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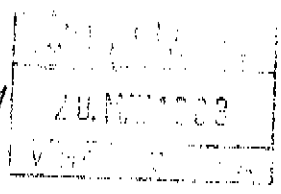


ORGANISATION MONDIALE DE LA SANTE  
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ВСЕМИРНАЯ ОРГАНИЗАЦИЯ ЗДРАВООХРАНЕНИЯ  
ЕВРОПЕЙСКОЕ РЕГИОНАЛЬНОЕ БЮРО

*HLT*

*Lead poisoning*  
*Lead-renal*  
*blood*  
*diagn*  
*Aluminum-renal*  
*blood*  
*in man, child*



EPIDEMIOLOGICAL STUDY PROTOCOL

ON

BIOLOGICAL INDICATORS OF LEAD NEUROTOXICITY IN CHILDREN



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## 1. Introduction

Lead is one of the most common pollutants in our outdoor air environment. Its quantities in homes where lead paints are used can be high and it is especially dangerous for young children with pica. Widespread pollution is found in areas of developed countries from lead in drinking water, from contamination of canned foods and from lead in petrol.

Concentrations of lead in blood considered safe for adults may be accompanied by neurological and behavioural abnormalities in children. Several epidemiological studies on children have tried to estimate the correlation between lead exposure and lead in blood level, lead in the dentine of shed teeth, and neurological and behavioural disorders, but these raised further questions in assessing the health risk of low-level, long-term exposure to lead.

A number of European countries and research groups are in the early stages of planning epidemiological studies of the effects of lead on children's health. Such studies could benefit from advice on methodology, especially in relation to the choice of biological markers, health measures and the measurement of potentially confounding social factors.

Cross-sectional cohort studies can answer many questions, provided they are well designed. Such studies can fill in existing gaps in our knowledge and improve the quality of information available. Longitudinal studies, although more expensive and time consuming, are potentially more powerful in dealing with confounding social variables and thereby in establishing the nature of causal relationships.

Investigators will, naturally, have to respond to different needs and conditions in different countries. New studies can take advantage of unique situations, but should, where feasible, incorporate standard methods and measures.

## 2. Measurement of lead in blood

### 2.1. Introduction

About 90 per cent 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 per cent of the lead is bound to the erythrocytes. The level of lead in blood is the best indicator of current exposure. Blood lead has a biological half-time of approximately 18 days. When exposure to lead changes, e.g. after occupational exposure, it takes approximately two months until the lead in blood correctly reflects the new exposure situation. Lead in the skeleton has a very long biological half-time, approximately 20 years (Task Group on Metal Toxicity, 1976; Chamberlain et al., 1978).

Procedures for sampling, sample handling, analysis, and data handling vary among laboratories. The purpose of the project should not be to standardize such methods. The participating laboratories can use methods of their own choice, provided they produce results acceptable according to the quality assurance criteria. Based on experience within a recent UNEP/WHO project "Assesment of Human Exposure to Lead and Cadmium through Biological Monitoring" (Vahter, 1982) it can be anticipated that the laboratories will use atomic absorption spectrophotometry (AAS). It is recommended to have a Coordinating Institution (CI) for the quality assurance programme. The CI should also have the responsibility to provide advice on suitable analytical methods, particularly in cases where the use of new analytical procedures will be started.

The monitoring of lead in blood should be accompanied by a quality assurance programme. Such a programme will deal not only with the accuracy of the analytical methods (analytical quality control) but will also take into consideration questions relating to sampling and avoidance of contamination during sampling and storage (preanalytical quality control). The experience from the UNEP/WHO project makes it imperative to implement a very rigid quality control programme. A review of published data shows that most published reports lack quality assurance data and that valid international comparisons therefore cannot be made. There is ample evidence as well that it is easy to introduce gross systematic errors (Vahter, 1982).

It seems advisable to divide the project into two main phases. The first phase will be devoted to training and if necessary technical assistance. The second phase will deal with the actual monitoring of lead in children's blood, with integrated quality assurance. No laboratory should start analysis of the children's blood until it has achieved satisfactory results in the quality control training phase. Criteria for acceptance of data will have to be agreed upon among the participating institutions, the CI and the principal investigator of the project.

The project will be cross-sectional, and it is anticipated that the study will be carried out on children at least four years old. It will therefore be possible to take blood samples via venopuncture and obtain at least 5 ml blood samples.

## 2.2. Training phase

### 2.2.1 Analytical quality control

The CI will distribute an appropriate number of quality control samples to the participating laboratories. Each quality control set will include both internal quality control samples (IQC samples; concentrations of lead known to the laboratories) and external quality control samples (EQC samples; concentrations not known to the laboratories). The IQC samples will be used as an integrated part of both the quality control analyses and the monitoring of children's blood. In Figure 1 an example is given of a flow sheet which illustrates the use of IQC samples in an analytical programme.

The number of EQC sets to be analysed during the training phase will depend on the skill of the participating laboratories. Experience from the UNEP/WHO project indicates that it will probably take a considerable amount of time until all participating laboratories meet satisfactory analytical requirements. In the UNEP/WHO project on an average 12 sets of quality control (QC) samples were distributed during the QC training phase (about 1 set each second month). Usually each QC set consisted of 6 blood samples prepared at the CI.

The concentrations of lead in the quality control samples will depend on the target populations studied. It seems reasonable to assume that the quality control samples should contain lead in concentrations between 50 or 100 up to 400 or 500 ug Pb/l blood.

### 2.2.2 Preamalytical quality control

There are many possibilities to contaminate biological samples through use of e.g. unsuitable blood collection 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 from certain vials and syringes in quantities which would invalidate any measurement of this metal in blood within the normal range of concentration (Nise & Vesterberg, 1978). Within the UNEP/WHO project a detailed protocol was set up to avoid contamination as far as possible. If the same procedures are followed, evacuated blood collection tubes (e.g. Venoject, Terumo Corp., Tokyo, Japan) with heparin from the same batch should be provided by CI after control of the metal content in a suitable number of tubes from the batch. If EDTA tubes with low metal content are available, the use of such tubes may have certain advantages. Before collecting blood the skin should be carefully washed and then cleaned with disposable napkins, saturated with e.g. 70% isopropyl alcohol (e.g. Medi-Swab, Pharmax Limited, Bexley, UK). These napkins should also be checked for metal content. Written instructions for the sampling of blood should be worked out and it might be advantageous to have a demonstration and some training when representatives of the different laboratories meet. The participating institutions should also prepare a protocol with information on e.g. procedures for collection, transport and storage of samples.

#### 2.2.3 Preparation of quality control samples

Quality control samples could obviously be prepared in different ways. Within the UNEP/WHO project the samples consisted of bovine blood with EDTA as anticoagulant, hemolyzed by ultrasonication and sterilized by gamma irradiation. The samples were spiked with lead nitrate. One major reason for using bovine blood was the requirement to obtain blood with a low background level; in the UNEP/WHO project about 25 ug Pb/l. In this programme considerable efforts had to be taken to make sure that the QC samples could tolerate fairly long transport time during which the outside temperatures might reach 30-40°C. They were sent deep-frozen in neopolyene containers via airfreight. It seems safe to take necessary precautions also for this project. For details, reference is made to Vahter (1982).

#### 2.2.4 Statistical procedure and criteria for acceptance or rejection of laboratory performance

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

A quality control programme can be implemented in different ways. Within a CEC programme (Yeoman, 1981) to be accepted a laboratory needed to obtain 80 per cent of its results in any series of tests within the interval A, B and C in Figure 2. 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  $100 \pm 15$  ug Pb/l to  $600 \pm 25$  ug Pb/l and for segments B and C  $100 \pm 30$  ug Pb/l to  $600 \pm 50$  ug Pb/l.

Within UNEP/WHO another procedure was used. The results of each set of QC samples were statistically assessed to form the basis for a decision in terms of accepting or rejecting the laboratory's current performance. The main feature was to guard against systematic errors along the range of values likely to occur during the operation.

In a diagram where y is the determined value and x is the "true" value, a recovery of 100 per cent would correspond to a straight line through the origin and regression of unity, i.e.  $y = x$ . It was decided that the regression lines calculated from the 6 results of each QC set with a certain deviation from the ideal,  $y=x$ , should be accepted. It was also considered necessary to allow a somewhat higher deviation at the lower x range. The limits for maximum allowable deviations (MAD-lines) were set as follows:

$$\text{for lead in blood (ug/l)} \quad y = x \pm (0.1x + 20)$$

In figure 3 the MAD interval for lead is indicated by the outer straight lines in the figure. With the statistical procedure used one could assure that the observed regression lines based on the values reported for each QC set, did not fall outside the MAD-lines with the statistical power of 80 per cent. The acceptance interval of the regression lines therefore had to be more stringent, as shown by the dotted lines in the figure. For details of the statistical procedure reference is made to Vahter (1982). In the proposed project the MAD-lines of course can be varied ad libitum.

#### 2.2.5 Reference values

It is not yet possible to obtain true concentrations of lead in biological tissue. 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). The use of IDMS for routine purposes is out of the question. If in connection with the quality assurance programme some analyses will be carried out with a reference method, the IDMS is suitable. The IDMS analyses were carried out within the UNEP/WHO project on a limited number of samples by the US National Bureau of Standards (NBS) and the CEC Joint Research Centre, Ispra, Italy (ISPRA).

#### 2.2.6 Analytical procedures

Because of the low concentration of lead in blood, the conventional AAS technique is not adequate. The Delves Cup technique or electrothermal atomization (ETA) will most probably be the method of choice. 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 in blood. In the UNEP/WHO report examples are given of some of the problems which may arise when using the AAS technique for monitoring low levels of lead in blood. In that programme three laboratories used the Delves Cup technique (Delves, 1970) modified according to Lind (1982). Seven laboratories used electrothermal atomization, ETA, originally reported by Matousek & Stevens (1971). Different modifications of the original methods were used (see sections 2.4 and 2.5).

#### 2.3. Monitoring phase

After collection of the blood samples (about 5 ml), the blood from each tube should be split into at least two portions (possibly three) and be deep-frozen. Suitable vials could be 5 ml tubes of polypropylene (washed with diluted nitric acid and deionized water). Such tubes should preferably be provided through the CI. One of the tubes should be stored to allow duplicate analyses at a possible reference laboratory at a later stage. Blood not used for the initial analysis should be stored to make possible reanalysis at the laboratory if necessary. A third portion could be saved for possible analyses in the future or for additional analyses if suspicion of contamination arises.

It seems advisable to carry out the analyses over one time period. IQC analyses should be monitored regularly. One set of EQC samples should be monitored before the analyses of children's blood and then for every 50 samples (if possible on a blind basis and mixed with the monitoring samples). Results of EQC analyses should be sent to CI for evaluation, if possible via telex. If the results do not meet criteria agreed upon, the analyses of children's blood should be temporarily interrupted in order to reconsider the problems and then employ appropriate measures.

The results of the EQC analyses during the monitoring phase should be published as an integral part of the results of the analyses of children's blood.

If needed, some analyses should be repeated at a reference laboratory.

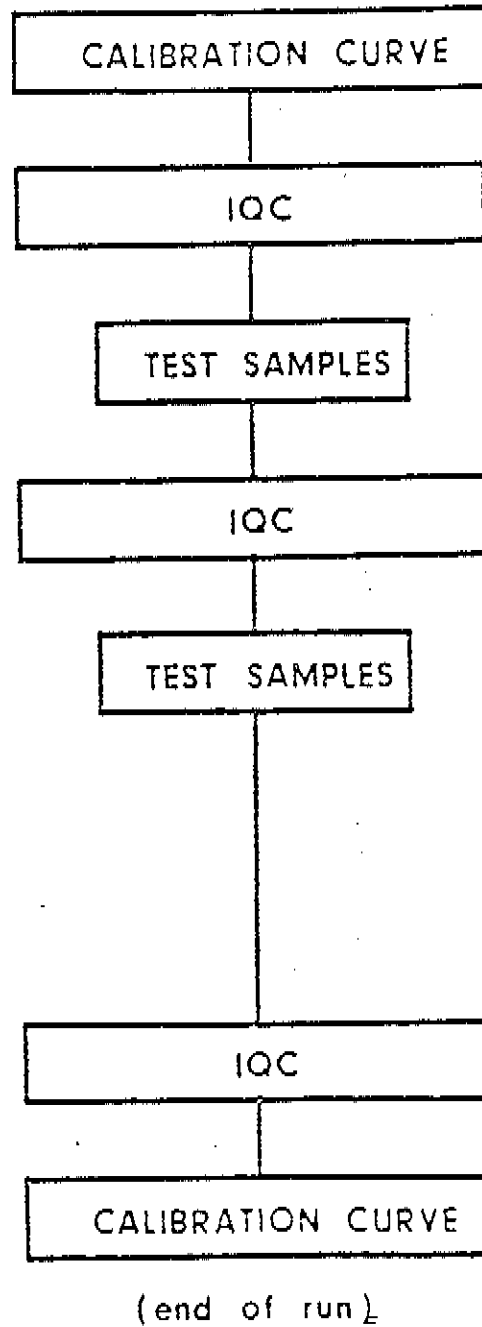


Figure 1. Flow sheet to illustrate use of internal quality control samples (IQC) in analytical programme. From: Vahter (1982)

