COUNCIL DIRECTIVE

of 29 March 1977

on biological screening of the population for lead

(77/312/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 235 thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Parliament [1],

Having regard to the opinion of the Economic and Social Committee [2],

Whereas one of the essential tasks of the European Economic Community is to promote throughout the Community a harmonious development of economic activities and a continuous and balanced expansion, neither of which can be achieved without combating pollution and nuisances and improving the quality of life and the protection of the environment;

Whereas the various uses of lead are at present causing contamination of many areas of the environment by this substance;

Whereas the many environmental sources of lead make it difficult to determine the total exposure of any one individual to this pollutant and therefore the protection of human health calls for the most accurate possible monitoring of the individual's total lead absorption;

Whereas biological screening of the population for lead should be carried out and the results of this screening evaluated so that, where appropriate, new proposals may be drawn up;

Whereas it is desirable to lay down the technical procedures and biological reference levels for such screening;

Whereas measurement of blood lead level is currently the best means of assessing the quantity of lead recently received by an individual as a result of exposure to environmental lead; whereas the enzymatic activity of delta-aminolevulinic acid dehydratase (ALAD) may be used as an indicative or supplementary test for determining exposure to lead;

Whereas the programme of action of the European Communities on the environment [3] provides for the coordination of national programmes having as their aim the improvement of the quality of life and for priority investigation into lead,

HAS ADOPTED THIS DIRECTIVE:

Article 1

The Member States shall take the necessary steps to apply a common procedure for biological screening in order to assess the exposure of the population to lead outside the working environment.

Article 2

This common procedure, the application of which shall be restricted to four years, shall be based on the measurement of blood lead levels.

As an indicative or supplementary test, the ALAD measurement may also be used in accordance with the procedures laid down in Annexes II and III.

[1] OJ No C 28, 9. 2. 1976, p. 31.
[2] OJ No C 50, 4. 3. 1976, p. 9.
[3] OJ No C 112, 20. 12. 1973, p. 3.

Article 3

1. The conditions for biological screening shall be fixed by means of:

— sampling and analysis procedures,

— frequency of sampling.

2. Blood sampling shall be carried out on volunteers.

Article 4

Sampling shall be carried out on:

— groups of at least 100 persons in urban areas with more than 500 000 inhabitants,

— groups of at least 100 persons, in so far as this is feasible, chosen from among people exposed to significant sources of lead pollution,

— critical groups determined by the competent authorities in the Member States.

In each Member State and during each campaign the number of analyses to be performed shall be 50 or more per million inhabitants.

Article 5

The sampling of the groups referred to in Article 4 shall be carried out during at least two campaigns in each area investigated during the period of operation of the programme, separated by at least 24 months. In the second campaign, samples shall not necessarily be taken from the same persons as in the first campaign.

Article 6

In assessing the results of the biological screening for the purposes of the action provided for in Article 8, the following blood lead levels, which take into account the relationships between dose and effect given in Annex I, shall be taken together as reference levels:

— a maximum of 20 μg of Pb/100 ml of blood for 50% of the group of people examined,

— a maximum of 30 μg of Pb/100 ml of blood for 90% of the group of people examined,

— a maximum of 35 μg of Pb/100 ml of blood for 98% of the group of people examined.

Article 7

Blood lead levels shall be determined as follows:

— Member States shall inform the Commission of the names of the laboratories taking part in the biological screening programme and of the methods of analysis used,

— the Commission, in conjunction with the Member States, shall organize inter-comparison programmes in which the abovementioned laboratories shall participate,

— the Commission, in conjunction with the Member States, shall examine the results of these programmes with a view to improving the comparability of the methods of analysis.

Article 8

Where the results of the analyses indicate that the reference levels set out in Article 6 have been exceeded in one or more cases Member States shall:

— check the validity of the results,

— take action to trace the exposure sources responsible for the levels being exceeded; this shall also include action on all individuals with a blood lead level over 35 μg/100 ml,

— take all appropriate measures at the discretion of their competent national authorities.

Article 9

1. Within six months of notification of this Directive, the Member States shall designate the competent national authorities which shall forward to the Commission:

— the data relating to the biological screening of the population groups referred to in Article 4, together with details of the methods of analysis, the population groups examined and the areas in which samples have been taken; complete anonymity shall be preserved as regards the persons examined; the Commission and the Member States shall agree on the procedures and the method whereby these data shall be forwarded,

— information on the causes or factors presumed to have resulted in the reference levels in Article 6 being exceeded.

2. The competent national authority shall also notify the Commission of the measures taken pursuant to the third indent of Article 8.

Article 10

At least twice a year the Commission shall convene a meeting of representatives of the Governments of the Member States, which shall, in particular:

— ensure that implementation of the biological screening and in particular of the provisions of Articles 4 and 5 is harmonized,

— see that the analyses carried out are comparable,

— examine the information and facilitate the exchange of information between the Member States on the results of the biological screening and on the measures taken pursuant to Article 8.

