MAXIMUM ALLOWABLE LIMITS IN BIOLOGICAL MATERIALS IN THE PREVENTION OF INORGANIC LEAD POISONING

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In order to prevent poisoning, it is necessary to have clearly in mind what one wants to prevent. There is great confusion concerning the concept of poisoning, especially in medical circles not in direct contact with occupational medicine. What one physician calls " intoxication ", " poisoning", " pathological state ", " disease ", is considered by another merely as a consequence of an allowable " high intake ", " impregnation ". Therefore it is necessary to specify that degree of reaction of the organism which is not allowable and which should be prevented.

POISONING

It is possible to distinguish three concepts of poisoning:

Pharmacological poisoning

The whole range of reactions from the earliest signs to the severest disturbances of functions is included.

Clinical poisoning

There is here a functional disturbance of the organism. This disturbance is observed either by the patient himself (colic, paralysis, *etc.*), or only by the examining physician, *e.g.* decrease in haemoglobin values without corresponding signs or symptoms. Disturbance of important functions conclusively indicates a pathological state, a " disease ".

Compensatable intoxication

This state of poisoning qualifies the sufferer for compensation under the laws relating to industrial diseases. According to the Accident Law of 1921 in the Netherlands, the Social Insurance Bank pays compensation for the loss of income and/or for the costs of medical treatment. With regard to industrial lead-poisoning, compensation is awarded in cases of inability to work, with very few exceptions.

It is evident that the maximum allowable limits (M.A.L.) in biological materials, and the maximum allowable concentration (M.A.C.) in air, will be different according to which of the above concepts of poisoning is adopted.

METHODS OF DETECTION

It is a well-known fact that a *decrease of haemoglobin* is one of the first signs of a functional disturbance: the homeostasis in the " milieu interieur " cannot be sustained. In most cases this decrease in haemoglobin precedes the occurrence of subjective symptoms. The prevention of lead-poisoning should be aimed at *prevention of this early functional disturbance* which can be assessed easily in industrial practice. In most cases the occurrence of subjective symptoms will also be completely prevented in that way (very acute poisoning excepted).

The prevention should be based upon methods which can be used by an occupational physician without requiring extensive laboratory equipment, but which still make it possible to assess early signs of pharmacological intoxication.

One can distinguish two groups of methods:

(a) those which assess haemopoiesis: determination of haemoglobin, basophilia, reticulocytosis, coproporphyrinuria;

(b) those which assess the extent of lead-excretion.

In our Dutch lead-processing industries, which are relatively small, it is not possible to base the prevention on serial quantitative determinations of lead or coproporphyrin in urine; this requires too high a standard of laboratory equipment and a highly specialized laboratory staff. This probably applies also to many industries in other countries.

The following methods are applicable for serial examinations in industrial conditions.

Determination of haemoglobin, Hb (with a Sicca-haemometer)

The reliability under laboratory conditions was investigated in our Institute; for $Hb > 10$ g per cent the standard deviation proved to be 0·28 g per cent.

Determination of basophilia and reticulocytosis per 1,000 erythocytes

An investigation into the methods of staining and counting has been published[1]. The conclusions drawn from this investigation included:

(a) standardization of methods is necessary;

(b) dark-field examination of a slide stained with methylene blue–sodium bicarbonate gives excellent, reproducible results, and makes it possible to count on one slide, at the same time, the basophilia and the reticulocytosis. This method is less tiresome and shorter than light-field methods;

(c) the reliability of counting is as good in dark-field as in light-field methods (staining according to Pappenheim, with $pH \geqslant 7{\cdot}2$): the variation-coefficients are relatively small.

Semi-quantitative determination of the coproporphyrin concentration of the urine

The determinations were carried out with the apparatus developed in our Institute by the late Professor Donath[2].

The standardization of the estimation of the degree of fluorescence, made possible with this apparatus, represents a considerable progress over previously used subjective-estimation procedures. It is possible in a very short time (10 urine samples examined in 15 min) to assess the coproporphyrin content with great reliability on a semi-logarithmic scale with values of 1–8, ranging from 0–50 μg/l. to 3000–5000 μg/l. In our experience this scale is easily read up to a value of 7 (1600–3000 μg/l.), but the difference between 7 and 8 proved to be too small to be distinguished accurately.

ESTABLISHMENT OF AN M.A.L. FOR INDIVIDUALS

The following investigations were carried out to establish the M.A.L.'s for basophilia and coproporphyrinuria, and also to find a correlation with the lead concentration in the air.