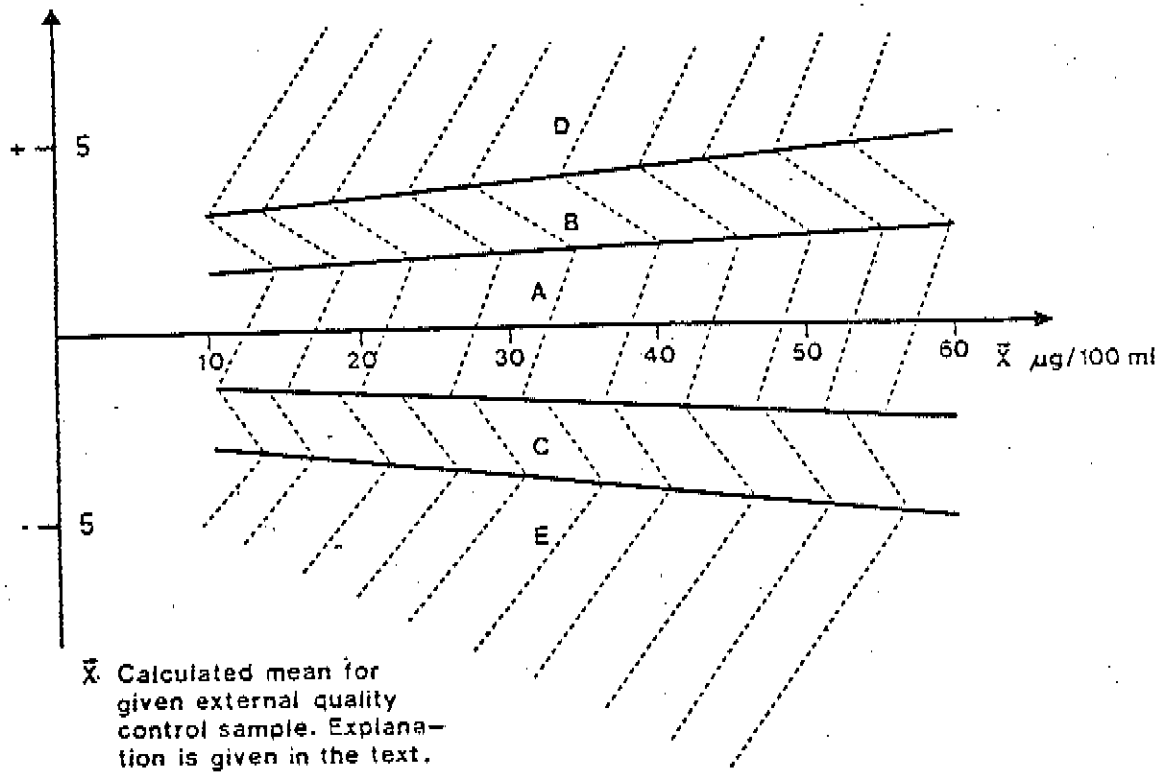


Figure 2. Acceptability criteria for lead in blood within the CEC Programme. Modified from: Yeoman (1981)  
For explanation, see the text

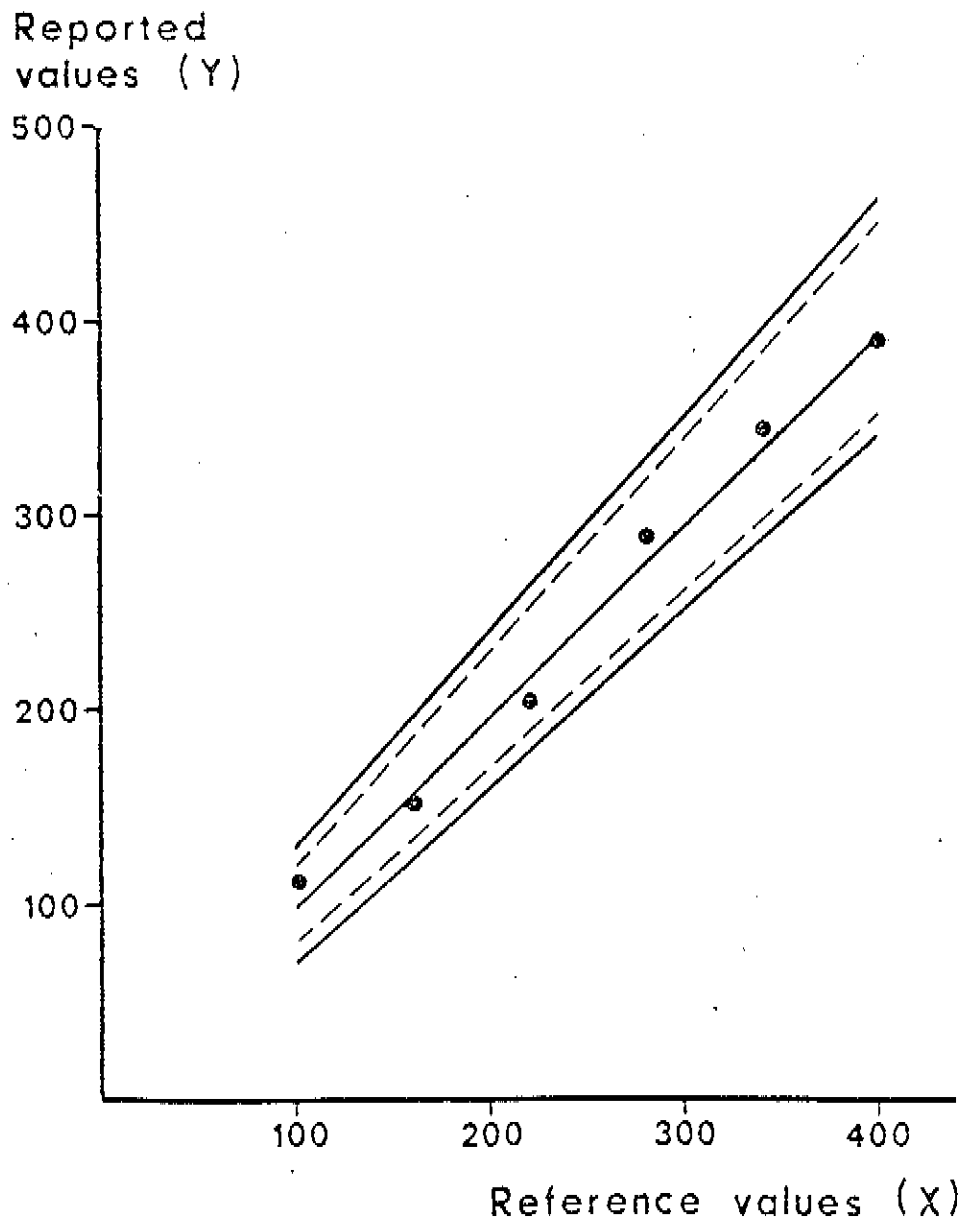


Figure 3. Regression line based on six reported values.  
The solid lines indicate the MAD-lines and  
the dotted lines the acceptance lines.  
From: Vahter (1982)  
For explanation, see the text

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## 2.4 Modified Delves Cup Technique for AAS-Analysis of Lead and Cadmium in Blood, by Birger Lind

### 2.4.1 Material

The acid washed tips used for blood dispensing are rinsed with n-heptan immediately before use. Eppendorf white tips are used instead of the yellow tips, since the latter contain cadmium (Salmila & Vuori, 1979; Skafte & Claesson, 1981, personal communication).

### 2.4.2 Standard

For standards, blood with low concentration of lead and cadmium is spiked with lead or cadmium. The following procedure is recommended.

Dilute the stock solutions (1000 ug/ml) of cadmium and lead with deionized water to achieve a range of 2.5 - 30 ug Pb/g and 0.05 - 7 ug Cd/g, using pasteur pipettes, a semi-balance with 1 mg accuracy, and 50 ml polyethylene bottles. Take 9.6 g of blood in a 25 - 50 ml polyethylene bottle and add 200 ul (record by balance) of a diluted cadmium or lead standard with an Eppendorf pipette. After mixing the standards should be left standing (overnight in refrigerator if possible) to equilibrate. The standards can then be kept up to a month if kept at +4°C and mixed before use. Normally the following combined standard additions (ng Pb/g blood - ng Cd/g blood) are made:

0 - 0, 50 - 1, 100 - 2, 200 - 4, 300 - 6, 400 - 8, 500 - 10 and 600 - 14. For details, see enclosed scheme 1. Standards are treated in the same way as samples throughout the method.

### 2.4.3 Procedure

After careful mixing of the blood, a set of five cups with 10 ug in each is used for every sample and standard according to Ulander & Axelsson (1974). A set of 20 cups is dried at 110-115°C for at least one minute and then analyzed by AAS utilizing a deuterium background compensation system.

Before the flame ignites a cup is inserted below the hole of the tube and the position of the cup is checked by placing a mirror on the three slot

burner head. The tube should previously be adjusted for minimum light loss. there must always be a cup below the hole when the flame is ignited. It should otherwise be turned off in order to prevent damage to the tube.

After drying, the cup is put into the loop and then pushed close to the flame in order to char the organic matrix according to Ediger & Coleman (1972). The distance between the cup and the flame is critical for achieving optimal charring, i.e. low background signal without losses especially of cadmium.

In order to perform this step in a reproducible way, a piece of brass with a width of 14 mm is held in front of the end stop of the system. The digital display of the AAS-instrument is set to show an integrated value of the signal area at each 10 seconds. When a new value appears on the display, the cup moves to the brass stop which thereafter is removed. After a few seconds, a flame in the cup will be seen. When it ceases and the next value is shown on the display, the cup moves into the flame and is kept there until the next integrated value is displayed. A recorder for registration of the signal (peak height) is essential for cadmium, since opposite to lead, the signal area does not give as good a precision as the peak height. Optimization of the distance between the cup and the tube hole is essential for the sensitivity and background signal (Fernandez, 1973). Both parameters will increase with decreasing distance. As a rule, a sensitivity giving a background signal too high to be fully compensated cannot be utilized. Normally at the optimal distance (1-2 mm) an increase in blood concentration with 600 ng Pb/g gives a net peak absorption signal of 0.22 absorbance (A) units and about 0.14 A for 14 mg Cd/g, if Perkin Elmer cups and tubes are used.

Tubes of  $Al_2O_3$  with an entrance slot for the cup are now available. For these tubes the optimization mentioned above is less critical since the cup is within the tube. Another advantage is that the life length of the tube is extended. The sensitivity achieved with such a tube is, however, less than with the other tube. It is important to carefully align the beam from the deuterium lamp with that of the metal lamp through the tube in order to achieve a correct compensation of the background signal according to Manning (1971).

The normal mode of analysis after optimization is a complete standard curve, four samples, a standard, four samples, another standard and so on.

Finally a complete standard curve is analyzed. The standard curve has to be parallelly moved to zero (if n-heptan does not give a signal) before evaluation of samples.

For efficient use of the Delves Cup technique two persons are needed, one to operate the AAS and the other to prepare the cups with blood.

#### 2.4.4 Accuracy and precision

Pools of human blood (including blood from occupationally exposed persons) containing < 2 ng/g, 2-5 ng/g, 5-10 ng/g, and 10-20 ng/g were collected. Aliquots of the samples were analyzed also by neutron activation analysis (NAA). The results from AAS-analysis and NAA are compared in Table 1.

Table 1. Results from cadmium analyses of blood using AAS and NAA (ug Cd/l)

		No. of analyses for each concentration	Method	Concentration (mean ± S.D.)			
				1.2	4.7	8.8	20.5
Stored at +4°C in glass bottles	17	AAS	1.3±0.5	4.8±0.9	9.1±1.3	19.5±2.2	
Stored frozen at -18°C in poly-ethylene tubes	15	AAS	1.5±0.8	4.3±0.7	8.6±1.3		

For lead in blood, comparison with NAA is not possible. The accuracy of the AAS-method was tested in an interlaboratory comparison organized by the Commission of the European Communities (CEC) in 1979. The agreement with the median values at CEC within the interval 90 - 550 ug/l was the following: Delves Cup = 1.02 x CEC - 24 ug Pb/l blood (n = 14; r = 0.993).

If the cups get stuck in the loop of the system and are difficult to remove, the acetylene gas is probably of poor quality (high PH<sub>3</sub> content). A way to test the quality of the gas is to prepare a 5% AgNO<sub>3</sub> solution and wet a filter paper with it. Move the paper into the acetylene gas flow and keep it there for 15-20 seconds. Let the gas tube be open with a low flow during 5 seconds before inserting the filter. A faint yellow colour indicates good quality (<2ppm H<sub>2</sub>S and hopefully low PH<sub>3</sub> content) and immediately brown/black colour indicates poor quality of the gas.

CADMIUM AND LEAD STANDARDS IN DEIONIZED WATER AND IN BLOOD

Cadmium standards in deionized water:

20	ug/g solution	1.000 g of	1000 ug/ml	diluted to	50.000 g	with deionized water
2	" "	2.500 g of	20	"	25.000	"
0.7	" "	7.000 g of	2	"	20.000	"
0.5	" "	5.000 g of	"	"	"	"
0.4	" "	4.000 g of	"	"	"	"
0.3	" "	3.000 g of	"	"	"	"
0.2	" "	2.000 g of	"	"	"	"
0.1	" "	1.000 g of	"	"	"	"
0.05	" "	0.500 g of	"	"	"	"

Lead standards in deionized water:

30	ug/g solution	0.600 g of	1000 ug/ml	diluted to	20.000 g	with deionized water
25	" "	0.500 g of	"	"	"	"
20	" "	1.000 g of	"	"	50.000	"
15	" "	15.000 g of	20	"	20.000	"
10	" "	10.000 g of	"	"	"	"
5	" "	5.000 g of	"	"	"	"
2.5	" "	2.5000 g of	"	"	"	"

50 ml acid washed polyethylene bottles (with cap of the same material) were used for these standards. The bottle was used for the same standard concentration all the time. New standards in deionized water were made the same day as they were added to the blood. A three decimal semi-balance was used to record the exact amounts added. The correct concentration value was then calculated.

Scheme 1 contd.

Blood standards

<u>ng Pb - ng Cd/g blood</u>	<u>Lead addition (g)</u>	<u>Cadmium addition (g)</u>	<u>Blood addition (g)</u>	<u>Total weight (g)</u>
0 - 0	0.200 g of deionized water	0.200 g of deionized water	9.600	10.000
50 - 1	0.200 g of 2.5 ug/g	0.200 g of 0.05 ug/g	9.600	10.000
100 - 2	" 5.0 "	" 0.10 "	" "	" "
200 - 4	" 10-0 "	" 0.20 "	" "	" "
300 - 6	" 15.0 "	" 0.30 "	" "	" "
400 - 8	" 20.0 "	" 0.40 "	" "	" "
500 - 10	" 25.0 "	" 0.50 "	" "	" "
600 - 14	" 30.0 "	" 0.70 "	" "	" "

50 ml (or smaller) acid washed polyethylene bottles (with cap of the same material) were used for these standards. The bottle was used for the same standard concentration all the time. Freshly made standards of cadmium and lead in deionized water were used for addition to the blood. Blood with low cadmium and lead content was used. A three decimal semi-balance was used to record the exact amount added. The correct concentration value was then calculated. After mixing, let stand overnight in a refrigerator (if possible) to equilibrate. The blood standards can be kept for a month if stored at +4°C (or less) and mixed before use.

