-frozen in liquid nitrogen, piece by piece, to avoid clotting.

Homogenization: Homogenization of the horse kidney cortex was performed by liquid nitrogen grinding using a cryogenic grinding machine (Spex Industries, Inc., N.J., USA; Shatterbox 8500, cryogenic grinding disc 8509). The grinding disc was cooled in liquid nitrogen for 40 minutes before use. About 100 g of deep-frozen horse kidney cortex was ground at a time. The ground material was freeze-dried and thoroughly mixed. It was kept in desiccators before dispensing.

Dispensing and storage: The freeze-dried horse kidney cortex powder was dispensed in 5 ml polypropylene tubes, previously washed in diluted nitric acid and deionized water. The samples were thereafter sterilized by gamma irradiation (2.5 Mrad) and stored at room temperature.

3.5 Statistical procedure and criteria for acceptance or rejection of laboratory performance

Results obtained for each set of QC samples from a participating laboratory were statistically assessed in order to decide whether to accept or reject the laboratory's current

performance. The main purpose of the procedure was to guard against systematic errors in the range of values likely to occur. Spiking of blood samples delivered to the laboratories ranged between 100–400 μg Pb/l in the case of lead, and between 1–15 μg Cd/l for cadmium. Cadmium in kidney cortex samples usually varied between 50–400 mg Cd/kg dry weight. In a diagram where y is the reported value and x is the "true" value, a recovery of 100% would correspond to a straight line through the origin and a regression of unity, i.e. $y = x$.

The most appropriate way of expressing a laboratory's performance is to calculate a regression line of the reported versus the "true" values—which can be thought of as an average of its current performance—and to establish an acceptance criterion based on how much the line may deviate from the ideal $y = x$. Another way would be to calculate all differences between y and x along the tested range and to stipulate a criterion for how much these differences would be allowed to deviate from zero. The former procedure has been employed in the present context since it lends itself more easily to making probability statements in terms of statistical power. Minor systematic errors are furthermore easier to observe and predict.

The concept of power is an essential ingredient of quality control, since it deals with the problem of evidencing "no effect", or rather the probability of neglecting a minor effect. The power expresses the probability of rejecting a null-hypothesis when it is false. A high power is essential in a control procedure. Accepting a laboratory that performs badly will lead to systematic over- or underestimation of the population averages that the method being evaluated aims to measure. Minor errors, however, cannot be totally avoided. Realistically, therefore, it was decided that regression lines with a certain deviation from the ideal, $y = x$, should be accepted. It was also considered necessary to allow for somewhat higher deviation as a proportion of x at the lower x-range than at the higher.

The limits for Maximum Allowable Deviations (MAD-lines) from the regression line $y = x$ were set as follows:

for lead in blood (μg/l)	$y = x \pm (0.1x + 20)$
for cadmium in blood (μg/l)	$y = x \pm (0.1x + 1)$
for cadmium in kidney cortex (mg/kg dry weight)	$y = x \pm 0.15x$

One basic parameter in the power calculation and hence in the quality control procedure is the random error of the method which can be estimated from each set of quality control results. As each quality control set consists of six samples only, the current calculation of the error of the method is largely influenced by one or two occasional gross errors, which are assumed to be random and unsystematic. Experience has shown that the error of the method centers around 10 μg Pb/l for lead in blood, around 0.5 μg Cd/l for cadmium in blood and around 3.0 mg/kg dry weight for cadmium in kidney cortex in well established laboratories. It has been judged as more appropriate to use these average values than to depend on estimates made each time. The use of such empirical estimates is generally regarded as bad practice when the number of observations are lower than about 30 since an erroneous estimation in this regard leads to faulty estimations of the power of the method. Using the average error estimation based on past experience, instead of an unusually high current error, may lead to an undue acceptance of the regression line. This is, however, quite rare and requires that the individual large deviations cancel out or else the line will certainly deviate to such an extent that the QC results will be rejected.

In the following a short account is given of the procedure used for acceptance or rejection of a quality control set. More detailed descriptions have appeared as working paper for the 1980 Stockholm meeting (Cederlöf, 1980), but are redundant here as the

procedures can also be obtained from statistical text-books. A few formulas are repeated, however.

The table below gives an account of the outcome of an assumed set of quality control analyses together with the reference values (μg Pb/l).

x (reference values)	100	160	220	280	340	400
y (reported values)	113	151	203	287	343	386

Calculation of the regression line reveals the function $y = 0.9643x + 6.095$. Some further statistics of interest are:

Mean of x-values ($\bar{x}$) = 250.0;
Mean of y-values ($\bar{y}$) = 247.2;
Error of method ($\sigma_{y/x}$) = 12.9 (in the acceptability calculations assumed to be = 10.0)

$$\sigma^2_{y/x} = \text{residual mean square} = \left(\Sigma(y-\bar{y})^2 - \frac{|\Sigma(x-\bar{x})\,(y-\bar{y})|^2}{\Sigma(x-\bar{x})^2} \right) \cdot \frac{1}{(n-2)}$$

where the Σ symbol indicates that the specified values from the six samples are to be summed, and

$$\Sigma(y-\bar{y})^2 = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$$

$$\Sigma(x-\bar{x})\,(y-\bar{y}) = \Sigma xy - \frac{\Sigma x \Sigma y}{n}$$

$$\Sigma(x-\bar{x})^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n}$$

Figure 3:2 shows the two MAD-lines $y = x \pm (0.1x + 20)$ and the current regression line with its data points. The line as well as the six points seem to be well accommodated between the MAD-lines. However, the line is only one sample out of an unlimited number of possible lines generated from an unlimited number of data which could be derived from six quality control specimens.

Any straight line in a coordinate system is completely defined by the regression equation $y = bx + a$. For closer scrutiny any arbitrary point along the line may be chosen, e.g. the point corresponding to $x = 100$. The line has here the function value

$$\hat{y}_{100} = 0.9643 \cdot 100 + 6.095 = 102.52$$

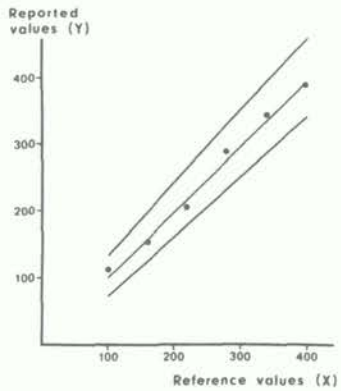


Figure 3:2. Regression line based on six reported values. Outer lines indicate the MAD-lines. For explanation, see text.

Like all sample statistics, however, also $\hat{y}_{100}$ has a sampling error, $\sigma_{\hat{y}}$, which is a function of

n = number of observations (6)
d = difference between x-value and x-mean (100−250 = −150)
σ_x = standard deviation of x-values (112.250)
$\sigma_{y/x}$ = error of method (assumed to be 10.000)

according to the formula

$$\sigma_{\hat{y}}^2 = \sigma_{y/x}^2 \left(\frac{1}{n} + \frac{d^2}{(n-1) \cdot \sigma_x^2} \right) = 10^2 \left(\frac{1}{6} + \frac{(-150)^2}{(6-1) \cdot 112.25^2} \right) = 52.4$$

The square root of this quantity, $\sigma_{\hat{y}}$, is often named "operating error" and equals 7.24. The operating error is the same also for the function of another x-value along the range, namely for x = 400, which is just as much above the x-mean (250) as 100 is below.

The philosophy behind the power calculation that determines the probability of excluding an unsatisfactory laboratory is revealed by figure 3:3. Three normal distributions are indicated in the chart, one with mean 70 (the function value of the lower MAD-line), another with mean 100 (the ideal line) and a third with mean 130 (the upper MAD-line). Of the two extreme curves each has one tail (shaded) between the vertical lines named C_L and C_U. The shaded tail occupies only 5% of its own curve. This implies that if the observed function value falls between C_L and C_U there is very little chance (low probability) that it belongs to a true line with true function value of 70 or lower or a true line with true function value of 130 or higher, which are the two points specified by the MAD-lines. The probability that an empiric function value within the interval C_L–C_U should belong to *either* the left *or* the right probability curve is in fact 10% only, implying that a test so constructed has a power of 100 − 10 = 90%. Probability theory tells us that line C_L cuts the abscissa at a point corresponding to 1.645 times the operating error right of the mean 70, and correspondingly, that the intersection of line C_U with the abscissa is 1.645 times the operating error left of the mean 130. Accordingly, the acceptance interval lies between

$$70 + 1.645 \cdot 7.24 \text{ and } 130 - 1.645 \cdot 7.24$$
$$81.9 - 118.1$$

Exactly the same calculations can be performed for the upper function value of 400, giving the interval

$$351.9 - 448.1$$

The empirical regression line chosen as example had the function values 102.5 and 391.8 at the evaluation points and is thus accepted.

If the points of the boundary lines C_L at the lower and upper evaluation points are connected with a straight line, one so-called "acceptance line" is constituted. If a similar operation is made for the points of the boundary lines C_U, an upper "acceptance line" is constituted. These acceptance lines are in reality not completely straight. The deviation is, however, of no relevance for the evaluation and has for simplicity not been indicated. Figure 3:4 shows the regression line with its data points, the MAD-lines (solid lines) and the acceptance lines (dotted lines). In the presentation of results of QC analysis only the acceptance lines, calculated for each QC run, are given.

Finally, it should be added that in employing two points on the curve for assessment, the combined power is $(1 - 0.1) \cdot (1 - 0.1) \cdot 100 = 81\%$. If one had wished to have a combined power of 90%, the interval for acceptance would have been somewhat decreased, in fact to 84 − 116 for the lower point and to 354 − 446 for the upper point.

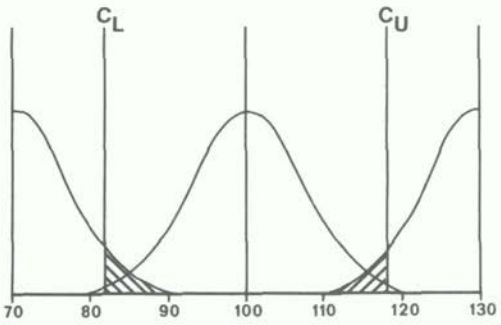


Figure 3:3. Hypothetical normal distribution curve of function value corresponding to x = 100, the ideal (y = x) relationship, and distribution curves corresponding to function values of two MAD-lines with mean of 70 (lower) and of 130 (upper). For further explanation, see text.

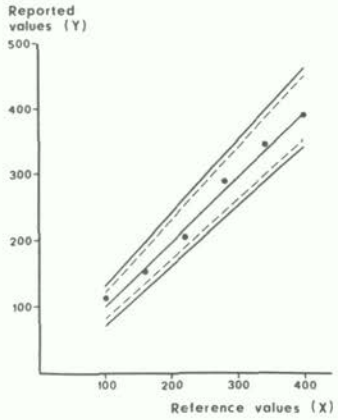


Figure 3:4. Regression line based on six reported values. The solid lines indicate the MAD-lines and the dotted lines the acceptance lines. For explanation, see text.

3.6 Reference values

3.6.1 Blood

3.6.1.1 General

To obtain the true concentrations of lead and cadmium analysis in biological tissue is not yet possible. The nearest approximation for blood analysis is probably attained with the Isotope Dilution/Mass Spectrometry (IDMS) method carried out in "ultra-clean" facilities (Barnes et al., 1973; Facchetti, 1978; Everson & Patterson, 1980).

At the planning meeting on quality control held in Geneva in 1979 (WHO, 1979a) it was agreed to use IDMS as reference method for both lead and cadmium in blood. It was decided to request the assistance of the U.S. National Bureau of Standards (NBS) and the CEC Joint Research Centre, Ispra, Italy (ISPRA). IDMS is extremely expensive both in time and cost per assay, and it was possible to get assistance only on a limited scale and at a rather late stage of the project. Lead analyses for reference purposes were performed also using atomic absorption spectrophotometry at ISPRA and at the Regional Toxicology Laboratory (RTL), Dudley Road Hospital, Birmingham, U.K.

The major problems with decisions on "true" values were related to the levels in unspiked blood. Estimations of the "true" values for unspiked blood were partly based on analyses of unspiked samples and partly on values from spiked samples after subtracting the spiked amount; this also made it possible to calculate the recovery of added lead and cadmium.

3.6.1.2 Lead

Reference values of the QC samples as a rule varied between 100–400 μg Pb/l blood. The basic lead level in unspiked bovine blood was estimated to be 24 μg Pb/l. This value was originally based on a limited number of analyses. Results from more extensive analyses did not justify a change of this value, which has thus been used as the "true" value for cow blood.

Table 3:1. Lead levels (µg Pb/l) in unspiked blood for CI and the different reference laboratories. Calculations obtained by subtraction of spiked lead from results at analysis

Laboratory	Method	Blood I			Blood II			Blood I+II
		Mean	SD	*n*	Mean	SD	*n*	Mean
CI	DC	16	10	*7*	33	12	*13*	24
	ETA	21	11	*10*	17	13	*13*	19
ISPRA	DC	29	7	*9*	41	9	*12*	35
	ETA	–	–	–	41	14	*9*	41
RTL	DC	–	–	–	26	14	*12*	26
NBS	IDMS	8	2	*8*	13	–	*2*	10
ISPRA	IDMS	11	7	*7*	31	18	*6*	21
Mean (total)		17			29			25
Mean (IDMS)		9			22			15

DC = Delves Cup
ETA = Electrothermal Atomization
IDMS = Isotopic Dilution/Mass Spectrometry

Table 3:1 gives results of analyses obtained at the CI and at laboratories that assisted the CI in establishing reference values. The values represent observed concentrations minus lead added through spiking. The mean value for all analyses was 25 µg Pb/l. The mean value for blood II, which was used for preparation of QC samples during the latter half of the programme, was 29 µg Pb/l.

There was a tendency towards lower values for IDMS at NBS. The reason for this is not known and IDMS analyses at ISPRA did not give such low values. At NBS all analyses were carried out in "ultra-clean" environments which may in part explain the low values. Such "ultra-clean" facilities did not exist in any of the other laboratories. It would be of interest to study the reason for the differences systematically, but they are of only minor importance for present purposes.

The mean recovery for the different laboratories and methods are presented in table 3:2. The recovery was approximately 100% for all methods. The low standard deviation for IDMS at NBS is noteworthy.

Table 3:2. Recovery expressed as mean of ratios (x100) between spiked lead and observed lead levels (mean base levels for each laboratory, method and blood batch have been subtracted)

Laboratory/method	*n*	Recovery %	Standard deviation
NBS/IDMS	*8*	101	2.9
ISPRA/IDMS	*13*	100	10.2
CI/DC	*18*	98	7.3
CI/ETA	*21*	98	8.1
ISPRA/DC	*19*	100	6.2
ISPRA/ETA	*9*	101	8.0
RTL/DC	*12*	97	7.9

Table 3:3. Cadmium levels (µg Cd/l) in unspiked blood for CI and NBS laboratories. Calculations obtained by subtraction of spiked cadmium from results of analysis

Laboratory	Method	Blood I			Blood II			Blood I+II
		Mean	SD	*n*	Mean	SD	*n*	Mean
CI	ETA	0.3	0.56	*6*	0.3	0.33	*24*	0.3
NBS	IDMS	0.4	0.21	*2*	0.4	0.54	*5*	0.4

3.6.1.3 Cadmium

The reference values of the QC samples as a rule varied between 1 and 15 µg Cd/l blood. The cadmium level for unspiked bovine blood was originally estimated to be 0.2 µg Cd/l. Later analyses indicated that the basic levels were slightly higher, but not significantly so. Throughout the project 0.2 µg Cd/l was used as the basic level.

Table 3:3 gives the results of QC analyses at CI and NBS. The values represent observed concentrations minus cadmium added through spiking. The mean values at CI and NBS were 0.3 and 0.4 µg Cd/l, respectively. In their comments NBS state that their cadmium analyses were not as reliable as their lead analyses because of the presence of high and varying blanks. NBS estimated that this introduced an uncertainty of about 0.2 µg Cd/l.

At NBS an experiment was carried out to determine whether cadmium had been adsorbed to the container walls. No cadmium above the analytical blanks was found in the container walls after the blood was taken out, indicating that the container material and the storage conditions were satisfactory. The observations confirm results obtained at the stability tests at CI (Appendix 1).

Table 3:4 gives the mean recoveries obtained with the ETA-S method at CI and the IDMS at NBS. The mean recovery was approximately 100% for both methods but with fairly large standard deviations.

3.6.2 Cadmium in kidney cortex

For cadmium in kidney cortex, where concentrations are high compared to those in blood, it is easier to obtain accurate results. At the planning meeting in Geneva (WHO, 1979a) it was agreed to use neutron activation as reference method and it was decided to request the assistance of the International Atomic Energy Agency (IAEA), Vienna, Austria, as a reference laboratory.

Ground, freeze-dried horse kidney cortex (see section 3.4.2) was used as reference material for human kidney cortex. Most concentrations ranged between 50 and 400 mg Cd/kg dry weight. No spiking was employed.

At CI kidney cortex was analyzed with a conventional flame AAS method. The samples were analyzed with neutron activation technique at the IAEA, at the Kern-

Table 3:4. Recovery expressed as mean of ratios (x100) between spiked cadmium and observed cadmium levels (mean base levels for each laboratory, method and blood batch have been subtracted)

Laboratory/method	*n*	Recovery %	Standard deviation
NBS/IDMS	*7*	105	12
CI/ETA	*30*	97	15

Table 3:5. Cadmium concentrations in different samples of horse kidney cortex (mg Cd/kg dry weight) and ratio between analysis at CI and reference laboratories

AAS	Neutron Activation (NA)					Ratio CI/Ref lab	
CI	IAEA	Jülich	Delft	TRC	Mean	CI/Mean (NA)	CI/IAEA
10	9			10	10	1.00	1.11
34	33			32	33	1.03	1.03
79	79	63		76	73	1.08	1.00
81	74	68		87	76	1.07	1.09
82	84	73		93	83	0.99	0.98
112	103			121	112	1.00	1.09
117	97	101	110	131	110	1.06	1.21
139	146	130		150	142	0.98	0.95
147	143			134	139	1.06	1.03
158	153			169	161	0.98	1.03
189	178	178	180	212	187	1.01	1.06
190	164	167	178	189	175	1.09	1.16
196	158	173		193	175	1.12	1.24
198	193			172	183	1.08	1.03
210	209	182		232	208	1.01	1.00
244	192	212	229	260	223	1.09	1.27
252	250	214	245	276	246	1.02	1.01
287	244	233		307	261	1.10	1.18
310	310			284	297	1.04	1.00
356	306			355	331	1.08	1.16
361	332			391	362	1.00	1.09
368	365	290		376	344	1.07	1.01
389	382	331		397	370	1.05	1.02
						Mean 1.04	Mean 1.08
						S.E. 0.009	S.E. 0.018

forschungsanlage in Jülich, Federal Republic of Germany and at the Interuniversity Reactor Institute in Delft, the Netherlands. CI also arranged for neutron activation analysis at the Tekniska Röntgencentralen (TRC), Stockholm, Sweden.

The results are given in table 3:5. On average there is good agreement and the values from CI have throughout the programme been used as reference values. They were the only ones available until late in the project. The agreement between AAS and NA was in fact even better than indicated by the figures in table 3:5 since the AAS measurements were made on re-dried specimens whereas the NA measurements were made on the specimens as received. The moisture content was probably of the order of 2–6%. Adjustment of the results to correct for this would have improved the agreement by the same percentage.

For individual values also, the differences among the CI and the reference laboratories as well as between the reference laboratories themselves as a rule were relatively minor although quite noticeable. They could not be explained by inhomogeneities between the samples as analysis at CI of different batches from the same samples showed good agreement (Appendix 1).

3.7 Quality control training phase. Results and analytical aspects

3.7.1 Introduction

Two main objectives of the project were to apply rigid quality control and, whenever needed, to provide technical assistance to the participating laboratories. The outcome of

Table 3:6. Reference values, acceptance intervals and function values as reported to the laboratories (example)

LEAD IN BLOOD						
Sample No	*13*	*14*	*15*	*16*	*17*	*18*
Reference value, x	100	160	220	280	340	400
Reported value, y	113	151	203	287	343	386

Acceptance intervals	Function value
for 100: 81.9–118.1	102.52
for 400: 351.9–448.1	391.81

Since the two function values are both within their respective acceptance intervals, the QC set is accepted.

the quality control training phase of the project thus constitutes an important part of the results of the whole project. The outcome of the training phase can be evaluated in different ways. In the section below results are presented with emphasis on the progress within each laboratory. Subsequent sections review analytical methods and discuss the analytical problems encountered at the laboratories as reported by a consultant.

3.7.2 Results of the quality control training phase

3.7.2.1 General

The results of the analyses of each set of 6 blood samples and 4 kidney cortex samples were reported to the CI. Results obtained on the internal quality control samples analyzed together with the external quality control samples were also reported.

The results were evaluated at the CI and sent back to the laboratory together with the reference values before the next quality control run was started. Criteria for acceptance were agreed upon at the meeting in Stockholm in May 1980 (section 3.5). Acceptance intervals and function values for the evaluation points ($x = 100$ and $x = 400$ μg Pb/l for lead in blood; $x = 1.5$ and $x = 12$ μg Cd/l for cadmium in blood; $x = 100$ and $x = 400$ mg Cd/kg dry weight for cadmium in kidney cortex) were calculated for each set of quality control results and reported to the laboratories. It was also stated whether the results were accepted according to the criteria or not. A feed-back could look like the example in table 3:6.

The results on lead and cadmium in blood were plotted in diagrams against the reference values. The regression line and the acceptance lines (see section 3.5) were calculated and included in the diagrams. Examples of such diagrams are given in figure 3:5. Diagrams A and B show results which are rejected (regression lines fall outside acceptance lines) while diagram C gives an example of an accepted QC run (regression line is within acceptance interval).

In order to obtain a quantitative estimate for easy comparison of results from different QC runs, ratios were formed based on the following calculations. The difference between the function values at each evaluation point and the "true" values (the value on the regression line $y = x$) were divided by the accepted deviation at the evaluation points. The procedure is illustrated in figure 3:6. The calculations can be expressed as

$$\frac{a_1}{b_1} \text{ and } \frac{a_2}{b_2}$$

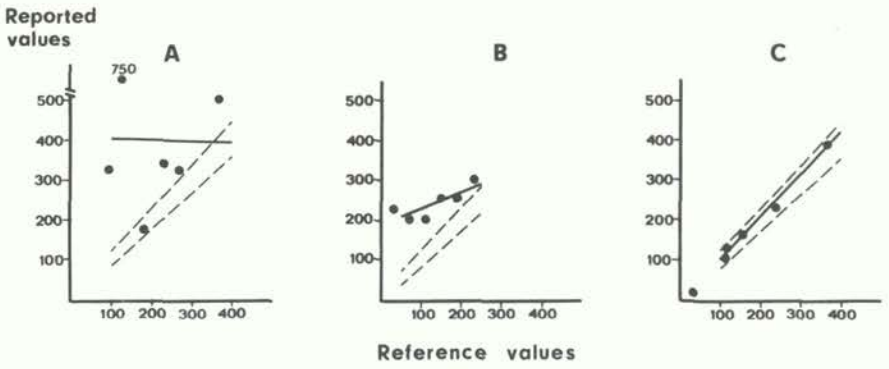


Figure 3:5. Diagrams with reported quality control results (µg Pb/l) plotted against the reference values. The solid line indicates the calculated regression and the dotted lines (acceptance lines) indicate the acceptance interval. A and B show rejected results and C accepted results.

where a_1 and a_2 in figure 3:6 indicate the differences between the end points of the calculated regression line and the "true"values, and b_1 and b_2 indicate the accepted deviation from the "true"value (differences between the acceptance lines and the "true" values).

In figure 3:6, A shows an accepted QC run, although the calculated regression line is just on the border for acceptance at the upper evaluation point (ratio −1.0). B shows a rejected QC run (ratio 6.7 at the lower evaluation point).

The calculated ratios have been indicated as bars in diagrams, an example of which is given in figure 3:7. The left bar of each pair represents the ratio at the lower evaluation point, while the right bar of each pair represents the upper evaluation point. The maximum allowable ratio for acceptance is 1. Negative ratios indicate that the results obtained are lower than the reference values, while positive ratios indicate that the results are higher. If one or both bars in each pair cross the acceptance lines (ratios higher than +1 or less than −1) the QC run is rejected. The height of the bar gives an estimate of how much reported results deviate from the reference values.

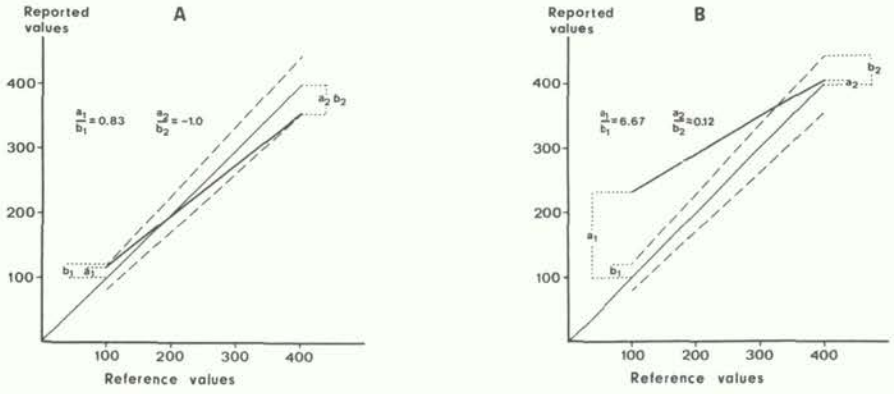


Figure 3:6. Illustrations of the procedure used for the calculation of ratios between obtained deviation from the reference value and the accepted deviation at the preset evaluation points (100 and 400 µg Pb/l). For explanation, see text. A gives an example of an accepted QC run and B of a rejected QC run.

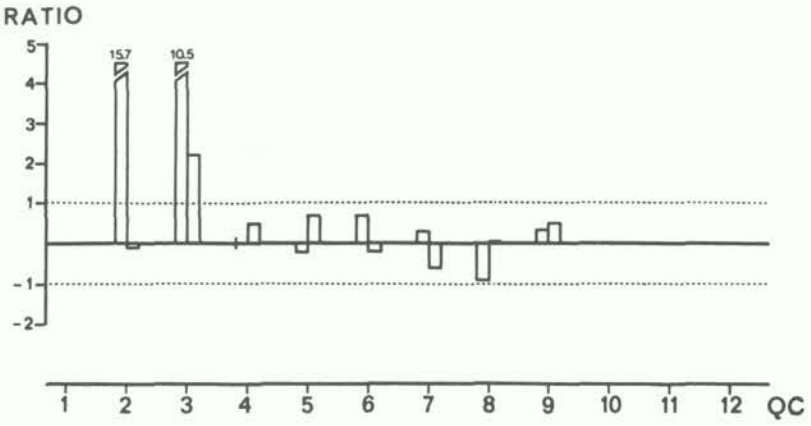


Figure 3:7. Diagram showing the ratios between the calculated deviations from the reference values and the accepted deviations at the lower (left bar) and upper (right bar) evaluation points. Dotted lines indicate acceptance lines.

3.7.2.2 Lead and cadmium in blood

Diagrams for all quality control results within the training phase of the project are given in Appendix 2 together with comments on the analytical performance at the laboratories.

Figure 3:8 and 3:9 show the overall results from the quality control training phase in condensed form. The results are expressed as ratios: reported value minus reference value divided by half the acceptance interval, as defined in section 3.7.2.1. The left bar of each pair represents the lower evaluation point (100 μg Pb/l for lead and 1.5 μg Cd/l for cadmium) while the right bar represents the upper evaluation point (400 μg Pb/l for lead and 12 μg Cd/l for cadmium).

It should be noted that the number of quality control runs do not correspond to a common time point during the project. The laboratories did not enter the project at the same time and some started the analyses of cadmium later than those of lead. Most countries, however, started with QC 1 in August–September 1979. On average, ten QC runs were performed by each laboratory; approximately one every second month.

Lead in blood. It can be seen from figure 3:8 that there was only one laboratory which met the criteria for acceptance throughout the training phase. In general, deviations from the reference values were most notable during the first quality control runs. The results improved significantly with time as shown by lower ratios. Part of the improvement coincided with visits of consultants.

Cadmium in blood. As can be seen from figure 3:9 none of the laboratories met the criteria for acceptance on all quality control sets. The situation in the initial phase of the project was in general worse than for lead analyses. The improvements were also slower. In fact, most of the laboratories had no experience at all in cadmium analysis at the start of the project (see Appendix 2). However, after assistance by a consultant and training, all laboratories eventually met the criteria for acceptance.

3.7.2.3 Cadmium in kidney cortex

QC samples prepared from horse kidney cortex were introduced from QC 4. Each quality control set consisted of four samples ranging between about 50 and 400 mg Cd/kg dry weight. In general, the results of these QC analyses were better than the corresponding

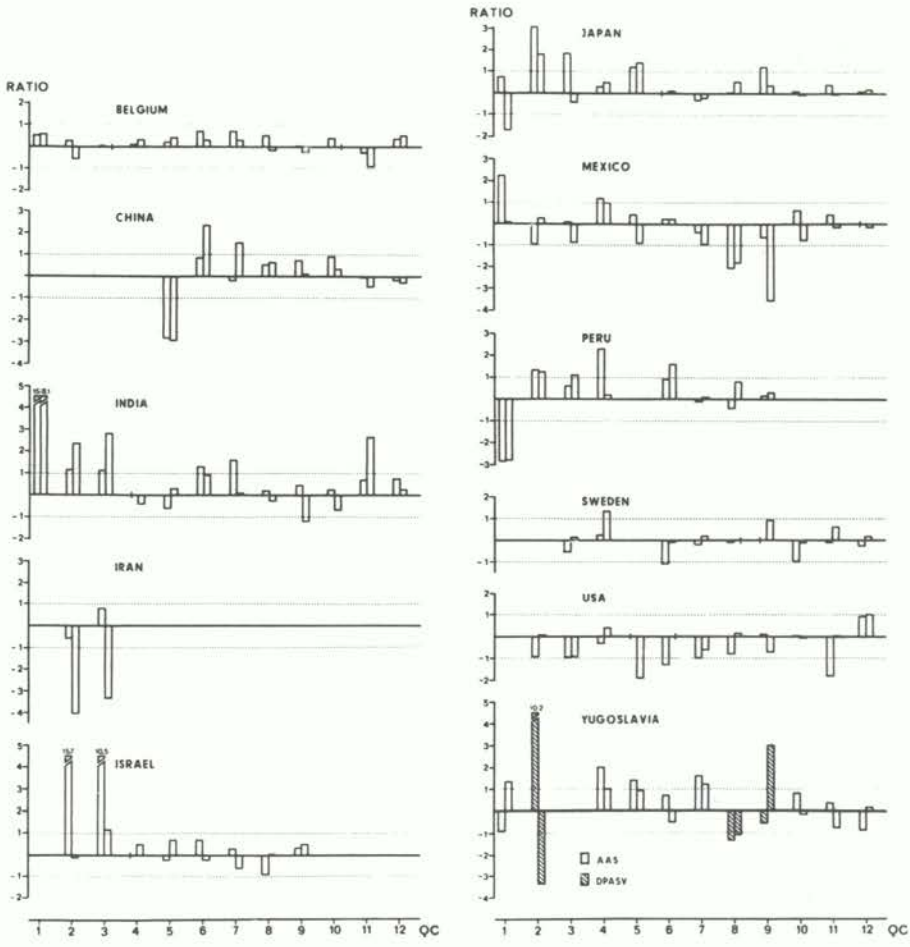


Figure 3:8. Results of analysis of lead in blood for quality control runs 1–12 (training phase) expressed as ratios between obtained and accepted deviations from "true" values (reference values). The QC runs are presented in the order they have been analyzed. For further explanation, see text and figure 3:7. Dotted lines represent the acceptance interval.