Article 11

On the basis of the information collected pursuant to Article 9, the Commission shall draw up in cooperation with the competent national authorities:

— a collated annual report on the implementation of the programme, which shall be forwarded to the Member States, the Council and the European Parliament,

— a general report at the end of the programme which will form the basis for drawing up any further proposals taking account of progress made in scientific and technical knowledge.

Article 12

Member States shall take the necessary measures to enable the procedure laid down by this Directive to enter into force within 12 months following its notification and shall immediately inform the Commission thereof.

Article 13

This Directive is addressed to the Member States.

Done at Brussels, 29 March 1977.

For the Council

The President

T. BENN

ANNEX I

RELATIONSHIPS BETWEEN DOSE AND EFFECT

The blood lead levels used in the assessment of the results of the biological screening are based on analysis of the scientific data concerning the various toxic effects of lead. With this analysis, which takes account of normal variations in biological values within the population, it is possible to establish quasi-quantitative relationships between dose and effect. The following relationships between dose and effect are used for references for the purposes of this Directive.

A decrease in ALAD activity in the red corpuscles as a result of exposure to lead may be tolerated for the population, provided that it does not interfere with haematopoiesis. For blood lead levels below 15 to 20 μg/100 ml, a decrease in ALAD activity is not currently considered to interfere with haematopoiesis.

An increase in the protoporphyrin content of erythrocytes in the blood (PPE) indicates interference with the body's use of iron and thus with synthesis of the haem. An increase in PPE has been found at blood lead levels over 20 to 30 μg/100 ml. An increase in PPE may however be due to other causes.

Interference with the glutathione synthesis may be tolerated only for a small fraction of the population and only if it is slight and does not result in any other sub-clinical symptoms. This unacceptable interference with glutathione synthesis is not found at blood lead levels below 30 μg/100 ml.

A significant increase in the excretion of delta-aminolevulinic acid in the urine (ALAU) is a significant sign of disturbance in the metabolism of porphyrins and thus a sign of health impairment. A statistically significant increase in ALAU begins to be found at blood levels above 35 μg/100 ml.

ANNEX II

CORRESPONDENCE BETWEEN BLOOD LEAD LEVELS AND ENZYMATIC ACTIVITY

For the purposes of Article 2 of this Directive, the relationship between blood lead levels and ALAD enzymatic activity measured in accordance with the European standardized method (Annex III) shall be as follows:

Blood lead level (μg/100 ml blood)	*ALAD* (units/litre)
35	20
30	25
20	35

If the ALAD values measured are significantly higher than the above limits for the different sections of population, confirmatory blood lead measurements shall not be required.

ANNEX III

TECHNICAL INSTRUCTIONS FOR MEASURING ALAD ACTIVITY

European standardized method for determining the activity of delta-aminolevulinic acid dehydratase

The principle of the method adopted to determine the activity of delta-aminolevulinic acid dehydratase is well known. It is based on incubation of the enzyme with an excess of delta-aminolevulinic acid substrate. The porphobilinogen (PBG) which forms after a certain length of time is mixed with the modified Ehrlich reagent and the colour obtained is measured against white using a photometer. The quantity of porphobilinogen produced constitutes a measurement of ALAD activity.

METHOD

Effect of light

Recent experiments have shown that PBG in particular is very sensitive to light. The entire analysis should thus be conducted with no direct sunlight at all in the laboratory (and not simply excluded from the point at which the analysis is carried out).

Phase one — Blood sampling and preservation before analysis

— Take a sample of 2 ml venous blood using a plastic syringe (not lead stabilized) in the presence of dried heparin (< 5 mg).

— Prepare immediately and cool to 4 °C four samples of 0·2 ml placed in plastic tubes (not lead stabilized). Graduated Marbourg-type pipettes should be used.

— If the samples are analyzed within three hours, there is no need to chill them.

— Samples should not be preserved at 4 °C for longer than 24 hours.

— Just before analysis, all samples should be placed in a bath of iced water for 10 minutes.

Note: Plastics which may be used include, for example, polythene, polystyrene and polypropylene. The preservation period of 24 hours at 4 °C is a cautious estimate. This interval is long enough for the sample to be removed for analysis from the point at which it was taken to a central laboratory. Three of the four blood samples are used to measure ALAD and the fourth as a blank.

Phase two — Haematocrit reading

This reading must be taken:

— at the same time as the blood sample,

— by a capillary method using two samples.

The sample should be centrifuged, after closing one end of the tube, at atspeed of a least 30 000 rev/minute and for not less than five minutes.

Note: This reading should preferably be taken at once, but in any case not later than 24 hours after the blood sample is taken. If possible a microhaematocrit centrifuge should be used.

Phase three — Haemolysis

— Take three blood samples which have been 'thawed' using 1·3 ml distilled water (preheated to 37 °C) and haemolyse for 10 minutes at 37 ± 0·2 °C.

— Use, for preference, a 2 ml graduated pipette for adding the water and then mix thoroughly.

— Do not shake the samples at this stage.

Note: It has been decided that water should be used rather than Triton X 100 for the haemolysis. Haemolysis experiments using Triton X 100 resulted in a considerable slowing in ALAD activity which has not yet been accounted for and which might be an artifact.