2.5 Modified deproteinization method according to Stoeppler et al. (1978) and Stoeppler & Brandt (1980) for electrothermal atomization analysis of lead and cadmium in blood by AAS, by Birger Lind

2.5.1 Material

All materials used including tips for pipettes are acid washed by soaking in 10%  $\text{HNO}_3$  overnight and rinsed several times with deionized water. After drying in an oven at  $70^\circ\text{C}$ , the materials should be stored under cover. Eppendorf white tips are used instead of the yellow tips, since the latter contain cadmium, (Salmilla & Vuori, 1979; Skafte & Claesson, 1981, personal communication). In this method it is important to have materials with low cadmium and lead content due to the low pH of the solutions used.

2.5.2 Standard

For standards, blood with a low concentration of lead and cadmium is spiked with lead or cadmium. The following procedure is recommended:

Dilute the stock solutions (1000  $\mu\text{g}/\text{ml}$ ) of cadmium and lead with deionized water to achieve a range of 2.5 - 30  $\mu\text{g Pb}/\text{g}$  and 0.05 - 0.7  $\mu\text{g Cd}/\text{g}$ , using pasteur pipettes, a semi-balance with 1 mg accuracy, and 50 ml polyethylene bottles. Take 9.6 g of blood in a 25 - 50 ml polyethylene bottle and add 200  $\mu\text{l}$  (record by balance) of a diluted cadmium or lead standard with an Eppendorf pipette. After mixing the standards should be left standing overnight in a refrigerator if possible to equilibrate. The standards can be kept for a month if stored at  $+4^\circ\text{C}$  and mixed before use. Normally the following combined standard additions  $\text{ng Pb}/\text{g blood} - \text{ng Cd}/\text{g blood}$  are made:

0 - 0, 50 - 1, 100 - 2, 200 - 4, 300 - 6, 400 - 8, 500 - 10 and 600 - 14.

For details see enclosed scheme 1. Standards are treated the same way as samples throughout the method.

2.5.3 Procedure

To Eppendorf reaction vessels of 1.5 ml, 600  $\mu\text{l}$  0.8 M  $\text{HNO}_3$  are added with an Eppendorf pipette after discarding the first injection in order to further clean the tip. This can preferably be done when the samples and standards are mixed on a Coulter mixer with rollers. Gloves should be used to

avoid contamination from the fingers especially of smokers. Add 200 ul of blood (sample or standard) by an Eppendorf pipette to the reaction vessel, close it and whirl mix or shake by hand vigorously and immediately. Always make duplicates in order to detect contamination and change tips between samples or standards. Make a chemical blank by adding 200 ul deionized water instead of blood to the vessel. When all tubes are ready, mix them once more with the whirl mixer for 5-10 seconds. Store overnight (if possible) in a refrigerator to extend the deproteinization time to achieve a better exchange. The next day the vessels are whirl mixed 5-10 seconds after some minutes of warming up. The vessels are placed in an Eppendorf centrifuge (Model 5413) with a capacity of 40 vessels. Spin for five minutes at 11,500 rpm. The clear solutions in the vessels are transferred to polyethylene cups which are placed in the tray of an automatic sampling system (Perkin Elmer AS-1 or AS-40). If no such system is available, the solutions are transferred to other vessels and 20 ul injected by hand into the electrothermal atomization (ETA) unit. Normally up to 4 trays are prepared which for AS-40 means 140 cups totally. This means that 42 samples are analyzed in duplicate, 4 internal quality control samples are analyzed in triplicate and the rest are standards and blanks. For details see enclosed scheme 2.

The same solutions are used for analysis of cadmium as well as lead. The cups are stored covered in a refrigerator for the next day's analyses.

#### 2.5.4 Instrumental analysis

A deuterium background compensation system is used for both elements. For lead the analytical line of 283 nm is used. Electrodeless discharge lamps (EDL) should be used if possible. The signal and preferably even the background signal should be recorded on a fast response recorder in order to receive the peak height. If integration facilities are available the area of the signal should be displayed and if possible printed. After several hours of analysis the graphite tube will change its porosity and hence the sensitivity. Normally this affects the peak height much more than the area. A mathematical model for compensation of this drift has been established by Lind (to be published). At least two equal injections of 20 ul have to be used for every solution when injections are done manually. For AS-1 and AS-40 three injections are normally selected automatically. In this case two

injections of each solution are used, since duplicates side by side give 4 injections per sample. The following programme can be used on the Perkin Elmer ETA system HGA-72:

	Step	Time	Temperature control units	°C	Comments
Cadmium	1	30	036	103	dry
	2	18	086	350	char
	3	11	332	1604	atomize
	4	5	770	2501	burn out
Lead	1	30	036	103	dry
	2	18	086	350	char
	3	11	440	1905	atomize
	4	5	770	2501	burn out

It could be that this programme has to be modified to some extent on other instruments due to variation in the contact with graphite tubes, the tubes themselves or the controller unit. For this programme Perkin Elmer's graphite tube No. 045057 was used.

An expansion of 10 times by concentration knob (335 units) is used on a Perkin Elmer 403 instrument and recorder full scale is set on 4A, giving a total expansion of 2.5 times on the recorder. The deproteinization method is especially useful for this kind of ETA unit, since the background signal will be very small despite problems with condensation effects at the ends of the cones for this unit.

For HGA-500 the following programme can be used:

	Dry	Dry	Char	Atomize	Burn out	Base level	End
Step	1	2	3	4	5	6	7
Temperature (°C)	100	200	450	2200 Cd 2400 Pb	2500	20	20
Ramp time (sec)	5	5	6	1	1	1	1
Hold time (sec)	20	10	4	6	1	6	0
Read (integration time= 7 sec, peak area)				0		0	
Recorder (in absorbance 5mV, 20 mm/min)			-3	0	0	0	0
Air internal flow ml/min					20		

Step 6 is needed when integration of the signal area is performed to receive a base level. A non-pyrolytic graphite tube is used, a new one every day and hence normally the tube stands  $4 \times 35 \times 2 = 280$  injections. The total time for one tray is one hour and 50 minutes which gives a total time of 7 hours and 20 minutes for the complete analysis.

#### 2.5.5 Comments

The standard curve has to be adjusted to the chemical blank value before evaluation of samples can be made.

To achieve maximum sensitivity for cadmium analysis it is recommended to use 500 ug 0.8 M  $\text{HNO}_3$  and an addition of 300 ul blood. The 2mV range is used on the recorder and step 3 in the programme for HGA-500 is then changed to the following: temperature ( $^{\circ}\text{C}$ ) = 500, ramp time (sec) = 6 and hold time (sec) = 14.

HGA 500

PE 373

Type of sample: \_\_\_\_\_

Date: \_\_\_\_\_

Serial No: \_\_\_\_\_

Element: \_\_\_\_\_

Programme: \_\_\_\_\_

Sample try No: 1

Comments: \_\_\_\_\_

1	<u>Chemical blank</u>	(18)	<u>600 - 16</u>
2	<u>"</u>	19	<u>0 - 0 (for carry over check)</u>
3	<u>0 - 0 (blood blank)</u>	20	<u>Brown IQC</u>
4	<u>"</u>	21	<u>"</u>
5	<u>50 - 1</u>	22	<u>Blue IQC</u>
6	<u>"</u>	23	<u>"</u>
7	<u>100 - 2</u>	24	<u>Black IQC</u>
8	<u>"</u>	25	<u>"</u>
9	<u>200 - 4</u>	26	<u>Red IQC</u>
10	<u>"</u>	27	<u>"</u>
11	<u>300 - 6</u>	28	<u>Sample 1</u>
12	<u>"</u>	29	<u>"</u>
13	<u>400 - 8</u>	(30)	<u>600 - 16 (for sensitivity check)</u>
14	<u>"</u>	31	<u>Sample 2</u>
15	<u>500 - 10</u>	32	<u>"</u>
16	<u>"</u>	33	<u>Sample 3</u>
17	<u>600 - 16</u>	34	<u>"</u>
		35	<u>Sample 4</u>

Note: Standard curve (position 3-18) should be parallely moved against the X-axis by decreasing all standard signals with the difference between mean of blood bank and chemical blank signals, before it is used for evaluation of the samples.

HGA 500

PE 373

Type of sample: \_\_\_\_\_

Date: \_\_\_\_\_

Serial No: \_\_\_\_\_

Element: \_\_\_\_\_

Programme: \_\_\_\_\_

Sample try No: 2

Comments: \_\_\_\_\_

1	<u>Sample 4</u>	18	<u>Sample 12</u>
2	<u>Sample 5</u>	19	<u>"</u>
3	<u>"</u>	20	<u>600 - 16 (for sensitivity check)</u>
4	<u>Sample 6</u>	21	<u>Sample 13</u>
5	<u>"</u>	22	<u>"</u>
6	<u>Sample 7</u>	23	<u>Sample 14</u>
7	<u>"</u>	24	<u>"</u>
8	<u>600 - 16 (for sensitivity check)</u>	25	<u>Sample 15</u>
9	<u>Blood bank (for calculation of</u> <u>detection limit)</u>	26	<u>"</u>
10	<u>Sample 8</u>	27	<u>Sample 16</u>
11	<u>"</u>	28	<u>"</u>
12	<u>Sample 9</u>	29	<u>Sample 17</u>
13	<u>"</u>	30	<u>"</u>
14	<u>Sample 10</u>	31	<u>Sample 18</u>
15	<u>"</u>	32	<u>"</u>
16	<u>Sample 11</u>	33	<u>600 - 16 (for sensitivity check)</u>
17	<u>"</u>	34	<u>Sample 19</u>
		35	<u>"</u>

HGA 500

PE 373

Type of sample: \_\_\_\_\_

Date: \_\_\_\_\_

Serial No: \_\_\_\_\_

Element: \_\_\_\_\_

Programme: \_\_\_\_\_

Sample try No: 3

Comments: \_\_\_\_\_

1	<u>Sample 20</u>	18	<u>Sample 27</u>
2	<u>"</u>	19	<u>Sample 28</u>
3	<u>Sample 21</u>	20	<u>"</u>
4	<u>"</u>	21	<u>Sample 29</u>
5	<u>Sample 22</u>	22	<u>"</u>
6	<u>"</u>	23	<u>Sample 30</u>
7	<u>Sample 23</u>	24	<u>"</u>
8	<u>"</u>	25	<u>600 - 16 (for sensitivity check)</u>
9	<u>Sample 24</u>	26	<u>Sample 31</u>
10	<u>"</u>	27	<u>"</u>
11	<u>600 - 16 (for sensitivity check)</u>	28	<u>Sample 32</u>
12	<u>Blood bank (for calculation of</u> <u>detection limit)</u>	29	<u>"</u>
13	<u>Sample 25</u>	30	<u>Sample 33</u>
14	<u>"</u>	31	<u>"</u>
15	<u>Sample 26</u>	32	<u>Sample 34</u>
16	<u>"</u>	33	<u>"</u>
17	<u>Sample 27</u>	34	<u>Sample 35</u>
		35	<u>"</u>

HGA 500

PE 373

Type of sample: \_\_\_\_\_

Date: \_\_\_\_\_

Serial No: \_\_\_\_\_

Element: \_\_\_\_\_

Programme: \_\_\_\_\_

Sample try No: 4

Comments: \_\_\_\_\_

1	<u>Sample 36</u>	18	<u>Brown IQC</u>
2	<u>"</u>	19	<u>"</u>
③	<u>600 - 16 (for sensitivity check)</u>	20	<u>Blue IQC</u>
4	<u>0 - 0 (for calculation of</u> <u>detection limit)</u>	21	<u>"</u>
5	<u>Sample 37</u>	22	<u>Black IQC</u>
6	<u>"</u>	23	<u>"</u>
7	<u>Sample 38</u>	24	<u>Red IQC</u>
8	<u>"</u>	25	<u>"</u>
9	<u>Sample 39</u>	26	<u>Chemical blank</u>
10	<u>"</u>	27	<u>"</u>
11	<u>Sample 40</u>	28	<u>0 - 0</u>
12	<u>"</u>	29	<u>"</u>
13	<u>Sample 41</u>	30	<u>200 - 4</u>
14	<u>"</u>	31	<u>"</u>
15	<u>Sample 42</u>	32	<u>400 - 8</u>
16	<u>"</u>	33	<u>"</u>
⑬	<u>600 - 16 (for sensitivity check)</u>	34	<u>600 - 16</u>
		35	<u>"</u>

Total positions = 4 trays with 35 cups each = 140 cups.

42 samples in duplicate = 42 x 2 = 84 cups; 60% of the total number of cups.

4 internal QC samples, before and after actual samples, and in duplicate  
= 4 x 2 x 2 = 16 cups; 11.4% of the total.

Standards and blanks = 40 cups; 28.6% of the total.

### 3. Measurement of lead in teeth

#### 3.1 Introduction

Determination of lead concentrations in shed deciduous teeth has become a useful measure of past lead exposures in children. The formation of deciduous teeth starts already in pre-natal life, and the average lead level in the shed teeth may be taken as an integrated measure of the total exposure during early life. Lead levels may vary, however, between different tooth tissues and between different tooth types. Thus, the highest lead concentrations are found in the inner and outer surfaces of the tooth, i.e. the outer layer of enamel and the circumpulpal dentine. Enamel appears to change very little after its formation, and lead concentrations are independent of age. On the other hand, the circumpulpal, or secondary, dentine which is formed after the eruption of the tooth continues to accumulate lead until the tooth is shed. Primary dentine contains much lower lead concentrations.

A significant variation in lead concentrations in whole teeth has been found in relation to tooth type. Thus, the lead concentration tends to decrease from the medial incisors to the premolars. Similar variations have not been found in secondary dentine levels. The possibility exists that the tooth-type related variation could be due to a differential proportion of dental tissues in the different tooth types. Thus, a premolar would contain comparatively more primary dentine with low lead levels than would a medial incisor. Conclusions cannot be made at this time, however, and tooth type remains a parameter which should be recorded and examined when tooth lead is used as an indicator of past lead exposure.