Table 3:7. Results on kidney cortex quality control samples QC 4–12. A = accepted; R = rejected

	Quality control run No								
Country	4	5	6	7	8	9	10	11	12
Belgium	A	R	R	R	R	R	A	A	
China	A	A	A	A	A	A	A	A	A
India	–	A	A	A	A	A	A	A	
Iran	–	–							
Israel	A	A	A	A	A	A	A	A	A
Japan	A	A	A	A	A	R	A		
Mexico	A	A	A	A	A	A	R		
Peru	A	A	A	A	R	A	A	A	A
USA	A	A	A	A	R	A			
Yugoslavia	R	R	A	–	A	R	A	A	A

– = the laboratory received samples but did not report any results.

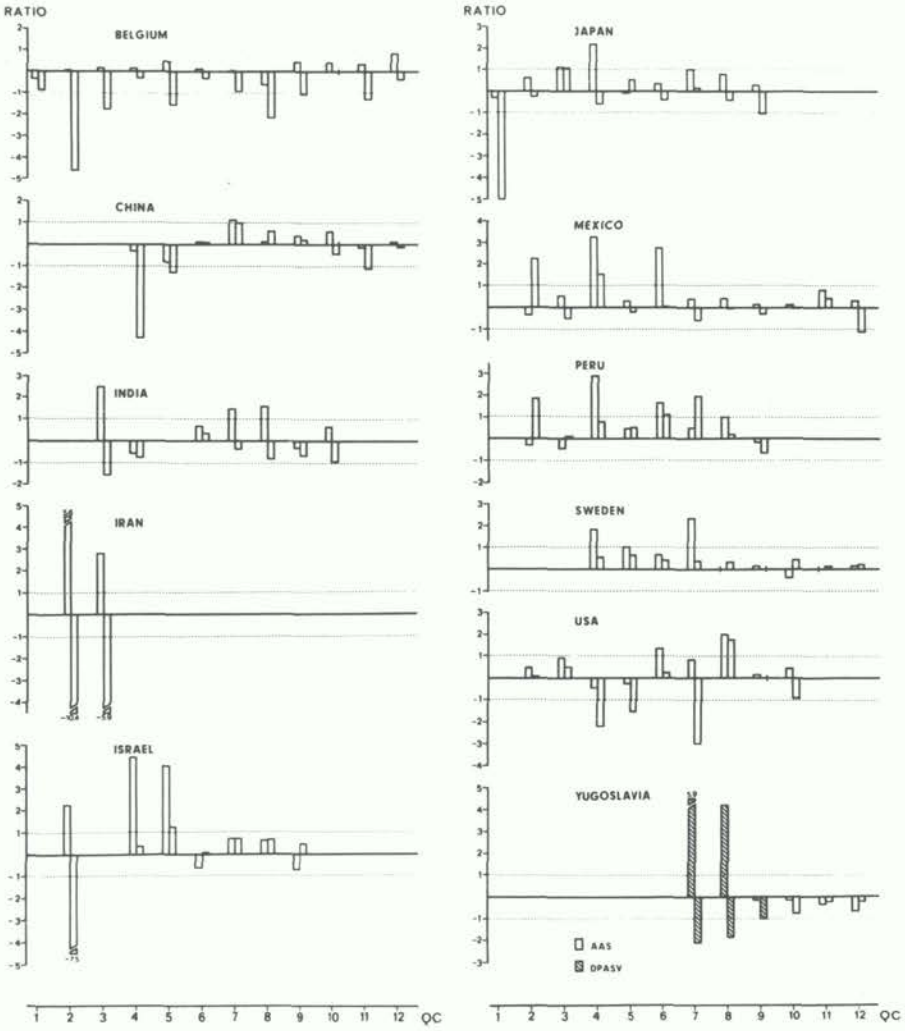


Figure 3:9. Results of analysis of cadmium in blood for quality control runs 1–12 (training phase) expressed as ratios between obtained and accepted deviations from "true" values (reference values). The QC runs are presented in the order they have been analyzed. For further explanation, see text and figure 3:7. Dotted lines indicate the acceptance interval.

ones for blood. As can be seen from table 3:7 most of the results were accepted according to the established criteria (section 3.5).

3.7.3 Analytical procedures

3.7.3.1 General

As a rule, the participating laboratories used atomic absorption spectrophotometry (AAS) with background correction (Price, 1979). Yugoslavia also used differential pulse anodic stripping voltammetry (DPASV; Siegerman & O'Dom, 1972; Stoeppler et al., 1979) for some QC runs.

For lead in blood three laboratories used the Delves Cup technique (Delves, 1970; Ediger & Coleman, 1972) modified according to Lind (1982a). Seven laboratories used electrothermal atomization, ETA, originally reported by Matousek & Stevens (1971). Three used the method by Fernandez (1975), ETA(F), involving dilution of blood with Triton X-100. The ETA methods used by the other four are specified below.

For cadmium in blood three laboratories used the Delves Cup technique (Ediger & Coleman, 1973; Ulander & Axelson, 1974) modified according to Lind (1982a) and seven used ETA. Four out of the seven with the ETA method used the method by Stoeppler & Brandt (1980) modified according to Lind (1982b) involving deproteinization of blood with nitric acid (ETA(S)). Two used a wet ashing procedure, and one dilution of blood with water (see below).

For cadmium in kidney cortex all laboratories used flame AAS. Five used dry ashing pretreatment (Kjellström et al., 1974; Elinder et al., 1976) and four used wet digestion pretreatment (Gorsuch, 1970).

3.7.3.2 Analytical methods used at the laboratories

Belgium

Lead in blood. AAS-ETA(F) modified in the sense that the standards were made in blood rather than in acid water solution.

Cadmium in blood. In the initial phase wet ashing-ETA and ETA(F). Due to contamination and background problems a deproteinization method, ETA(S), was used from QC 3 onwards.

Cadmium in kidney cortex. In QC 4–9, samples of 10 mg were treated in a low temperature asher. After dilution with water, the final solution was analyzed with AAS-ETA. For increased accuracy a method involving larger samples, wet ashing and flame AAS was introduced from QC 10.

China

Lead and cadmium in blood. Initially wet ashing ETA method. Due to background problems, ETA(S) was used for the major part of the project.

Cadmium in kidney cortex. AAS flame after wet digestion.

India

Lead and cadmium in blood. For the major part of the project a modified Delves Cup method was used.

Cadmium in kidney cortex. AAS flame after dry ashing pretreatment.

Iran

Lead and cadmium in blood. Due to lack of equipment, QC 2 and 3 were analyzed by AAS-ETA outside the participating laboratory in Teheran. A modern ETA unit, PE 400, was provided by WHO, but no more QC analyses have been performed.

Cadmium in kidney cortex. No analyses performed.

Israel

Lead in blood. AAS-ETA(F) was used for the major part of the training phase and AAS-ETA(S) during the monitoring phase.

Cadmium in blood. Initially AAS-ETA(F). Introduction of AAS-ETA(S), used for the major part of the project, increased the accuracy.

Cadmium in kidney cortex. AAS-flame after dry ashing.

Japan

Lead and cadmium in blood. AAS-ETA after wet digestion with HNO_3 and $HClO_4$.

Cadmium in kidney cortex. AAS-flame after wet digestion as for blood.

Mexico

Lead and cadmium in blood. Delves Cup technique according to Barthel et al. (1973) without background correction and recording facilities gave low accuracy for QC 1. Installation of background corrector, phase button, current stabilizer, recorder and modification of the Delves Cup method improved the accuracy. Due to problems with gas and spare parts the ETA(S) method was introduced at the end of the training phase.

Cadmium in kidney cortex. AAS-flame after dry ashing.

Peru

Lead and cadmium in blood. Modified Delves Cup method.

Cadmium in kidney cortex. AAS-flame after dry ashing.

Sweden

Lead in blood. The Delves Cup and ETA(F) methods were used for QC 1–12. The ETA(S) method was used for QC 3–12. This method was also used during the monitoring phase.

Cadmium in blood. The Delves Cup and ETA(S) methods were used for QC 1–12. The latter method was used also during the monitoring phase.

Cadmium in kidney cortex. AAS-flame after dry ashing.

USA

Lead and cadmium in blood. AAS-ETA(F) and a direct method involving dilution of blood with deionized water. Due to high background, the ETA(S) method was introduced at the end of the training phase.

Cadmium in kidney cortex. AAS-flame after wet digestion.

Yugoslavia

Lead in blood. AAS-ETA(F) using calibration by standard addition in blood. DPASV was also used but was rejected owing to sample treatment difficulties.

Cadmium in blood. DPASV for QC 7–9 substituted with a modified AAS-ETA(S) method from QC 10 for the same reason as for lead. The AAS-ETA(S) method was modified in the sense of nitric acid concentration, blood dilution ratio and preparation of blood standards for the calibration.

Cadmium in kidney cortex. DPASV for QC 4 and 5. AAS-flame after dry ashing for the remaining part of the project.

The analytical procedures (AAS) used at the laboratories during the monitoring phase are summarized in table 3:8.

Table 3:8. AAS procedures used at the laboratories during the monitoring phase

Country	Blood Pb	Blood Cd	Kidney cortex Cd
Belgium	ETA(F)	ETA(S)	Flame, wet ashing
China	ETA(S)	ETA(S)	Flame, wet ashing
India	Delves Cup	Delves Cup	Flame, dry ashing
Israel	ETA(S)	ETA(S)	Flame, dry ashing
Japan	ETA wet ashing	ETA wet ashing	Flame, wet ashing
Mexico	Delves Cup ETA(S)	Delves Cup ETA(S)	Flame, dry ashing
Peru	Delves Cup	Delves Cup	Flame, dry ashing
Sweden	ETA(S)	ETA(S)	Flame, dry ashing
USA	ETA(S)	ETA(S)	Flame, wet ashing
Yugoslavia	ETA(F)	ETA(S)	Flame, dry ashing

AAS = Atomic absorption spectrophotometry
ETA = Electrothermal atomization
ETA(F) = Method by Fernandez (1975), dilution with Triton X-100, calibration in blood
ETA(S) = Method by Stoeppler et al. (1978) and Stoeppler & Brandt (1980), deproteinization with HNO_3, in most cases modified according to Lind (1982b)
Delves Cup method, modified according to Lind (1982a)

3.7.4 Comments

Some comments related to analysis of trace metals in biological samples are given below based on consultants' visits and the results of the quality control analyses. For more details see Appendix 2.

3.7.4.1 Lead and cadmium in blood

Because of the low concentration of lead and especially of cadmium in blood, the conventional AAS technique is not adequate. The Delves Cup technique or electrothermal atomization (ETA) were used by the laboratories for these analyses. The basic principle for both methods is that the atom cloud formed is kept inside a tube in order to increase the time for photon bombardment from the metal lamp. The sensitivity of the methods is rather high. However, the non-atomic absorption (background) is also high, and therefore a simultaneous background correction system and a fast recording of the signal must be used. Otherwise, it is not possible to accomplish a correct analysis of lead or cadmium in blood (see e.g. QC 1 for lead, India and Mexico, figure 3:8).

The alignment between the light beam from the hollow cathode lamp and the deuterium lamp (for background correction) is critical. Bad alignment was probably the reason why results were rejected on QC 2 and 3 for lead and on QC 2 for cadmium, Israel. When blood is diluted with water or Triton X-100, ETA methods generate a large background signal compared to the metal signal, especially for the older type of ETA units (PE HGA 70–72). This impairs accuracy and precision (e.g. QC 2 and 3 for lead and cadmium, Iran; QC 2 and 4 for cadmium and QC 2 and 3 for lead, Israel; QC 1, 4, 5 and 7 for lead, Yugoslavia). Wet digestion before ETA analysis normally also gives a high background signal, due to the present acids (e.g. QC 4 for lead and cadmium, China; QC 2 for cadmium, Belgium; and QC 1 for lead and cadmium, Japan).

Impurities (PH_3) in the acetylene gas produced problems in Peru when the Delves Cup system was used the first time. The cups stuck in the loop assembly and were very difficult

to remove. The critical distance between the cup and the tube was then affected (QC 1 for lead, Peru).

As a consequence of the low concentration of lead and cadmium in blood contamination problems can be severe. Improper acid washing of laboratory wares and use of material which had previously been used for analysis in the high concentration range were found to cause contamination (e.g. QC 2 and 3 for lead, Israel). Another reason for cadmium contamination may have been that smokers did not use gloves when performing the analyses. Some laboratories had to change to other buildings or to rebuild (India, Mexico and USA) to meet problems arising from improper basic installations, e.g. contamination from the air and hoods (Mexico and Yugoslavia), impurity of gases used, pressure and quality of water and air used and exhaust from instruments and furnaces.

Instrument breakdown (India, USA) and change of personnel (Mexico, USA) considerably delayed progress and affected the quality of the analyses (e.g. QC 5–9 for lead and cadmium, USA). The lack of service, spare parts and manuals in a language spoken by the technicians were matters of great concern, especially in developing countries.

Standards prepared in water were found to be the reason for some of the rejected results (e.g. QC 1 for lead and cadmium, Japan). Standards made by addition of lead and cadmium to blood with low metal content improved the results significantly. Standard curves with points scattering around the line (e.g. QC 9 for lead and cadmium, USA) were improved by using a balance for the preparation of the standards.

Internal quality control (IQC) samples were supposed to have been analyzed together with the EQC samples and the actual monitoring samples to check the present condition of the analytical performance. Sometimes IQC was used to create the standard curve. This gave good results but such a procedure should not be used since the underlying problems have not been solved.

3.7.4.2 Cadmium in kidney cortex

Analyses of cadmium in horse kidney cortex as a rule did not constitute a problem, mainly because the concentrations of cadmium in these samples were high. The conventional AAS technique with flame was used after sample pretreatment. Use of a background compensation system and its alignment was of lesser importance, since the background signal is small compared to the cadmium signal. Since contamination problems are related to the original concentration of the element within the sample, they are less likely to be of importance when analyzing kidneys. Possible losses of cadmium during pretreatment were controlled and minimized by temperature calibration of the furnaces used.

Problems related to low accuracy of the analytical balances were encountered in several countries, since very small quantities of the quality control material were used for the analyses. Only a few laboratories had solid weights of good quality for regular checkups. It was also found that keeping the QC samples in a desiccator after redrying was important since the samples were hygroscopic. Other common problems were related to the adjustment of the atomizer (spray unit) of the AAS instrument and to the preparation of standards.

4. Monitoring phase

4.1 Target populations

4.1.1 Lead and cadmium in blood

The ultimate aim of the present pilot project was to assess human exposure to metals through biological monitoring. It was felt that with respect to the average exposure to the metals under study, the target population could be, e.g. urban or rural without any further restrictions. An alternative approach was to study certain occupational groups not extensively exposed to lead or cadmium, e.g. teachers, high-school students or policemen. This would restrict the possibilities of drawing generalized conclusions, but it would provide reasonably good comparability between countries. The possibility to use blood donors was also discussed. However, they would be difficult to use since they are highly selective in certain countries. Certain types of emergency cases could be a suitable group for comparisons among countries. It was recognized, however, that it would require large efforts to motivate the health personnel involved and to control for possible bias.

The two main alternatives, random sample representing the general population or selected occupational groups, were both considered acceptable. However, it was felt that, at least in some countries, it would be very difficult to find a group of 100–200 persons representing the general population, even for a limited area. Therefore, it was felt that for this pilot project a certain occupational group was preferable and that teachers in certain areas would be an acceptable target population.

Teachers offer several advantages:

- They probably constitute a rather homogeneous group, making it possible to compare different geographical areas within a country, although there may not be total comparability between countries;
- Individual teachers are fairly easy to select for monitoring from lists in the chosen cities and areas;
- Teachers represent some form of middle-class and both sexes would be represented;
- Samples could probably be obtained at their working places, schools, without any particular risk of contamination; and
- Teachers could also be useful contacts for possible future studies of children.

At the meeting in Stockholm (WHO, 1980a) it was decided to choose teachers (200 teachers in each country) as the target population for the monitoring of lead and cadmium in blood. It was agreed that the teachers would be chosen from a single urban area in each country. They should be employed full-time in elementary schools. If possible, the ratio male/female should be 1. No age limits were fixed. Data to be reported to the CI included:

- Sample identification number
- Area where the schools were located
- Sex

- Date of birth
- Smoking habits
- Date of sampling
- Date of analysis
- Lead and cadmium concentrations in blood (μg/l)

It was agreed that the selection of the individuals within chosen areas should be random as far as possible. In countries with population registries, the procedure was straight-forward and did not offer any problems. Where such registries were not at hand, the sampling system had to be devised in accordance with local possibilities.

The participating institutions were requested to prepare a detailed protocol for the design of the study including definitions of target population, sample size, sampling procedures and location for collection of blood specimens. In addition, each participating institution was asked to prepare a general characterization of the area(s) and population(s) studied. Such information would include social structure of the country and the area studied, population density, climate, traffic intensity, food habits (especially certain restrictions typical for the area monitored), prevailing religion, alcohol and drug consumption and smoking habits. Problems of representative sampling have to be carefully considered in studies of selected segments of the population. Most of the countries designed their monitoring studies so as to obtain a representative sample of the population of teachers in the study areas. Details for each country on the design and its implementation are provided in Appendix 3.

4.1.2 Cadmium in kidney cortex

It was decided that the target population for determination of cadmium levels in kidney cortex should consist of cases of "sudden unexpected death" without any obvious kidney disease. Samples of kidney cortex from about 50 subjects were to be collected at autopsy with as wide an age range as possible covered. Data to be reported to the CI included:

- Sample identification number
- Area of residence
- Sex
- Date of birth
- Smoking habits
- Underlying and contributory cause of death
- Kidney weight
- Date of sampling
- Date of analysis
- Cadmium concentrations in kidney cortex (mg/kg, both on dry and wet weight basis)

A detailed protocol for the design of the study, similar to that for the teachers, was to be prepared by each participating institution (Appendix 3).

4.2 Results

4.2.1 Lead and cadmium in blood

4.2.1.1 General

Tables 4:1–4:4 show the concentrations of lead and cadmium in blood from the populations studied in the participating countries. Data for Mexico represent partly samples

analyzed at the laboratory in Mexico City and partly samples analyzed at the CI. The analysis at the CI was performed in order to increase the data pool for Mexico since time did not permit the presentation of more data accompanied by accepted QC runs from the Mexican laboratory. It can be expected that more data will be available from Mexico at a later stage. Sweden did not officially participate in the project, but data referring to a random sample of the population in Stockholm have been included for comparison.*

Median values and 90-percentiles are given for the total number of subjects in each area as well as separately for males and females subdivided into current smokers (C) and nonsmokers, including former smokers (N+F). It should be noted that the number of subjects in some subgroups is small. The 90-percentile is not given when the number of subjects is less than 10. The median values for lead and cadmium in blood of male and female teachers, subdivided into smokers and nonsmokers, are also presented in figures 4:1 and 4:2. In cases where data have been reported to be below the detection limit of the analytical method, half the detection limit has been used in the statistical calculations. This procedure was of importance only for results on cadmium in blood among nonsmokers in Israel, Mexico (for samples analyzed at the CI) and Sweden, where a substantial number of results were reported to be low and below detection limit. The detection limit for cadmium in blood was 0.2–0.3 μg Cd/l at CI and 0.5 μg Cd/l at the laboratory in Israel. In other countries median values, even among nonsmokers, were 0.5 μg Cd/l or higher and only in a few cases values were reported to be below detection limit. The procedure at CI for calculation of detection limit was mean blank value plus 3 standard deviations. This was recommended by the consultant from the CI to be used also at other laboratories.

Data for the different subgroups, including median values, 90-percentiles, arithmetic mean values, standard deviations, geometric mean values and geometric standard deviations for each country are given in Appendix 4.

4.2.1.2 Lead in blood

Table 4:1 and figure 4:1 show the concentrations of lead in blood. The spread in blood lead levels among the countries is shown to be considerable. Median values for the total number of teachers ranged from about 60 μg Pb/l in Beijing and Tokyo to 220 μg Pb/l in Mexico City. The median values were below 100 μg Pb/l also in Baltimore, Jerusalem, Lima, Stockholm and Zagreb, and between 100 and 200 μg Pb/l in Brussels and the Indian cities. The 90-percentiles ranged from 89 μg Pb/l in Tokyo to 346 μg Pb/l in Mexico City.

In general, there was a reasonably good agreement between the median and the arithmetic mean values (see Appendix 4), although with some exceptions. The arithmetic mean values ranged from about 65 μg Pb/l in Beijing and Tokyo to 236 μg Pb/l in Mexico City. They were also below 100 μg Pb/l in Baltimore, Jerusalem, Lima and Stockholm, and between 100 and 200 μg Pb/l in Brussels, the Indian cities and Zagreb. Mean values plus 2 S.D. (below which about 95% of the observations would be found) varied from about 110 μg Pb/l in Beijing and Tokyo to 411 μg Pb/l in Bangalore.

In all populations studied, males showed higher blood lead levels than females (figure 4:1). On the average, the median blood lead levels of male teachers were 1.29 times (range 1.07–1.65) higher than those of female teachers. There was furthermore a difference in blood lead levels between smokers and nonsmokers. Except for male teachers in Mexico City and in the Indian cities, smokers had somewhat higher blood lead values than non-

* This study was supported by grant No. 5972600–0 from the National Swedish Environment Protection Board.

Table 4:1. Lead in blood (µg Pb/l) of teachers. Median values, 90-percentiles and number of teachers in each group indicated.

Country/Area		Males		Females		Males and females
		C	N+F	C	N+F	C+N+F
Belgium	Median	181	168	127	114	152
Brussels	90-perc.	241	217	245	164	213
	n	*29*	*57*	*15*	*40*	*143*[1]
China	Median	78	66	58	55	64
Beijing	90-perc.	112	98	–	87	102
	n	*68*	*52*	*2*	*118*	*240*
India	Median	116	138	–	129	131
Ahmedabad	90-perc.	254	303	–	245	260
	n	*17*	*83*	*0*	*100*	*200*
Bangalore	Median	148	207	–	171	183
	90-perc.	456	382	–	288	311
	n	*13*	*16*	*0*	*44*	*73*
Calcutta	Median	101	106	–	97	101
	90-perc.	162	194	–	149	164
	n	*16*	*34*	*0*	*50*	*100*
Israel	Median	102	90	82	70	86
Jerusalem	90-perc.	129	129	132	110	127
	n	*37*	*96*	*17*	*51*	*201*
Japan	Median	69	64	59	51	60
Tokyo	90-perc.	92	86	123	73	89
	n	*64*	*36*	*15*	*85*	*200*
Mexico[2]	Median	244	259	226	198	220
Mexico City	90-perc.	394	395	369	302	346
	n	13	*23*	*14*	*35*	*85*
Peru	Median	120	105	96	93	95
Lima	90-perc.	–	149	134	124	135
	n	*9*	*36*	*20*	*141*	*206*
Sweden[3]	Median	89	78	78	60	73
Stockholm	90-perc.	130	145	113	101	123
	n	*42*	*62*	*42*	*64*	*212*[1]
USA	Median	106	93	76	67	75
Baltimore	90-perc.	259	123	134	91	125
	n	*20*	*35*	*39*	*85*	*180*[4]
Yugoslavia	Median	139	123	84	77	90
Zagreb	90-perc.	260	206	122	126	169
	n	*27*	*33*	*45*	*87*	*192*

[1] Includes a few with unknown smoking habits.
[2] Including 50 samples analyzed at CI.
[3] Not officially participating. Random sample of the total population.
[4] Includes one with unknown sex.
C = current smoker; N = nonsmoker; F = former smoker; *n* = number of teachers.

smokers. On the average the values for smokers were 1.09 times (range 0.69–1.34) higher than those for nonsmokers.

In Brussels blood samples were collected also from blood donors. Table 4:2 compares blood lead levels of teachers with those of the blood donors. The overall median value for

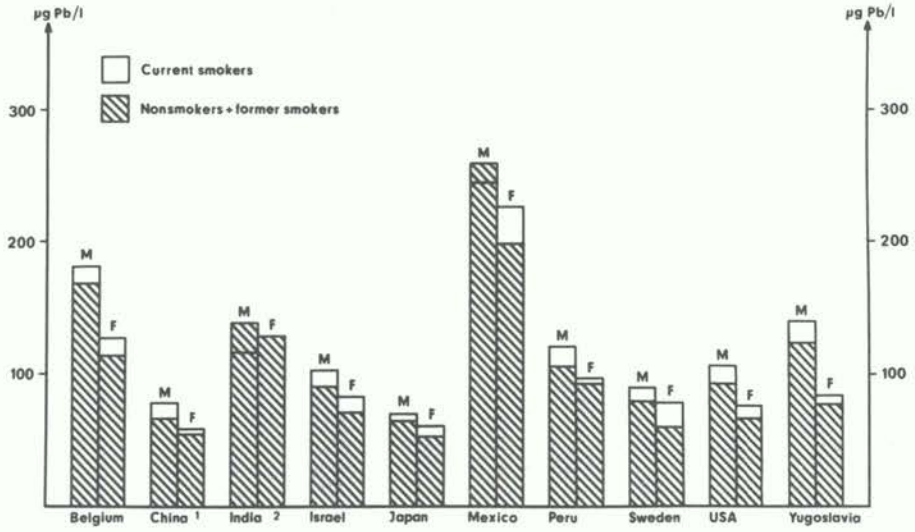


Figure 4:1. Concentrations of lead in blood (median values) for male (M) and female (F) teachers, subdivided into smokers and nonsmokers (including former smokers). Indian data represent teachers in Ahmedabad. Swedish data represent a random sample of the total population in Stockholm. 1) Only 2 female smokers; 2) No female smokers.

teachers (152 µg Pb/l), was about the same as that for blood donors (145 µg Pb/l). Within the population studied in Sweden there were 15 teachers (8 males and 7 females). The median value for lead in blood was 97 µg Pb/l, which should be compared with a median value of 73 µg Pb/l for the total population studied. The combined results indicate that teachers do not differ substantially from the general population with regard to lead exposure.

4.2.1.3 Cadmium in blood

The blood cadmium levels for the different populations studied are shown in table 4:3 and figure 4:2. Due to the skewed distribution of the blood cadmium values within the populations studied, the arithmetic mean values were in general considerably higher than the median values.

Table 4:2. Comparison of teachers and blood donors from Brussels, Belgium, with respect to lead in blood (µg Pb/l).

Population		Males		Females		Males and females
		C	N+F	C	N+F	C+N+F[1]
Teachers	Median	181	168	127	114	152
	90-perc.	241	217	245	164	213
	n	*29*	*57*	*15*	*40*	*143*
Blood donors	Median	195	158	115	121	145
	90-perc.	249	248	168	185	240
	n	*41*	*53*	*9*	*74*	*179*

[1] Including 2 teachers and 2 blood donors with unknown smoking habits.
C = current smoker; N = nonsmoker; F = former smoker; *n* = number of subjects.

Table 4:3. Cadmium in blood (μg Cd/l) of teachers. Median values, 90-percentiles and number of teachers in each group indicated.

Country/Area		Males		Females		Males and females
		C	N+F	C	N+F	C+N+F
Belgium	Median	2.0	1.1	2.0	0.9	1.2
Brussels	90-perc.	5.5	1.7	5.3	1.8	3.1
	n	*29*	*57*	*15*	*40*	*143*[1]
China	Median	1.8	0.6	2.6	0.8	0.9
Beijing	90-perc.	3.4	1.5	–	1.5	2.4
	n	*68*	*52*	*2*	*118*	*240*
India	Median	1.1	0.9	–	0.8	0.9
Ahmedabad	90-perc.	1.5	1.5	–	1.6	1.5
	n	*17*	*83*	*0*	*100*	*200*
Bangalore	Median	1.1	0.7	–	0.9	0.8
	90-perc.	1.6	1.6	–	1.4	1.5
	n	*13*	*16*	*0*	*44*	*73*
Calcutta	Median	0.7	0.7	–	0.8	0.7
	90-perc.	1.2	1.5	–	1.3	1.3
	n	*16*	*34*	*0*	*50*	*100*
Israel	Median	1.6	<0.5	1.1	<0.5	<0.5
Jerusalem	90-perc.	3.5	1.0	5.1	1.0	1.9
	n	*37*	*96*	*17*	*51*	*201*
Japan	Median	1.5	1.0	1.1	1.2	1.2
Tokyo	90-perc.	2.8	1.8	3.5	2.1	2.3
	n	*64*	*36*	*15*	*85*	*200*
Mexico[2]	Median	3.9	0.3	2.1	0.4	1.0
Mexico City	90-perc.	9.0	3.2	8.9	1.7	5.2
	n	*12*	*19*	*13*	*31*	*75*
Peru	Median	2.9	1.0	1.2	0.8	0.9
Lima	90-perc.	–	2.3	4.9	1.4	1.7
	n	*9*	*36*	*20*	*142*	*207*
Sweden[3]	Median	1.8	0.2	1.5	0.3	0.5
Stockholm	90-perc.	4.3	0.8	3.0	0.7	2.5
	n	*42*	*62*	*42*	*64*	*212*
USA	Median	1.1	0.6	1.0	0.5	0.6
Baltimore	90-perc.	5.0	1.6	2.2	1.0	1.6
	n	*20*	*35*	*39*	*85*	*180*[4]
Yugoslavia	Median	3.6	0.6	2.7	0.5	0.7
Zagreb	90-perc.	10.0	1.3	7.0	1.0	5.3
	n	*27*	*33*	*45*	*87*	*192*

[1] Includes a few with unknown smoking habits.
[2] Including 50 samples analyzed at CI.
[3] Not officially participating. Random sample of the total population.
[4] Includes one with unknown sex.
C = current smoker; N = nonsmoker; F = former smoker; *n* = number of teachers.