Phase four — Addition of the ALA solution to the haemolysate

— Prepare the ALA solution.

— It should not be prepared more than five hours beforehand.

— Heat this solution to 37 °C for at least 10 minutes before adding it.

— Add 1 ml of this solution to the haemolysate, preferably using a 1 ml volumetric pipette, and mix.

Note: A pH value of 6·4 has been taken, since experiments have shown that it is at this pH that there is the best correlation between ALAD activity and blood lead level in normal populations. It is not necessary to obtain the maximum activity (which can be done by raising the pH), since the activities to be measured are already fairly high.

Phase five — Preparation of the blank

— Take 0·2 ml of blood treated as when measuring ALAD up to the point where the ALA solution is added; in place of this, add 1 ml of TCA-$HgCl_2$ solution, 1 ml of ALA solution, and then proceed as when measuring ALAD (see below).

— Add the solutions using volumetric pipettes.

Note: Only one blank is compared against a series of three tests for each blood sample. If the OD obtained for the blank is very high, the operation should be repeated to check it.

Phase six — Incubation

— 60 minutes at 37 ± 0·2 °C in a water bath.

— Incubation time runs from addition of the ALA solution.

Note: The incubation period of 60 minutes was chosen in order to increase ALAD activity by natural means, given that no other phase leads to any artificial increase in activity. Experiments have shown that the incubation time/activity ratio is linear for intervals over two hours.

The incubation temperature has been kept at 37 °C for practical reasons.

Phase seven — Halting the PBG reaction

— Add 1 ml of TCA-$HgCl_2$ solution to the incubation mixture, preferably using a 1 ml volumetric pipette.

Phase eight — Centrifugation and filtration

— 30 000 rev/minute.

— Filtration using Whatman No 54 paper or equivalent (acid-resistant).

Note: Centrifugation should last about 10 minutes. The filtration phase is included in order to avoid the transfer by pipette of small particles on to the surface of the supernatant liquid. These particles seem to produce a colour reaction with Ehrlich's reagent. A number of tests have shown that inclusion of the filtration phase leads to better reproducibility. This phase can if necessary be replaced by a second centrifugation.

Phase nine — Reaction with Ehrlich's reagent

— Mix 1 ml supernatant liquid with 1 ml modified Ehrlich's reagent, using a 1 ml volumetric pipette.

— Use a Vortex-type mixer to ensure that the mixture is homogeneous.

— Allow the reaction to continue for five minutes before measuring extinction.

Note: To obtain a homogeneous mixture it is very important to ensure that the filtered supernatant liquid is well mixed with the Ehrlich's reagent.

Phase ten — Extinction measurement

— Calibrate the spectrophotometer using a phenolphthaline solution in a basic buffer.

— Compare the extinction measurement of the sample with that of the blank at 555 nm in a 1 cm cell (or 2 cm if the absorption is very low).

Phase eleven — Calculation of enzyme activity

— The equation for calculating enzyme activity is as follows:

$$\frac{\text{OD corr.} \times 35 \times 2 \times 100 \times K}{\text{Hct\%} \times 60 \times 62} = \mu\text{moles ALA/mn/ml RBC} = \text{U/ml}$$

where:

OD = extinction measured,
60 = incubation time,
35 = dilution factor,
62 = molal extinction coefficient in $cm^2/\mu mol$,
K = spectrophotometric correction coefficient.

Note: The units proposed are consistent with the recommendations of the International Union of Biochemistry on enzyme nomenclature.

SOLUTIONS

1. Preparation of an ALA solution

Solution A:
1·78 g $Na_2HPO_4 \cdot 2H_2O$ dissolved in 100 ml distilled water (preferably deionized).

Solution B:
1·38 g $NaH_2PO_4 \cdot 1H_2O$ dissolved in 100 ml distilled water.

29 ml of solution A + 71 ml of solution B give a buffer of 0·1 M sodium phosphate at pH 6·4.

This buffer strength is necessary to prevent any fluctuation of pH value in the solution undergoing the reaction.

167·6 mg ALA-HCl are dissolved in solution B (which must always be acid); the pH is adjusted to 6·4 on the basis of solution A. The volume is then made up to 100 ml using the buffer solution of 0·1 M sodium phosphate at pH 6·4. This preparation gives a solution of 0·01 M ALA.

2. TCA-$HgCl_2$ solution

1·35 g $HgCl_2$ are dissolved in 100 ml 10 % trichloracetic acid.

3. Ehrlich's reagent solution

Reagents:

2·5 g *p*-dimethylaminobenzaldehyde (pDMAB),

0·25 g $HgCl_2$ dissolved in 10 ml glacial acetic acid,

perchloric acid SG 1·7,

glacial acetic acid.

Preparation:

Dissolve the pDMAB in 50 ml acetic acid. Add 24·5 ml perchloric acid and 4 ml $HgCl_2$ solution. Mix, cool and make up to 100 ml with the glacial acetic acid in a graduated flask (1). Store in a dark bottle.

Calibration:

Community-level calibration should be carried out every year by a laboratory jointly appointed by the Member States.

(1) If a brown coloration appears at this point, the reagent should be discarded.