Two recent studies have mentioned considerable variation in lead content between two teeth of the same type from the same individual or between two samples of secondary dentine from the same tooth (1, 2). Under most circumstances, this variation is within the normal range of analytical variation, but the occurrence of significant proportions of "outliers" suggests the presence of a biological variation. At this time, no basis or mechanism for such biological variation can be proposed, and some of the "outliers" could certainly be explained by a random occurrence of contamination. Under these circumstances, it seems advisable to analyse more than one tooth from each individual.

These questions were addressed and discussed at a recent consultation meeting in London, 23-25 September 1982. The present proposal for a quality assurance programme for tooth lead measurements follows the recommendations from that meeting.

### 3.2 Analytical quality requirements

As indicated above, differences in tooth-type and tooth-tissue are major sources of variability of the results obtained. Analytical variability has been studied to a limited degree. Thus, Lockeretz (3) obtained a reproducibility of  $\pm 20\%$  (probably coefficient of variation) for an internal standard material of powderized whole tooth. A more recent study utilizing anodic stripping voltametry found a coefficient of variation varying between 7% and 28% (4). A large-scale project in the USA found a coefficient of variation for secondary dentine lead averaging 6-7% (1). In a similar project in the Federal Republic of Germany, the average coefficient of variation for an internal standard was 5%, while reproducibility of duplicate samples averaged 6% (5). Similar results were obtained in a British study, where external quality assessment suggested a coefficient of variation of 10% (2). The external quality assessment study showed that results for a homogeneous tooth powder varied between 4.3 and 15.5 ug/g while most laboratories obtained values between 6 and 10 ug/g (2). Thus, past experience indicates that within-run and day-to-day variation can be limited to 10-15% or less. Data on accuracy are scarce, but the data available suggest considerable variation and that errors of up to 50% may not be uncommon.

Such variability may hamper the possibility of comparing results from different countries of an international collaborative project. In addition, if considerable analytical variation is present, biological sources of variation related to tooth-type and different dental tissues may be difficult to characterize. Thus, stringent quality requirements for the tooth lead analysis are considered a prerequisite.

As judged from published and unpublished experience, good quality performance of a valid analytical method for tooth lead concentrations should result in a day-to-day variation of 10% or less. At lower lead concentrations, this variation may be somewhat higher, but stringent control of laboratory contamination must ensure that this variation never exceeds 20%. Results for an external standard material should be within  $\pm 30\%$  of the assigned value for the material. At very low levels, results within  $\pm 50\%$  may

be acceptable. Such requirements appear realistic given at the present state of the art. Stricter requirements would have the advantage of better comparability between the results, but such requirements would probably preclude the inclusion of data from several laboratories. Thus, the proposed limits for acceptability should be considered a realistic compromise. However, as indicated below, these limits should be agreed upon by all participating laboratories before the initiation of the quality control programme. At this point it should also be mentioned that considerable improvement of laboratory performance is expected during the training phase, and laboratories which do not comply with the acceptability criteria at the beginning of the programme may have the possibility of improving considerably during the following stages.

### 3.3 Sampling strategies

At the consultation meeting in London, a recommendation was made that incisors should be used, and that the whole tooth should be analysed. Also, the participants recommended that at least two teeth should be analysed from each individual and only those results showing "a good level of agreement" should be used as indices of lead exposure. However, the participants acknowledged the potential bias which will be introduced in the study if only subjects giving two incisors for examination may be included in the study population.

The problems associated with such selection bias may be partly overcome if secondary dentine is used instead of whole tooth, because secondary dentine lead levels are little, if at all, associated with tooth type (6, 7). Should secondary dentine be preferred for lead analysis in any part of the collaborative study, a correlation study between lead levels in whole tooth and secondary dentine must be included.

The presence of caries, fillings or other specific dental treatment could interfere with the lead concentration of whole teeth, while secondary dentine lead levels would be less subject to changes. However, the inclusion of only healthy whole teeth is recommended. Each tooth should be placed in a small plastic bag which should be marked with the subject's code and the presumed tooth-type. Other data, e.g. the date of tooth shedding, should be indicated on a data sheet along with the name of the child and other information.

### 3.4 Pretreatment of teeth

The sampling and storage conditions are not critical factors with regard to lead contamination of the teeth, because teeth are easy to clean. Various methods have been proposed, but the cleaning should include soaking in hydrogen peroxide to remove organic material on the surface of the dental tissues. Additional cleaning may include soaking in an ultrasonic bath with a detergent or solvent with low surface tension in order to remove contamination from both smooth and irregular surfaces. No standard method for cleaning is recommended, because comparative studies have not been performed, and no individual cleaning method has been proved to be better than others. Attention is called to the fact that clean laboratory techniques must be performed from the initiation of cleaning of the teeth. Thus, contamination of samples during pretreatment, as indicated by poor precision at low lead concentrations, may not be related to any particular method for pretreatment but is conceivably associated with poor laboratory practice and general contamination in the laboratory.

Following the cleaning stage, each tooth should be dried and weighed. If the whole tooth is analysed, no further stage should be included before dissolution in acid. Each additional stage will always increase the risk of contamination. Under certain circumstances, however, e.g. when a solid sample is analysed by electrothermal atomic absorption spectrophotometry, the tooth must be powdered and homogenized before a subsample is taken out for analysis. Powdering is easier after ashing overnight at 450°C, but the ashing process itself could be a source of contamination, and lead may be lost if the ashing temperature is too high. Alternatively, whole teeth may become more brittle if soaked in liquid nitrogen before homogenization. Past studies have used various lead-free mortars for crushing individual teeth. Care should be taken to avoid any residual lead contamination (memory effect). However, in general such elaborate pretreatment of the teeth is discouraged.

For analysis of secondary dentine, two different approaches for pretreatment are available. According to the method (8) employed in the subsequent study by Needleman et al. (1), each tooth is embedded in self-curing acrylic, and 600- $\mu$ m thick longitudinal sections are prepared on a sectioning machine with two diamond cutting disks. Under a dissecting microscope, the circumpulpal dentine is rigidly clamped, and by means of pressure, a fracture is produced to obtain a 300- $\mu$ m wide sample of circumpulpal dentine. Two such samples could be obtained from each section.

According to the original procedure, lead was subsequently detected by anodic stripping voltametry, and a trained laboratory technician was then able to prepare and analyse about 40 teeth per week. A somewhat simpler method was described by the same authors in another publication (9). The secondary dentine was removed from each tooth by means of dental reamers. The powder produced was collected, dried and then dissolved before analysis. This method has shown surprisingly little variability (7, 9). Despite its simplicity, contamination of the tooth powder may easily occur, and the method should only be used in a clean laboratory.

The tooth analysis comprises both pretreatment and lead detection. Most detection methods are based on a liquid solution of the sample to be analysed. The tooth material therefore needs to be dissolved in a strong acid. Concentrated nitric acid is preferred, because this acid only interferes with detection methods to a limited degree, and because ultra-clean nitric acid is readily available. In order to speed-up the dissolution process, the acid is usually heated. Preferably this process should be carried out in polytetrafluoroethylene-lined bombs. For one gram of tooth material, 5 ml of concentrated nitric acid may be used, and the material is totally dissolved after 1.5 hours at 150°C. A small amount of perchloric acid may be added, if a clear solution is not obtained, but perchloric acid may interfere with atomic absorption detection. A dilution, e.g. 5-fold, is then prepared for the final detection of lead. For anodic stripping, the diluent is usually a sodium acetate solution in redistilled water, while pure water is used in case of atomic absorption. Some authors have discovered a matrix interference from sulphate when electrothermal atomic absorption is used, and this interference may be reduced by the addition of lanthanum in the diluent (10).

### 3.5 Detection methods

The detection methods most frequently used are atomic absorption spectrometry (AAS) and anodic stripping voltametry (ASV). Various modifications of AAS may be applied, e.g. solvent extraction, Delves' cup or electrothermal AAS. Minor variations of ASV techniques have also been described. In a single instance, isotopic dilution mass spectroscopy has been applied. No reference method has been agreed upon, and all the detection methods mentioned may be acceptable. The validity of a method depends not

only on the validity of the detection method, but the reliability of the pretreatment procedures and the avoidance of contamination in the laboratory are extremely important determinants of the validity of results obtained.

With regard to AAS, standard addition should be used to assess the possible presence of matrix interference. If a significant and variable effect is found, all samples should be analysed by the method of standard addition. In particular, in case of electrothermal AAS, calcium from the tissue samples may deposit on the walls of the graphite tube and may eventually decrease the lead signal. This problem could be minimized by using a very high atomization temperature or by applying maximum temperature following the atomization phase (20). Nitric acid as such may cause some matrix interference, but the method of standard addition is only necessary if this interference is found to vary. The effect can be minimized by dilution of the sample.

For the characterization of control and standard materials described below, an electrothermal AAS method will be applied, and a general average and confidence limits will be defined by this method. In addition, specific samples will be analysed by other methods. On the basis of such studies, an assigned value will be determined as a basis of comparison.

### 3.6 Preparation of control materials

Two kinds of control materials will be necessary in order to assess both precision and accuracy. As described below, main emphasis is placed on a powdered, homogenized tooth material which will be prepared at different lead concentrations. In addition, a smaller number of samples will be prepared as half teeth.

A large quantity (at least 100 g) of tooth powder containing a typical lead concentration will have to be prepared as common internal quality control material. A major requirement for this material is that sufficient supplies should be available in case the material is used up in any one laboratory. The need in each laboratory will depend on the number of tooth determinations to be made and the size of each sample of control material for a single determination. Supposedly, each laboratory will need at least 10 g of the material for internal quality control purposes. The need should be assessed in detail by communication with the participating laboratories before initiation of the programme.

In addition, two large samples should be prepared, one containing a very low lead concentration, the other containing a very high lead concentration. Each sample should be carefully homogenized and the lead concentrations characterized in detail. From these two samples, smaller samples with lead concentrations between the two extremes can be easily mixed. Spiking of the samples should be avoided, unless a lead-containing commercially available hydroxyapatite can be added to the samples. A large number of teeth with high lead levels are available from 200-year old skeletons recently excavated. Although such historic samples of teeth may have a slightly changed composition due to the destruction of organic matter, the use of these samples would be preferable to that of a lead-spiked sample as standard material.

The control materials described above will not be able to detect analytical errors due to the early stages of sample pretreatment. Thus, as an additional external quality control sample, one half tooth will be included. Each laboratory will analyse the sample (or the secondary dentine part) by its routine procedure, and the coordinating institute will analyse the other one-half (or the secondary dentine part). Although biological variations in lead distribution within teeth may hamper the possibility of interpreting these results, this part of the quality control programme is considered a necessary supplement.

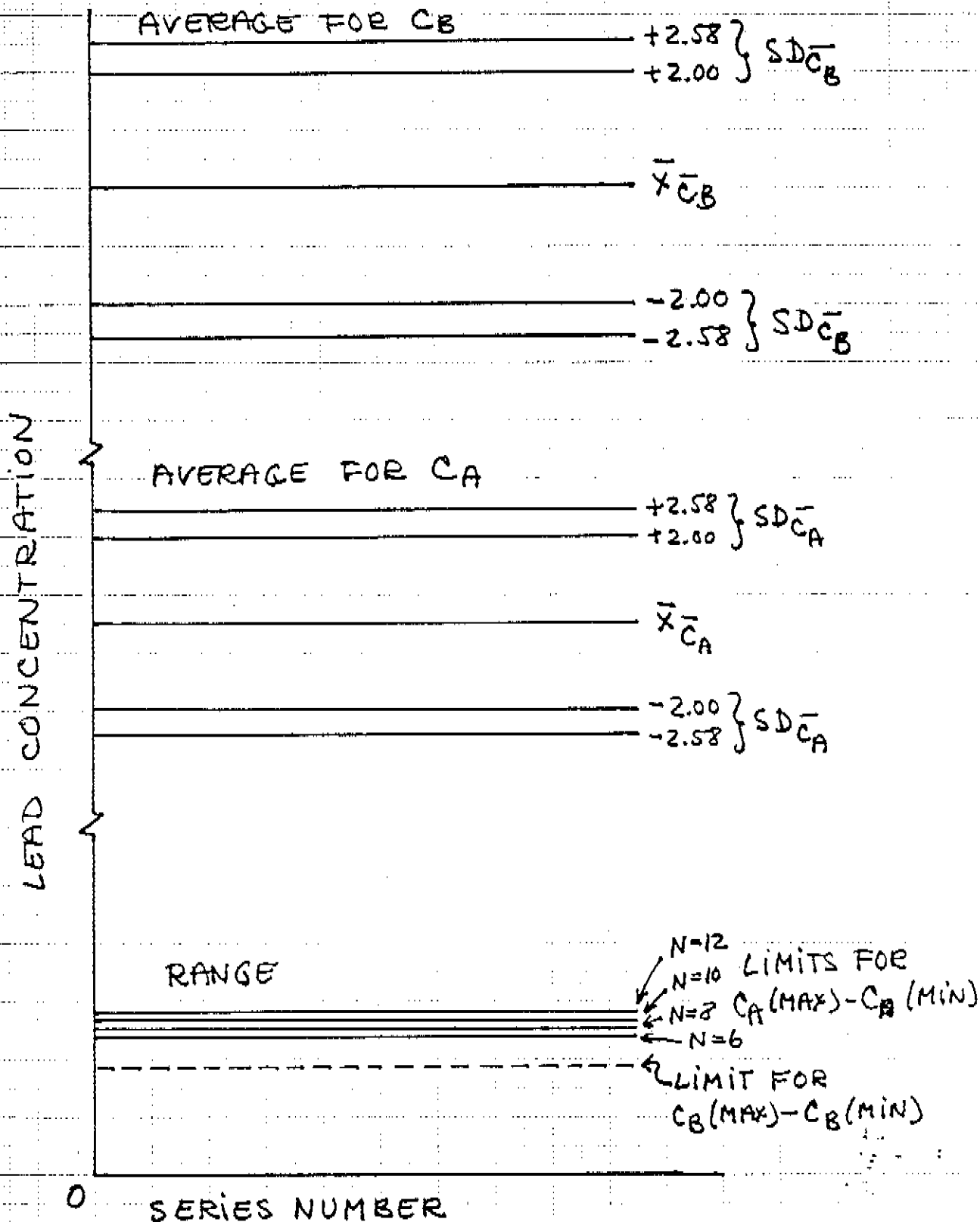
### 3.7 Design of the quality assurance programme

Participating laboratories will be permitted to use their own procedures, as already established. This fact makes the need for a carefully planned quality assurance programme obvious. The programme consists of the design of the quality procedures as part of measuring procedures and the materials used within the programme.