Except for the Indian populations and the female teachers in Tokyo, smokers showed considerably higher cadmium values than nonsmokers (figure 4:2). Median values for nonsmokers (including former smokers) ranged from less than 0.5 μg Cd/l in Jerusalem, Mexico City and Stockholm to 1.2 μg Cd/l in Tokyo (female teachers), while median

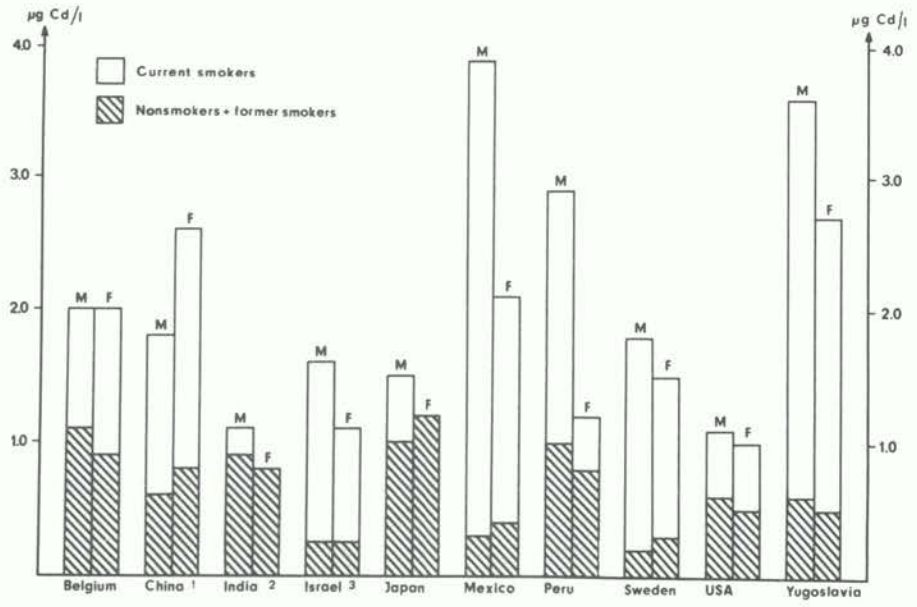


Figure 4:2. Concentrations of cadmium in blood (median values) for male (M) and female (F) teachers, subdivided into smokers and nonsmokers (including former smokers). Indian data represent teachers in Ahmedabad. Swedish data represent a random sample of the total population in Stockholm. 1) Only 2 female smokers; 2) No female smokers; 3) The values for nonsmokers are given as 0.25 µg Cd/l, i.e. half the detection limit.

values for smokers ranged from about 1 µg Cd/l in Baltimore and the Indian cities to 3.9 µg Cd/l among male teachers in Mexico City. On an average current smokers (males and females) had 3.6 times (range 0.9 (Calcutta) – 12.3 (Mexico City)) higher blood cadmium values than nonsmokers. The 90-percentile values also show that the difference between smokers and nonsmokers is considerable. The 90-percentiles varied between 0.7 and 3.2 µg Cd/l for nonsmokers and between 1.2 and 10.0 µg Cd/l for smokers.

There was no obvious difference in blood cadmium levels between nonsmoking males and nonsmoking females. Among the smokers, however, there was a tendency towards higher values for males than for females, possibly due to differences in smoking habits.

In table 4:4 blood cadmium levels of teachers in Brussels are compared with those of blood donors from the same city. It can be seen that the overall cadmium value for blood donors is somewhat higher than that for teachers, especially nonsmokers.

The median blood cadmium value of the 15 teachers (8 males and 7 females) included in the group studied in Sweden was 0.4 µg Cd/l which should be compared with the median value of 0.5 µg Cd/l for the total group studied. There was thus no indication that teachers are differently exposed to cadmium compared to the population in general.

4.2.1.4 QC analysis

QC samples (as a rule 5 sets of 6 samples each) were run in parallel with the monitoring to enable evaluation of the accuracy of the final results. Figures 4:3 and 4:4 show the QC results from the participating laboratories. The empirical regression lines were based on all QC samples run in parallel with the monitoring samples. The data on lead and cadmium in blood reported from Mexico (35 for lead in blood and 25 for cadmium in blood) were ac-

Table 4:4. Comparison of teachers and blood donors from Brussels, Belgium, with respect to cadmium in blood (µg Cd/l).

Population		Males		Females		Males and females
		C	N+F	C	N+F	C+N+F[1]
Teachers	Median	2.0	1.1	2.0	0.9	1.2
	90-perc.	5.5	1.7	5.3	1.8	3.1
	n	*29*	*57*	*15*	*40*	*143*
Blood donors	Median	2.4	1.5	2.0	1.5	1.7
	90-perc.	4.4	2.2	5.1	2.2	2.7
	n	*41*	*53*	*9*	*74*	*179*

[1] Including 2 teachers and 2 blood donors with unknown smoking habits.
C = current smoker; N = nonsmoker; F = former smoker; *n* = number of subjects.

companied by one set of accepted QC results (see figure 4:3 and 4:4). The Mexican samples analyzed at CI were run in parallel with two accepted QC sets.

The regression lines, except that for the Mexican cadmium analyses, were well within the acceptance intervals indicating that the median values of lead and cadmium in blood obtained by the respective laboratories are valid. Also the analyses of cadmium in Mexico were considered satisfactory taking into consideration that the results were close to acceptance and the laboratory had shown good results on previous QC runs (see figure 3:9). Furthermore, the accuracy of the monitoring results were checked by duplicate analyses at the CI (see below). In general, there was a spread of the points around the regression lines which has to be considered when evaluating individual values as well as the 90-percentile values. Table 4:5 shows the errors of method as reflected by the results from the quality control analysis. As can be seen from the table, all errors for cadmium were about the same as could be expected in laboratories performing well (0.5 µg Cd/l; see section 3.5). For lead, an error of method of 10 µg Pb/l was used in the statistical evaluations. Table 4:5 shows that the errors at the participating laboratories varied from 8 to 31 µg Pb/l. In retrospect, one might have wanted to include more reference values below 1 µg Cd/l,

Table 4:5. Errors of method for analysis of quality control samples (as a rule 30 QC samples) during the monitoring phase. Values used in the statistical evaluations were 10 µg Pb/l for lead and 0.5 µg Cd/l for cadmium (see section 3.5)

Country	Lead in blood µg Pb/l	Cadmium in blood µg Cd/l
Belgium	8.4	0.4
China	12.5	0.3
India	17.8	0.9
Israel	20.5	0.4
Japan	18.8	0.7
Mexico[1]	15.3	0.4
Peru	23.0	0.8
Sweden	15.2	0.4
USA	18.1	0.6
Yugoslavia	30.7	0.5

[1] Calculated from one QC set (6 samples).

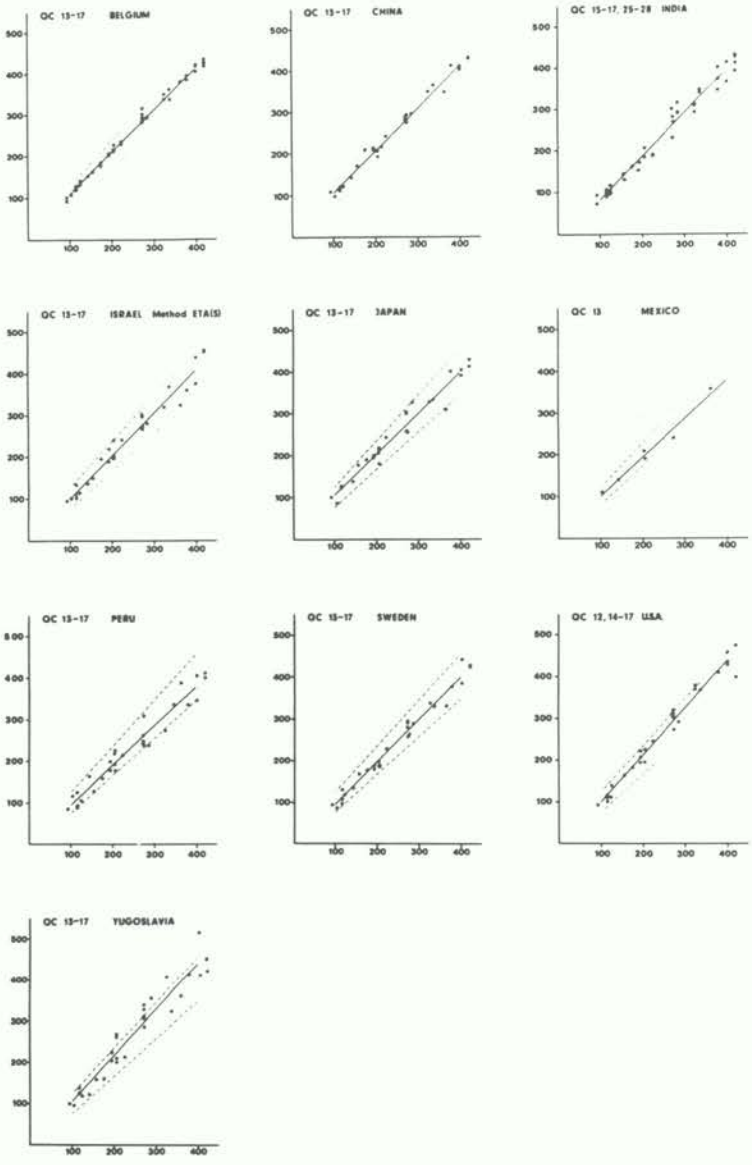


Figure 4:3. Results on lead in blood (μg Pb/l) for QC samples analyzed together with the monitoring samples. Y-axis: reported values, X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

since nonsmokers usually showed such low cadmium levels. Results from QC runs during the training phase, when unspiked samples were also analyzed (see data on cadmium in figures A2:1–A2:13) indicate, however, that analysis within the concentration range used was sufficient to predict systematic errors in the low concentration range. As both systematic and random errors, on a relative scale, will be of greater importance the lower the "true" value, caution should be exercized when comparing levels below 0.5–1 μg Cd/l.

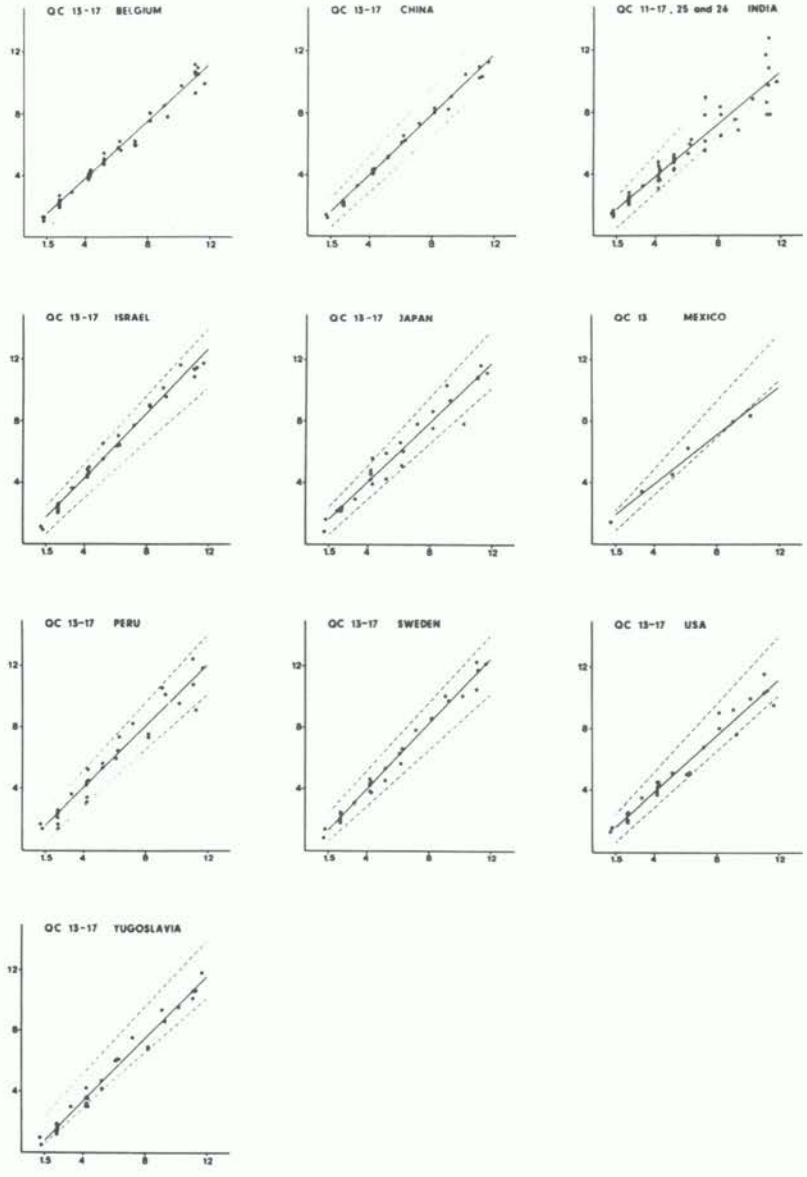


Figure 4:4. Results on cadmium in blood (µg Cd/l) for QC samples analyzed together with the monitoring samples. Y-axis: reported values, X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

As additional quality control, some duplicate analyses were performed at the CI. Fifteen samples were randomly selected from all 200 Japanese blood samples, 23 from the samples analyzed in Mexico and 15 from the first 50 Peruvian samples. Table 4:6 shows that the agreement between values obtained at the laboratories and at the CI was good.

The Mexican lead values showed a fairly wide concentration range. It was possible to compare the values obtained by the CI with those obtained by the Mexican laboratory in

Table 4:6. Comparison of results of lead and cadmium in teachers' blood obtained at the participating laboratories and the CI. The figures represent mean values (µg/l) and standard errors of mean (µg/l) based on analyses of a number of samples from teachers. For lead is also given the correlation coefficient, r. For cadmium this parameter has not been included as the monitoring values are close to detection limit and the error of method large relative to the range of values.

Country	LEAD						CADMIUM				
	n	Part. lab.		CI			*n*	Part. lab.		CI	
		$\bar{x}$	S.E.	$\bar{x}$	S.E.	r		$\bar{x}$	S.E.	$\bar{x}$	S.E.
Japan	*15*	64	3.6	62	4.4	0.88	*15*	1.3	0.13	1.4	0.15
Mexico	*23*	234	17.1	239	18.1	0.75	*11*	1.7	0.48	1.3	0.42
Peru	*15*	99	5.2	103	7.5	0.92	*15*	1.3	0.26	0.8	0.23

more detail. In figure 4:5 the CI values are plotted on the X-axis and those from the laboratory in Mexico City on the Y-axis. It can be seen that there is a good agreement over the whole concentration range.

4.2.2 Cadmium in kidney cortex

Concentrations of cadmium in kidney cortex in the different countries are shown in table 4:7. The studies were carried out in the same cities as those on lead and cadmium in blood except for Belgium where the kidney cortex samples were collected in Liège and the blood samples were collected in Brussels. Data on cadmium in kidney cortex were not reported from Mexico and Peru. For comparison data from an earlier study carried out in Sweden (Elinder et al., 1976) have been included.

The values in table 4:7 are given in relation to age and expressed as geometric mean values (G.M.) and standard deviation of the geometric means (G.S.D.). Values are derived from the distribution of the logarithms of the original data, but transformed back to com-

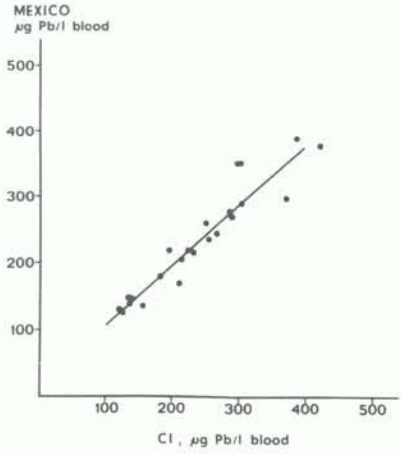


Figure 4:5. Comparison between results on lead in blood obtained at CI and the laboratory in Mexico City.

Table 4:7. Cadmium in kidney cortex (mg Cd/kg wet weight). Geometric mean, G.M., geometric standard deviation, G.S.D. and number of samples in each group, n, indicated.

Country		Age (years) –19	20–29	30–39	40–49	50–59	60–	Total
Belgium	G.M.	9.0	16.9	20.8	39.3	38.4	29.7	30.5
Liège	G.S.D.	1.3	1.8	1.7	1.7	1.6	1.7	1.8
	n	*4*	*2*	*11*	*16*	*35*	*89*	*158*[1]
China	G.M.	12.6	12.4	10.3	19.0	12.0	25.7	13.0
Beijing	G.S.D.	1.4	1.4	1.5	1.1	–	1.8	1.5
	n	*2*	*13*	*6*	*2*	1	*2*	*26*
India	G.M.	15.5	18.5	18.6	15.7	22.6	8.5	17.8
Ahmedabad	G.S.D.	1.4	1.3	1.3	1.4	1.2	–	1.4
	n	*4*	*23*	*14*	*7*	*2*	*1*	*51*
Bangalore	G.M.	4.4	9.1	10.3	24.5	20.9	13.0	9.0
	G.S.D.	1.9	1.6	2.3	–	1.5	1.6	2.1
	n	*10*	*11*	*11*	*1*	*3*	*6*	*42*
Calcutta	G.M.	19.0	16.0	13.2	18.4	24.1	14.8	16.2
	G.S.D.	1.1	1.6	1.7	1.8	1.3	2.0	1.7
	n	*2*	*10*	*10*	*11*	*2*	*4*	*39*
Israel	G.M.	6.2	15.5	23.9	25.6	22.9	13.3	15.1
Jerusalem	G.S.D.	2.0	1.6	1.7	2.3	2.1	1.6	2.0
	n	*7*	*10*	*7*	*3*	*8*	*16*	*51*
Japan	G.M.	–	25.0	59.9	67.0	61.1	61.8	56.2
Tokyo	G.S.D.	–	1.6	1.5	1.5	1.6	1.4	1.7
	n	*0*	*6*	*6*	*12*	*11*	*15*	*50*
Sweden[2]	G.M.	5.1	10.6	18.0	21.7	18.3	12.0	13.1
Stockholm	G.S.D.	1.9	1.9	1.9	1.8	2.3	2.0	2.0
	n	*31*	*32*	*34*	*40*	*43*	*111*	*291*
USA	G.M.	32.9	17.1	35.2	29.2	23.9	38.5	26.1
Baltimore	G.S.D.	5.0	2.4	1.8	1.2	1.7	1.3	2.0
	n	*2*	*8*	*3*	*3*	*7*	*3*	*29*[3]
Yugoslavia	G.M.	8.0	14.8	17.9	32.6	30.7	20.0	24.2
Zagreb	G.S.D.	–	2.6	1.9	1.8	1.8	1.9	2.0
	n	*1*	*6*	*4*	*13*	*15*	*11*	*50*

[1] Including one with unknown age.
[2] Data from Elinder et al. (1976).
[3] Including three with unknown age.

mon units. Since the distribution of original values shows a skewness which is corrected by the logarithmic transformation, confidence limits and statistical tests should be performed on the logarithmic statistics. A detailed description of the procedure to be used is given in Appendix 4, together with diagrams showing the distribution of all results in relation to age for each country.

It is obvious from table 4:7 that the concentration of cadmium in kidney cortex varies with age. In general, the highest concentrations were found in the age group 40–59 years, while younger and older subjects had lower levels. Figure 4:6 shows a comparison of the mean cadmium levels of the 40–59 year age group in the different countries. In the Chinese results all subjects over 40 years of age were included and the subjects from the three Indian cities were pooled in order to increase the number of subjects. The levels

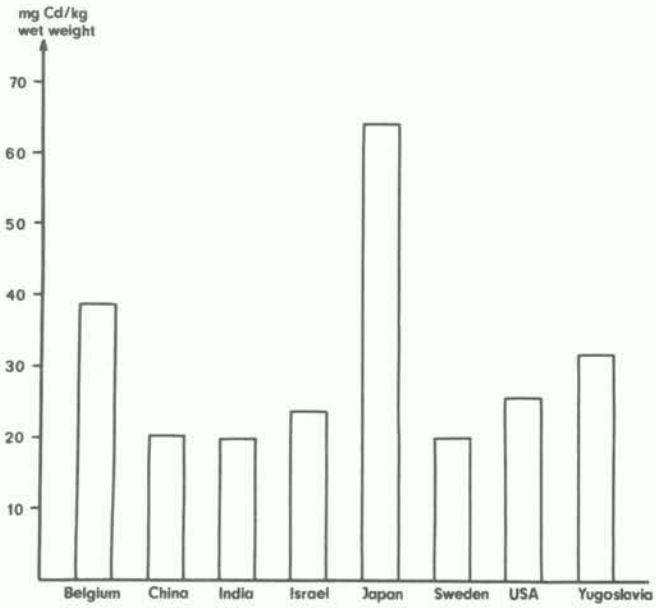


Figure 4:6. Concentration of cadmium in kidney cortex (geometric mean values) for the age group 40–59 years of the groups studied in the different countries. For Beijing, China, all subjects above 40 years have been included and for India data from Ahmedabad, Bangalore and Calcutta have been pooled in order to increase the number of subjects. Swedish data from Elinder et al. (1976). The number of subjects in each group is given in table 4:7.

varied from 19–25 mg Cd/kg wet weight in Baltimore, Beijing, the Indian cities, Jerusalem and Stockholm, to as much as 64 mg Cd/kg in Tokyo. Subjects in Liège and Zagreb had 30–40 mg Cd/kg.

The number of subjects in each age group was generally too low to allow a comparison between males and females. Therefore the geometric mean for all males was compared with that for all females in each city. The comparison is shown in figure 4:7. Since differences in age could be a serious confounding factor in a comparison of values for males with those for females, the mean age for the total number of males and females in each city has been given below the bars. The number of subjects in each subgroup is also indicated. It can be seen that cadmium levels in kidney cortex were not systematically related to sex.

In several countries it was difficult to obtain information on smoking habits for the subjects included in the study, since the information had to be collected from relatives of the deceased. Data on smoking habits were, however, reported for most of the subjects of the Indian, Japanese and Yugoslavian populations. Due to the relatively large number of subjects in the Belgian population, it was possible to divide these subjects into smokers and nonsmokers, although data concerning smoking habits were reported for about 50% of the subjects only. There were no data on smoking habits from Israel and USA and only for 13 of the 26 subjects in the Chinese population.

However, even in populations with fairly complete information on smoking habits it was not possible to divide the age groups presented in table 4:7 into smokers and nonsmokers. Therefore the data for smokers within the age range 30–69 years from Belgium, India (pooled data from Ahmedabad, Bangalore and Calcutta), Japan and Yugoslavia

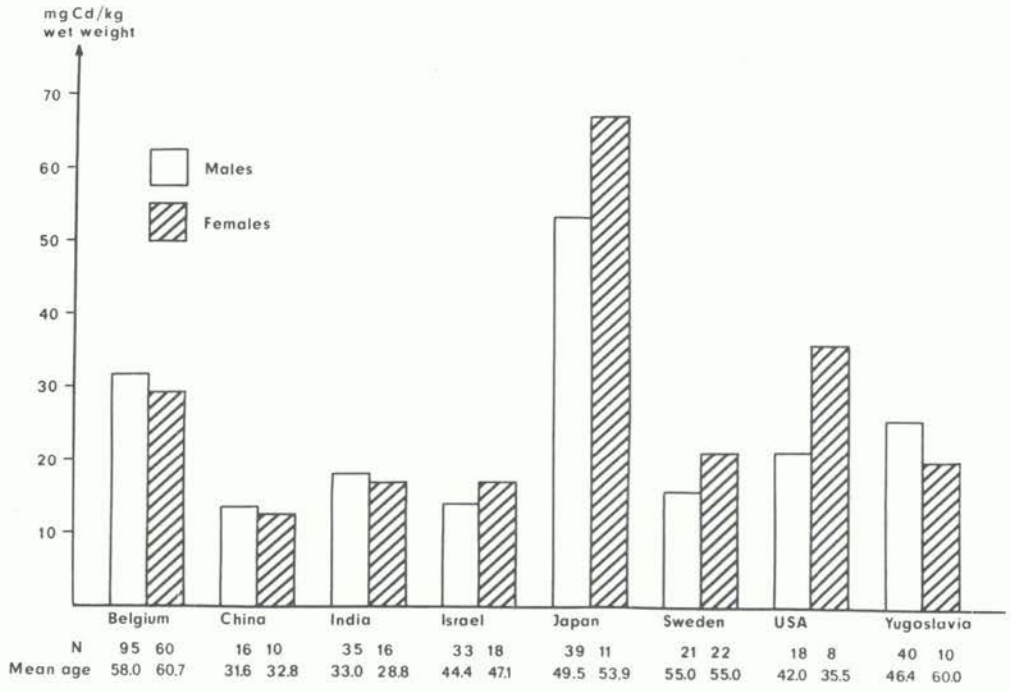


Figure 4:7. Comparison of cadmium levels in kidney cortex (geometric mean values) between males and females of the groups studied in the different countries. Indian data represented by Ahmedabad. Swedish data from Elinder et al. (1976). Number of subjects (N) and mean age of subjects in each subgroup are indicated under the bars.

were compared with those for nonsmokers of the same age in respective country. The results are presented in figure 4:8, showing the geometric mean values with 1.28 times the geometric standard deviations (corresponding approximately to the 90-percentiles) indicated. Since the age interval is fairly wide, there could still be a difference in age between smokers and nonsmokers of importance for a comparison. The mean age for each subgroup was calculated and also reported in figure 4:8. Data on former smokers were pooled with those on smokers since the concentration of cadmium in kidney cortex represents lifetime accumulation.

The data given in figure 4:8 indicate that smokers in general have higher values than nonsmokers, which is in accordance with the results on cadmium in blood. However, in the Indian population there was no obvious difference between smokers and nonsmokers with regard to cadmium in kidney cortex. Nor was there, as mentioned in section 4.2.1.3, any major difference between smokers and nonsmokers with regard to cadmium in blood.

Three sets (in some cases more) of quality control samples were analyzed together with the monitoring samples for evaluation of the accuracy of final results. Results of the analyses of the quality control samples (in general 12 altogether) were plotted against the reference values with the acceptance interval indicated. As shown in figure 4:9 the empirical regression lines are within the acceptance intervals for all laboratories which reported data on cadmium in kidney cortex, indicating that the mean values for cadmium in kidney cortex are valid.

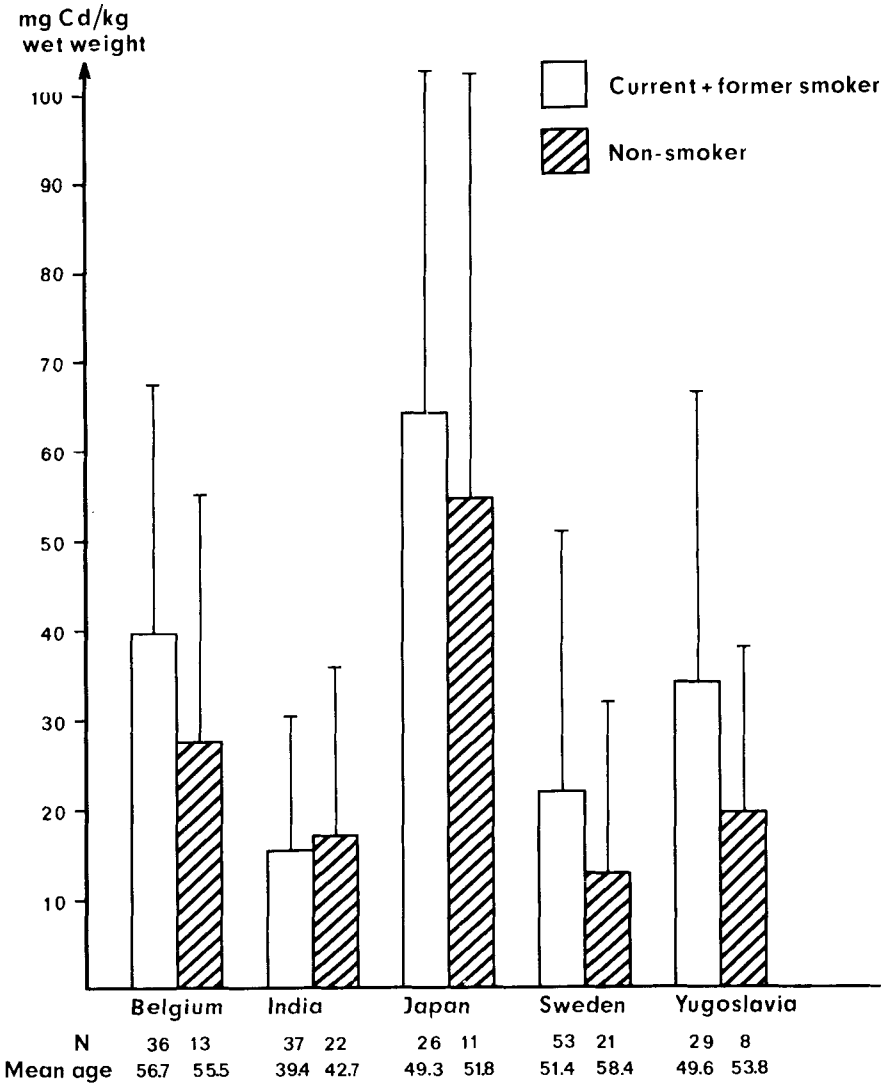


Figure 4:8. Concentration of cadmium in kidney cortex (geometric mean values with 1.28 times the geometric standard deviations indicated) in relation to smoking habits among the subjects (30–69 years of age) studied in Belgium, India (data from Ahmedabad, Bangalore and Calcutta pooled), Japan and Yugoslavia. Swedish data from Elinder et al. (1976). Number of smokers (including former smokers) and nonsmokers as well as mean age of subjects in each subgroup are indicated under the bars.

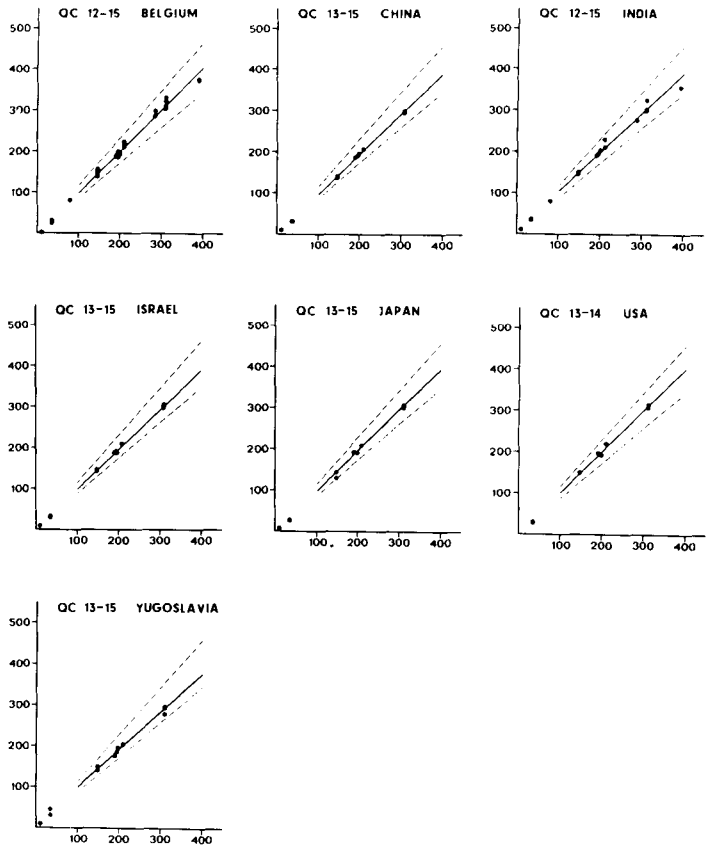


Figure 4:9. Results on cadmium in kidney cortex (mg Cd/kg dry weight) for QC samples analyzed together with the monitoring samples. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

5. Discussion and conclusions

5.1 Introduction

The objectives of the UNEP/WHO project included the design and implementation of a programme for quality control, technical advice and training, and implementation of a number of pilot studies on selected segments of the population in different countries. These objectives have been successfully completed. In the following the results are discussed under two main headings, the quality control training phase and the monitoring phase.

5.2 Quality control training phase

The need for a rigid quality assurance programme, particularly for the measurement of lead and cadmium in blood was confirmed by the results of the quality control analyses. It was rare that a laboratory met the criteria for acceptance throughout the entire training phase of the project. In several cases problems were identified at an early stage and considerable improvement was achieved rapidly. There are data, however, which indicate that certain problems remained despite repeated consultant's visits and training. In developing countries, such problems were partly due to difficulties to get spare parts and service from instrument manufacturers. CI has pointed out these problems to certain manufacturers of instruments as well as to the Scientific Apparatus Makers Association (SAMA), a central organization for manufacturers within the USA. They are aware of the problems and are working to improve the situation.

It is obvious from the results of the QC analyses that an accepted performance in a limited number of quality control runs was no guarantee for a continuous good performance. Furthermore, correct results reported for the internal quality control samples did not always guarantee accurate results of the external quality control samples. This emphasized the need to include not only internal quality control samples but also external quality control samples in the monitoring phase of the project. It is recommended that future programmes also recognize this need and design their protocols accordingly. Even well experienced laboratories showed substantial problems at times. One obvious conclusion is that such laboratories must also continuously check their results by means of quality control analysis, performed *in parallel* with actual monitoring activities. The problems encountered within the present project are not surprising considering the difficulties in trace metal analysis and the results of the interlaboratory comparisons that have been reported earlier. Unfortunately, however, it is still more an exception than the rule to include valid quality assurance data when publishing results from trace metal analysis. Analysis of cadmium in kidney cortex was in general carried out without any major problems, mainly due to the high concentration of cadmium in kidney cortex.

Analysis of quality control samples is only one part of a quality assurance programme. The preanalytical part of such a programme, primarily avoidance of contamination, is also of great importance. It was not possible, however, to check if a high standard in this

respect had been maintained. Several measures were taken to avoid the risks for contamination (see section 3.3), and hence the risk for errors due to this and similar factors has probably been brought down to a minimum. It is still not possible to exclude that individual high values might occasionally have been caused by contamination. It seems highly unlikely, however, that such factors have to any major extent influenced the results.

As has been discussed in section 3.7.2 and in Appendix 2, technical assistance has been an important part of the project. It has been provided through training and consultant's visits. As it goes without saying that adequate analytical equipment is a prerequisite for the performance of accurate analysis, such equipment has been provided to some participating institutions in developing countries, where possible. Also when such equipment has been available, technical advice has been of great importance. There are many pitfalls in trace element analysis, of which the analysts are not fully informed by the instrument manufacturers. For laboratories which have not studied the methodological aspects in detail, such pitfalls are not easily recognized.

Basically there are two ways to improve the performance at a laboratory. One is to send personnel to experienced laboratories for training, another is to have consultants visit the laboratory. Both ways are meaningful and important. The experience from the present project points towards having a consultant visit the laboratory as a first step. Specific problems at the laboratory can then be identified and major improvements can be achieved rapidly. It would seem more meaningful to send personnel for training to other laboratories or to arrange special training courses following this first step.

5.3 Monitoring phase

Data from the present project constitute the only international comparison on a global scale of lead and cadmium concentrations in blood and cadmium levels in kidney cortex where a rigid quality assurance programme has been implemented. The reporting of the results of the QC analyses, performed in parallel with the analyses of the actual monitoring samples, makes it possible to evaluate the validity of the results for each separate laboratory. The results together with the "preanalytical" quality control programme have made it highly probable that the results from the actual monitoring are valid and comparable. Furthermore, the target populations studied, teachers, seem to be comparable with the general population with regard to exposure to lead and cadmium, thus making an international comparison meaningful.

The results show that the exposure to lead and cadmium varies substantially among the areas studied. For *lead in blood* the median values thus ranged from about 60 μg Pb/l for teachers in Beijing and Tokyo to 220 μg Pb/l for teachers in Mexico City. The median values were below 100 μg Pb/l also in Baltimore, Jerusalem, Lima, Stockholm and Zagreb and between 100 and 200 μg Pb/l in Brussels and the Indian cities.

The results may be compared with those of the CEC project on lead in blood (CEC, 1981; see section 2.2.1.2), where quality assurance procedures were also applied. It is clear that most of the population groups studied within the UNEP/WHO project had considerably lower median values than was usually found in the CEC project. The overall median value for the CEC survey comprising 17,600 subjects was 130 μg Pb/l (Berlin, 1982), and the median values for the different countries studied ranged from 100 to 210 μg Pb/l for males and from 80 to 160 μg Pb/l for females.

The results of the present study confirm earlier data that males have higher blood lead levels than females (see e.g. Berlin, 1982). On an average, the median blood lead levels of male teachers were 1.3 times higher than those of female teachers. This can partly be ex-

plained by the higher number of red blood cells and higher hemoglobin levels among males compared to females. There was furthermore a tendency towards higher blood lead levels among smokers than among nonsmokers, although it varied considerably from country to country. On an average, the median values for smokers were 1.1 times higher than those for nonsmokers, which is in good agreement with data reported for smokers (about 20 cigarettes a day) and nonsmokers in a recent British study (Shaper et al., 1982).

Differences in sex distribution and smoking habits between areas did not to any significant extent explain the observed differences in blood lead levels between countries.The reason for the differences among the different areas included in the UNEP/WHO project and the differences between this project and that of CEC is not known. Since it is likely that the samples are comparable and that the differences are real, it is an urgent task for future programmes to investigate such differences. Industrialization, food and drinking habits, use of lead in gasoline as well as other environmental and social factors, including nutritional status, may be of importance.

Some tendencies noticed in the present project, which are worth persuing, relate to blood lead levels and exposure to lead from gasoline. Mexico, showing the highest median blood lead level, also has the highest concentration of lead in gasoline among the participating countries. Mexico City is furthermore a city with extremely heavy traffic. The groups studied in Beijing and Tokyo showed the lowest blood lead levels. Tokyo, like Mexico City, is a large city with heavy traffic, but almost all gasoline used at present is unleaded. Beijing is a city with low traffic intensity and, furthermore, about 75% of the gasoline used is unleaded. The other participating countries have intermediate concentrations of lead in gasoline and also intermediate blood lead levels. A detailed evaluation of the correlation between blood lead levels and lead in gasoline was not possible to perform within the present project. It must be emphasized, however, that many factors, other than lead in gasoline, may have influenced the blood lead levels in the different areas. Thus it is not possible to explain the relatively high blood lead levels found in some of the Indian cities by exposure to lead in gasoline.

It would have been of great interest to compare the results of the present study with earlier published data from the different countries. Unfortunately, very few reliable data exist. One international study on lead in blood, also sponsored by WHO, was performed in 1967 (Goldwater & Hoover, 1967). The median blood lead values for Peru and Sweden were in fairly good agreement with those of the present project, while for some other countries the blood lead levels were considerably higher. The median value in Japan was 210 μg Pb/l in the 1967 study compared to 60 μg Pb/l in the present study, that for Israel 150 μg Pb/l in 1967 and 86 μg Pb/l in 1981, that for the three areas in the USA about 180 μg Pb/l in 1967 and 75 μg Pb/l in 1981 and that for Yugoslavia 150 μg Pb/l in 1967 and 90 μg Pb/l in 1981. Thus, provided that the data from 1967 are correct, there is a general trend for decreasing blood lead levels. For USA this is confirmed when comparing data from a nationwide study with adequate quality control (Mahaffey et al., 1979; median values 130–180 μg Pb/l) with the present data for Baltimore (median value 75 μg Pb/l). A decrease in blood lead levels is further confirmed by results of another nationwide US study (Annest et al., 1982) being part of the second National Health and Nutrition Examination Survey (NHANES II). During a 4-year period (1976–1980) there was a decrease in mean blood lead levels from 158 μg Pb/l to 100 μg Pb/l. A decrease was found for both black and white races, all age groups and both sexes.

It was agreed upon that it should not be the aim of this report to make a detailed assessment of the health implications of the data obtained from the project. The differences between areas, however, are of such a magnitude that it must be considered highly important from the health point of view.

The median values for *cadmium in blood* among nonsmokers ranged from less than 0.5 μg Cd/l in Jerusalem, Mexico City and Stockholm to 1.2 μg Cd/l in Tokyo (female teachers). The results may indicate a difference in exposure to cadmium also among countries with low blood cadmium concentrations. Due to the analytical difficulties in the low concentration range, such differences have to be evaluated with great caution. The levels of cadmium in blood were closely correlated to smoking habits but the values from Brussels and Tokyo were the highest also among nonsmokers. Smokers had in general considerably higher concentrations than nonsmokers while former smokers had values close to those of nonsmokers. The differences in blood cadmium levels among the areas studied were obvious for smokers, indicating that the type of tobacco used and/or the tobacco consumption is of great importance for the exposure to cadmium. Smokers in Mexico City and Zagreb had for example about 4 times higher values than smokers in the Indian cities. Analysis of cadmium in cigarettes from different countries has shown considerably lower levels in Indian cigarettes compared to those in most other countries (Elinder et al., 1982). This may explain why there were no differences in blood cadmium levels between smokers and nonsmokers in India. 90-percentile values in the range 5–10 μg Cd/l were noticed among smokers in several countries. It can be mentioned that a value of 10 μg Cd/l in blood has been considered as an individual critical level for the development of low molecular weight proteinuria from the long-term exposure to cadmium (WHO, 1980b).

All countries except Mexico and Peru presented data for *cadmium in kidney cortex*. As could be expected, based on earlier data, the concentrations of cadmium in kidney cortex varied with age (see section 2.2.2). In general, the highest concentrations were found in subjects 40–59 years of age. The geometric mean values for these age groups varied considerably among the areas studied: from 19–25 mg Cd/kg wet weight in Baltimore, Beijing, the Indian cities, Jerusalem and Stockholm (data from an earlier study included for comparison) to more than 60 mg Cd/kg in Tokyo. High levels in kidney cortex from the Japanese subjects are in agreement with earlier studies (Ishizaki et al., 1970; Kitamura et al., 1970; Tsuchiya et al., 1972; Kjellström, 1979).

The critical level of cadmium in the kidney cortex, according to a WHO Task Group (WHO, 1977b), for tubular proteinuria is between 100 and 300 mg Cd/kg wet weight with the most likely estimate of about 200 mg Cd/kg.

It could not automatically be expected that there should be a very good correlation between cadmium levels in blood and cadmium levels in kidney cortex. Blood levels reflect to a great extent recent exposure while kidney levels reflect the accumulation over several years. Nevertheless, it can be seen that the countries with the lowest cadmium levels in blood also had low levels in kidney cortex. Correspondingly, in countries with rather high mean cadmium levels in blood, kidney cortex levels were also high.

As was noticed for cadmium levels in blood there was no obvious difference between males and females with regard to cadmium levels in kidney cortex. There was a clear tendency towards higher cadmium levels in kidney cortex among smokers than among nonsmokers, although not to the same extent as in blood. The data from the Indian cities showed no such difference between smokers and nonsmokers. On the other hand, there was no obvious difference between Indian smokers and nonsmokers with regard to cadmium in blood.

In conclusion, it can be stated that the results of the present project have strongly emphasized the need for an adequate quality assurance programme. They also showed that there is a considerable variation in exposure to lead and cadmium among the different study areas. Since the project was a pilot study that involved only a few participating countries, it would be useful to expand biological monitoring of the type carried out in this

project to other areas, in order to obtain a more complete picture of the exposure situation concerning lead and cadmium. Such an expansion should include integrated monitoring of pollutants in different environmental media, e.g. food, drinking water and air, which would give valuable data for the evaluation of reasons for increased exposure levels. Integrated monitoring has been recommended by the participating institutions and endorsed by a UNEP/WHO Government Expert Group in 1982. Such studies will be of immediate importance for areas with known high exposure levels. Also countries presently classified as low exposure areas will greatly benefit from such studies, since trends of rising exposure which ultimately may reach levels of direct implication for human health may be prevented.

Appendix 1

Studies on the preparation of quality control samples for cadmium in blood and kidney cortex

1. Introduction

An important part of the quality control programme has been the preparation of quality control samples (QC samples) with specified concentrations of lead and cadmium to be used for training purposes as well as in the actual monitoring phase of the project.

It was decided that the QC samples should consist of internal quality control samples (IQC; concentrations of lead and/or cadmium known to the laboratories) and external quality control samples (EQC; concentrations of metals known only to the CI). Furthermore, they should have a matrix as similar as possible to the material used for the actual monitoring (human blood and kidney cortex). Concentrations of lead and cadmium should cover a range which could be anticipated in the actual monitoring. The samples had to remain homogeneous and stable (without loss of metals) for a period of several months. A number of special problems had to be examined in relation to long range transport to countries which vary in climate and development.

Some standard reference material with certified concentrations of several trace elements is commercially available, e.g. orchard leaves and bovine liver from the U.S. National Bureau of Standards (NBS) and fish solubles and lake sediments from the International Atomic Energy Agency (IAEA), Vienna. Bovine liver was used in an early phase of the project but was not considered suitable as QC material for kidneys, partly because only one concentration of cadmium was available and partly because the concentration was too low. Reference material which could be used for blood analysis was not commercially avialable.

For QC samples for cadmium in blood no information whatsoever was available from the literature. Within the CEC programme, where blood samples for quality control purposes had been used, the stability of lead in blood samples had been studied in a series of experiments (WHO, 1979a). Human or bovine blood samples with EDTA added as anticoagulant, hemolyzed by ultrasonication and sterilized by gamma irradiation were kept at −20°C, +4°C, +20°C and +37°C for up to four weeks. Lead concentrations were analyzed by AAS, Delves Cup. Both human and cow blood seemed to remain reasonably stable. The recovery of added lead was about 100% for samples stored four weeks at −20°C, +4°C and +20°C, while samples stored at +37°C gave unreliable results after six hours of storage, i.e. 70–90% recovery and thereafter clotted.

The importance of the choice of blood vials was shown by Moore & Meredith (1977) who studied the stability of ^{203}Pb-labelled lead in blood (0.6 μmol Pb/l and 1.9 μmol Pb/l). They had a recovery between 96 and 105% after two weeks of storage in vials of polystyrene, polypropylene or pyrex glass at 0°C and +20°C, while samples stored in soda glass tubes at +4°C gave a recovery of only 70%. After an additional two weeks of

storage most blood samples had clotted and the recovery of lead could not be measured. Thus, available data indicated that blood could be used for preparation of QC samples for lead analysis within the UNEP/WHO project. It was felt necessary, however, to study in detail the stability of cadmium particularly as it occurs in much lower concentrations in blood than lead. At the same time studies on the stability of blood as such were carried out. In most of the stability experiments cadmium was added to blood *in vitro*. At long-term low level exposure, cadmium in blood is mainly localized in the red cells (Friberg, 1952), where it is bound to a protein of low molecular weight, metallothionein (Nordberg & Nordberg, 1975; Friberg et al., 1979). Therefore, studies were also carried out using blood from rabbits subcutaneously injected with cadmium.

Horses accumulate cadmium in the kidneys to a greater extent than humans (Piscator, 1976). Cadmium concentrations in kidneys increase with age, and thus by selecting kidneys from horses of different age from slaughter houses it was possible to obtain samples with varying concentrations of cadmium. Procedures for preparation of QC samples consisting of freeze-dried horse kidney cortex were worked out.

2. Material and methods

2.1 Stability of blood

The *first experiment* was carried out using blood from human volunteers (collected from the cubital vein), rabbits (New Zealand White), pigs and cows (blood from the two latter species was collected at the slaughter house). Dipotassium-EDTA (1.5 mg EDTA/ml blood) or heparin (14.3 u.s.p. units/ml) was used as an anticoagulant. The blood was hemolyzed by addition of Triton X-100 (Carl Roth K.G. Chemische Fabrik, Karlsruhe) 1 ml/100 ml blood or by ultrasonication (MSE 150W Ultrasonic Disintegrator equipped with a titanium probe, 19 mm in diameter) at an amplitude of 8 μm, a frequency of 20 kHz and 10 minutes/50 ml blood. Sterilization of the blood samples was made by gamma irradiation with a total dose of 2.5 Mrad (Silverman & Sinskey, 1968; IAEA, 1973).

In a *second experiment* the stability of human blood was studied in relation to temperature, time of storage and anticoagulant. EDTA or heparin was added to the blood which was then mixed with Triton X-100 for hemolysis. The blood was dispensed in 1.5 ml polypropylene tubes (in duplicate). Half the samples were sterilized by gamma irradiation. The samples were stored at +4°C, +22°C and +35°C for up to two months.

A *third experiment* was designed to investigate the effect of sterilization by gamma irradiation on blood hemolyzed by different methods. Human blood with EDTA as anticoagulant was hemolyzed by addition of Triton X-100, ultrasonication or by deep-freezing the blood twice at −70°C. The blood was dispensed in 1.5 ml polypropylene tubes and sterilized by gamma irradiation.

In a *fourth experiment*, carried out in collaboration with Dr. B. Yeoman at the Regional Toxicology Laboratory, Dudley Road Hospital, Birmingham, the effect of different hemolyzing methods on the blood was studied. Samples of human blood with added EDTA were hemolyzed by addition of Triton X-100 or by ultrasonication, whereafter the remaining number of red cells were counted using a Bürker cell counting chamber or with a Coulter-S counter.

2.2 Recovery of cadmium from blood

Spiked samples. Hemolyzed cow blood was spiked with ^{109}Cd-labelled cadmium and stored for up to 32 weeks in different types of vials. The blood was taken directly at the

slaughter house and poured into a beaker containing dipotassium-EDTA to give a final concentration of 1.5 mg EDTA/ml blood. After a thorough mixing, the blood was divided into two parts, A and B. Blood A was hemolyzed by ultrasonication while blood B was hemolyzed by addition of Triton X-100.

Both lots of blood were spiked with ^{109}Cd-labelled cadmium nitrate (2 or 10 μg Cd/l blood) mixed over night and dispensed in vials of polypropylene, polycarbonate or glass. After dispensing, blood A was sterilized by gamma irradiation (2.5 Mrad). All samples were stored at +4°C or +22°C and subsamples were taken out for measuring ^{109}Cd activity (Searle, Nuclear Chicago gamma counter, model 1195 with a 2 in. diameter NaI(Tl) crystal).

Blood from rabbits exposed to cadmium in vivo. A rabbit was administered ^{109}Cd-labelled cadmium at a dose of 0.1 mg Cd/kg body weight intravenously once a week for three weeks. Blood samples were taken from the ear vein once a month after the last injection. After four months, when the concentration of labelled cadmium had decreased from 62 μg Cd/l to 17 μg Cd/l, the rabbit was anaesthetized with ether and blood collected directly from the heart using a syringe, containing EDTA. Part of the blood was hemolyzed by ultrasonication, the rest by addition of Triton X-100. The blood was dispensed in 1.5 ml polypropylene tubes (in duplicate) and stored at +4°C or +22°C. Samples of 0.1 g of blood were taken for gamma counting after 1, 2, 4, 8 and 12 weeks of storage. When subsamples were taken out, the foam produced by shaking the samples was carefully avoided.

To study whether the cadmium in the samples was unevenly distributed between the foam and the rest of the blood, about 10 ml of each batch of blood (hemolyzed in different ways) was transferred to 25 ml polypropylene vials and shaken vigorously to produce much foam. One gram of the foam was taken out and measured for ^{109}Cd activity.

Samples stored for 1 and 12 weeks at +4°C were analyzed for cadmium concentration also by atomic absorption spectrophotometry (Delves Cup) (Elinder et al., 1978).

2.3 Kidney cortex

Quality control samples for cadmium in kidney cortex were prepared from horse kidneys. The kidneys were obtained from a slaughter house (Kalmar county slaughter house, Kalmar, Sweden) where they had been deep-frozen immediately after slaughter. The kidneys were thawed just enough to enable cutting in 2–3 mm slices. The cortex was separated from the medulla and cut into 5 mm pieces. These were deep-frozen in liquid nitrogen, piece by piece, to avoid clotting.

Homogenization of the horse kidney cortex was performed by liquid nitrogen grinding using a cryogenic grinding machine (Spex Industries, Inc., N.J., USA; shatterbox 8500, cryogenic grinding disc 8509). The grinding disc was cooled in liquid nitrogen for 40 minutes before use. About 100 g of deep-frozen horse kidney cortex was ground at a time. The ground material was freeze-dried (EF 4 freeze dryer, Edwards, Crawley, England), mixed thoroughly and dispensed in 5 ml polypropylene tubes, previously washed in diluted nitric acid and deionized water. The kidney cortex powder was sterilized by gamma irradiation (2.5 Mrad) and stored at room temperature.

The particle size of the ground and freeze-dried material was determined with a Lanameter (Reichert, Austria). The homogeneity was checked by analyzing the cadmium content in subsamples of one and the same batch. Fourteen subsamples (7 of 0.05 g and 7 of 0.1 g) were collected and analyzed by flame atomic absorption spectrophotometry after dry ashing (Kjellström, 1979). In addition six subsamples (3 of 0.05 g and 3 of 0.1 g) from each of the prepared 24 bathes of freeze-dried kidney cortex have been analyzed by AAS.

All samples were kept in desiccators after drying to avoid absorption of water from the air which would change the dry weight of the samples.

3. Results and discussion

3.1 Stability of blood

Results of the *first experiment*, concerning the stability of blood from different species in relation to storage time and temperature are given in table A1:1. Pig blood clotted or became gelatinous after one day of storage, independent of temperature, and was thus not suitable for preparation of quality control samples. Human, cow and rabbit blood were stable during the whole period when kept at +4°C, and for at least four days at +22°C.

The stability of human blood in relation to storage time, temperature, type of anticoagulant and sterilization by gamma irradiation, studied in the *second experiment*, is shown in table A1:2. Samples kept at +35°C clotted within 24 hours. Nonsterilized blood with EDTA as anticoagulant remained unclotted during the whole time period when kept at +4°C. When kept at +22°C it clotted after three weeks of storage. Nonsterilized samples with heparin as anticoagulant became slightly gelatinous after two days at +4°C. All samples sterilized by gamma irradiation clotted rapidly. Based on data in table A1:2 EDTA seems to be a better anticoagulant than heparin under the present conditions. However, even EDTA may give rise to clotting at +22°C.

Table A1:3 shows the stability of human blood in relation to type of hemolyzation in combination with sterilization by gamma irradiation (*third experiment*). It is obvious that hemolyzation by addition of Triton X-100 or freezing cannot be used when the samples have to be sterilized. This is also apparent from the second experiment where blood treated either with EDTA or heparin and thereafter mixed with Triton X-100 clotted upon gamma irradiation. Heparin was not used as anticoagulant in this experiment since studies within the CEC programme had shown that ultrasonication of heparinized blood led to clotting (Yeoman, personal communication).

The *fourth experiment* concerning the hemolyzing efficiency of Triton X-100 and ultrasonication showed that less than 1% of the original red cells remained intact, independent of method used for the hemolyzation, when analyzed with the Bürker cell counting

Table A1:1. Stability of blood samples in relation to type of blood, time and storage temperature

Type of blood	Storage temperature °C	Days of storage 1	4	21	35
		EDTA + Triton X-100			
Human	4	N	N	N	N
	22	N	N	C	C
Cow	4	N	N	N	N
	22	N	N	N	N
Rabbit	4	N	N	N	N
	22	N	N	C	C
Pig	4	N	G	G	G
	22	N	G	G	G

N = no clots; C = clots present; G = gelatinous

Table A1:2. Stability of human blood in relation to time, storage temperature, type of anticoagulant and sterilization by gamma irradiation

Temperature °C	Days of storage 1	2	4	22	36	44	58
			EDTA; Triton X-100				
4	N	N	N	N	N	N	N
22	N	N	N	C	C	TC	TC
35	TC	TC					
			EDTA; Triton X-100; gamma irradiated				
4	C	C	C	C	C	C	C
22	C	C	C	C	TC	TC	TC
			Heparin; Triton X-100				
4	N	G	G	C	C	C	C
22	G	G	G	C	TC	TC	TC
35	TC	TC					
			Heparin; Triton X-100; gamma irradiated				
4	C	C	C	C	C	C	TC
22	C	C	C	C	TC	TC	TC

N = no clots; C = clots present; TC = totally clotted; G = gelatinous

chamber. When the number of cells were counted with the Coulter-S counter, less than 1% of the original red blood cells were detected in the ultrasonicated blood whereas no significant decrease in the number of red blood cells was observed in the blood treated with Triton X-100. Probably Triton X-100 gives hemolysis mainly by disrupting the cells but leaves big pieces of the cell walls which can cause clotting. The ultrasonication obviously breaks the cells into very small fragments.

3.2 Recovery of cadmium from blood

Table A1:4 shows the recovery of ^{109}Cd from cow blood spiked with ^{109}Cd-labelled cadmium nitrate in relation to time of storage, temperature, concentration of cadmium added and tube material. Essentially no loss of cadmium was observed. Recovery ranged between 90 and 107% of the initial ^{109}Cd activity.

Table A1:5 shows the recovery of ^{109}Cd from samples of blood from rabbits administered ^{109}Cd-labelled cadmium nitrate. Recovery varied between 95 and 101%. The absorption of cadmium to the walls of the containers was thus negligible even after storage

Table A1:3. Stability of human blood in relation to type of hemolyzation. All samples had EDTA as anticoagulant and were gamma irradiated for sterilization

Hemolyzation	Days of storage at +4°C 2	7	14
Triton X-100	C	C	C
Ultrasonication	N	N	N
Freezing	C	C	C

N = no clots; C = clots present

Table A1:4. Recovery of ^{109}Cd (% of initial concentrations) in blood samples spiked with ^{109}Cd-labelled cadmium nitrate in relation to type of blood, time, temperature, concentration of cadmium added, and tube material (mean of two determinations).

Blood	Tube material	Cd added µg/l	Storage temperature °C	% of initial activity at time (weeks)					
				2	4	8	16	24	32
A. Cow blood; EDTA, ultrasonicated, gamma irradiated	Polypropylene	2	+ 4	99	97	105			
	"	2	+22	98	91	105			
	"	10	+ 4	97	96	105			
	"	10	+22	98	94	107			
	Polycarbonate	2	+ 4	101	98	101	99	100	101
	"	2	+22	99	96	103			
	"	10	+ 4	99	97	102	101	100	101
	"	10	+22	96	96	97	97		
	Glass	2	+ 4	100	94	98	102	100	99
	"	2	+22	99	95	102	102	100	99
	"	10	+ 4	100	98	103	101	101	101
	"	10	+22	101	96	104			
B. Cow blood; EDTA, Triton X-100	Polypropylene	2	+ 4	96	93	102			
	"	2	+22	103	95	104			
	"	10	+ 4	101	96	99			
	"	10	+22	99	103	96			
	Polycarbonate	2	+ 4	97	90	98	102	97	99
	"	2	+22	95	98	100	101		
	"	10	+ 4	102	94	99	102	100	101
	"	10	+22	102	94	102			
	Glass	2	+ 4	97	95	99	102	100	99
	"	2	+22	103	94	103	97	100	98
	"	10	+ 4	97	93	98	101	97	100
	"	10	+22	97		98	102	97	98

Table A1:5. Recovery of ^{109}Cd (% of initial activity) in blood with EDTA and hemolyzed by Triton X-100 or ultrasonication after storage at +4°C or +22°C for up to 12 weeks

Sample treatment	Temperature °C	Time of storage (weeks)					
		0	1	2	4	8	12
EDTA, ultrasonication	+ 4	100	101	98	97	100	101
	+22	100	99	99	99	97	97
EDTA, Triton X-100	+ 4	100	100	97	97	99	98
	+22	100	97	101	95	96	96

Table A1:6. Concentration of cadmium (µg Cd/kg; AAS-Delves Cup) in blood samples stored at +4°C for 1 or 12 weeks. Samples with and without foam.

Sample treatment		After storage for 1 week	12 weeks
EDTA, ultrasonication	no foam	15; 17	15
	foam	15; 18	16
EDTA, Triton X-100	no foam	17	15
	foam	16	15

for 12 weeks, independent of temperature (+4°C or +22°C). No difference was found in relation to method of hemolyzation. The ^{109}Cd activity per gram of foam was the same as in the blood. It should be noted, however, that the amount of cadmium recovered in blood with foam may be different if subsamples are taken by volume and not by weight as in the present experiment.

Results from the analysis of cadmium concentrations with AAS shown in table A1:6 do not indicate any change in the concentration of total cadmium with time. No variation in relation to foam formation was observed.

3.3 Homogeneity of kidney cortex samples

Analysis of the 14 subsamples from the bulk batch of freeze-dried horse kidney cortex showed a mean cadmium concentration of 224 mg/kg dry weight with a coefficient of variation of 2.3%. Analysis of the other 24 batches (3 samples of 0.05 g and 3 samples of 0.1 g from each batch) gave a mean coefficient of variation of 1.9% (range 0.3—6.1%), indicating a high degree of homogeneity.

The method used for the grinding was the same as that used by the U.S. National Bureau of Standards for preparation of bovine liver (Barnes, personal communication). Three minutes of grinding produced a powder with particles of 1—2 µm diameter.

4. Procedures for preparation of quality control samples

Based on the results of the stability studies the following procedure for preparation of blood quality control samples was considered the most suitable.

Due to the large quantities of blood required it was decided to use cow blood collected at the slaughter house. Cow blood was found to be as stable as human blood. An advantage of the cow blood as compared to the human blood was the much lower concentrations of lead and cadmium.

EDTA was used as anticoagulant since it maintained a higher stability of the blood. Furthermore, it had earlier been recognized that heparin could not be used in combination with ultrasonication. This procedure was chosen for the hemolysis since addition of Triton X-100 caused clotting of blood upon sterilization by gamma irradiation.

The samples were spiked with cadmium nitrate and lead nitrate after ultrasonication. The volume of the added standard solutions was always less than 2% of the total volume. Results of experiments with blood spiked with ^{109}Cd-labelled cadmium nitrate showed that the recovery of spiked cadmium was satisfactory even after 32 weeks of storage at +4°C or +22°C.

The blood samples used in the major part of the project were dispensed in 5 ml polypropylene tubes with screw-on-caps, previously washed in diluted nitric acid and deionized water.

The procedure used for the preparation of quality control samples for analysis of cadmium in kidney cortex was the same as described under "Material and methods". Kidney cortex from horses of various age was used.

Appendix 2

Quality control training phase. Results and analytical aspects

1. Introduction

Altogether 12 sets of quality control samples were distributed to the laboratories for analysis of lead and cadmium in blood during the QC training phase. The results reported from the laboratories were evaluated for acceptance according to the criteria decided upon at the meeting in Stockholm 1980 (see main report, section 3.5). The results on lead and cadmium in the QC blood samples were plotted against the reference values in diagrams. The regression line and the acceptability lines for each QC run were calculated and included in the diagrams.

For analysis of cadmium in kidney cortex QC samples consisting of freeze-dried horse kidney cortex (4 samples in each QC set) were distributed to the laboratories from QC 4 onwards. The training phase of the QC programme thus consisted of 9 QC runs for kidney cortex. Due to the limited number of samples in each QC run, these results have not been plotted in diagrams like the blood samples.

In the following section the results obtained on lead and cadmium in blood during the training phase are given in diagrams together with some comments on the analytical problems encountered at the laboratories.