The aim of the quality control programme is to ensure results that fulfil the clinical needs. This subject has already been discussed above under "analytical quality requirements", and general agreement on this issue must be ensured before the quality programme is initiated.

In the proposed programme we have closely followed the definitions and concepts as recently described in Interim Document No 4 on laboratory quality control (11). Additional useful information has been obtained from the GEMS project on "assessment of human exposure to lead and cadmium through biological monitoring" (12). The phases of the quality assurance programme have been outlined on the attached flow chart.

# GRAPHICAL REPRESENTATION OF INTERNAL QUALITY CONTROL RESULTS



## Outline of the three phases

### Establishment phase

In this initial phase, the laboratory will make exercises with lead-containing materials received from the coordinating institution (CI). Two objectives must be met: (a) to establish the internal quality control system and (b) to assess the analytical bias through a preliminary external quality control round. The outcome of this phase will include useful information concerning the analytical accuracy of the individual laboratory, as compared to other participating laboratories, and an overall information concerning analytical performance in terms of accuracy.

### Training phase

In this phase an assessment of analytical performance will be made with regard to the quality requirements agreed upon. The individual laboratories will use the internal quality control procedure established at this time to ensure a stable analytical performance. A validation of the results obtained using external quality control materials will then be made. Corrections and adjustments should, if necessary, be instituted before the training phase is finished.

### Project phase

In this phase test materials will be analysed within the framework of both internal and external quality assurance programmes. Rules for rejecting and accepting test results within the laboratory exist as part of the internal quality control system. In addition, further evaluation of results from each laboratory will be included in an external quality control programme.

#### 3.7.1 Standardization (establishment phase)

Aqueous standards are used (along with a blank sample) in several runs in each laboratory planning to analyse teeth. Also, the two samples of tooth powder ( $C_A$  and  $C_B$ ) are analysed at this stage. The results are submitted

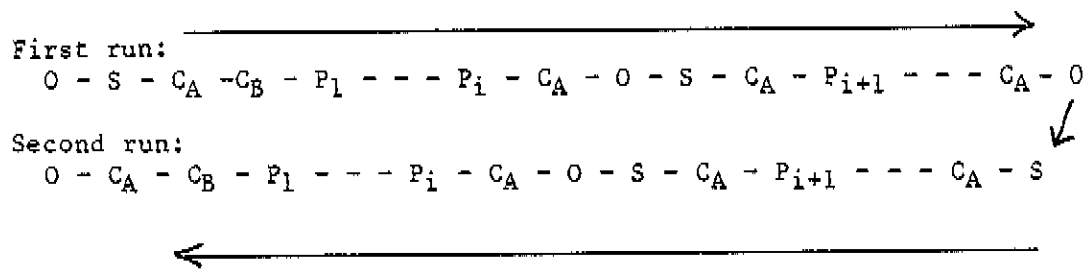
to the coordinating laboratory and will be used to assess any interlaboratory bias by regression analysis, as described by Vahter (12).

Internal quality control programme

Procedures

Materials used (see attached table) will be an aqueous standard with known lead content (S), two internal quality control samples of tooth powder ( $C_A$  and  $C_B$ ), and a blank sample (O).

The analytical set-up should be as follows: all test samples (P) will be analysed in duplicate with standards and control materials placed as suggested below:



The duplicates should be analysed separately. Recalibration may be necessary following the standard (S). The number "i" may vary from 5 to 20. Each run should include 6  $C_A$  quality control samples and 2  $C_B$  samples. The results calculated for  $C_A$  and  $C_B$  are used after the completion of the whole series to accept or reject the results obtained for the P samples.

Quality control rules

The calculations are based on the average results for  $C_A$  and  $C_B$ , respectively, and the differences between the lowest and highest results obtained for each sample. The results should comply with the following three rules (assuming that  $C_A$  has been analysed 6 times and  $C_B$  has been analysed twice).

$$\text{Rule 1: } \bar{C}_A \gtrless \bar{X}_{C_A} \pm 2.58 \cdot SD_{\bar{C}_A}$$

$$\bar{C}_B \gtrless \bar{X}_{C_B} \pm 2.58 \cdot SD_{\bar{C}_B}$$

$$\text{Rule 2: } C_A (\text{max}) - C_A (\text{min}) > 4.76 \cdot SD_{C_A}$$

$$C_B (\text{max}) - C_B (\text{min}) > 3.64 \cdot SD_{C_B}$$

The probability for false rejection ( $P_{FR}$ ) is 1% for each rule. In addition, the following rule should be included:

Rule 3:  $\bar{C}_A$  and  $\bar{C}_B$  outside  $\bar{X} \pm 2 \cdot SD$  to the same side.

These rules carry a  $P_{FR}$  of about 5%. The results should be plotted graphically, and a copy should be submitted to the coordinating institute (CI) at the termination of each phase of the programme. A suggestion for graphical representation is attached.

### 3.7.2 Assessment of contamination (training phase)

One half tooth with a low lead content (sample T in the attached table) will be pretreated and analysed in each laboratory as if it were one of the teeth obtained in the project. The other one half is analysed at the CI, and the results obtained are compared. If the project laboratory submits a result which is more than 50% above the result obtained in the CI, a careful examination of possible sources of contamination should be performed, and a repeat assessment should be done before proceeding to the project phase.

### External quality control programme

The materials used (see attached table) will be two powdered tooth materials,  $C_D$  and  $C_E$ . These samples should each be run in every second (or third) series as regular test samples in duplicate. The results should be mailed to the CI.

The evaluation of results from each individual laboratory is based on "rule 1" for the averages of the internal quality control. The standard deviation will be proposed as 10% of the mean, as adjusted according to the results of serial measurements (and determination of material homogeneity) at the CI. The project laboratory will be contacted if  $P_{(FR)} < 0.05$  and a repeat analysis will be suggested. The results will be rejected if  $P_{(FR)} < 0.01$ . Results from all laboratories are plotted on a Youden plot with tolerance limits of 0.05 and 0.01 for overall evaluation of comparability.

### 3.7.3 Quality assessment (project phase)

The materials used (see attached table) will be tooth powder samples  $R_K$ ,  $R_L$  and  $R_M$ . One sample is incorporated in each run (alternating). Results are submitted to the CI at the end of each month during the project phase. The analytical quality is assessed at each of the three levels, as described above.

#### Suggested schedule for the programme

The programme outline should be submitted to all participating laboratories for comments in order to ensure that all details, including quality criteria, are acceptable.

The three phases of the programme may then be implemented. According to the design suggested, all project laboratories need not carry out the analyses at the same time, and the laboratories may be in different phases of the programme. However, the programme must continue until the last laboratory has completed its analyses.

The results of this quality control programme will be published if found of general interest. Each laboratory will be identified under a code name. This report will be of concial importance for comparing results obtained in projects performed in different countries.

#### Acknowledgements to:

P. Hyltoft, M. Hørdér and P.J. Jørgensen of the Department of Clinical Chemistry, Odense University Hospital, Søndre Boulevard, Odense, Denmark

MATERIALS

Matrix	Code	Suggested lead content <sup>1</sup>	Application <sup>2</sup>	Phase <sup>3</sup>	Amount
Water	S <sub>1</sub>	1/2xL	Standardization	E	10 ml
-	S <sub>2</sub>	1 x L	-	-	-
-	S <sub>3</sub>	4 x L	-	-	-
Half tooth	T	<1/10xL	Assessment of contamination	T	1 g
Tooth powder	C <sub>A</sub>	1 x L	Internal QC	E+T+P	10 g
-	C <sub>B</sub>	4 x L	-	-	-
-	C <sub>D</sub> <sup>4</sup>	3/4xL	External QC	T+P	1 g
-	C <sub>E</sub> <sup>4</sup>	2 x L	-	-	-
-	R <sub>K</sub> <sup>4</sup>	1/2xL	External QA	P	-
-	R <sub>L</sub> <sup>4</sup>	1,1/2xL	-	-	-
-	R <sub>M</sub> <sup>4</sup>	3 x L	-	-	-

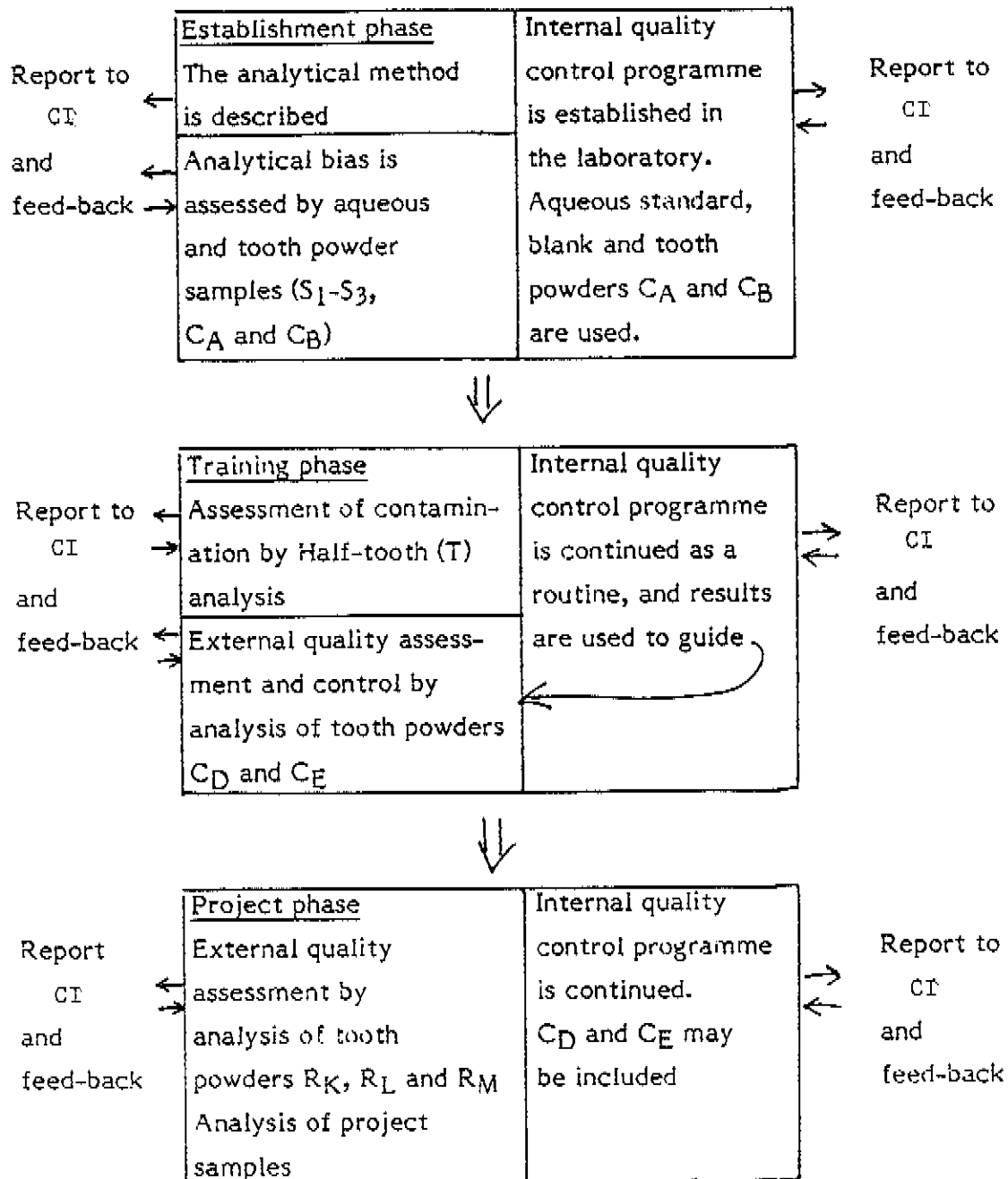
<sup>1</sup> L = "critical limit"

<sup>2</sup> QC = Quality Control, QA = Quality Assessment

<sup>3</sup> E = Establishment, T = Training, P = Project

<sup>4</sup> Distributed under different code

### FLOW CHART FOR QUALITY ASSURANCE PROGRAMME



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#### 4. Health effects measurements

##### 4.1 Introduction

This section of the protocol is concerned with the measurement of psychological, neuropsychological and social factors in relation to studies of biological indicators of lead neurotoxicity in children. The assessment of psychological factors - as opposed to biochemical factors such as levels of lead in blood - is complicated by two major considerations: (a) children are rapidly developing in psychological terms so that different functions are measured in different ways at different ages; (b) psychological development is multi-factorially determined, and is especially sensitive to social factors, some of which also relate to lead exposure. Therefore, in studying lead neurotoxicity in children, no one protocol can cover all age groups, and all protocols must investigate social factors.

The present draft protocol is designed to apply to children aged 6 to 12 years. In most countries children of this age are in full-time primary schooling and therefore are readily accessible for study. Moreover, most psychometric tests are well standardized on children of this age range. Later sections of the draft protocol will indicate how this protocol needs to be altered for use with younger children.

##### 4.2 Aims

Increasing evidence from human and animal studies suggests that there may be adverse effects of lead on children's development even at low exposures corresponding to blood lead levels below 30 ug/100 ml. There is a need to study dose-response relationships between lead and health indicators at such relatively low exposure levels.

Whilst many of the confounding social factors can best be controlled in longitudinal studies, such studies are expensive and time-consuming. Well designed cross-sectional, cohort studies can answer many questions.

This protocol is designed to apply to a cross-sectional study of children aged 6 to 12 years with blood lead levels below 35 ug/100 ml. The aim is to examine the psychological and neuropsychological correlates of lead exposure while controlling for important, relevant, confounding social factors.

#### 4.3 Choice of health indicators

In accordance with the WHO Regional Office for Europe Planning Meeting on Monitoring and Epidemiological Studies for Toxic Chemicals Control (Copenhagen 5-8 May 1981) and the Planning Meeting on Biological Indicators of Lead Neurotoxicity in Children (London 21-23 September 1982) the protocol includes measures under the following four headings:

- (a) Psychometric measures
- (b) Behavioural measures
- (c) Neuropsychological measures
- (d) Confounding social factors.

Measures are selected in accordance with the following principles:

- (a) Any test used should satisfy the usual standards with respect to reliabilities, validity, and should preferably have been standardized in the country in which it is being used.
- (b) Measures should be comparable to those used in other studies.
- (c) Tests should be appropriate for the age of the children.
- (d) Tests should only be included where there is clear empirical or theoretical justification for suspecting that the function measured is affected by lead.

#### 4.4 Psychometric measures

##### General cognitive development

Many recent studies have found that moderately increased lead exposure in school children is associated with a drop of about 5 points in general intelligence (standardized intelligence tests express scores in standard form to yield a population mean of 100 with SD of 15).

Early studies had assumed that a neurotoxin would have similar effects as general brain damage. It was widely believed, without adequate empirical evidence, that brain damaged children performed less well on performance IQ tests than on verbal IQ tests. Similar effects were expected in relation to lead. However the results of the early studies did not support this view. There was no consistent pattern of deficits.

Studies published over the last few years tend to be more consistent and indicate that verbal IQ may be more affected by lead.

It is therefore necessary to measure both verbal and performance aspects of general cognitive functioning.

The Wechsler Intelligence Scale for Children - Revised (WISC-R) (Wechsler, 1974) is strongly recommended for use with children aged 6-12 years.

The Full Test - i.e. consisting of 10 subscales - should be administered according to the standard procedure by well trained psychologists who are supervised by qualified clinical or educational psychologists. On average, this will take 1 1/2 hours to complete. It has to be individually administered. See Appendix 1 for scoring protocol.

#### 4.5 Specific cognitive functions

Where differences in global IQ are found to be related to lead exposure, this does little to help understand what mechanisms are involved. The interpretation of differences on the shorter, less-reliable subtests of WISC-R is a risky business. There is a need to examine more specific functions.

## Language

Since language functioning is currently a focus of interest, consideration should be given to including tests of language.

There is, at present, no consensus as to which language functions may be most sensitive to lead, so that premature specification of tests to be used may inhibit experimentation and impede research.

With that proviso in mind, Needleman et al. (1979) found the Sentence Repetition Test (Vogel, 1975) to be simple to administer and to discriminate between their high and low lead groups. Even though to date this finding has not been replicated, consideration should be given to including this test. (See Appendix 2.)

### 4.6 Visual-motor integration

Different studies have implicated visual motor integration, although this has not always been replicated. Tests of visual-motor integration must be scored using detailed, standardized methods of scoring as was done in the Winneke et al. (1982) study.

Consideration should be given to using the Bender-Gestalt (Bender, 1938) with Schlange et al's. (1971) objective scoring system, as used by Winneke et al. (1982). (See Appendix 3)

### 4.7 "Attention"

It is widely recognized that a variety of different tests and ratings of attention suggest that this is affected by lead. There is less agreement on which paradigm to follow for assessing attention.

Needleman et al. (1979) used a version of Shakow's (1962) delayed reaction time paradigm. A similar paradigm is currently being used by Smith, Graham, Lansdown et al. at the Institute of Child Health in London, by Harvey in Birmingham and by Yule, Lansdown et al. at the Insititute of Psychiatry in London. The latter investigators have developed a micro-processor controlled system for administering and scoring the test. (See Appendix 4.)

Winneke et al. (1982) are using the Wiener Determinationsgerät (Klebensberg 1960). This, too, has proved useful in differentiating different lead levels. (See Appendix 5.)

Various research laboratories recommend the use of a Continuous Performance Test (e.g. Sykes et al. 1973; Levy 1980) as a measure of vigilance or sustained and focussed attention. One such test, suitable for this age range, is described in Appendix 6.

Different laboratories in Europe are currently trying to standardize these and other measures of attention for use in future lead research. Until the measures have been further developed and standardized, it is impossible to recommend which to use, but at least one of the measures described should be included in the protocol.

#### 4.8 Scholastic attainment

Tests of academic attainment - in reading, spelling and mathematics - should be included, partly because previous studies have found differences in relation to lead exposure and partly because of a general concern to describe the functioning of the children studied.

More than tests of cognitive functioning, tests of academic attainment will vary according to the school systems in the country in which the children are living. Tests must be selected according to their appropriateness for the school system, and should be adequately standardized.

Appendices 7, 8 and 9 provide examples of tests appropriate for use in the United Kingdom. The test of reading, the Neale Analysis of Reading Ability (Neale, 1958), must be administered individually. The tests of spelling (Vernon, 1977) and of mathematics (Vernon and Miller, 1976) can be administered in small groups by experienced examiners. The examples are provided to give an impression of the range covered in the tests.

#### 4.9 Neuropsychological batteries

Test batteries, such as the Halsted-Reitan battery (Ball, 1980; Ball and Booth, 1980), measure some of the functions and behaviours tapped by the tests considered earlier. The neuropsychological test batteries are often less well standardized, require skilled administration and interpretation and are of

limited value with children under the age of 9 years. Hence, they are not recommended for inclusion in the current protocol, but may be more useful with children aged over 9 years.

#### 4.10 Behavioural measures

Teachers' ratings of children's behaviour have been found to be related to lead exposure. Behaviours indicative of attention and activity level are particularly involved. For theoretical and practical reasons related to the pervasiveness of effects across different situations, ratings should also be made by parents.

It is difficult to judge what happens to rating scales when translated and used in different cultures. Ideally, new validity studies should be conducted. Because these are rarely done, it is difficult to recommend which rating scales to use in any particular study. Instead, examples will be given in the appendices.

The 11-item forced choice scale used with teachers in the Needleman et al. (1979) study is described in Appendix 7.

The Conners scales - both for teachers and parents - are described in Appendix 8.

The Rutter scales - again in both the teacher and parent versions - are given in Appendix 9.

The Werry-Weiss-Peters Activity Scale for completion by parents is given in Appendix 10.

Direct observational methods have been used in some recent, unpublished studies. Needleman (1982) reported that high lead children were more often observed to be "off-task" than were low level children. More complex ratings of behaviour during psychological testing and in free play sessions have been suggested, but no standardized formats have yet been reported on.

#### 4.11 Neurophysiological measures

Where possible, EEG measures should be included in any cohort study. It is recognized that these require expert administration and add greatly to the cost of the study.

Quantitative measures should be taken. Power spectral analyses of spontaneous EEG records should be used.

Evoked potential techniques should be considered. These are probably less affected by confounding social factors than are traditional psychometric techniques, although they may be confounded with dietary and other non-social factors.

EMG studies of nerve conduction velocity should be considered for inclusion.

#### 4.12 Confounding social factors

Many social factors affect children's intelligence and behaviour. Some of these social factors may also be related to the child's risk of exposure to lead. It is clearly important to be able to sort out which of the many observed relationships are causal. It is also important to remain alert to the possibility of over-controlling lead exposure when statistically controlling for the effects of social factors.

Different societies hold differing cultural values which make it difficult to equate the measurement of social factors across studies undertaken in different countries. For this reason, the "measurement of social factors" section of this protocol is even less prescriptive than earlier sections. Instead, the rationale for enquiring into each social area will be given and possible means of measurement will be described.

As a result of recent research, it can be agreed that it is desirable to measure the intelligence of fathers and mothers. The London studies have used very short versions - only two subtests - of the Wechsler Adult Intelligence Scale (WAIS), whereas American studies have used the Ammon's Quick Test. Other investigators use the Raven's Progressive Matrices and Mill Hill Vocabulary Scale. These are described in Appendix 11.

Demographic data. Family size, birth order, twin status and the marital status of the child's natural parents should be determined. The child's age and sex should be recorded. The composition of the current social unit should be determined. All these factors are relevant to the child's emotional and cognitive function.

Pregnancy and neonatal period. In some studies, it may be possible to obtain reliable pregnancy and birth records. Otherwise, one has to rely on retrospective data with all the inaccuracies which that implies. Careful questioning of factual data can reduce unreliability. Birth weight should be recorded. Examples of other questions are given in Appendix 12, pages 2 to 3.

Early development. Exposure to lead through artificial feeds in infancy may be important in cases of high plumbo-solvent water. Early indications of severe developmental delay could be critical in ascertaining when damage to CNS was sustained - Appendix 12, page 4.

Child's current health. This can be estimated both by general impression from the informant and by the number of visits to doctor or dentist. The numbers of immunisations may be an index of the parent's reasonable concern for the child's well-being. (It is argued that parents who care less for their children's development also are less restrictive regarding where children play, thus allowing the children to get contaminated. Such parental attitudes and practices are difficult to pin-point, especially in attitudinal questionnaires. At present, the reliability of this particular item has yet to be established). Appendix 12, page 4.

Children's behaviour. In accordance with the techniques developed for the epidemiological studies of childhood psychiatric problems (Rutter, Tizard and Whitmore, 1970), parents are asked to answer Appendix 12, pages 5-6.

Pica. All children put things in their mouths when babies. If this persists and when it occurs with unusual severity, it is referred to as Pica. Our concern is with trying to establish the self-exposure to lead by this behaviour. One set of questions is given in Appendix 12, page 4.

Diet. The child's current diet is of interest for two reasons: (a) the adequacy of the diet may be related to the moderation of any effects of lead; (b) the diet may expose the child to undue amounts of lead. See Appendix 12, page 2.

Pre-school experience. This will be related to the child's overall adaptation and attainment. In countries where it is voluntary, it may indicate the parents' interest in the child's attainment.

Current schooling. Again, a discussion of the child's current schooling affords an opportunity to gauge the parents' interest in their child's academic progress.

Parental involvement. The amount that the parents are involved in their child's activities at home may also indicate their interest. This is related to both emotional and cognitive development. Restrictions placed on where the child plays may be related to exposure to lead contamination areas (this is cross checked from a knowledge of the surrounding area, preferably including measures of lead in dust, etc.).

Lead related hobbies. Some household activities and hobbies may release lead into the immediate environment. It is important to enquire into this. See Appendix 12, page 4, point 5.6.

Housing. Type and age of housing can indicate a variety of social and other factors. In the UK, older housing often has higher lead risks associated both with lead in paint and in plumbing. The latter can be checked by direct question and observation. The amenities of the house allow one to estimate the adequacy of the living environment. The location of the house in relation to the main roads and factories may allow one to build up an index of exposure to airborne lead. It must be remembered that families are often mobile so that current circumstances may be an inadequate indicator of previous exposure. Earlier housing should also be investigated.

Social class or socioeconomic status. Relatively simple indices of social factors based on a knowledge of the status of the occupation of the fathers are often used to partial out social factors. Simple social class is indeed related to child's IQ. However, it is likely that more sophisticated measures based on the status of occupations held by both parents, their education and their income may prove more useful. Sometimes, genetic questions are being implicitly investigated and so it is important to differentiate between biological and social parents. See Appendix 12, pages 1-2.

Occupation-related lead risk. It is important to get sufficient information about parental occupation to be able to judge whether it involves any elevated risk of exposure to lead - particularly one in which contamination may be brought into the home. See Appendix 12, page 2.

Parental health. Parental health in general, and mental health in particular, is related to the child's emotional adjustment. Following from methods developed at the Institute of Psychiatry, Denmark Hill, London, for use in epidemiological studies, it is possible to elicit sufficient information from a standard interview to allow a rating of mental health to be made.

Marriage and family life. Since some studies have found that children's behaviour difficulties are associated with increased exposure to lead and since it is known that difficulties in marriage are also related to increased behaviour problems in children, it is necessary to investigate the independent contributions of these factors to children's adjustment. Standard methods of assessing the quality of marriage have been developed at the Institute of Psychiatry, Denmark Hill, London. Other measures are also available.

State of house. During the interview, the interviewer has an opportunity for directly inspecting the cleanliness of the house. This can be recorded as in Appendix 12, page 4, point 5.6.

#### 4.13 Practical considerations

In undertaking any study, a number of practical considerations need to be borne in mind.

The psychologists undertaking the psychometric assessment, the teachers rating the children's behaviour and the social scientists interviewing the parents must remain ignorant of the child's blood lead level until after all the data are collected.

Parents' informed consent must be obtained, preferably in writing, for their child to participate in the study. Permission has also to be obtained from the appropriate education, health and social services to ensure goodwill and collaboration throughout the study.

Close contact should be maintained with parents and teachers throughout the study in order to keep the number of refusals and later drop-outs to a minimum. A good liaison research officer can coordinate the data collection and foster good relations with parents and schools.

The reading difficulty of letters to parents can be estimated using techniques such as the Flesch Reading Ease Formula.

In recruiting samples through schools, it is advisable to have parents actively opt out of the study rather than opt in - i.e. letters and invitations to participate should be couched on the assumption that most parents will wish to participate. If they wish to opt out, they must take active steps to do so. Non-response to letters should be followed by personal contact, always respecting the parents' clearly stated wish to refuse to participate further.

Testing in schools can introduce uncontrolled distractions; testing in a laboratory can be expensive in travel. As far as possible, individual testing of children should be undertaken in quiet surroundings. If future quality assessment exercises indicate that particular testing circumstances yield better data, this factor should be included in the design of the study.

#### 4.14 Study population and research design

Recent research indicates that with respect to general intelligence, one is probably dealing with a small relationship between lead and IQ of the order of a difference of 5 points or one-third of a standard deviation between "high" and "low" exposure groups. This largely determines the number of children to be studied.

About 400 children are needed for the study to have adequate power to detect an association between moderately elevated lead exposure and neurotoxic effects.

A two-stage process is recommended in which about 1000 school children are screened for body lead levels in the first stage and then 400 are selected for further intensive study.

The elevated group should consist of the 200 children with the highest blood leads.

The "control" group should consist of 200 of the remainder selected at random. Note that it is important to select children across the whole range of exposure in order to examine for any dose-response relationships. Sampling of two extreme groups does not allow for this and may produce misleading results.

Matching of "elevated" and "control" children on key social variables may be considered, provided care is taken to ensure that one is not over-controlling for lead effects.

Care must be taken to avoid bias introduced by selective refusal to participate or post hoc exclusion of subjects. Where subjects are excluded, the rationale must be clearly stated. There is an ever present danger of confusing cause and effect by selective exclusion.

Because of the large number of statistical comparisons made (at least implicitly), researchers should separate out strong predictions from tentative comparisons before data analysis. Then, multiple comparisons can be taken into account statistically by techniques such as multiplying P-values by the total numbers of tests performed.