The atomic absorption spectrophotometry (AAS) methods referred to below are the following:

ETA = Eletrothermal atomization
ETA(F) = Method by Fernandez (1975), dilution with Triton X-100
ETA(S) = Method by Stoeppler et al. (1978) and Stoeppler & Brandt (1980), deproteinization with HNO_3, in most cases modified according to Lind (1982b)
Delves Cup technique, modified according to Lind (1982a).

2. Results of quality control training phase

The results of the QC training phase are given in figures A2:1–A2:13, country by country, followed by comments. From the diagrams it is possible to make a more thorough evaluation of the results and the progress made than from figures 3:8 and 3:9 in the main report. Note that the diagrams are presented in the order the QC runs were analyzed. The analytical equipment available at the laboratories is presented in table A2:1.

Belgium

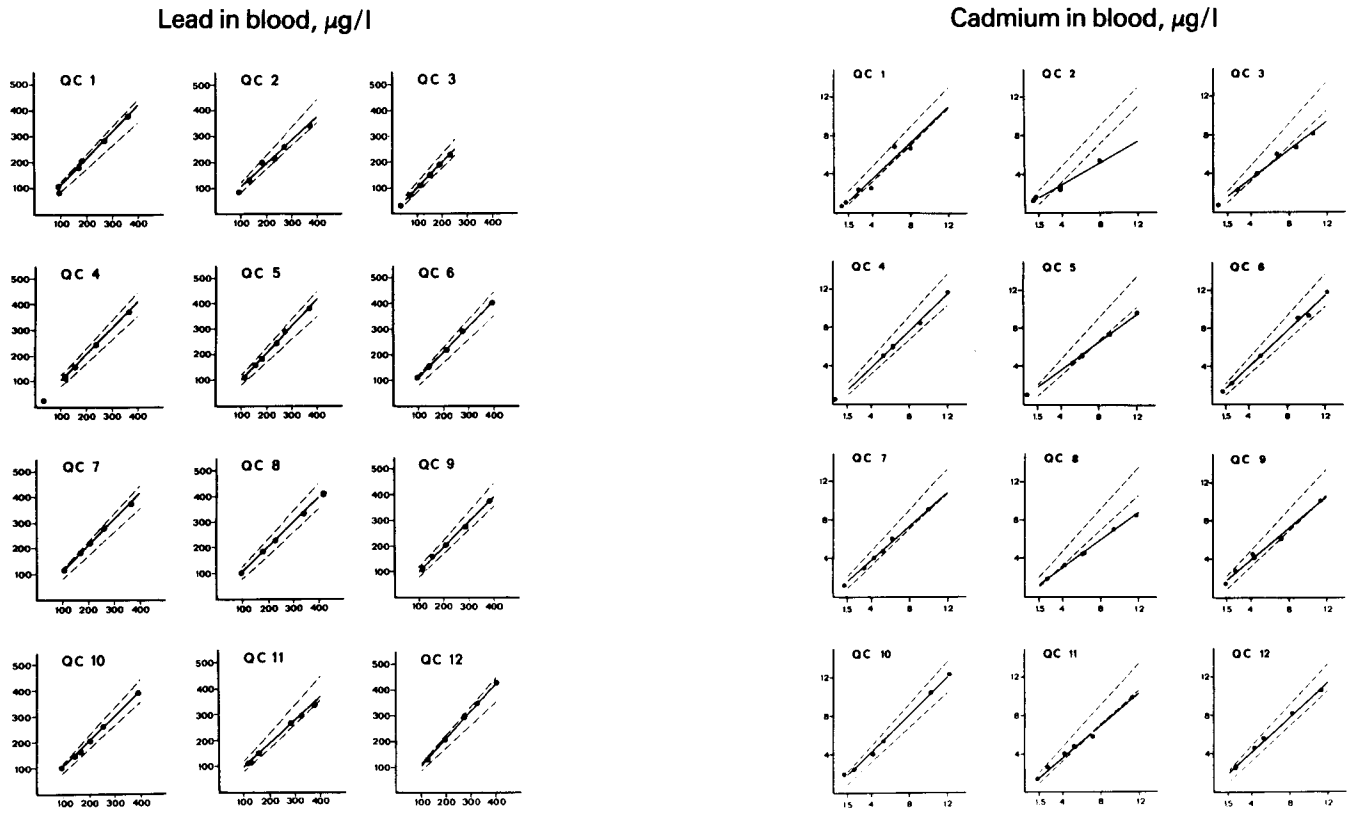


Figure A2:1. Results from quality control runs in Belgium during training phase—lead and cadmium in blood. Y-axis: reported values: X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

China

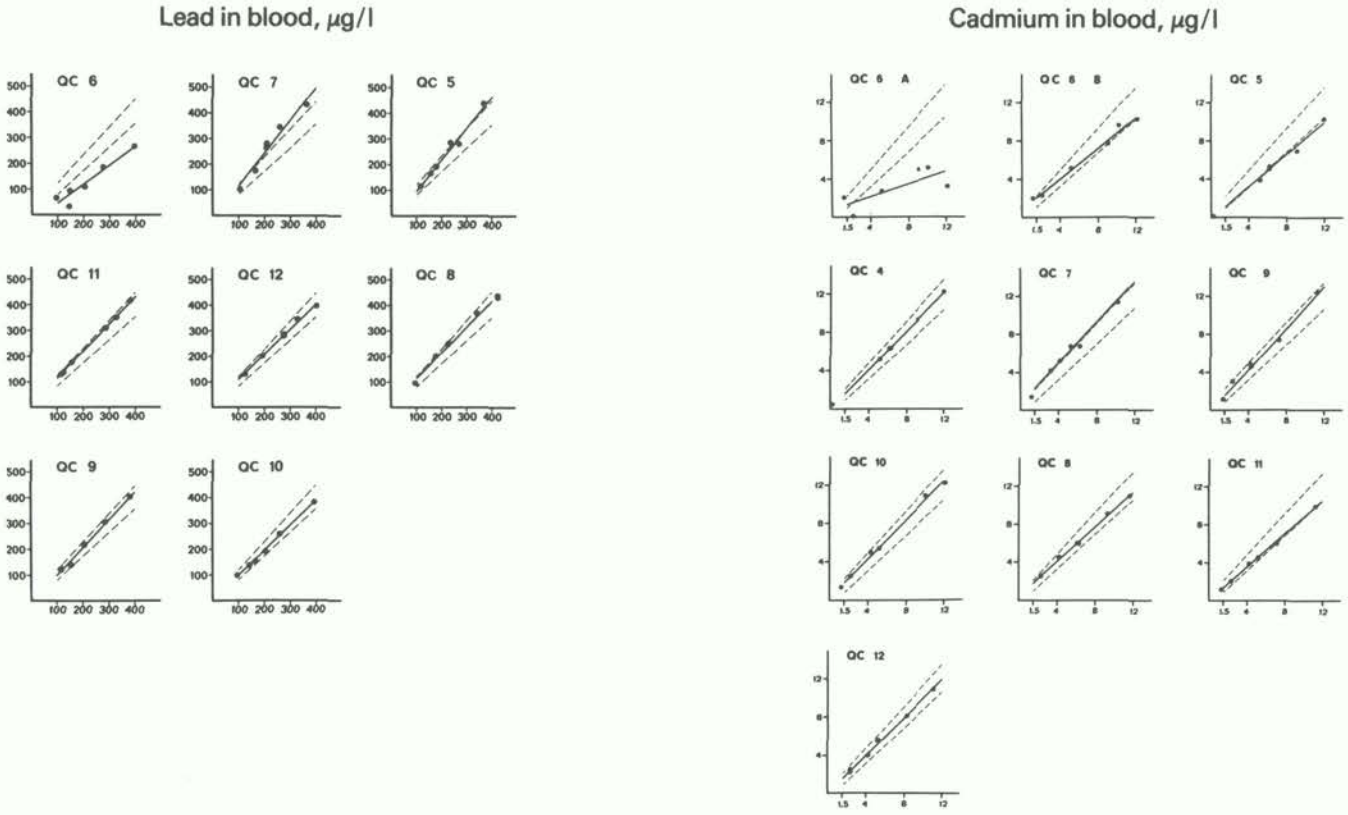
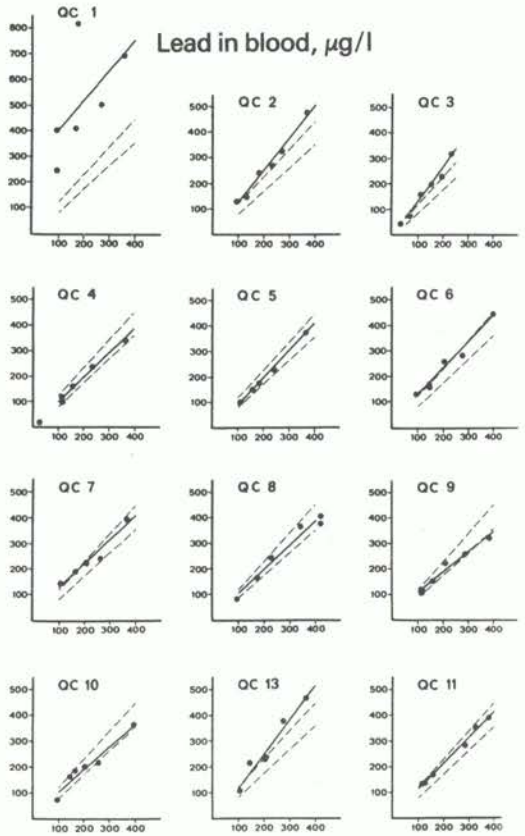


Figure A2:2. Results from quality control runs in China during training phase–lead and cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval. For cadmium in blood: A = before adjustment of the AAS instrument; B = after adjustment of the AAS instrument.

India



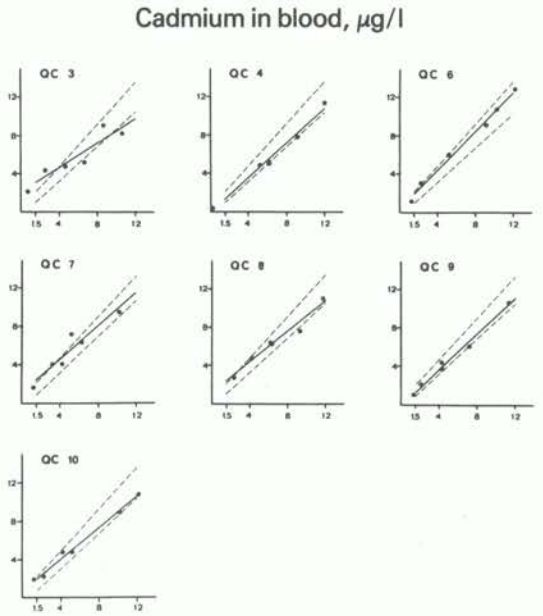


Figure A2:3. Results from quality control runs in India during training phase—lead and cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

Iran

Figure A2:4. Results from quality control runs in Iran during training phase—lead and cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

Israel

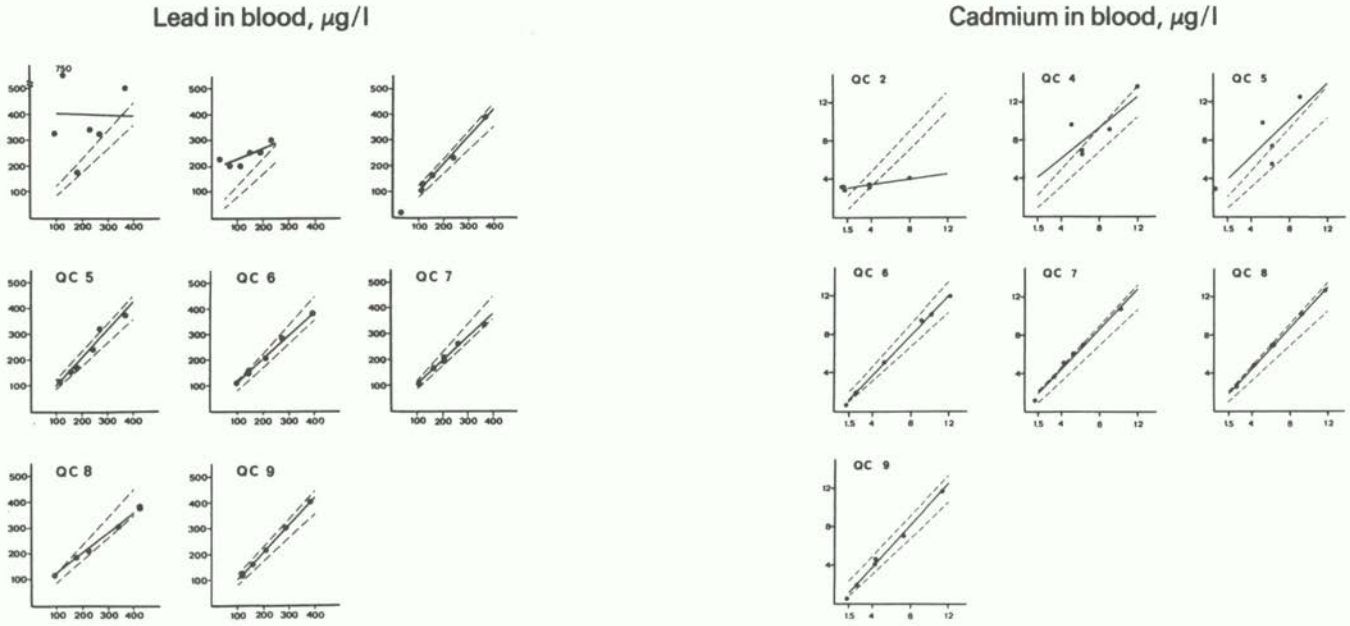


Figure A2:5. Results from quality control runs in Israel during training phase—lead and cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

Japan

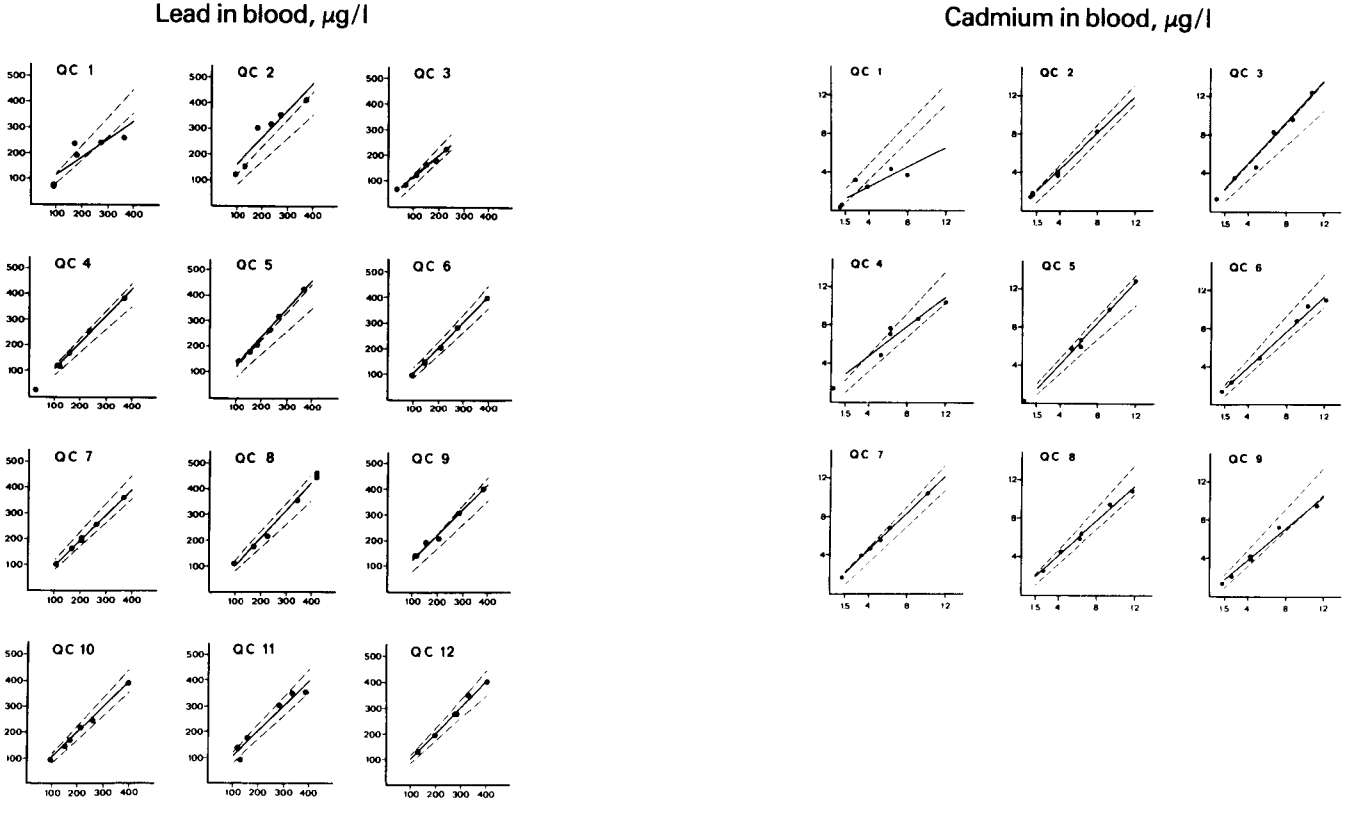
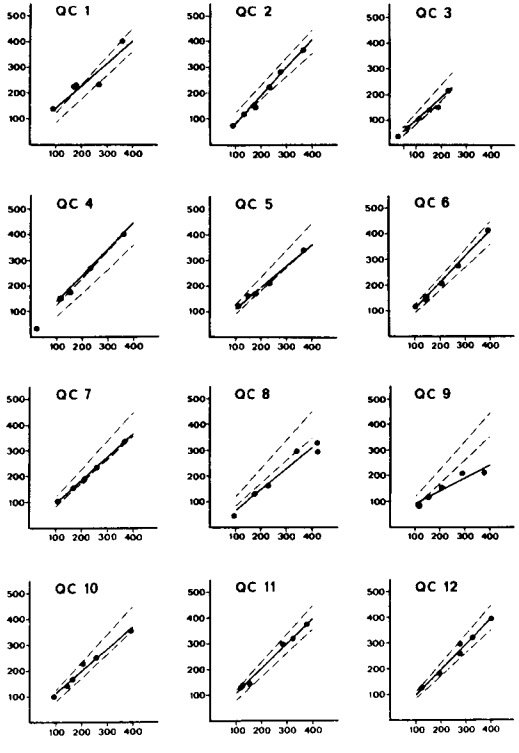


Figure A2:6. Results from quality control runs in Japan during training phase—lead and cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

Mexico

Lead in blood, µg/l



Cadmium in blood, µg/l

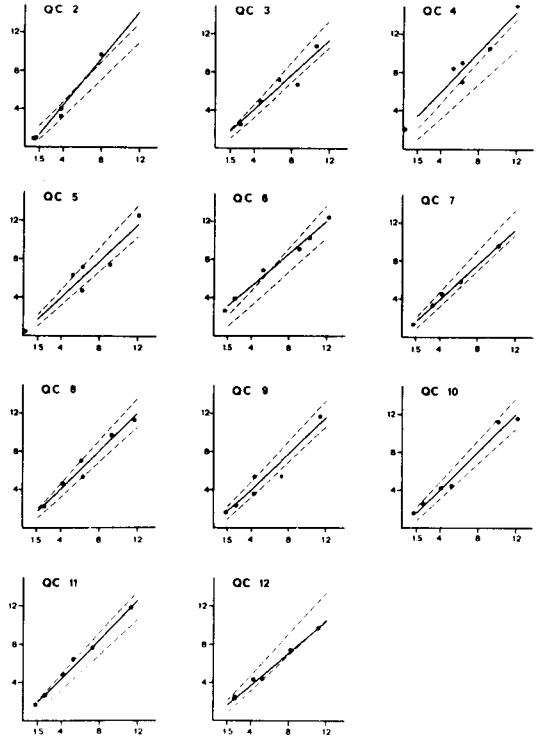


Figure A2:7. Results form quality control runs in Mexico during training phase–lead and cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

Peru

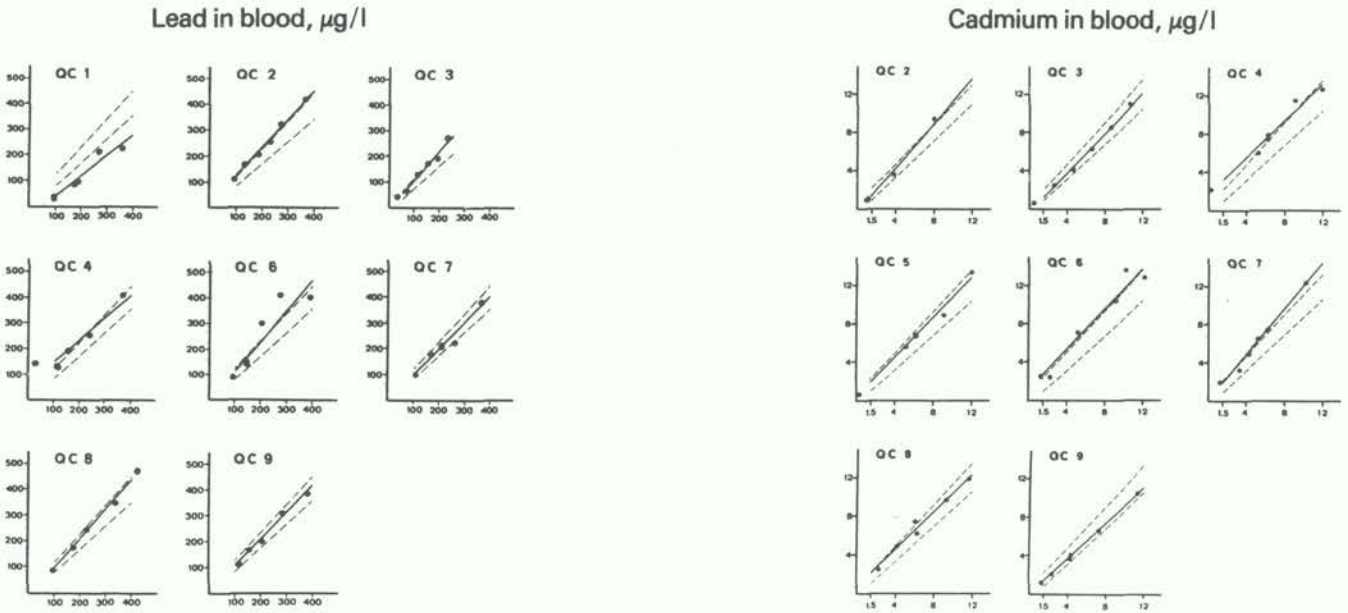
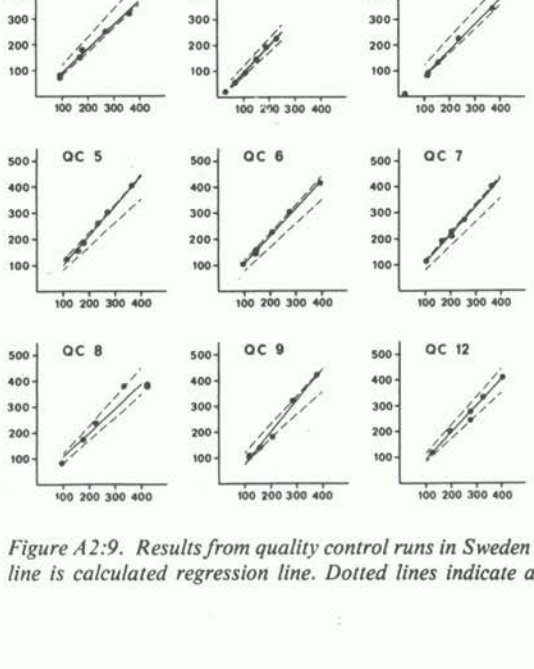


Figure A2:8. Results from quality control runs in Peru during training phase—lead and cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

Sweden

Lead in blood, µg/l Method Delves cup



Lead in blood, µg/l Method ETA(S)

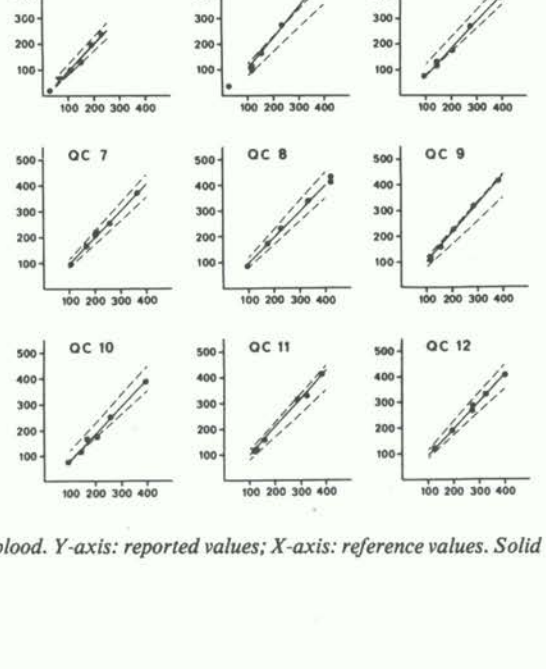
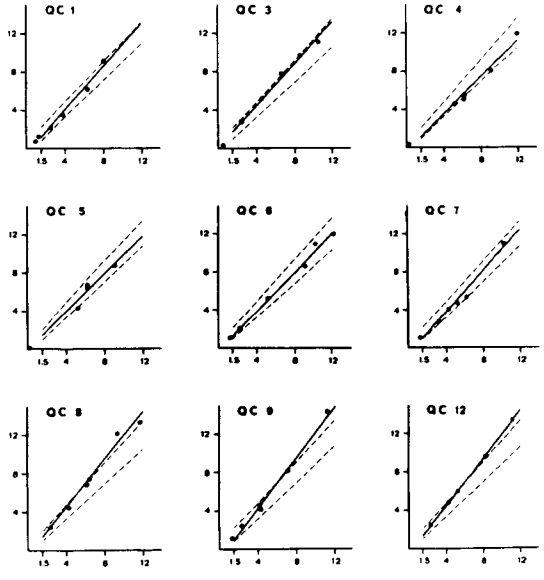


Figure A2:9. Results from quality control runs in Sweden during training phase—lead in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

Sweden

Cadmium in blood, µg/l Method Delves cup



Cadmium in blood, µg/l Method ETA(S)

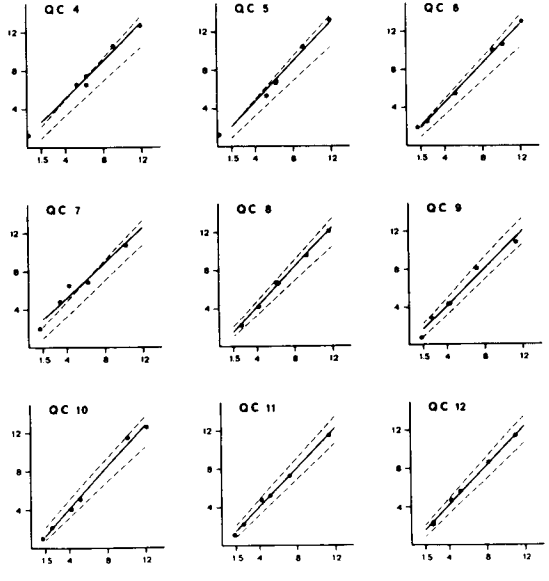


Figure A2:10. Results from quality control runs in Sweden during training phase–cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

USA

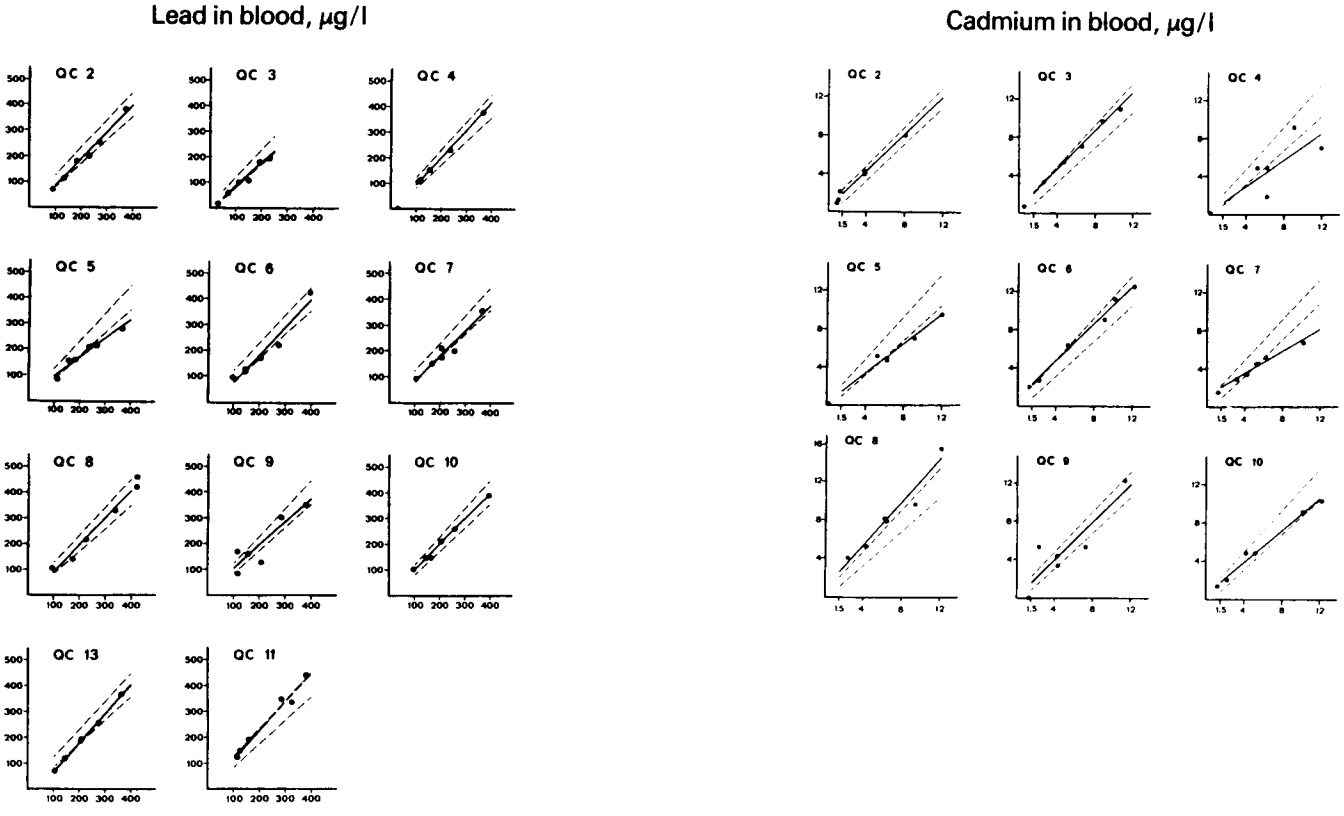


Figure A2:11. Results from quality control runs in USA during training phase—lead and cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

Yugoslavia

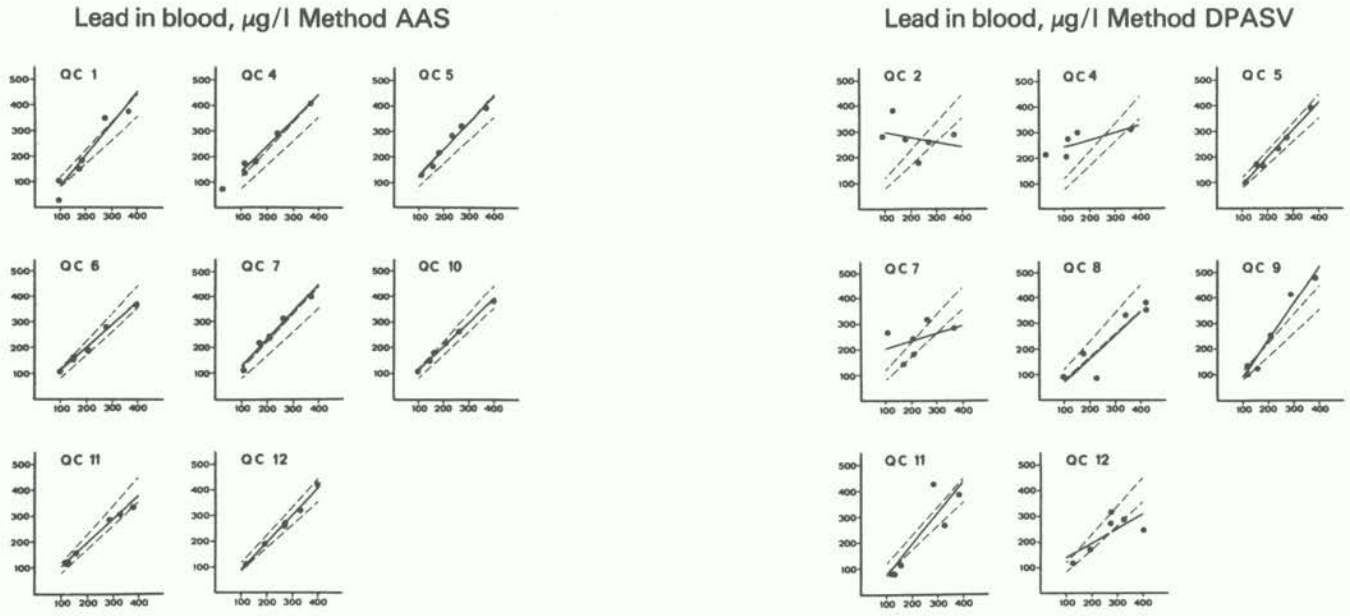


Figure A2:12. Results from quality control runs in Yugoslavia during training phase—lead in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

Yugoslavia

Cadmium in blood, μg/l

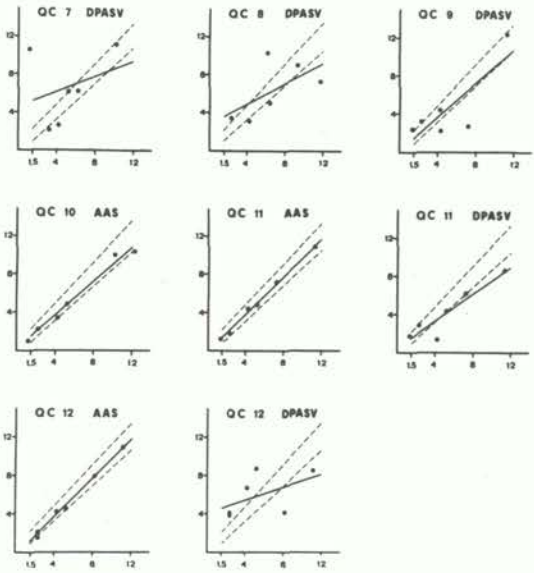


Figure A2:13. Results from quality control runs in Yugoslavia during training phase—cadmium in blood. Y-axis: reported values; X-axis: reference values. Solid line is calculated regression line. Dotted lines indicate acceptance interval.