#### 4.15 Quality control of psychological measures

There is a need to build in some form both of intra- and inter-study quality control of psychological, neuro-psychological and social interview measures.

Most standardized psychometric tests will have known inter-tester reliability, but it is still desirable to have some assessment of this within each study.

For non standardized methods, such checks are essential.

Lead researchers are meeting to discuss ways of promoting inter-study quality control of data and as recommendations are published from such groups they should be incorporated into studies.

In the mean time the following suggestions should be considered:

- (a) children's and parents' verbatim responses should be recorded in extenso, with audio tape recorders being used for a proportion of interviews;
- (b) inter-scorer exercises should be undertaken on samples of scripts/tapes;
- (c) consideration should be given to videotaping some performance tasks with a view to intra- and inter-laboratory comparability exercises;
- (d) wherever possible, test procedures should be automated both in presentation and in scoring using micro-processor technology.

#### Acknowledgements

Dr G. Winneke (Düsseldorf), Mr P. Harvey, (Birmingham) and Dr R. Lansdowne (London) - for comments on early draft.

Appendix 1

Wechsler Intelligence Scale for Children - Revised (WISC-R)

This is the latest of the tests of general intelligence devised by Wechsler and used since the early 1940's. Ten short subtests are given to each child individually. Five of the tests are predominantly "verbal" in nature and five are predominantly non-verbal, or "performance". Raw scores are transformed into age-corrected standard scores (Mean of 10; SD of 3); and the scaled scores are summed to yield three estimates of intelligence - Full Scale (or general) IQ; Verbal Scale IQ; and Performance Scale IQ. These are standardised to yield a population mean of 100 (SD = 15).

Earlier versions of WISC have been thoroughly investigated and found to be readily usable in other English speaking countries. A German version is available, although it has been found to yield higher mean scores. A revised German version will shortly be available.

The interpretation of the pattern of sub-scale scores is controversial since the small sub-scales are much less reliable than the overall three IQ scales. The reliability of the Full Scale IQ is 0.96.

The test is standardised for use with children aged 6-17 years. A great deal of information is available showing how scores on WISC-R relate to scores on, for example, tests of scholastic attainment.

WISC and WISC-R have been used in most recent studies of the effects of lead on children's development (Needleman et al, 1979; Winneke et al, 1982; Yule et al, 1981).

The content of the sub-test can be appreciated by examining the record forms available for inspection from Dr Yule, Institute of Psychiatry, London.

Reference

Wechsler, D. Wechsler Intelligence Scale for Children - Revised: Manual. New York: Psychological Corporation (1974).

Winneke, G. et al. Neuropsychological studies in children with elevated tooth lead concentrations - pilot study. Occ. Env. Health. 51: 169-183 (1982).

Yule W. et al. The relationship between blood lead concentrations, intelligence and attainment in a school population: a pilot study. Develop. Med. Child Neurolo. 23: 567-576 (1981).

Appendix 2

Sentence repetition test

This test consists of 20 sentences of increasing syntactic complexity. Nine sentences are 8 words in length; 11 are 9 words in length. The level of difficulty of the vocabulary was controlled. Syntactic complexity increases both developmentally and transformationally. The number of underlying sentences increases from 2 to 4 sentences.

The instructions appear below. The children's responses are tape-recorded for later transcription and scoring. "A response was scored as correct if the child repeated the sentence accurately without any additions, substitutions or changes of word order. Repeating more than once, one or more words within a sentence was not considered an error. Similarly, if a word such as 'its' was repeated as 'it is', or vice versa, and not other changes were made, the response was scored as correct".

The test was used with 20 normal and 20 dyslexic children aged 7 1/2 to 8 1/2 years. It was found to have an internal consistency coefficient (Hoyt r) of + 0.85. The mean score of the normal children was 15.80 (range 13-19; SD = 2.04). The difference between normal and dyslexic children was highly significant.

Needleman et al. (1979) used the test with their high and low lead groups aged approximately 7 1/2 years. The scores obtained were 12.6 for low lead and 11.3 for high lead (difference significant at P = 0.04). It is not clear why Needleman's children score so much lower than Vogel's sample, but it may be that they were somewhat younger.

Reference

Vogel, S.A. Syntactic abilities in normal and dyslexic children. Baltimore: Univ. Park Press (1975).

Sentence repetition test - instructions

I am going to say something to you. When I get all through, you say just what I said.

---

1. Will you stop to watch the game now?
2. This is mine, but that one is his.
3. When are they going to start the game?
4. She isn't going to the store because it's closed.
5. What can I do to help you now?
6. Who will come to fix what is broken?
7. He isn't coming now but he wants to come.
8. He wants to go only if she will go.
9. You will go to play when you are well.
10. He wants to jump higher than everybody else.
11. He did what was asked of him to do.
12. Nobody began working until the bell rang loudly.
13. I know what to do whatever may happen.
14. Many children knew where to find those old books.
15. Shouldn't children who aren't well go to sleep early?
16. I can't fix everything that needs fixing today.
17. Whoever went skating knows that it's cold and windy.
18. They hurt themselves badly in falling but continued playing.
19. When can someone tell us why it stopped ringing?
20. Don't begin to serve until everyone who's coming arrives.

Appendix 3

Bender Gestalt Test

This test first appeared in 1938 and is said to be suitable for children aged 4 years and over. Children are asked to look briefly at a simple design and then to reproduce it from memory. It is claimed that children with neurological disorders, learning difficulties and behaviour problems perform less adequately than normal children. The original test was freely scored, and not found to be very reliable. More detailed scoring systems have been proposed. Winneke et al. (1982) used a more recent, detailed, objective scoring system (Schlange et al. 1971) and found some differences between high and low lead children.

References

Bender, L. A Visual Motor Gestalt Test and its Clinical Use. Res. Monogr. No 3: American Orthopsychiatric Association, (1938).

Schlange, H., et al. Göttinger Formproduktions Test (G-F-T): Zur Diagnose der Hirnschädigung in Kindersalter. Gottingen: Hogrefe, (1971).

Appendix 4

Delayed reaction time

Needleman et al. (1979) found interesting differences between "high" and "low" lead children on a version of Shakow's (1962) delayed reaction time task. This task has been automated for presentation and scoring by Hunter (1982) and is currently being used in studies in the United Kingdom. The instructions for administration are given below to illustrate the sort of tasks presented to children.

Reaction time under varying intervals of delay

The child is seated in front of a response box which has a red push button and a brass key, about 7 cm apart, both connected to microswitches. There is room to rest the hand on the box. The experimenter controls the test using the TV monitor screen and the computer keyboard. The child's preferred hand is established before beginning the test by asking him to write his name on a list.

Each trial begins when the child is asked to press the red button with one finger of the preferred hand on a verbal "Ready" signal from the tester. The pre-stimulus interval of either 3 or 12 seconds is simultaneously initiated by the tester and ends with the presentation of a tone, the auditory stimulus. The child must respond to this (and thereby switch it off) by releasing the red button and pressing the brass key with the same finger. The delay between stimulus presentation and the release of the first button, together with the total time to press the second button, are both recorded. The first corresponds to Needleman et al. 1979s "reaction time".

Trials are presented according to the following, preceded by a practice session to make sure the child understands the task: 6 trials with a 3 second PS1; 6 trials with 12 second PS1; 6 trials with a 12 second PS1 and 6 trials with a 3 second PS1.

References

Hunter, J. Personal communication (1982).

Shakow, D. Segmental set. Arch. gen. Psychiatr. 6, 1-17, (1962).

Appendix 5

Wiener Determinationsgerät

This apparatus has been used by Winneke et al (1982) to test for simple and complex reaction times. The task is best described as an experimenter-paced serial reaction time task. After pre-training, lengthy series of signals are presented. The examiner notes the number of correct, late and false reactions. Children with higher lead levels were found to give more false responses than children with low lead.

This paradigm is still experimental and few normative data are available on children.

References

Klebensberg, D. Wiener Determinationsgerät. Diagnostica (1960).

Appendix 6

Continuous performance task

The continuous performance task is a vigilance task said to be sensitive to attentional deficits. (Rosvold et al, 1965.) A series of stimuli - letters or pictures - is presented to the child who has to press a button only when a particular sequence occurs. For example, Harvey (1982) has developed a micro-processor presentation of a series of letters on a visual display unit. The child has to detect, say, the sequence "x - o". Pressing when this occurs allows detection of reaction time as well as accuracy. Incorrect responses occur when sequences are missed or when the button is pressed after seeing an "o" which has not been preceded by an "x".

As yet, there are no agreed standardised methods for presenting the continuous performance task, and few normative data are available. The task has not been found sensitive to the effects of head injury (Chadwick et al. 1981) but it appears sensitive to the effects of methylphenidate in hyperactive children (Taylor et al, 1982).

References

- Chadwick, O, et al. A prospective study of children with head injuries: II. Cognitive sequelae. Psychol. Med. 11: 49-61 (1981).
- Harvey, P. Relationship between blood lead levels and behavioural measures in young children: Protocol - 5 1/2 year old sample. Univ. Birmingham (1982).
- Rosvold, H.E., et al. A continuous performance test of brain damage. J. consult. Psychol. 20: 343-350 (1965).
- Taylor, E. et al. Personal communication (1982).

Appendix 6A

Tests of educational attainment

Tests of educational attainment must be selected to reflect the educational systems and goals of particular countries. The record forms illustrate three tests found useful in the United Kingdom. (Record forms are available for inspection from Dr Yule<sup>1</sup>.)

(a) Reading

The Neale Analysis of Reading Ability (Neal, 1958) is a prose-reading test suitable for children aged 6 to 12 years. It has test-retest reliability over one year of  $\pm 0.95$  for its measures of Accuracy of Reading and Comprehension of Reading. Children have to read short paragraphs out aloud (scores for accuracy) and then answer questions on the content (score for comprehension).

(b) Spelling

Increasingly, it is realised that the ability to spell, while related to the ability to read, nevertheless, involves different skills and psychological functions. There are many lists of words to be spelt, the one illustrated is Vernon's Graded Word Spelling Test (Vernon, 1977). This has been recently standardized in both England and Canada, and is suitable for children aged 5 1/2 to 17 1/2 years. The internal consistency of the test is 0.94.

(c) Mathematics

Mathematical ability is again different from reading and spelling ability. It encompasses more than merely understanding the basic rules of arithmetic. The Vernon Graded Arithmetic-Mathematics Test (Vernon and Miller, 1976) has also been recently standardized in England and Canada. It is suitable for children aged 6 to 17 years. Its reliability is approximately 0.90.

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<sup>1</sup> Dr W. Yule, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London.

References

Neal, M.D. Neal Analysis of Reading Ability Manual. London, Macmillan (1958).

Vernon, P.E. Vernon Graded Word Spelling Test. London, Hodder and Stoughton (1977).

Vernon, P.E. & Miller, K.M. Vernon Graded Arithmetic-Mathematics Test. London, Hodder and Stoughton (1976).

Appendix 7

Needleman et al's scale for teachers

The eleven-item forced-choice rating scale for completion by teachers and used by Needleman et al. (1979) is reproduced below. In the Boston study, dose response relationships were found between tooth lead levels and behaviour ratings on a majority of items. This was largely replicated in the smaller London pilot study (Yule et al. 1982). In neither study were social factors or other related variables controlled. Yule et al. (1982) report that the ratings on the Needleman items are closely related to ratings on Rutter's B. Scale and to intelligence, but not to social class. Otherwise, no data are available on the psychometric properties of the scale.

The scale is:

1. Is this child easily distracted during his/her work?
2. Can he/she persist with a task for a reasonable amount of time?
3. Can this child work independently and complete assigned tasks with minimal assistance?
4. Is his/her approach to tasks disorganized (constantly misplacing pencils, books, etc.)?
5. Do you consider this child hyperactive?
6. Is he/she over-excitabile and impulsive?
7. Is he/she easily frustrated by difficulties?
8. Is he/she a daydreamer?
9. Can he/she follow simple directions?
10. Can he/she follow a sequence of directions?
11. In general, is this child functioning as well in the classroom as other children his/her own age?

References

Needleman, H.L. et al. Deficits in psychological and classroom performance of children with elevated dentine lead levels. New. Eng. J. Med. 300: 679-695 (1979).

Yule, W. et al. Teachers' ratings of children's behaviour in relation to blood lead levels, (1982). (In press).

Appendix 8

Conner's teaching questionnaire

The Conners (1969) IQ was developed to evaluate drug trials with hyperactive children. It consists of 29 items rated by teachers on a 4 point scale. A number of factor analytic studies show reasonably good agreement in identifying four underlying factors - aggressive disorder, inattentiveness, anxiety and hyperactivity. The scale has repeatedly been found sensitive to drug administration. Satisfactory inter-rater reliability and high (0.7 to 0.97) test-retest reliability are reported by Taylor and Sandberg (1981).

The scale has been used in David's studies of lead in New York and by Yule et al. in London.

The scales are available for inspection from Dr Yule<sup>1</sup>.

References

Conners, C.K. A teacher rating scale for use in drug studies with children. Amer. J. Psychiat. 126: 884-888 (1969).

Taylor, E. & Sandberg, S. Classroom behaviour problems and hyperactivity: A questionnaire study in English schools. (In preparation) (1981)

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<sup>1</sup> Dr W. Yule, Institute of Psychiatry, Denmark Hill, London.

Appendix 9

Rutter's behaviour rating scales

These were originally developed for use as screening instruments in epidemiological surveys. The parents' scale (A2 Scale) consists of 31 items and has a re-test reliability of 0.74.

Teachers (B2) scale consists of 26 items and has re-test reliability of 0.89. Both scales have good validity (Rutter, Tizard and Whitmore, 1970). Items are scored on a 3 point scale and summed. Children scoring above the empirically determined cut-off point are defined as "deviant". Sub-scales determine whether the content of the deviancy is primarily neurotic or anti-social. In addition, recent factor analytic studies (Schachar, Rutter and Smith, 1981) have identified a factor of overactivity. Yule et al. (1982) found that his/her lead levels were associated with a significantly increased rate of overactivity.

The scales are available for inspection from Dr Yule.<sup>1</sup>

References

Rutter, M. et al. (Eds) Education, Health and Behaviour. London, Longmans (1970).

Schachar, R. et al. The characteristics of situationally and pervasively hyperactive children: Implications for syndrome definition. J. Child Psychol., Psychiat. 22: 375-392. (1981)

Yule, W. et al. Teachers' ratings of children's behaviour in relation to blood lead levels. (1982) (In press)

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<sup>1</sup> Dr W. Yule, Institute of Psychiatry, Denmark Hill, London.

Appendix 10

Werry-Weiss-Peters Activity Scale

This consists of 22 items to be completed on a three point rating scale by parents (Werry, 1968).

It has been used in the Birmingham study of 5 1/2 year olds. However, its author states "... offered as an experimental device, it has received uncritical acceptance ... (it) has generally proven drug sensitive (Werry, 1978). Psychometric analyses have failed to substantiate the construct validity of the scale as a measure of activity (Routh et al, 1974; Shaffer et al, 1974)". (Conners and Werry, 1979)

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	NO	SOME	MUCH
<u>During meals</u>			
Up and down at table			
Interrupts without regard			
Wiggles			
Fiddles with things			
Talks too much			
<u>Television</u>			
Gets up and down during programmes			
Wiggles			
Manipulates objects or body			
Talks constantly			
Interrupts			
<u>Play</u>			
Inability for quiet play			
Constantly changing activity			
Seeks parental attention			
Talks too much			
Disrupts others' play			
<u>Sleep</u>			
Difficult settling down for sleep			
Inadequate amount of sleep			
Restless during sleep			
<u>Behaviour away from home (except at school)</u>			
Restlessness during travel			
Restlessness during shopping (including touching everything)			
Restlessness during church/cinema			
Restlessness while visiting friends, relatives, etc.			

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References

- Conners, C.K. & WERRY, J.S. Pharmacotherapy, Ch.9. In: A.C. Quay and J.S. Werry (EDS) Psychopathological Disorders of Childhood (2nd Ed). New York: Wiley. (1979).
- Routh, D. et al. Development of activity level in children. Devel. Psychol. 10: 168-173 (1974).
- Shaffer, D. Et al. Controlled observation on patterns of activity, attention and impulsivity in brain damaged and psychiatrically disturbed boys. Psychol. Med. 4: 4-18 (1974).
- Werry, J.S. Developmental hyperactivity. Ped. Clin. An. Amer. 15: 581-599 (1968).
- Werry, J.S. Pediatric Psychopharmacology. New York, Brunner Mazel (1978).

Appendix 11

Measurement of parental intelligence

It is highly desirable to measure the intelligence of both parents. To administer a complete individually administered test of general intelligence would take approximately 1 1/2 hours per parent. This is probably too expensive at present and so shorter tests are currently used. A short form of the Wechsler Adult Intelligence Scale has been used by both the Institute of Child Health and the Institute of Psychiatry studies in London.

Other investigators (Ernhart, Landa and Schell, 1981) have used shorter measures of a more pencil-and-paper type, the Quick Test (Ammons and Ammons, 1962).

Initially, some investigators worried that parents might not cooperate with this aspect of the study. Recent experiences in London and Leeds show that 94% of mothers and 79% of fathers cooperated.

References

Ammons, R.B. and Ammons, C.H. the Quick Test (QT): Provisional Manual. Psychol. Rep. 11: 111 (1962).

Ernhart, C.B. et al. Subclinical levels of lead and developmental deficit - a multivariate follow-up reassessment. Pediatrics. 67: 911-919 (1981).

Appendix 12

Case History Questionnaire

Serial No.

Questions and answers		Code
Interview with Mrs. _____	on _____	
Child: Name _____	m	
First name _____	f	
Address _____		
_____		
Date of birth _____	Age _____	
School _____		
Location _____		
Form _____	School year _____	
Questions and replies		Code
1. Sociodemographic data		
1.1 Total number of children _____		No
Sibling rank (eldest etc) _____		Rank
1.2 Civil status of mother	1. Single 2. Married 3. Separated/Divorced 4. Widowed	Civil Status
1.3 Age of parents (present, in years)	1. -29      4. -59 2. -39      5. -69 3. -49      6. 70+	Mother Father
1.4 Occupational status of parents (current)	1. Fully employed 2. Employed part-time 3. Family member working without payment 4. Unemployed 5. Housewife 6. Pensioner	Mother Father
1.5 Occupations of parents	0. No training/undergoing training 1. Unskilled/semiskilled 2. Skilled 3. Salaried employee (e.g. salesman) 4. Professionally qualified employee (e.g. accountant) 5. Higher-level employer (e.g. graduate) 6. Civil servant 7. Higher official 8. Senior official 9. Self-employed artisan (e.g. farmer, tradesman) 10. Professional (e.g. businessman)	Mother Father

1.6 Education of parents	1. Still at school 2. Primary School 3. Middle school 4. Full secondary school (without diploma) 5. Full secondary school (with diploma)	Mother Father	
1.7 Training	1. No training 2. Undergoing training 3. Practice period 4. Apprenticeship 5. Technical/vocational school 6. Technical/engineering college 7. Higher educational institution/university	Mother Father	
1.8 Monthly net income (After tax deductions)	1. up to 1000      4. up to 2500      7. 3500+ 2. up to 1500      5. up to 3000 3. up to 2000      6. up to 3500 Grouping based on FRG income levels in DM	Mother Father	
1.9 Sector in which the father works	1. Metal industry: lead 2. Metal industry: other 3. Asbestos industry 4. Fertilizer industry (e.g. guano) 5. Metal industry (shipping, machinery) 6. Other industry (construction) 7. Farming, forestry, fishing 8. Energy, water supply 9. Trade 10. Transport, communications 11. Service (e.g. hotels) 12. Other		
1.10 Fruit and vegetables from the garden?	generally <input type="checkbox"/> at the moment <input type="checkbox"/> no <input type="checkbox"/>		
1.11 Play pattern of the child	Generally indoors?	Generally outside?	
No. of hours spent outside in good weather during the summer			
1. -2 hours	2. 2-4 hours	3. 4-6 hours	4. +6 hours <input type="checkbox"/>
2. Pregnancy	Positive	No Symptoms	Negative
2.1 Multiple birth			
2.2 Diseases up to the 12th week			
- Flu with fever	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Rubella contact			
- Other identified virus infections			
2.3 Diseases after the 13th week			
- Identified virus infections	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Identified bacterial infections			
- Rubella/rubella contact			
2.4 Bleeding during early pregnancy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

	Positive	No Symptoms	Negative	
2.5 Haemorrhage during the third trimester?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
2.6 Anaemia during the third trimester ( 8g%)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
2.7 EPH-gestosis? - Edema (lower/upper extremities/joints) - Proteinuria ( ++ / > 1%) - Hypertension (BP > 140/90)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
2.8 Smoking during pregnancy?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
2.9 Quantity	under 5	5-10	10-20	20
2.10 Drugs? (Please ask further details)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
2.11 Alcohol?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3. Delivery				
3.1 _____ before term _____ after term				
3.2 Duration of delivery (after contractions at regular 10-15 min. intervals)				_____
3.3 Induced labour _____ days before term because of _____				
3.4 Abnormal presentation - Breech, transverse, oblique	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3.5 Assisted delivery? - Vacuum - Forceps - Section - Extraction	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3.6 Umbilical complications - Prolapse/knot	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3.7 Other complications (e.g. arrest)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3.8 Narcosis subpartu (Was the child's crying heard?)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3.9 Premature delivery - Labour before 38th week (were inhibitors taken)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
3.10 Birth weight				_____
3.11 Apgar score	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

4. First year of life								
4.1 Development								
- Walking at age:	_____							
- First words (except mummy, daddy) at age:	_____							
4.2 Diseases								
4.3 Accidents								
5. Subsequent development								
5.1 Preschool/school development								
- Continent (day and night) at age:	_____							
- Satisfactory school development up to now	<table border="0"> <tr> <td></td> <td style="text-align: center;">Yes</td> <td style="text-align: center;">No</td> </tr> <tr> <td></td> <td style="text-align: center;"><input type="checkbox"/></td> <td style="text-align: center;"><input type="checkbox"/></td> </tr> </table>		Yes	No		<input type="checkbox"/>	<input type="checkbox"/>	
	Yes	No						
	<input type="checkbox"/>	<input type="checkbox"/>						
	Always	sometimes	seldom	never				
Help with the homework	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
Checking of homework	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
5.2 Illnesses and susceptibility to infections								
5.3 Accidents								
5.4 Hospitalizations								
5.5 Thumb-sucking	<input type="checkbox"/>	No	up to age _____					
Nail-biting	<input type="checkbox"/>	<input type="checkbox"/>	up to age _____					
Putting objects in mouth	<input type="checkbox"/>	<input type="checkbox"/>	up to age _____					
5.6 Other								

QUESTIONNAIRE ON YOUR CHILD'S PERFORMANCE OF HOMEWORK

Name _____ Date of birth _____		
First enrolled in 19__ Now in form _____ School _____		
For each question please check the reply you consider most applicable to your child ( <input checked="" type="checkbox"/> )		
Questions	Replies	
Child's application during homework	<input type="checkbox"/> Very good <input type="checkbox"/> good <input type="checkbox"/> fairly good <input type="checkbox"/> average <input type="checkbox"/> some <input type="checkbox"/> scarcely any <input type="checkbox"/> none	
1. Perseverance (e.g. works steadily, can keep going for quite a long time without pausing)	<input type="checkbox"/> Very good <input type="checkbox"/> good <input type="checkbox"/> fairly good <input type="checkbox"/> average <input type="checkbox"/> some <input type="checkbox"/> scarcely any <input type="checkbox"/> none	
2. Attention (e.g. does not do anything else at the same time, does not draw on the table or in exercise books, does not daydream)	<input type="checkbox"/> Very good <input type="checkbox"/> good <input type="checkbox"/> fairly good <input type="checkbox"/> average <input type="checkbox"/> some <input type="checkbox"/> scarcely any <input type="checkbox"/> none	
3. Ability to work without constant urging	<input type="checkbox"/> Very good <input type="checkbox"/> good <input type="checkbox"/> fairly good <input type="checkbox"/> average <input type="checkbox"/> some <input type="checkbox"/> scarcely any <input type="checkbox"/> none	
4. Ability to resist distraction (e.g. does not take any notice of noise or other people, does not start playing)	<input type="checkbox"/> Very good <input type="checkbox"/> good <input type="checkbox"/> fairly good <input type="checkbox"/> average <input type="checkbox"/> some <input type="checkbox"/> scarcely any <input type="checkbox"/> none	
5. Ability to cope with setbacks (i.e. is not immediately discouraged by difficulties)	<input type="checkbox"/> Very good <input type="checkbox"/> good <input type="checkbox"/> fairly good <input type="checkbox"/> average <input type="checkbox"/> some <input type="checkbox"/> scarcely any <input type="checkbox"/> none	
6. Concentration	<input type="checkbox"/> Very good <input type="checkbox"/> good <input type="checkbox"/> fairly good <input type="checkbox"/> average <input type="checkbox"/> some <input type="checkbox"/> scarcely any <input type="checkbox"/> none	

<p>7. Awareness of tasks (e.g. knows what must be done for homework)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	
<p>8. Ability to sit still (e.g. does not fidget, does not tap on the table)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	
<p>9. Readiness to start homework immediately once seated (e.g. does not dawdle, does not leaf through books)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	
<p>10. Knowledge of what went on at school (e.g. can state what was said at school)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	
<p>11. Ability to do homework independently (e.g. needs little help)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	
<p>12. Child's absorption in games</p>	<p>0 always very absorbed in games 0 sometimes very absorbed in games 0 average absorption 0 starts many games but does not play any for long</p>	
<p>13 Is the child alexic</p>	<p>0 no 0 yes, noticed by 0 suspected</p>	

QUESTIONNAIRE ON YOUR CHILD'S BEHAVIOUR IN CLASS

Name (Pupil) _____ Completed on _____	
Name (Teacher) _____	
Form/School _____	
For each question kindly check the reply you consider most applicable to your child (X)	
For approximately how long have you known the child as a pupil? _____	
Questions	Replies
Child's application during lessons	<input type="radio"/> Very good <input type="radio"/> good <input type="radio"/> fairly good <input type="radio"/> average <input type="radio"/> some <input type="radio"/> scarcely any <input type="radio"/> none
1. Perseverance (e.g. works steadily, can keep going for quite a long time without pausing)	<input type="radio"/> Very good <input type="radio"/> good <input type="radio"/> fairly good <input type="radio"/> average <input type="radio"/> some <input type="radio"/> scarcely any <input type="radio"/> none
2. Attention (e.g. does not do anything else at the same time, does not draw on the table or in exercise books, does not daydream)	<input type="radio"/> Very good <input type="radio"/> good <input type="radio"/> fairly good <input type="radio"/> average <input type="radio"/> some <input type="radio"/> scarcely any <input type="radio"/> none
3. Ability to work without constant urging	<input type="radio"/> Very good <input type="radio"/> good <input type="radio"/> fairly good <input type="radio"/> average <input type="radio"/> some <input type="radio"/> scarcely any <input type="radio"/> none
4. Ability to resist distraction (i.e. is not distracted by trivialities or disturbances)	<input type="radio"/> Very good <input type="radio"/> good <input type="radio"/> fairly good <input type="radio"/> average <input type="radio"/> some <input type="radio"/> scarcely any <input type="radio"/> none
5. Ability to cope with setbacks (i.e. is not immediately discouraged by difficulties)	<input type="radio"/> Very good <input type="radio"/> good <input type="radio"/> fairly good <input type="radio"/> average <input type="radio"/> some <input type="radio"/> scarcely any <input type="radio"/> none
6. Concentration	<input type="radio"/> Very good <input type="radio"/> good <input type="radio"/> fairly good <input type="radio"/> average <input type="radio"/> some <input type="radio"/> scarcely any <input type="radio"/> none

<p>7. Awareness of teacher's instructions (e.g. knows what task has been set)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	
<p>8. Ability to sit still (e.g. does not fidget, does not tap on the table)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	
<p>9. Readiness to obey instructions, immediately and rapidly (e.g. does not dawdle over starting work, leaf through books, or sharpen pencils continually)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	
<p>10. Ability to follow the lesson (e.g. knows what the teacher has just said)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	
<p>11. Ability to do homework independently (e.g. needs little help)</p>	<p>0 Very good 0 good 0 fairly good 0 average 0 some 0 scarcely any 0 none</p>	

Other comments