BELGIUM

Lead in blood was analyzed by the AAS-ETA(F) method with standards made of blood. The method had been used previously. The results were in very good agreement with reference values throughout the project.

Cadmium in blood had not been analyzed before. In the initial phase a wet ashing-ETA method was used, but due to contamination problems this method was discontinued. The AAS-ETA(F) method, used for QC 1 and 2, gave a high operating error due to high background level. Still after dilution of the blood ten times, the accuracy was low, probably because cadmium concentrations were too small to give an acceptable cadmium signal. From QC 3 onwards, the AAS-ETA(S) method was used. Several QC runs were rejected due to low results in the high concentration range. It was discovered after QC 11 that the cadmium stock solution (1000 ppm) was contaminated.

Cadmium in kidney cortex had not been analyzed before. In QC 4–9, subsamples of 10 mg were taken and treated in a low temperature asher. After dilution with water, the final solution was analyzed with AAS-ETA. Larger subsamples, wet ashing and flame AAS, introduced from QC 10 resulted in higher accuracy.

CHINA

Lead and cadmium in blood. In the middle of September, 1980, the first analysis of lead in blood was performed with ETA(F). Later it was found that ETA analysis after addition of

phosphoric acid to the blood and wet ashing with HNO_3 and $HClO_4$ gave better results. This method was used for the first QC run. However, the accuracy was not acceptable due to a high background level. Furthermore, there was no optical kit installed in the instrument to protect the photomultiplier from stray light. Another problem emanated from unsatisfactory electrical contacts in the HGA-72. After modification of the AAS equipment, the ETA(S) method was used for the major part of the project with high accuracy.

Cadmium in kidney cortex. AAS flame analysis after wet digestion with HNO_3 and $HClO_4$ previously used for biological samples gave accepted results for all QC runs.

INDIA

Lead and cadmium in blood. At the first visit of the consultant in 1978 a wet ashing method followed by flame AAS was used for metal analysis. Since no background correction was used, the values were generally too high. A direct extraction method according to Westerlund-Helmerson (1970) was used in the beginning of the project, but gave too low results for the internal QC samples. The Delves Cup method, also suitable for the cadmium analysis, was introduced and a background correction system and recorder were installed in November 1979 (after the first QC run). Improvement was achieved after further training (including one fellowship), receipt of spare parts, and change of laboratory facilities.

There have been some problems with the background correction system throughout the project. Difficulties in obtaining service and spare parts have apparently delayed the analyses considerably.

Cadmium in kidney cortex was analyzed by AAS-flame after wet ashing. A dry ashing method, introduced after temperature calibration of the available muffle furnace, has been used with accurate results for the major part of the project.

IRAN

Lead and cadmium in blood had not been analyzed before. No cadmium or lead lamps were available. The Perkin Elmer AAS instrument, model 306, was not equipped with background correction system or recorder and thus not suitable for the analysis of lead and cadmium. QC 2 and 3 were analyzed by ETA at another institute in Teheran but the accuracy was not acceptable. A modern ETA unit, PE 400, was delivered but no further results have been reported.

Cadmium in kidney cortex had not been analyzed before and no analyses have been performed on QC samples.

ISRAEL

Lead in blood had been analyzed only occasionally before the start of the project. For the first QC samples the ETA(F) method was used but gave too high values, probably due to a combination of nonatomic absorption, improper standards and contamination. At the consultant's visit it was found that the AAS instrument was highly overcompensating when the deuterium background correction system was used (poor alignment between light beam from the hollow cathode lamp and deuterium lamp due to incorrect position of mirror in front of the hollow cathode lamp). After modification of the ETA programme and changing from water standards to blood standards, the method gave satisfactory results. Contamination problems were solved mainly by changing routines for acid washing and sample handling.

Cadmium in blood had not been analyzed before. The method used for lead was used for the first QC samples but due to severe overcompensation, results on the internal QC samples were not in agreement with reference values. The ETA(S) was introduced for cad-

mium analysis and after adjustment, as per lead, the analysis gave results of much higher accuracy. A fellowship was provided for further training in trace metal analysis.

Cadmium in kidney cortex had not been analyzed before. A wet ashing method had been used occasionally for analysis of hair and results were compared with those with a dry ashing method introduced after temperature calibration of a Heraus MR-170 muffle furnace. Both methods gave acceptable results. The dry ashing method was used for the major part of the project because of less risk for contamination.

JAPAN

Lead and cadmium in blood. This laboratory had some experience in lead analysis and analysis of other metals in biological material.

ETA analysis was performed after wet digestion of the samples with HNO_3 and $HClO_4$ in a dry block heater. Minor problems were encountered at the first three QC runs, but after checking the standard solutions and introducing additional IQC samples, prepared at the laboratory, the analyses were performed with high accuracy.

Cadmium in kidney cortex was throughout the project analyzed by flame (with background correction) after wet ashing of the samples as described for blood samples.

MEXICO

Lead in blood. Before the start of the present project an AAS method of Barthel et al. (1973) utilizing Delves Cup without background correction and recorder facilities had been used routinely. This method gave too high values in the low concentration range for QC 1. Problems related to contamination from air were encountered. After moving to a new laboratory, installation of background corrector, phase button, current stabilizer, recorder and modification of the Delves Cup method, the accuracy was improved (consultant's visits June and December 1979). Other problems, related to electrical installations, purity of acetylene gas and need for AAS spare parts, caused rejection of some QC runs. After receiving an automatic injector (PE AS-I), the ETA(S) method was introduced in the end of the training phase (QC 13).

Cadmium in blood had not been analyzed before. The same method as for lead in blood was used after the improvements mentioned above. Contamination problems were related mainly to the automatic pipette (model 25000 from Micromedic System) and the 0.5 M nitric acid used for sample treatment. ETA(S) was introduced at QC 10.

Cadmium in kidney cortex had not been analyzed before. At a consultant's visit in November 1980 a muffle furnace and an analytical balance were installed and calibrated. A dry ashing method as well as a wet digestion method followed by flame AAS were introduced. The dry ashing method, used for the main part of the project, gave results in good agreement with reference values.

PERU

Lead and cadmium in blood had not been analyzed before and experience in AAS analysis was limited. A consultant visited the Institute for one week in June 1979 and provided Delves Cup equipment. The main problem was related to impurities in the acetylene gas (cups stuck in the loop assembly and were very difficult to remove), but this was solved after contact with the producer. A consultant visited the laboratory again for three weeks in November 1979. The results of lead and cadmium analyses from QC 2 showed considerable improvement. For cadmium in blood some problems arose after QC 3 due to changes of deuterium lamps in the instrument. Since only one holder for the hollow cathode lamps was in operation it was sometimes difficult to align the light beam from the hollow cathode lamp with that of the deuterium lamp (critical for correct background

compensation since cadmium absorbs light of low wave-length and is sensitive to sodium chloride absorption). At a consultant's visit in December 1980 some old lamp holders were repaired and introduced with the result of higher accuracy for QC 9.

Cadmium in kidney cortex had not been analyzed before. In November 1979 a new Heraus MR-170 muffle furnace was received and a new method of analysis introduced as a result of a consultant's visit (AAS-flame/dry ashing). The results obtained were in good agreement with the reference values.

SWEDEN

Lead in blood was rejected when using Delves Cup technique for QC 5 due to contamination by repipetting of the sample containing the highest concentration. QC 9, which was analyzed together with the accepted QC 8 and 12, was rejected due to variation of some sample signals during the determination. For ETA(S) method QC 4 was rejected due to repeated analyses with a decrease in concentration of standards giving too high values for the samples. QC 6 was rejected due to incorrect calculation of the standard curve.

Cadmium in blood was analyzed the same day with Delves Cup technique for QC 8, 9 and 12. They were all rejected due to a decrease in concentration of standards (made in blood and stored in glass tubes from the previous day) resulting in too high values for the samples. For ETA(S) method QC 4 was rejected due to contamination of some samples and a change in sensitivity during the analysis giving problems to evaluate the results. QC 7 was rejected due to contamination and background problems caused by storage of the prepared solutions in sample tray cups over the weekend causing evaporation.

USA

Lead in blood. The laboratory had experience with trace element analysis of environmental samples, such as soil, water and vegetable crops. Experience with human tissues and fluids was limited. ETA(F) was used for QC 2–4. Renovation of the laboratory from October 1980 to January 1981, breakdown of instrument and change of personnel considerably delayed the project. The results of QC 5–9 showed high operating error due to high background levels. The ETA(S) method was introduced at the end of the training phase.

Cadmium in blood. A method of direct injection after dilution of blood with 0.1% HNO_3 was first used. Results were accepted for QC 2–3, using an AAS instrument from Hitachi equipped with a Zeeman background compensation system, despite a high background signal. For QC 3–9 all results were rejected, probably due to the high background signal. The ETA(S) method was introduced at the same time as for lead.

Cadmium in kidney cortex. A wet digestion method, followed by flame AAS has been used throughout the project with high accuracy.

YUGOSLAVIA

Lead in blood. The Institute had extensive experience in analysis of lead in blood, urine and tissues and was participating in the CEC and the British Quality Assurance Programmes at the start of this project. The ETA(F) method (PE HGA-72) was used, but there were problems with condensation effects at the end of the graphite tube causing high background signals. (The temperature programme for an ETA unit is critical for maximal charring without losses of lead before atomization and for lowest possible background signal). There were problems in handling large number of samples due to poor stability (more than 10% variation) of the electrical current, affecting the temperature programme and hence the background signal as well as the sensitivity. These were probably the reasons for few accepted results for QC 1–7. In February 1981 the stability problem was

solved by two additional servo stabilizers for HGA-72 unit (the AAS instrument was stabilized already in 1973, when it was installed). At a consultant's visit (two weeks in March 1981) the ETA(S) method, which is less sensitive to problems with background signals, was introduced. Alignment of hollow cathode lamps against deuterium lamps and preparation of standards were also demonstrated. Glass (instead of polyethylene) containers had to be used throughout the analysis, due to custom restrictions. Due to the contamination problems caused by the use of strong acid in ETA(S) method, the ETA(F) method was used throughout the project. After QC 17 the problems were solved by washing glassware in EDTA solution following 2x24 h soaking in 30% HNO_3. The results of QC 13–17 were accepted when evaluated together, but showed large operating errors.

Differential pulse anodic stripping voltammetry (DPASV) was also used for lead and cadmium in blood but the accuracy was low, probably due to difficulties related to sample treatment. A fellowship was provided for one month's training.

Cadmium in blood. DPASV was used for QC 7–9 with low accuracy. The ETA(S) method, proposed by CI was introduced from QC 10 and was used with success for the rest of the project.

Cadmium in kidney cortex. QC 4 and 5 were analyzed by DPASV with low accuracy and therefore flame AAS after dry ashing was utilized for the remaining part of the project. This method, previously used for analysis of cadmium in tissues, gave accepted results for QC 6. QC 8 was rejected, because the stock standard solution was contaminated, resulting in too low values. A balance was introduced for preparation of standards and all results for QC 10–17 were accepted.

Table A2:1. AAS equipment available at the laboratories

Country	Instrument	ETA/Delves Cup	Auto sampler	Used for
Belgium	PE 5000	PE HGA-500	PE AS-I	blood
	PE 360			kidney
		PE HGA-76	PE AS-I	not used
China	PE 403	PE HGA-72		kidney+blood
India	PE 373	PE Delves Cup		kidney+blood
		PE HGA-2200		not used
Iran	PE 306	PE HGA-400		not used
Israel	PE 460	PE HGA-2200		kidney+blood
	CZ M20+PMQ 11			not used
Japan	JA AA-8500	JA FLA-10		kidney+blood
	PE 4000	PE HGA-400	PE AS-40	not used
Mexico	PE 403	PE Delves Cup		kidney+blood
		PE HGA-2200+ramp	PE AS-I	blood
	IL 251	IL 555		kidney
Peru	PE 305 A	PE Delves Cup		kidney+blood
Sweden	PE 373	PE HGA-500	PE AS-40	blood
	PE 403	Delves Cup		kidney+blood
		PE HGA-72	PE AS-I	not used
	PE 303	V CRA-63		not used
		PE HGA-70		not used
USA	H ZAA 170-70			blood
	PE 403	PE HGA-500		kidney+blood
	PE 306			not used
	PE 303			not used
Yugoslavia	PE 403	PE HGA-72	PE AS-I	blood
		PE Delves Cup		not used
	V 375			kidney
		V CRA-90		not used

PE = Perkin Elmer
CZ = Carl Zeiss
JA = (Japanese) Jarrell Ash
IL = Instrumental Laboratory
H = Hitachi (Z = Zeeman)
V = Varian

Appendix 3

Epidemiological designs, characterization of areas and procedures for sampling of blood and kidney cortex

BELGIUM

1. General description of the area and population monitored

The survey was carried out in the agglomeration of Brussels (19 communities), capital of Belgium. Brussels is located at 51° north-latitude and 2° east-longitude, in the center of the country, which has a total population of 9,868,000 with a density of 323 persons/km^2. Brussels has 1,009,000 inhabitants, i.e. slightly more than 10% of the total population. Brussels covers an area of 162 km^2, with 6,228 persons/km^2.

The climate is of the temperate-maritime type with four seasons: spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). The average daily temperature is about 16°C in summer and in winter 3°C. The average annual precipitation is about 780 mm.

Traffic density is high. In 1979 there were about 3,100,000 private motor-cars and 470,000 commercial vehicles (trucks, tractors, etc.), i.e. about one vehicle for three inhabitants. The lead content in gasoline was limited to 0.55 g/l in March 1977 and to 0.45 g/l in May 1978. Controls indicate that the limit is respected in Belgium. Before 1977, no limit was enforced and levels as high as 1 g/l or 1.5 g/l had been observed.

Belgium has complete freedom of religion, catholicism being prevailing. There is no special restriction in food habits. Foreign residents were not included in the survey.

The inhabitants of Brussels live primarily on commercially produced food. A recent survey estimated the median daily dietary intake of cadmium and lead as 15 μg Cd and 97 μg Pb in the adult Belgian population.

A rough estimation of smoking and drinking habits can be made on the basis of the results of surveys carried out by the Institute of Hygiene and Epidemiology since 1978:

- The cigarette smokers in Belgium represent 26% of the population (31% males and 18% females);
- 31% of the population (41% males and 16.5% females) is drinking beer daily;
- 15% of the population (18% males and 9.5% females) is drinking wine daily; and
- 7% of the population (11.5% males and 1.8% females) is drinking alcoholic beverages daily.

Brussels is the administrative center of the country, and there are a number of industries, producing articles such as laces, gloves, furniture, car parts, musical instruments, jewellery, printworks, and lithography. There are also breweries and distilleries.

2. Outcome of the monitoring

Two kinds of populations were used in the study:

- primary and middle school teachers
- blood donors living in Brussels.

For the *teacher's survey*, the design is rather complicated:

- contacts by personal visit, letter, phone call, etc. with authorities of the Ministry of Education to obtain the necessary authorization
- subdivision of the area into four quarters
- listing of the schools
- random selection of 20 schools (5 in each quarter of the area) representing about 2000 teachers
- contacts, by personal visit, letter, phone call, etc. with the principals of the schools
- listing of teachers and contacts to encourage participation
- sampling.

For the *blood donor's survey*, the design is more simple:

- contact with the medical director of the blood bank and random selection of donors (Brussels residents) when they come to give blood.

The design worked very well for the blood donors, but not as well for the teachers. Principals of certain schools refused participation and the schools had to be replaced by others situated in the same quarter. Furthermore, teachers were reluctant to participate. As a whole, only 15% of the teachers agreed to participate. The reasons for the high number of non-respondents are multiple, such as:

- Teachers are too frequently requested to participate in various surveys;
- Fear for giving blood;
- Participation in other health programmes such as prevention of cardiovascular disease and cancer detection;
- Campaigns for blood donation.

2. Sampling of blood and kidney cortex

Blood. The sampling of blood donors was made at the blood bank. A member of the laboratory staff, well aware of the contamination problems, went to the blood bank and made a random selection of donors. Interviews were carried out at the blood bank. Controlled blood collection tubes were used.

The sampling of teachers was made at the school infirmary by a physician with the help of an assistant. Both were members of the laboratory staff and well aware of the contamination problems. The same precautions as for blood donors were made to avoid contamination.

All blood collection tubes were transported by car to the laboratory immediately after sampling (within 3–4 hours), where they were stored at +4°C. The analyses were performed in duplicate, on different days but within the same week. All samples were finally stored deep-frozen (–60°C) for eventual future analyses.

Kidney cortex. The kidney cortex samples were collected at the University Hospital of Liège. The film on collection procedures was shown. Samples were kept deep-frozen in plastic screw-capped bottles controlled for possible cadmium contamination.

CHINA

1. General description of the area and population monitored

Beijing, capital of the People's Republic of China, is located at 39.6° north-latitude, 116.2° east-longitude with a total area of 16,800 km^2 on the north-western edge of the north China plain. There are mountains to the north and west, and the inclining plain stretches south-east towards Bo Sea which is about 150 km away. The city is far inland on the alluvial plain of Yong-Din river and lies 44 m above the sea level. The Guan-Tin and Mi-Yuin reservoirs are located in the north-west and north-east suburbs, and the Yong-Din river runs through the south-west suburbs.

The survey was carried out within urban Beijing, an area of 87.1 km^2. The population of the urban area is 2.3 million with a density of 26,000 residents/km^2.

The climate of Beijing is of the temperate-continental type. Spring (April and May) and autumn (September and October) are rather short, while winter (November to March) is rather long. It is windy and dry in spring, hot and rainy in summer, sunny and less rainy in autumn and cold and dry in winter. According to records over the past 31 years, the average annual precipitation is 696 mm. The average annual temperature is 11.6°C, with summer and winter temperatures of 24.5° and −3°C, respectively.

Underground water resources are rich, and constitute the main source of tap water. The Guan-Tin and Mi-Yuin reservoirs are supplement sources.

Major industries, such as chemical, metallurgical, engineering and textile, are mostly located in the suburbs, but there are numerous small factories and workshops scattered in the urban area of Beijing.

Beijing is the most important hub of communications of China. It is connected with all parts of the country by air, buses and trains. In the urban district, buses, trolleys and bikes are the main transportation vehicles. The total number of automobiles is about 150,000 and increasing year by year. The number of motor-bikes has increased in the past one or two years and is now about 18,000. The consumption of gasoline increased by a factor 3 between 1971 and 1981. Of the gasoline consumed in 1971, 2.1% was non-leaded. Corresponding value for 1977 was 75%.

2. Sampling of teachers and collection of blood specimens

Subjects for the survey were middle school teachers in Beijing. There are 187 schools in the urban area. The total number of full-time teachers is 15,041 (6,159 males and 8,882 females). The age range of students is 13–19 years.

Two hundred and forty school teachers were selected from the total number of teachers employed in the urban area of Beijing. Through contacts with officials at the municipal and the regional Bureau of Education, detailed information about schools and the number of teachers in each school was obtained. At first, several schools were selected by random sampling. Then the selected schools were contacted for information about name and sex of the teachers. Statisticians were consulted for the sampling procedure.

Two hundred and forty samples were taken proportionally to the population in four regions. The number of samples for each region was 60, 72, 48 and 60. Ten teachers were selected from each school. Both teachers and schools were selected randomly.

An official of the municipal Bureau of Education informed the schools about the purpose of the project and asked for co-operation. To increase response, the importance of the project was stressed and a small reward given to participants. If subjects still did not wish to participate, alternatives were selected randomly.

Experienced nurses were employed for the collection of blood samples. This was done in the school infirmary according to the procedure recommended by the CI. At the same time interviews were carried out and the questionnaires completed. Two or three schools per day were sampled. Immediately after the sampling, the blood samples were put in an ice box and sent to the laboratory, where they were divided into 3 tubes and stored deep-frozen.

3. Collection of kidney cortex samples

Kidneys were collected at the autopsy room of the legal medical office and hospital. Direct contact was established with pathologists and legal medical experts. Collected samples were put into acid-washed polyethylene bags and stored in a freezer until analysis.

INDIA

1. General description of the areas and population monitored

Three cities, Ahmedabad, Bangalore and Calcutta, were monitored in the project.

Ahmedabad. Ahmedabad, the main city of Gujarat, is located in the western part of India (23°01′ north-latitude) at about 50 m above the sea level. It has an area of 93.2 km^2. The river Sabarmati which runs from north to south divides the city into two parts. According to the latest census (1981), the total population of Ahmedabad city is about 3.8 million. The highest population density of 88,577 persons/km^2 is within the walled city. Outside the wall the density is 15,137 persons/km^2 on the east side and 6,720 persons/km^2 on the other side of the river.

The four seasons in Ahmedabad are the cold weather period (December-February), the hot weather period (March-June), the monsoon season (July-September) and the post-monsoon season (October-November). Ahmedabad has an average rain fall of about 650 mm, most of which occurs during the monsoon season. Nearly one half of the city water supply is soft river water. The other half is medium hard groundwater.

Ahmedabad is a textile centre. There are other industries such as engineering, chemicals, printing, leather works, situated in and around the city.

In Ahmedabad, the Gujarati language is spoken and most people follow the Hindu religion. Some follow the Jain or the Muslim religion. People are mostly vegetarians and eat a diet high in carbohydrates and lipids. People mainly eat commercially produced food. Alcohol and drugs are not used. People (mainly men) smoke cigarettes and bidi (tobacco leaves) and they chew tobacco and betel.

The number of motor vehicles in Ahmedabad has risen phenomenally in the last few years. Ahmedabad is also connected with the rest of the country by road, railway and air. Lead is added to gasoline in the range of 0.15–0.3 g/l.

Bangalore. Bangalore is the capital city of Karnataka state in the southern part of India (12°58′ latitude and 77°35′ longitude) and lies at about 900 meters above the mean sea level. The area of Bangalore city is about 150 km^2. The lastest census showed that the population of Bangalore is over 2.5 million and the density about 16,666 persons/km^2.

There are four seasons, summer (March-May), monsoon (June-September), post-monsoon (October-November) and winter (December-February). Bangalore has an average rain fall of about 925 mm most of which falls in the monsoon season.

Bangalore is famous for manufacturing incense sticks. Three big industrial complexes are located in Bangalore. There are also many small industries, e.g. chemical and battery manufacturing units.

The city water supply consists of an artificial tank about 30 km from the city and the Cauvery river. Suburban areas take water from local wells.

The Kannad language is spoken in Bangalore and most of the people follow the Hindu religion. People are mostly vegetarians and consume mainly rice but also some wheat products. People (mainly men) in Bangalore smoke cigarettes and bidi. They also chew tobacco and betel. Very few persons use alcohol.

Calcutta. Calcutta is the capital of the state of West Bengal. It is situated on the eastern bank of the river Hooghly (latitude 22°32′ north and longitude 88°22′ east) at about 6.4 meters above the sea level. Calcutta metropolitan district covers an area of 1,380 km^2, with the city proper covering about 104 km^2. The population of Calcutta metropolitan district is about 8.3 million. The population density is about 30,276 persons/km^2.

There are three seasons in Calcutta, summer season (March-June) with a humidity of about 74% and wide fluctuation in temperature. Rainfall is about 100 mm. During the monsoon season (July-October) humidity is about 82% and monthly rainfall about 300 mm. Winter season (November-February) has a humidity of about 75% and the temperature goes down to about +4°C.

Calcutta is still handling the major part of all cargo leaving India, why export transactions is by far the biggest business. There are different types of industries, such as jute works, chemical, engineering, glass and ceramics, paper-boards, textile, rubber and power plants in the city. Calcutta, being one of the major ports of industrial and commercial activities, has had a rapid urbanisation. Poor planning and uncontrolled growth of the city has resulted in overcrowding and that not even minimum sanitary requirements are met in the slums.

Bengalee language is spoken in Calcutta and most people follow the Hindu religion. The Muslim and Christian religions have some followers. People are mostly non-vegetarians and consume much fish and rice. The teachers do not take alcohol which is restricted to upper social status and few of them are addicted to drugs. Cigarette smoking and pan-chewing (a kind of leaf taken with lime, betel and tobacco) are common practices.

Drinking water is supplied from an overhead water reservoir known as "Talah tank" situated on one side of the city proper. The tank holds purified Ganges water collected at Palta, about 40 km outside the city, where sedimentation and chlorination is being done. It is supplied throughout the city. In addition, there are tube-wells in some areas as a supplement.

2. Sampling of teachers and collection of blood specimens

Ahmedabad. The target population consisted of 200 primary and secondary school teachers employed in the Ahmedabad school system. A total of 218 secondary schools (children 13–19 years of age) and 434 primary schools (children 5–13 years of age) are situated in Ahmedabad, in which 9,570 teachers are employed (16.3% male primary teachers, 40.9% female primary teachers, 29.6% male secondary teachers, 13.1% female secondary teachers). A complete list of primary and secondary schools was obtained from the Department of Education, State of Gujarat and Ahmedabad Municipal Corporation. The number of teachers employed in each school, their name, age, sex and address were collected.

The area stratification was implemented by dividing the total area of Ahmedabad into

three areas: residential, walled city and industrial area. The elementary unit of sampling for the first stage unit (f.s.u.) was a cluster of schools consisting of one or more schools. At the first stage of sampling there were two distinct populations of f.s.u., one with the schools employing male teachers and the other with the schools employing female teachers because the ratio males/females differed considerably for the schools. Ten f.s.u. for male teachers and ten f.s.u. for female teachers were allocated among the three areas in proportion to the number of teachers (male or female) in the respective areas. At both stages of sampling random number tables were used.

Detailed information describing the aim and objective of the project was circulated and small seminars were held with the teachers in the schools. The teachers were contacted individually and interviewed. Only twelve teachers, out of 200 teachers did not give blood samples. Five teachers refused to give blood even after further information about the project (afraid to give blood). Another six teachers had left the school for different reasons and one teacher was on long sick leave. Substitutes were chosen from the alternate sampling scheme.

Blood samples were collected by a team of laboratory personnel at the selected schools after prior appointment with the teachers. After collection the samples were kept in an ice-box and transported to the laboratory within 1–2 hours, where they were stored in a freezer until analysis.

Bangalore. One hundred blood samples were collected after statistical evaluation of the total number of teachers (primary and secondary schools) employed in Bangalore. Information regarding the total number of schools and teachers employed was obtained from the Deputy Director of Public Instruction (DDPI), Government of Karnataka. A proforma giving necessary information was sent to the head masters of all 775 schools in the north and south districts of Bangalore. Response was received only from 384. The majority of the schools in the suburban and rural areas did not respond.

Area stratification was the same as that followed in Ahmedabad, i.e. Bangalore was divided into three areas, residential area, densely populated commercial area and industrial area. The first stage unit of sampling (f.s.u.) was a cluster of schools consisting of one or more schools. The first and second stages of sampling were performed as in Ahmedabad. The schools were contacted directly as well as through government and municipal agencies and information about the project was given to the teachers.

A team consisting of one technician (for blood sampling), one research assistant (trained for the interview) and one officer involved in this project went to Bangalore with complete kits for collection of blood samples. Three teachers of the 100 selected were absent at the time of sampling. Samples were kept in an ice-box after collection and were transported by air to Ahmedabad packed in a specially made ice-box (transport time about 10 hours). In Ahmedabad the samples were kept in a freezer at NIOH.

Calcutta. One hundred blood samples were collected after statistical evaluation of the total number of teachers (primary and secondary schools) employed in Calcutta.

Information about the total number of schools and teachers employed was collected from the Department of Health and Education of Calcutta Corporation, the secretary of the Department of Education, Government of West Bengal; the president of the Board of Secondary Education, the director of Public Instruction, Government of West Bengal and also the district inspector of schools, primary and secondary of Calcutta District.

There are about 1900 schools in Calcutta city proper (Howrah schools are not included). Letters asking for participation were sent to the principals of all schools. Response was received from 1646 schools.

Calcutta was divided into five zones according to residential, commercial and industrial characteristics. The first stage of sampling gave two distinct populations, one with the

schools employing male teachers and the other schools with female teachers. Ten first stage units for male teachers and ten for female teachers were allocated among the three areas in proportion to the number of teachers in each area. Random number tables were used for the sampling.

Procedures for contacting schools and teachers as well as blood sample collection were the same as for Bangalore. There were altogether 32 nonrespondents. The major reasons were transfer to other schools, religion and sick leave. Eight teachers refused to give blood. Substitutes were selected from the alternate sampling scheme.

3. Collection of kidney cortex samples

Kidney cortex samples were collected from autopsies. These samples were collected by pathologists and brought to the laboratory by a trained technician. The procedure for collection of kidney cortex samples at autopsies followed the recommendations set up at the meeting in Stockholm (WHO, 1980a). The samples were collected in contamination-free plastic bottles. The bottles were sent to the NIOH laboratory from Civil Hospital in Ahmedabad every week. In Bangalore and Calcutta, the samples were stored in freezer of ROHC Laboratories. When collection was completed, the samples were transported to Ahmedabad by air in ice-boxes.

ISRAEL

1. General description of the area and population monitored

Israel lies on the eastern sea-board of the Mediterranean between Asia and Africa. Generally the climate is hot with humid summers and mild winters. Rain falls only in winter season. Average daily minimum and maximum temperatures are 5 and 12°C in January and 19 and 28°C in August.

Total population is around 3,750,000 consisting of 3,150,000 Jews, 460,000 Moslems, 85,000 Christians and 47,000 Druzes. The people reside in 888 localities with an average density of 434,3 persons per square mile. Eighty-six percent live in 103 towns, including 33 major urban centers, 13% in Moshavim (co-operative villages) and 3% in kibbutzim (collective villages). Two percent are transient or nomadic Bedouins. All Jews share one faith. The communities may be divided into three main groups, those born in Israel (54.2%), those born in Europe or America (25.4%), and those born in Asia or Africa (20.4%). The national language is Hebrew.

The Government is responsible for the national education. The educational network includes 7470 schools employing about 60,000 teachers, around 23,000 of which are elementary school teachers.

Over 10,000 km of highway stretches across the country. Public transportation is primarily by bus. There are more than 5000 buses and 4200 taxis. Only 5% of the freight transport is hauled by rail, the rest is trucked. The lead content of gasoline is 0.4 g/l.

The consumption of milk, dairy products, bread, cereals, vegetables and fruits, is high. The percentage of vegetarians is very low. No basic religious food restrictions are imposed except for pork and certain sea foods. The general population consumes mainly commercially produced foods. The alcohol consumption is very low, and a significant decrease in smoking has been noted. Generally no significant dietary differences, related to lead or cadmium, exist between different groups of the population.

Regulatory requirements have been issued regarding lead content in food. The total daily oral intake from dietary sources is about 200–300 μg Pb/day. Generally foodstuffs with high lead residues, e.g. sesame paste (about 2 ppm) are very rare. Legal requirements have been set for lead in toys and ceramic ware.

2. Sampling of teachers and collection of blood specimens

The population to be sampled consisted of public elementary school teachers from one specific urban area–Jerusalem Central District. The age of the children was between 7 and 16 years. The percentage of female teachers was very high, more than 75%.

The frames used in the design of the survey were the following:

1. Division of large localities in Israel into statistical geographic areas, based on the 1972 Census of Population and Housing.
2. Lists of schools in the national education system including data on code number of school, location, name, address, teaching posts and number of pupils (1980).
3. Lists of teachers by name–the monthly payroll.

In the first stage, 60 schools in the area were chosen from the list, after which direct contact was established with the school manager to prepare the final list of teachers and make the necessary arrangements for blood collection.

In order to obtain a sample with an approximately even number of males and females, it was decided to treat males and females as separate populations. The population was selected without stratification because the teacher population in the area was quite homogeneous with respect to socio-economic and demographic data.

The total number of blood samples was 213, of which 201 were fit for analysis. Non-response in the population was around 50%, which can be considered quite satisfactory and corresponding to the general trend in this kind of project. A number of explanations can be considered: natural hesitaiton; the population had previously been requested to participate in various surveys; venous blood is taken for health checks (pregnancy). To decrease the non-response it would be necessary to make repeated visits to the schools, and give more information, which called for more personnel and an adequate budget.

A protocol based on the recommendations proposed during the Stockholm meeting, May 27–30, 1980, was prepared in order to assure uniformity in blood collection procedures, storage and transportation to the laboratory. Medical personnel was specially trained for the blood collection and instructed on trace aspects of lead and cadmium in blood and the importance of avoiding contamination during the sampling. Evacuated collection tubes (Venoject) were used and the skin carefully cleaned by disposable towels containing alcohol (Medi Swab). Generally, a member of the Institute was present during the blood collection.

The blood specimens were kept in a refrigerator after collection, then immediately transported to the Institute under cooled conditions. Each blood sample was divided into 3 tubes of polypropylene. One sample was analyzed, the other two tubes stored deep-frozen.

3. Collection of kidney cortex samples

The target population was cases of sudden death. The samples were obtained from the National Institute of Forensic Medicine. Direct contact was established with pathologists at the Institute. The importance of non-contamination was stressed and the film on

procedures for collection of kidney cortex samples at autopsies, produced by WHO/IAEA in relation to the project of trace elements in cardiovascular diseases, was shown.

The kidney cortex was generally collected 12 hours and not more than 20 hours after death. The samples were stored in cadmium-free plastic boxes and kept deep-frozen. Monthly, the samples were transported to the Institute under cooled conditions and then transferred to a freezer until the analysis.

JAPAN

1. General description of area and population monitored

Tokyo, the capital of Japan, is located between 35°30′ and 35°33′ north-latitude and between 138°58′ and 138°54′ east-longitude. It is situated midway along the Pacific coast of Honshu (main island in Japan). Edo and Tama rivers run through the east and west area of Tokyo and empty into Tokyo bay. Tokyo, consisting of 23 wards, and the Tama and Island areas cover 2154 km^2 with a population of 11.6 million. The area corresponding to the prewar city of Tokyo (23 wards) covers about 590 km^2 and has a population of 8.4 million.

The climate is mild in spring and autumn, hot and rainy in summer, and cold and dry in winter. Total precipitation was 1453 mm in 1979. Drinking water is supplied by Tama, Arakawa, Sagami and Tone rivers.

The transportation system includes national railways, private railways, subways and buses. The number of motor vehicles registered in 1979 was about 3 million and the total amount of sold petroleum was 14 million kl. Regular gasoline constituted about 85% of all gasoline consumed in 1970, and more than 97% in 1980. The lead content in 1970 was 0.18 g/l. It has continuously decreased and is less than 0.0001 g/l since 1976.

2. Sampling of teachers and collection of blood specimens

Full-time teachers in public elementary schools (pupils 7—12 years of age) and junior high schools (pupils 13—15 years of age) in the Tokyo metropolitan area were designated as the target population.

For area stratification of the schools, the Tokyo metropolitan area was first divided into six blocks according to geographic features, average city tax (reflecting socio-economic status) and density of population.

Following area stratification, 13 schools were selected according to the number of teachers, using random number tables. The selection satisfied the following requirements: at least 2 schools in each block; a proportional number of teachers; sufficient number of schools for 200 samples, a male/female ratio of one to one; and selection of elementary schools and junior high schools in the proportion one to one as far as possible. The sex ratio in each group was highly skewed. About 60% of the teachers in elementary schools and about 30% in junior high schools were female.

The number of schools to be selected in each block was allocated by the following formula: (the number of teachers in each block/51,592 = total number of teachers in 5 blocks) x 13 = the number of schools to be selected in each block. All teachers in the chosen schools were regarded as monitoring subjects and asked to co-operate in the project. The subjects were divided into male and female groups in each block, and 200 in-

dividuals were finally selected in proportion to the number of teachers in each block. This sampling procedure is different from the one advised by UNEP/WHO, but believed to be practical and satisfying to a certain extent as a random sampling.

After selecting 13 schools, excluding the rural area, the Board of Education was contacted through the Tokyo Metropolitan Government. Information on the purpose of the project was given to principals and teachers. A clinical examination was included in the project. The sampling response rate was over 94%. Almost all teachers who refused to participate had reasonable excuses, such as anemia or chronic disease.

The teachers appear to be omnivorous in dietary habits according to the questionnaire. The main foods are rice, bread, fish and meat.

All blood samples were collected in the school infirmary. They were immediately brought to the laboratory under cooled conditions and stored at −20°C until used.

3. Collection of kidney cortex samples

All samples were collected at the Tokyo Metropolitan Examiner's Office. The kidney cortex was cut down to about 1 g and weighed. After drying in an oven at 80–100°C for 24 hours, the samples were reweighed and subjected to wet ashing with nitric acid and measured for cadmium by flame AAS.

MEXICO

1. General description of the area and population monitored

Mexico City is the capital of the country. The metropolitan area has 14,000,000 inhabitants on an area of 1500 km^2 and a population density of 9400 persons/km^2. It is situated 2240 m above sea level. The climate is moderate with a minimum of −2°C in winter and a maximum of +29°C in summer; humidity is low and rainy season is in summer. Catholicism is the predominant religion.

Traffic is very heavy. Most people must travel 30–60 minutes to go from home to work, and there are 1,600,000 automobiles. The lead content of regular gasoline (97% of the total production) is about 0.9 g/l.

Primary state school teachers are considered as lower middle class in the socio-economic structure. Their food habits are fairly good without restrictions. There is not a very high consumption of canned foods. Inhabitants in Mexico City usually live on commercially produced food. Alcohol and drug consumption is considered low, but tobacco smoking is common.

2. Sampling of teachers and collection of blood specimens

State primary school teachers of both sexes and varying ages, working in Mexico City and registered in 1980 were selected as the target population for collection of blood specimens. The total population consisted of 38,000 teachers, 20,000 males and 18,000 females. Since the size of the sample was set at 200 with 50% males and 50% females, and a non-response of 33% was estimated, it was decided to sample 305 teachers, 153 males and 152 females, i.e. 0.8% of the total population. Selection of individuals was done at random after stratification of teachers according to sex.

Sampling of blood was performed during four days with around 50 samples taken a day in a clean room of the Institute. A team of 6 nurses worked simultaneously, supervised by one chemist and two doctors. Samples were collected in Vacutainers supplied by the CI. A presentation was given to explain the project to the teachers, who were asked to complete the questionnaires. Samples were set in a wire rack at room temperature during sampling (about 1 hour), and then transported to the laboratory in polyurethane boxes with ice in the bottom (transport time about 30 minutes). Each sample was transferred into three plastic vials and stored frozen until analysis.

A total of 200 blood samples were collected. The non-response was 33% as estimated and there was no explanation to it.

Each respondent was notified of the lead and cadmium level in the blood. In addition respondents were informed of the results of ECG and blood count whenever they wished to undergo these additional tests free of charge. All information was kept confidential.

PERU

1. General description of area and population monitored

Peru is a country in South America of more than 1,000,000 km^2 and nearly 18 million inhabitants. Because of the Pacific Ocean and the Andes it is a country with many different climates. It is mainly a mining and fishing nation. Many food products are imported, particularly meat, milk, wheat, oil, some vegetables and fruits, but in general people do not eat much commercially produced food. No basic religious food restrictions exist. In general the diet is high in carbohydrates and poor in lipids. Protein comes mainly from plant origin although Peru is a fishing nation. The alcohol consumption is constituted mostly of beer and a distilled wine beverage (pisco). A significant increase in cigarette smoking has been noted lately among women. No legal requirements have been set for lead in toys (national or imported) and native ceramic ware.

Peru has not a very extensive net of transportation. A programme is under way to integrate the country by roads. There are two principal railways (Centre and South). Most of the transportation today is by airlines. Generally speaking Peru is a country rich in natural resources and progressing towards industrialization.

Lima, the capital of the Republic, was selected as the area to be monitored. It is a cosmopolitan city with more than 4 million inhabitants. Lima is the core of Peru, and has problems with industrialization, immigration and transportation. Major industries, such as chemical, engineering and textile, are located in the suburbs but some small factories and workshops are scattered in the urban area. Transportation between the 39 districts is by bus, automobiles (some are taxis, others are "colectivos", i.e. 5 people share the same car), and trucks. The total number of motor vehicles can be estimated to about 200,000. The traffic is heaviest down-town Lima and on the principal highways. The lead content of gasoline is in general 0.2–0.6 g/l.

The area to be studied included what is called Lima Metropolitana with the exception of the Constitutional Province of Callao. The city is located in the Central Coast section, very close to the Pacific Ocean with an altitude in some parts of no more than 30 m above sea level. In the Eastern part it is surrounded by the western part of the Andes. This influences the climate and the temperature ranges from 10°C in winter to 28°C in summer. It is generally cloudy from April to November, and sunny from December to March. What sometimes is called rain consists of small drops during summer, and common drizzle during winter.

Unfortunately, much agricultural land has disappeared during the last 30 years due to urbanization. There are now plans to reforest the arid areas surrounding the city.

2. Sampling of teachers and collection of blood specimens

The study was carried out within Lima Metropolitana including urban and suburban areas. The Primary Sample Unit was selected in collaboration with the Direccion General de Informatica y Estadistica from the Ministry of Education. By random 22 districts were selected. Only primary public shools, open in the morning turn, with teachers of both sexes teaching either female or male students from 6 to 14 years of age were taken into account. When the schools had been selected, a list of selected teachers was worked out for each school. Alternate lists of selected teachers were also prepared.

Because of many factors, e.g. natural hesitation, participation in various other blood examinations, feelings of being over-requested for duties and obligations to programmes different from their field, many teachers did not agree to participate in the study despite further motivation and recommendations of the high authorities. Three out of the 22 districts had to be excluded and whole schools in 5 districts had to be replaced. Only 23 of the 204 selected teachers in the selected schools agreed to participate. The rest (181 teachers) were volunteers from the alternate lists. When there was non-respondents at a school, the field team took the next-door school of the same district,

A total number of 46 males and 158 female teachers were obtained. The large non-response occurred in spite of written information about the purpose of the project and the importance of collaboration from the Vice-Minister of Health, the Vice-Minister of Education and the Director of the National Institute of Nutrition to the Regional Education Director, the Directors of the selected schools, and the teachers. The samples were achieved mainly by providing food packages and diplomas to the teachers.

The blood samples were collected in the field, i.e. in the classrooms of the schools with precautions to avoid contamination. Full instructions, based on the recommendation proposed during the Stockholm meeting, 1980, were given in order to assure uniformity in blood collection procedures, storage and transportation of samples to the Central Laboratory. There were two teams of field workers consisting of one research assistant and one technician, trained in collecting blood specimens with Venoject tubes. The questionnaires were filled at the same time as the collection of blood.

Each blood sample was divided into 3 tubes provided by the CI. One aliquot of the first 50 samples were sent to the CI. The rest of the tubes were deep-frozen. The samples were analyzed for lead and cadmium.

SWEDEN

1. General description of the area and population monitored

The survey was carried out in Stockholm, the capital of Sweden. Stockholm is located at 59° north-latitude and 18° east-longitude on the coast of the Baltic Sea. The total population in Stockholm, including suburbs, is 1,375,000 with 650,000 in the down-town area. About 8% of the inhabitants in Stockholm is of foreign origin. The total population in Sweden according to the 1980 census is 8.2 million of which 82% lives in densely built-up areas.

The warm Gulf Stream in the Atlantic Ocean gives Sweden a milder climate than other areas equally far north. The summers are fairly warm with an average temperature in Stockholm of 17.8°C in July. The average temperature in February is −3.1°C. Annual precipitation is around 550 mm. The ground is covered with snow about 100 days per year.

The occupational distribution in percent of the total number of gainfully employed persons is: agriculture 6.4, industry 37.8, trade and transport 27.2 and public services 28.4.

About 95% of the population belongs to the Church of Sweden (Lutheran). There are no food restrictions for religious reasons. Mainly commercially produced foods are consumed. The highest calorie intake is via cereals, meat and milk products.

There are about 150,000 private cars in Stockholm corresponding to about 220 cars per 1000 inhabitants. In addition, there is a well developed public transportation system with buses, subways and railways as well as cars and trucks for commercial transport. Lead content in gasoline has decreased from about 0.8 g/l in 1963 to 0.15 g/l in 1981 (both regular and premium gasoline).

2. Sampling of teachers and collection of blood specimens

The population studied was sampled in connexion with a project aiming at investigating attitudes and medical risks related to exposure to motor exhausts and traffic noise. The initial phase of the project comprised a detailed postal enquiry directed to about 8000 persons in various areas of Stockholm and two other Swedish cities. One of the selected groups consisted of a random sample of persons living in the inner city of Stockholm, and data from this group have been included in the present project. The sampling was performed randomly from a register of the total population in the inner city.

The second phase of the project involved a personal interview with the individuals, together with a medical check-up and blood sampling. The subjects included in the present study were randomly sampled from the respondents belonging to the first stage sample of subjects living in the inner city. These were called to the Institute for the test session. Totally 212 blood samples were collected from individuals representing the inner city (3 were excluded due to medical reasons). There were 104 males and 108 females.

Blood samples were collected at the CI by trained nurses. About 10 ml of blood was drawn from the cubital vein using evacuated blood collection tubes (Venoject) after cleaning the injection site with disposable swabs containing alcohol. Each blood sample was divided into 7 polypropylene tubes, previously washed with diluted nitric acid and deionized water. All samples were coded and stored deep-frozen at −30°C until analysis.

USA

1. General description of the area and population monitored

The city of Baltimore is a large industrial city approximately 60 km northeast of Washington, D.C. The population, according to 1980 census figures, is 780,000. Located at approximately 76°7′W longitude and 39°3′N latitude, the city is a port on the Atlantic coast of the U.S. and enjoys four seasons, with annual temperatures ranging from +30°C in the summer to −4°C in the winter. The average annual precipitation for the city was recorded as 1,028 mm for a recent 30-year period. Drinking water for the city is supplied by several open reservoirs. An industrial city, steel and chemical manufacturing as well as petroleum refining are major activities.

In 1975 unleaded gasoline constituted about 17% of all gasoline sold in Baltimore. The regular and premium gasoline contained 0.4–0.5 g/l of lead. The use of unleaded gasoline has increased steadily and constituted in 1980 almost 60% of all gasoline. In about the same time the lead content of regular and premium gasoline has been decreased to 0.25–0.35 g/l.

2. Sampling of teachers and collection of blood specimens

For purposes of selecting the sample, the city was divided into three strata, based on the density of potential point source discharges of lead and cadmium. A list of facilities whose manufacturing processes and industrial activities could be expected to generate lead and/or cadmium-containing waste products was derived from data maintained by various Federal and State Offices. These sites were plotted on a topograhic map of the city, revealing an area of high density in the south-west and south-east quadrants of the city, an area of moderate density in the north-west quadrant, and an area of low density in the north-east quadrant. From among the 193 schools (7036 teachers) in the city, 20 schools were originally selected for participation, with probability of selection proportional to faculty size in 3 geographic areas of the city. Ten schools were selected from the Southern administrative region of the school district, 5 from the North-east region, and 5 from the North-west region.

Because school district officials required that teacher selection be from among volunteers only, a roster of names of faculty members in the selected schools was not made available. Rather, solicitation of teacher volunteers was done by letter to each faculty member in the 20 schools, as well as in 7 alternate schools, with hopes of attaining a high volunteer response rate from which to select a random sample of teachers. The actual response rate was just under 10%, despite the offer of remuneration made to all volunteers whose names would be selected. A second recruiting effort was conducted, which resulted in an overall response rate of 25%. Schools with fewer than 10 teachers volunteering were dropped for replacement by alternate schools, appropriately selected from within the same geographic area. Ten teachers per school (20 in two of the large schools) were selected at random from among the teachers volunteering in each school for the study. In some cases, fewer than 10 teachers actually arrived at the study room the day of the sampling. In others, teachers who had not previously registered for the study, but arrived to participate that day, were taken as "walk-in's" and treated as alternates, where necessary.

In the final count, the actual sample consisted of 180 teachers from 18 schools, representing over 17% of the faculty in the sampled schools. Basic demographic information obtained for the teacher population shows that approximately 76% of the teachers are female, 24% male, 66% are Blacks and 33% Whites. The volunteer sample was comprised of 70% females, 30% males, 56% Blacks, and 44% Whites.

Blood collection was carried out at the schools over a 2-week period. A brief introduction was given describing the project. During the time the self-administered questionnaire on health and occupational history was being completed, individual participants were selected for blood pressure checks and venipuncture, which was performed by licensed phlebotomists or nurses. The blood samples were stored in wet ice after 10–15 inversions.

After collection, the blood samples were brought to the local laboratory and split into three portions of about 3 ml each. These portions, in plastic, screw-top vials sealed with plastic tape, were maintained at dry ice temperature until shipment to the Toxicant Analysis Center.

3. Collection of kidney cortex samples

Kidney cortex samples were obtained from the Medical Examiner's Office in Baltimore. They were collected from cases of acute traumatic death. The samples were collected in plastic bottles and frozen. Demographic characteristics were recorded for each case.

YUGOSLAVIA

1. General description of the area and population monitored

The city of Zagreb, capital of Croatia, one of the six federal states of SFR Yugoslavia, is situated at 15°59′ E longitude and 45°49′ N latitude between the south-west slopes of the 1035 m high Mt. Medvednica and the banks of the river Sava, expanding more and more beyond the south bank of the river. The city is well protected from the north-west winds, but is exposed to the west air flow and completely open to the winds from the south, east and north-east where the spacious Pannonian plain is expanding. The city center lies 122 m above the sea level, other parts are built on the slopes of Mt. Medvednica up to 160 m above the sea level.

According to the Conrad's climatic classification based on dry cooling power values, Zagreb has a mild but bracing climate during the cold part and a relaxing climate during the warm part of the year. The average annual precipitation is 896.6 mm. The yearly average temperature is 11.6°C. There are 73.5 days with temperatures higher than 25°C, 268 days with temperatures higher than 5°C and 60 days with temperature below freezing point. In a period of 120 years (1862–1981) the highest temperature was recorded on 5 July 1950 (40.3°C) and the lowest on 24 January 1942 (–22.2°C).

Zagreb has 800,000 inhabitants in an area of 497.95 km^2 with a population density of 1607 persons/km^2. Fourty-seven percent of all inhabitants are males. The male/female ratio tends to decrease with age: the extremes are 1:0.8 in the 0–2.99 years (age) group and 1:1.99 in the over 65 years group.

Tap water comes mainly from underground resources, supplemented by springs in the surrounding hills with adjoining reservoirs.

The city is connected with other parts of the country and abroad by air, trains and buses. It is a cross-road between Central Europe and the Adriatic Sea. The urban traffic is very heavy at times, particularly during rush hours. Trams, buses and cars are the main transportation vehicles. There are more than 400,000 cars. The concentration of lead in gasoline is 0.6 g/l.

2. Sampling of teachers and collection of blood specimens

Full-time employed teachers from the city of Zagreb (10 inner boroughs), teaching at the elementary and secondary level (age of students 6–18 years) constituted the target population.

First sample frame. Twenty schools and five replacements were selected at random from the official list of all 99 schools (totally 6000 teachers), containing the number of teachers and the sexes in each school. Replacements were selected in case a sufficient number of male teachers could not be found in some schools. The school selection was done on probability proportional to size (p.p.s.) and stratified according to sex. Selection was made with random number tables.

A letter of information written jointly by the Institute for Medical Research and Occupational Health and appropriate school authorities was sent to the directors of the selected schools. Members of the Institute staff also contacted the directors personally and, if necessary, the selected teachers.

Only one of the selected schools refused participation and one did not participate due to administrative difficulties (schools No. 7 and 15). Therefore, schools No. 22 and 24 were taken, again at random, as replacements.

For the *second sampling frame* 10 techers and 5 replacements were selected at random in each school. In some schools there would not be enough male teachers even if all male teachers present had been taken without the random sampling procedure.

The blood specimens were taken at the Outpatient Department of the Institute for Medical Research and Occupational Health by the same personnel according to the agreed procedure described earlier. The teachers were transported from their schools to the Outpatient Department and back to school by the Institute's wagoon car.

3. Collection of kidney cortex samples

The target population consisted of cases of sudden deaths (accidents, cardiovascular arrest or suicide) occurring within the city of Zagreb and surroundings during 45 days. The whole left kidney was obtained at autopsy from the Institute for Forensic Medicine, Medical Faculty, Zagreb. Autopsies were performed within 24 hours after death. The specimens were transferred to the Institute for Medical Research and Occupational health, where the kidney cortex sampling was performed immediately according to instructions given by the WHO. After weighing the whole organ, portions of about 1 g of fresh kidney cortex were put into acid-washed glass beakers, weighed and stored at −18°C. Before analysis samples were dried at 105°C overnight, then weighed and dry ashed at 405°C. Cadmium analysis was performed by flame AAS.

Appendix 4

Results of the monitoring phase

1. Lead and cadmium in blood

Tables A4:1–A4:13 show concentrations of lead and cadmium in blood from teachers from selected areas in the different countries. For Belgium the concentrations in blood from blood donors in Brussels are given separately. The Swedish material consists of a random sample of the general population in Stockholm.

In addition to the concentration for the total groups studied, results are given in relation to sex and smoking habits (nonsmoker, former smoker, current smoker). Data in the tables describe number of subjects in each subgroups, median values, 90-percentiles, arithmetic means and standard deviations, as well as geometric means and standard deviations for the geometric means. Occasionally the number for "totals" slightly exceeds the sum of the subgroups due to unknown smoking habits. It should be observed that the number of cases in some subgroups is rather small. Data have nevertheless been presented in the tables, except for the 90-percentile which is not given when the number of cases is less than 10. In cases where data have been reported to be less than detection limit, they have been set to half the reported detection limit in the statistical calculations.

The results of the monitoring phase of the project are discussed in chapter 4 of the main body of the report.

Comments to the tables. The geometric means and standard deviations are derived from the distribution of the logarithms of the original data, but transformed back to common units. As the distribution of common units tends to show a moderately positive skewness which corrects by the logarithmic transformation, confidence limits and statistical tests should be performed on the logarithmic statistics. This involves a simple mathematical exercise described in the following example taken from table A4:1.

		Common units		Logarithmic units	
		M	F	M	F
1 Number of cases	N	50	39	50	39
2 Geometric mean	G.M.	160	119 →	2.204	2.076
3 Geometric stand. dev.	G.S.D.	1.277	1.277 →	0.106	0.106
				↓	↓
4 Geometric stand. error	G.S.E.			0.015	0.017
5 1.96 x G.S.E.	C.D.			0.029	0.033
6 Low limit G.M.–C.D.	L.L.	150	110 ←	2.175	2.043
7 High limit G.M.–C.D.	H.L.	171	129 ←	2.233	2.109
8 Difference M–F				0.128	
9 S.E. (difference)				0.023	
10 t				5.646	
11 Degrees of freedom (D.F.)				87	

Lines 1–3 give data on number of cases (N), the calculated geometric mean (G.M.) and its standard deviation (G.S.D.). The two last mentioned statistics are in the result tables (tables A4:1–A4:13) expressed in common units for easier comprehension, but are here transformed back to logarithmic units. Arrows in the table demonstrate this direction from common to logarithmic units.

Line 4 shows a downward direction: The standard error has been calculated by the common expression

$$\text{S.E.} = \frac{\text{S.D.}}{\sqrt{\text{N}}}$$

Line 5 shows the critical distance (C.D.) from the logarithmic mean to either of the two z-points, which between them cover the confidence intervals. The multiplication factor is set to 1.96 which for a large sample denotes 95% confidence.

Lines 6 and 7 give, in logarithmic units, the low and high points of this confidence interval. The arrows showing a left-ward direction denote that these two points may be transformed back to common units.

Lines 8–11 show the procedure used for a conventional testing of the null-hypothesis that no difference exists between males and females with respect to the studied parameter. Also here the logarithmic data have to be used. The expression for solving the test statistics is the usual, and is here only presented for the actual figures.

$$t = \frac{2.204 - 2.076}{\sqrt{(0.015)^2 + (0.017)^2}} = \frac{0.128}{0.023} = 5.565$$

The one-tailed critical value for t at the significance level 0.05 is 1.645 for large samples*. Hence the null-hypothesis is rejected and the alternative that males have a higher value accepted.

* In practice, a sample is considered "large" when the degree of freedom ($N_1 + N_2 - 2$) is 30 or more. In the present case where D.F. equals 87, the critical value is in fact 1.663.

Table A4:1. Concentrations of lead and cadmium in blood from teachers in Brussels, Belgium (for further details, see text below).

		Pb, µg/l			Cd, µg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*50*	*39*	*89*	*50*	*39*	*89*
	Median	162	111	141	1.1	0.9	1.0
	90-percentile	217	159	207	1.8	1.8	1.8
	Mean	165	123	146	1.1	1.0	1.1
	S.D.	38.8	29.9	40.8	0.48	0.60	0.54
	Geom. mean	160	119	141	1.0	0.9	1.0
	G.S.D.	1.3	1.3	1.3	1.64	1.72	1.68
Former smoker	Number	*7*	*1*	*8*	*7*	*1*	*8*
	Median	179	217	179	1.1	0.7	1.0
	90-percentile	–	–	–	–	–	–
	Mean	179	217	184	1.0	0.7	1.0
	S.D.	34.4	–	34.5	0.42	–	0.40
	Geom. mean	177	217	181	0.9	0.7	0.9
	G.S.D.	1.2	–	1.2	1.58	–	1.54
Current smoker	Number	*29*	*15*	*44*	*29*	*15*	*44*
	Median	181	127	165	2.0	2.0	2.0
	90-percentile	241	245	232	5.5	5.3	5.1
	Mean	180	148	169	2.5	2.4	2.5
	S.D.	37.5	52.5	45.1	1.76	1.62	1.69
	Geom. mean	176	141	163	2.1	2.2	2.1
	G.S.D.	1.2	1.4	1.3	2.00	1.77	1.91
Total*	Number	*88*	*55*	*143*	*88*	*55*	*143*
	Median	172	125	152	1.3	1.0	1.2
	90-percentile	217	194	213	3.1	2.6	3.1
	Mean	171	131	156	1.6	1.4	1.5
	S.D.	38.0	40.2	43.4	1.25	1.16	1.21
	Geom. mean	167	126	150	1.3	1.1	1.2
	G.S.D.	1.3	1.3	1.3	1.93	1.97	1.95

* Including a few with unknown smoking habits

Table A4:2. Concentrations of lead and cadmium in blood from blood donors in Brussels, Belgium (for further details, see text below).

		Pb, µg/l			Cd, µg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*44*	*72*	*116*	*44*	*72*	*116*
	Median	160	123	135	1.6	1.6	1.6
	90-percentile	259	187	226	2.3	2.2	2.2
	Mean	181	131	150	1.6	1.6	1.6
	S.D.	71.4	47.1	62.1	0.50	0.42	0.45
	Geom. mean	171	125	141	1.5	1.6	1.5
	G.S.D.	1.4	1.4	1.4	1.41	1.33	1.36
Former smoker	Number	*9*	*2*	*11*	*9*	*2*	*11*
	Median	155	108	153	1.5	1.5	1.5
	90-percentile	–	–	220	–	–	2.2
	Mean	164	108	154	1.6	1.5	1.6
	S.D.	38.9	7.1	41.6	0.35	0.00	0.31
	Geom. mean	160	108	149	1.6	1.5	1.6
	G.S.D.	1.3	1.1	1.3	1.24	1.00	1.21
Current smoker	Number	*41*	*9*	*50*	*41*	*9*	*50*
	Median	195	115	170	2.4	2.0	2.4
	90-percentile	249	–	243	4.4	–	4.4
	Mean	191	124	179	2.7	2.5	2.7
	S.D.	51.2	26.6	54.1	1.70	1.09	1.60
	Geom. mean	184	121	171	2.4	2.3	2.4
	G.S.D.	1.3	1.2	1.4	1.73	1.43	1.68
Total*	Number	*96*	*83*	*179*	*96*	*83*	*179*
	Median	169	120	145	1.8	1.6	1.7
	90-percentile	245	175	240	3.6	2.3	2.7
	Mean	183	130	158	2.1	1.7	1.9
	S.D.	60.4	44.8	59.8	1.29	0.59	1.04
	Geom. mean	175	124	149	1.8	1.6	1.7
	G.S.D.	1.3	1.3	1.4	1.63	1.37	1.52

* Including a few with unknown smoking habits

Table A4:3. Concentrations of lead and cadmium in blood from teachers in Beijing, China (for further details, see text below).

		Pb, μg/l			Cd, μg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*43*	*118*	*161*	*43*	*118*	*161*
	Median	65	55	59	0.6	0.8	0.7
	90-percentile	98	87	94	0.9	1.5	1.5
	Mean	71	59	62	0.6	0.9	0.8
	S.D.	20.7	18.7	19.9	0.48	0.54	0.54
	Geom. mean	68	56	59	0.5	0.7	0.7
	G.S.D.	1.3	1.4	1.4	1.88	1.77	1.84
Former smoker	Number	*9*	*0*	*9*	*9*	*0*	*9*
	Median	72	–	72	0.8	–	0.8
	90-percentile	–	–	–	–	–	–
	Mean	74	–	74	1.1	–	1.1
	S.D.	22.3	–	22.3	0.77	–	0.77
	Geom. mean	70	–	70	0.8	–	0.8
	G.S.D.	1.4	–	1.4	2.74	–	2.74
Current smoker	Number	*68*	*2*	*70*	*68*	*2*	*70*
	Median	78	58	76	1.8	2.6	1.8
	90-percentile	112	–	112	3.4	–	3.4
	Mean	80	58	80	2.0	2.6	2.0
	S.D.	22.4	19.1	22.5	0.92	0.92	0.92
	Geom. mean	77	57	77	1.8	2.5	1.8
	G.S.D.	1.3	1.4	1.3	1.63	1.45	1.63
Total	Number	*120*	*120*	*240*	*120*	*120*	*240*
	Median	75	55	64	1.2	0.8	0.9
	90-percentile	106	87	102	2.9	1.6	2.4
	Mean	76	59	68	1.4	0.9	1.2
	S.D.	22.1	18.7	22.2	1.00	0.59	0.86
	Geom. mean	73	56	64	1.1	0.8	0.9
	G.S.D.	1.4	1.4	1.4	2.29	1.80	2.10

Table A4:4. Concentrations of lead and cadmium in blood from teachers in Ahmedabad, India (for further details, see text below).

		Pb, μg/l			Cd, μg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*76*	*100*	*176*	*76*	*100*	*176*
	Median	137	129	131	0.9	0.8	0.9
	90-percentile	261	245	251	1.4	1.6	1.5
	Mean	155	147	150	1.0	0.9	0.9
	S.D.	70.9	72.6	71.8	0.41	0.44	0.43
	Geom. mean	141	133	137	0.9	0.8	0.9
	G.S.D.	1.5	1.6	1.5	1.46	1.50	1.49
Former smoker	Number	*7*	*0*	*7*	*7*	*0*	*7*
	Median	293	–	293	0.8	–	0.8
	90-percentile	–	–	–	–	–	–
	Mean	229	–	229	1.0	–	1.0
	S.D.	116.0	–	116.0	0.62	–	0.62
	Geom. mean	197	–	197	0.9	–	0.9
	G.S.D.	1.9	–	1.9	1.58	–	1.58
Current smoker	Number	*17*	*0*	*17*	*17*	*0*	*17*
	Median	116	–	116	1.1	–	1.1
	90-percentile	254	–	254	1.5	–	1.5
	Mean	144	–	144	1.1	–	1.1
	S.D.	69.1	–	69.1	0.35	–	0.35
	Geom. mean	131	–	131	1.0	–	1.0
	G.S.D.	1.5	–	1.5	1.40	–	1.40
Total	Number	*100*	*100*	*200*	*100*	*100*	*200*
	Median	137	129	131	0.9	0.8	0.9
	90-percentile	298	245	260	1.4	1.6	1.5
	Mean	158	147	153	1.0	0.9	1.0
	S.D.	76.1	72.6	74.4	0.41	0.44	0.43
	Geom. mean	143	133	138	0.9	0.8	0.9
	G.S.D.	1.6	1.6	1.6	1.45	1.50	1.48

Table A4:5. Concentrations of lead and cadmium in blood from teachers in Bangalore, India (for further details, see text below).

		Pb, μg/l			Cd, μg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*9*	*44*	*53*	*9*	*44*	*53*
	Median	215	171	194	1.0	0.9	0.9
	90-percentile	–	288	311	–	1.4	1.5
	Mean	238	198	205	1.0	0.9	0.9
	S.D.	91.2	101.5	100.1	0.52	0.38	0.40
	Geom. mean	224	178	185	0.8	0.8	0.8
	G.S.D.	1.4	1.6	1.6	1.94	1.50	1.57
Former smoker	Number	*7*	*0*	*7*	*7*	*0*	*7*
	Median	199	–	199	0.7	–	0.7
	90-percentile	–	–	–	–	–	–
	Mean	188	–	188	0.7	–	0.7
	S.D.	93.5	–	93.5	0.15	–	0.15
	Geom. mean	169	–	169	0.7	–	0.7
	G.S.D.	1.6	–	1.6	1.25	–	1.25
Current smoker	Number	*13*	*0*	*13*	*13*	*0*	*13*
	Median	148	–	148	1.1	–	1.1
	90-percentile	456	–	456	1.6	–	1.6
	Mean	187	–	187	1.0	–	1.0
	S.D.	136.0	–	136.0	0.42	–	0.42
	Geom. mean	160	–	160	0.9	–	0.9
	G.S.D.	1.7	–	1.7	1.63	–	1.63
Total	Number	*29*	*44*	*73*	*29*	*44*	*73*
	Median	196	171	183	0.7	0.9	0.8
	90-percentile	359	288	311	1.5	1.4	1.5
	Mean	203	198	200	0.9	0.9	0.9
	S.D.	112.9	101.5	105.4	0.41	0.38	0.39
	Geom. mean	180	178	179	0.8	0.8	0.8
	G.S.D.	1.6	1.6	1.6	1.64	1.50	1.55

Table A4:6. Concentrations of lead and cadmium in blood from teachers in Calcutta, India (for further details, see text below).

		Pb, μg/l			Cd, μg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*31*	*50*	*81*	*31*	*50*	*81*
	Median	106	97	98	0.7	0.8	0.8
	90-percentile	188	149	170	1.6	1.3	1.3
	Mean	132	108	117	0.9	0.8	0.8
	S.D.	128.5	34.6	84.0	0.36	0.30	0.33
	Geom. mean	110	104	106	0.8	0.7	0.7
	G.S.D.	1.7	1.3	1.5	1.45	1.52	1.50
Former smoker	Number	*3*	*0*	*3*	*3*	*0*	*3*
	Median	152	–	152	0.6	–	0.6
	90-percentile	–	–	–	–	–	–
	Mean	150	–	150	0.6	–	0.6
	S.D.	47.0	–	47.0	0.15	–	0.15
	Geom. mean	145	–	145	0.6	–	0.6
	G.S.D.	1.4	–	1.4	1.27	–	1.27
Current smoker	Number	*16*	*0*	*16*	*16*	*0*	*16*
	Median	101	–	101	0.7	–	0.7
	90-percentile	162	–	162	1.2	–	1.2
	Mean	112	–	112	0.7	–	0.7
	S.D.	31.2	–	31.2	0.27	–	0.27
	Geom. mean	108	–	108	0.6	–	0.6
	G.S.D.	1.3	–	1.3	1.46	–	1.46
Total	Number	*50*	*50*	*100*	*50*	*50*	*100*
	Median	105	97	101	0.7	0.8	0.7
	90-percentile	172	149	164	1.3	1.3	1.3
	Mean	127	108	117	0.8	0.8	0.8
	S.D.	103.1	34.6	77.1	0.34	0.30	0.32
	Geom. mean	111	104	107	0.7	0.7	0.7
	G.S.D.	1.5	1.3	1.4	1.47	1.52	1.49

Table A4:7. Concentrations of lead and cadmium in blood from teachers in Jerusalem, Israel (for further details, see text below).

		Pb, μg/l			Cd, μg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*92*	*51*	*143*	*92*	*51*	143
	Median	90	70	84	<0.5	<0.5	<0.5
	90-percentile	127	110	123	1.0	1.0	1.0
	Mean	93	70	85	0.5	0.5	0.5
	S.D.	26.3	27.9	29.1	0.38	0.38	0.38
	Geom. mean	89	64	79	0.4	0.4	0.4
	G.S.D.	1.4	1.6	1.5	1.80	1.81	1.80
Former smoker	Number	*4*	*0*	*4*	*4*	*0*	*4*
	Median	106	–	106	<0.5	–	<0.5
	90-percentile	–	–	–	–	–	–
	Mean	107	–	107	0.2	–	0.2
	S.D.	30.4	–	30.4	0.00	–	0.00
	Geom. mean	104	–	104	0.2	–	0.2
	G.S.D.	1.3	–	1.3	1.00	–	1.00
Current smoker	Number	*37*	*17*	*54*	*37*	*17*	*54*
	Median	102	82	94	1.6	1.1	1.3
	90-percentile	129	132	128	3.5	5.1	3.6
	Mean	102	82	95	1.6	1.9	1.7
	S.D.	27.6	32.5	30.4	1.28	1.84	1.46
	Geom. mean	98	74	90	1.1	1.2	1.1
	G.S.D.	1.3	1.6	1.5	2.72	2.69	2.69
Total	Number	*133*	*68*	*201*	*133*	*68*	*201*
	Median	94	70	86	<0.5	<0.5	<0.5
	90-percentile	129	113	127	2.0	1.7	1.9
	Mean	96	73	88	0.8	0.8	0.8
	S.D.	26.9	29.4	29.8	0.90	1.14	0.98
	Geom. mean	92	66	82	0.5	0.5	0.5
	G.S.D.	1.4	1.6	1.5	2.39	2.42	2.39

Table A4:8. Concentrations of lead and cadmium in blood from teachers in Tokyo, Japan (for further details, see text below).

		Pb, μg/l			Cd, μg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*17*	*77*	*94*	*17*	*77*	*94*
	Median	64	51	52	1.0	1.2	1.1
	90-percentile	99	74	78	1.5	2.3	2.0
	Mean	67	55	57	1.0	1.3	1.2
	S.D.	19.7	18.1	19.0	0.39	0.61	0.58
	Geom. mean	65	52	54	0.9	1.1	1.1
	G.S.D.	1.3	1.3	1.4	1.66	1.62	1.64
Former smoker	Number	*19*	*8*	*27*	*19*	*8*	*27*
	Median	64	50	59	1.0	1.1	1.0
	90-percentile	82	–	81	2.1	–	2.0
	Mean	64	51	60	1.2	1.1	1.2
	S.D.	12.6	10.6	13.3	0.54	0.35	0.49
	Geom. mean	62	50	58	1.1	1.0	1.1
	G.S.D.	1.2	1.2	1.2	1.55	1.42	1.50
Current smoker	Number	*64*	*15*	*79*	*64*	*15*	*79*
	Median	69	59	68	1.5	1.1	1.5
	90-percentile	92	123	99	2.8	3.5	3.0
	Mean	72	69	72	1.7	1.5	1.6
	S.D.	27.1	30.9	27.7	0.80	0.99	0.83
	Geom. mean	69	64	68	1.5	1.3	1.5
	G.S.D.	1.3	1.5	1.4	1.55	1.84	1.61
Total	Number	*100*	*100*	*200*	*100*	*100*	*200*
	Median	66	51	60	1.4	1.2	1.2
	90-percentile	92	75	89	2.4	2.2	2.3
	Mean	70	56	63	1.5	1.3	1.4
	S.D.	23.9	20.6	23.3	0.75	0.6	0.71
	Geom. mean	67	54	60	1.3	1.2	1.2
	G.S.D.	1.3	1.4	1.4	1.63	1.64	1.64

Table A4:9. Concentrations of lead and cadmium in blood from teachers in Mexico City, Mexico (including 50 samples analyzed at CI; for further details, see text below).

		Pb, μg/l			Cd, μg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*21*	*33*	*54*	*17*	*30*	*47*
	Median	255	198	212	<0.3	0.4	0.3
	90-percentile	401	304	325	1.7	1.5	1.5
	Mean	269	201	227	0.6	0.6	0.6
	S.D.	77.1	59.8	74.4	0.64	0.59	0.60
	Geom. mean	259	193	216	0.3	0.3	0.3
	G.S.D.	1.3	1.3	1.4	3.49	3.20	3.26
Former smoker	Number	*2*	*2*	*4*	*2*	*1*	*3*
	Median	268	197	237	4.3	2.1	3.2
	90-percentile	–	–	–	–	–	–
	Mean	268	197	233	4.3	2.1	3.6
	S.D.	13.4	26.9	44.8	1.56	–	1.68
	Geom. mean	268	196	229	4.2	2.1	3.3
	G.S.D.	1.1	1.1	1.2	1.45	–	1.61
Current smoker	Number	*13*	*14*	*27*	*12*	*13*	*25*
	Median	244	226	243	3.9	2.1	3.7
	90-percentile	394	369	365	9.0	8.9	8.3
	Mean	275	236	255	3.9	3.4	3.6
	S.D.	70.4	76.9	75.1	2.73	3.08	2.86
	Geom. mean	267	224	244	2.6	2.1	2.3
	G.S.D.	1.3	1.4	1.3	3.31	2.99	3.08
Total	Number	*36*	*49*	*85*	*31*	*44*	*75*
	Median	257	202	220	1.4	0.7	1.0
	90-percentile	387	307	346	5.6	4.4	5.2
	Mean	271	211	236	2.1	1.5	1.7
	S.D.	71.5	65.3	74.0	2.43	2.13	2.26
	Geom. mean	262	201	225	0.8	0.6	0.7
	G.S.D.	1.3	1.3	1.4	5.12	4.32	4.65

Table A4:10. Concentrations of lead and cadmium in blood from teachers in Lima, Peru (for further details, see text below).

		Pb, µg/l			Cd, µg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*12*	*89*	*101*	*12*	*89*	*101*
	Median	107	92	93	0.7	0.8	0.8
	90-percentile	165	118	127	1.2	1.3	1.3
	Mean	115	94	96	0.7	0.9	0.8
	S.D.	30.8	20.6	22.9	0.29	0.38	0.37
	Geom. mean	111	92	94	0.6	0.8	0.8
	G.S.D.	1.3	1.2	1.3	1.53	1.50	1.51
Former smoker	Number	*24*	*52*	*76*	*24*	*53*	*77*
	Median	104	95	99	1.2	0.9	1.0
	90-percentile	142	134	136	3.4	1.6	1.7
	Mean	105	98	100	1.5	1.0	1.2
	S.D.	27.0	22.9	24.3	1.17	0.52	0.81
	Geom. mean	102	96	98	1.2	0.9	1.0
	G.S.D.	1.3	1.3	1.3	1.89	1.58	1.71
Current smoker	Number	*9*	*20*	*29*	*9*	*20*	*29*
	Median	120	96	99	2.9	1.2	1.5
	90-percentile	–	134	149	–	4.9	5.1
	Mean	122	100	107	2.7	1.7	2.0
	S.D.	31.6	24.8	28.6	1.55	1.40	1.50
	Geom. mean	119	97	103	2.5	1.4	1.6
	G.S.D.	1.3	1.3	1.3	1.95	2.05	2.11
Total	Number	*45*	*161*	*206*	*45*	*162*	*207*
	Median	107	93	95	1.1	0.8	0.9
	90-percentile	152	124	135	4.0	1.7	1.7
	Mean	111	96	99	1.5	1.0	1.1
	S.D.	29.2	21.9	24.4	1.29	0.68	0.87
	Geom. mean	107	94	96	1.2	0.9	0.9
	G.S.D.	1.3	1.3	1.3	2.11	1.65	1.78

Table A4:11. Concentrations of lead and cadmium in blood from a random sample of the population in Stockholm, Sweden (for further details, see text below).

		Pb, µg/l			Cd, µg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*31*	*45*	*76*	*31*	*45*	*76*
	Median	77	58	66	<0.2	0.3	0.2
	90-percentile	147	110	117	0.3	0.6	0.6
	Mean	83	64	72	0.2	0.3	0.3
	S.D.	37.7	25.3	32.1	0.36	0.19	0.28
	Geom. mean	75	59	65	0.1	0.3	0.2
	G.S.D.	1.6	1.6	1.6	1.99	1.99	2.10
Former smoker	Number	*31*	*19*	*50*	*31*	*19*	*50*
	Median	86	64	72	0.4	0.3	0.3
	90-percentile	146	96	137	0.9	1.0	0.9
	Mean	90	66	81	0.5	0.4	0.5
	S.D.	32.4	29.7	33.3	0.39	0.39	0.39
	Geom. mean	85	60	75	0.3	0.3	0.3
	G.S.D.	1.4	1.6	1.5	2.53	1.88	2.28
Current smoker	Number	*42*	*42*	*84*	*42*	*42*	*84*
	Median	89	78	86	1.8	1.5	1.6
	90-percentile	130	113	124	4.3	3.0	3.3
	Mean	90	77	84	2.0	1.7	1.8
	S.D.	31.0	28.7	30.3	1.31	0.88	1.12
	Geom. mean	85	72	78	1.6	1.5	1.5
	G.S.D.	1.4	1.5	1.4	2.44	1.75	2.09
Total*	Number	*104*	*108*	*212*	*104*	*108*	*212*
	Median	85	65	73	0.4	0.5	0.5
	90-percentile	136	107	123	2.8	2.5	2.5
	Mean	88	69	78	1.0	0.9	0.9
	S.D.	33.4	28.1	32.1	1.21	0.87	1.05
	Geom. mean	82	63	72	0.5	0.5	0.5
	G.S.D.	1.5	1.5	1.5	3.77	2.81	3.27

* Including a few with unknown smoking habits

Table A4:12. Concentrations of lead and cadmium in blood from teachers in Baltimore, USA (for further details, see text below).

		Pb, μg/l			Cd, μg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*24*	*64*	*88*	*24*	*64*	*88*
	Median	89	65	67	0.6	0.5	0.6
	90-percentile	127	88	100	1.5	1.0	1.1
	Mean	87	65	71	0.7	0.6	0.6
	S.D.	25.5	19.7	23.4	0.42	0.28	0.33
	Geom. mean	83	62	67	0.5	0.5	0.5
	G.S.D.	1.4	1.4	1.4	1.89	1.85	1.86
Former smoker	Number	*11*	*21*	*32*	*11*	*21*	*32*
	Median	102	74	80	0.6	0.5	0.6
	90-percentile	124	130	124	3.7	1.2	1.5
	Mean	99	79	86	1.1	0.6	0.8
	S.D.	22.1	29.9	28.8	1.10	0.40	0.74
	Geom. mean	97	73	81	0.8	0.4	0.5
	G.S.D.	1.3	1.5	1.4	1.98	2.38	2.35
Current smoker	Number	*20*	*39*	*60**	*20*	*39*	*60**
	Median	106	76	85	1.1	1.0	1.0
	90-percentile	259	134	150	5.0	2.2	2.6
	Mean	134	80	97	1.7	1.1	1.3
	S.D.	69.7	31.9	54.4	1.47	0.76	1.06
	Geom. mean	121	73	85	1.2	0.8	1.0
	G.S.D.	1.5	1.5	1.7	2.14	2.20	2.20
Total	Number	*55*	*124*	*180**	*55*	*124*	*180**
	Median	96	68	75	0.8	0.6	0.6
	90-percentile	148	105	125	2.1	1.5	1.6
	Mean	106	72	82	1.1	0.7	0.8
	S.D.	50.3	26.6	39.0	1.12	0.55	0.79
	Geom. mean	98	67	75	0.8	0.6	0.6
	G.S.D.	1.5	1.5	1.5	2.17	2.15	2.19

* Including one with unknown sex

Table A4:13. Concentrations of lead and cadmium in blood from teachers in Zagreb, Yugoslavia (for further details, see text below).

		Pb, μg/l			Cd, μg/l		
		Males	Females	Total	Males	Females	Total
Nonsmoker	Number	*30*	*84*	*114*	*30*	*84*	*114*
	Median	125	76	83	0.6	0.5	0.5
	90-percentile	213	127	158	1.3	1.0	1.0
	Mean	138	81	96	0.6	0.5	0.6
	S.D.	49.3	33.4	45.4	0.37	0.35	0.35
	Geom. mean	129	76	87	0.5	0.4	0.4
	G.S.D.	1.4	1.5	1.6	2.11	2.07	2.09
Former smoker	Number	*3*	*3*	*6*	*3*	*3*	*6*
	Median	104	94	99	0.5	0.5	0.5
	90-percentile	–	–	–	–	–	–
	Mean	102	89	95	0.8	0.6	0.7
	S.D.	22.6	19.6	20.2	0.70	0.46	0.54
	Geom. mean	100	87	93	0.6	0.5	0.5
	G.S.D.	1.3	1.3	1.2	2.36	2.35	2.18
Current smoker	Number	*27*	*45*	*72*	*27*	*45*	*72*
	Median	139	84	94	3.6	2.7	3.2
	90-percentile	260	122	208	10.0	7.0	7.3
	Mean	170	84	116	4.4	3.6	3.9
	S.D.	106.5	23.9	79.2	3.10	2.70	2.87
	Geom. mean	150	81	102	3.2	2.6	2.8
	G.S.D.	1.6	1.3	1.6	2.54	2.68	2.62
Total	Number	*60*	*132*	*192*	*60*	*132*	*192*
	Median	135	78	90	0.9	0.7	0.7
	90-percentile	238	122	169	6.4	5.1	5.3
	Mean	150	82	104	2.3	1.6	1.8
	S.D.	81.2	30.1	60.5	2.81	2.15	2.39
	Geom. mean	137	77	92	1.2	0.8	0.9
	G.S.D.	1.5	1.4	1.6	3.48	3.34	3.43

2. Cadmium in kidney cortex

Figures A4:1—A4:2 show concentrations of cadmium in kidney cortex from subjects of "sudden unexpected death" in selected areas of the participating countries. The reported values expressed as mg Cd/kg wet weight have been plotted against age in the diagrams. Data on cadmium in kidney cortex have not been obtained from Mexico and Peru.

The results of the monitoring of cadmium in kidney cortex are discussed in section 4.2.2 in the main body of the report.

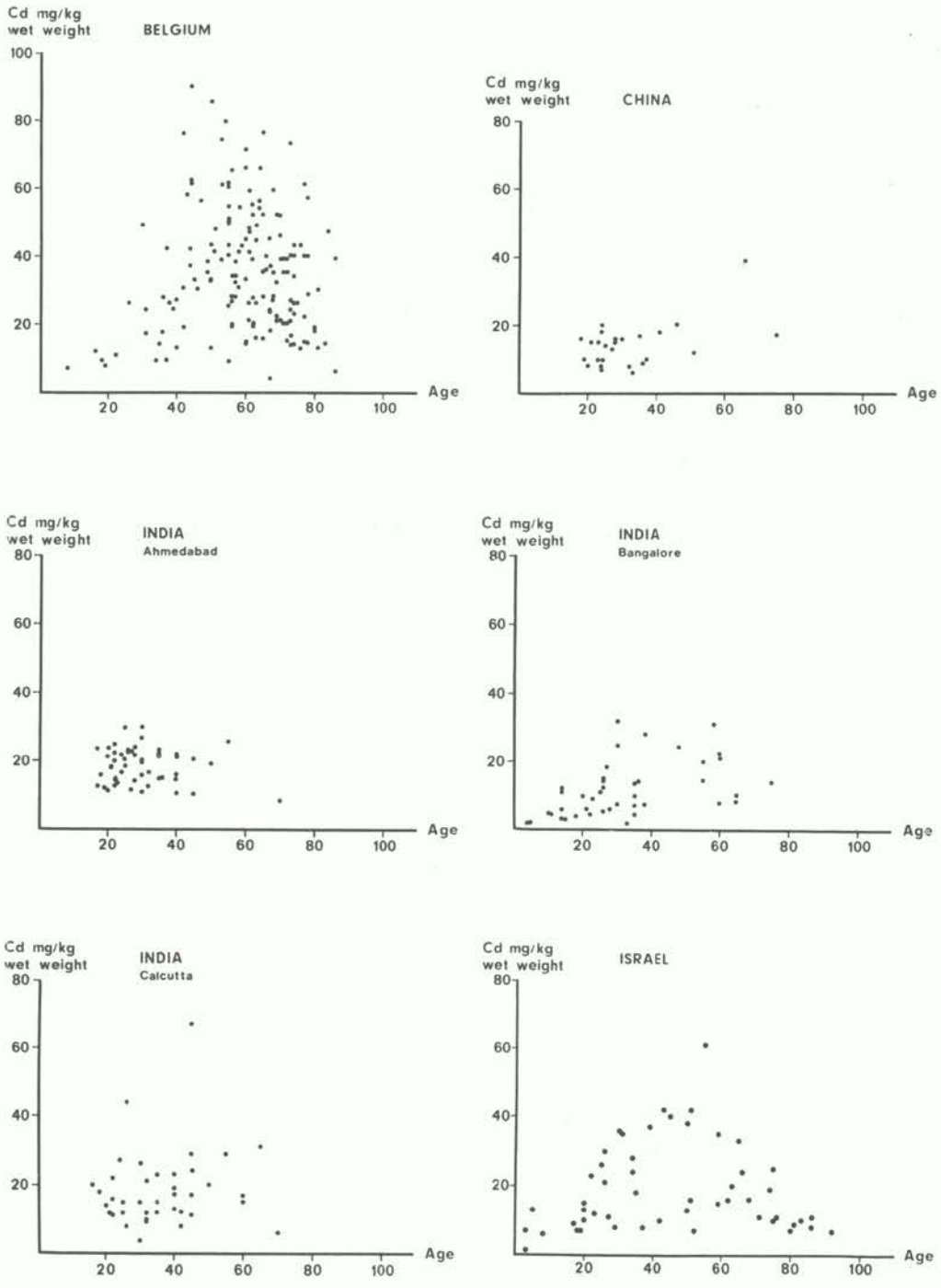


Figure A4:1. Cadmium concentrations in kidney cortex from subjects in Liège, Belgium; Beijing, China; Ahmedabad, Bangalore and Calcutta, India; and Jerusalem, Israel, in relation to age.

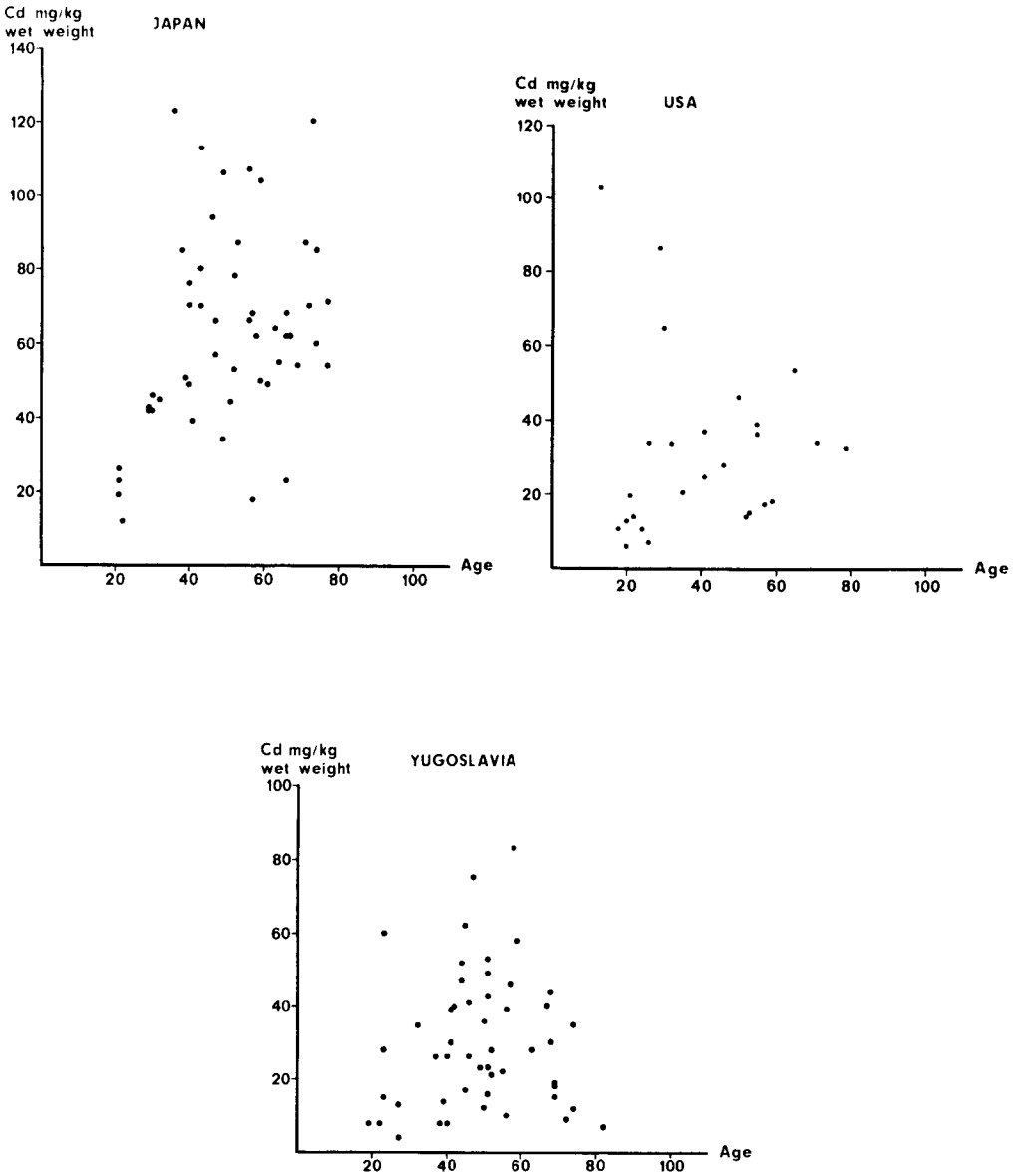


Figure A4:2. Cadmium concentrations in kidney cortex from subjects in Tokyo, Japan; Baltimore, USA; and Zagreb, Yugoslavia, in relation to age.

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