Investigation of 117 non-exposed male persons, in order to establish " normal " values

The following " normal " values were established:

Hb: 12·8–17·0 g per cent; average: 15·5 g per cent
5 per cent of values: 12·8–13·7 g per cent

Basophilia: 80 per cent of values: 0·0 per thousand; 20 per cent of values: 0·1–0·5 per thousand

Reticulocytosis: 0·5–9·0 per thousand; average : 2·0 per thousand

Coproporphyrinuria: 70 per cent: 1 (0–50 μg/l.); 20 per cent: 2 (50–100 μg/l.); 10 per cent: 3 (100–200 μg/l.)

It should be understood that these " normal " values do not represent " ideal " values, but those that are generally found on examination of subjectively healthy, non-exposed, male persons from 20–60 years of age. In other investigations it became evident that male persons of a low social scale (unexposed, unskilled workers in lead-processing industries), tended to have a somewhat lower haemoglobin (average 14·0 g per cent) with the same minimum values (12·8 g per cent) as in the above-mentioned group, partly consisting of skilled workers. The other criteria were independent of social class.

Serial investigation in lead processing industries

The industries investigated consisted of a white-lead factory (32 workers) and a red-lead paint factory (7 workers) over a period of 1½ years. The concentrations of lead in air were not measured during the investigation, but, based upon earlier measurements and experience of work-hazard, the exposures of the examined persons could be estimated qualitively. From this investigation the following conclusions could be drawn.

Exposure to low concentrations—This resulted in an increase of coproporphyrin excretion, sometimes also of reticulocytosis and, slightly, of basophilia; there was no decrease in haemoglobin.

Exposure to moderate concentrations—This resulted in a rapid increase of coproporphyrin excretion up to a constant level of (4)–5–7; later, there was an increase of basophilia and reticulocytosis, followed sometimes, depending on the exposure-intensity, by a decrease in Hb-values.

Exposure to high concentrations—There was a rapid increase of coproporphyrin excretion to 5–7, of basophilia and reticulocytosis, and a decrease of haemoglobin; the changes began to occur almost simultaneously.

Level of basophilia—This was inversely related to the level of haemoglobin, and the trend of the basophilia followed the trend of Hb-values.

Level of coproporphyrin excretion—This showed a much less evident correlation with the level of haemoglobin; the coproporphyrin excretion increased initially and then remained at a constant level (5–7) for long periods, also after a decrease in basophilia and an increase in haemoglobin resulting from a transfer to lower exposure; a coproporphyrin excretion value of 1 or 2 was repeatedly found and nearly always excludes a high lead-intake; a value of 3 may indicate a normal as well as too high a lead intake.

Level of reticulocytosis—This generally increases before the basophilia, but as a criterion is less useful, because it is less specific, and because the increase in reticulocytosis is less clear and less rapid than the increase in basophilia; the reticulocytosis is less specific and less sensitive for high exposures.

Intensity of exposure—Disregarding the first few months of exposure, the levels for haemoglobin, basophilia and coproporphyrinuria could not be correlated with the time of exposure, but only with the intensity of exposure; the coproporphyrin excretion, being the most sensitive criterion, only varied in the lower range of exposure-intensities.

In order to prevent a decrease in haemoglobin in an individual, a *maximum allowable limit for the basophilia* could be established; if the level of basophilia remained <1–$1{\cdot}5$ per thousand, there was hardly any substantial decrease in Hb. A temporary increase of basophilia $>1{\cdot}5$ per thousand requires further attention, and can only be tolerated for a short time. In the first few months of exposure the basophilia-response is probably higher, and, in that case, somewhat less stringent limits should be applied. Regarding the examination of an individual, the coproporphyrin excretion is too sensitive a criterion to be used as a practical guide. An elevated coproporphyrin excretion indicates only an increased lead-intake, but gives hardly any evidence as to the extent of the increase. It should be understood that these conclusions are relevant only with regard to excretions determined with the apparatus of Donath: the semi-logarithmic scale tends to level off at a large increase in concentrations, and reaches a maximum at 7 ($\geq$ 1600 μg/l.).

Investigation in different occupations

These occupations represent different exposures to lead. Up to October 1958 about 400 male workers were examined once. None of the workers showed any subjective symptoms necessitating interruption of work. The overall picture showed a considerable increase of rather low Hb-values; 16 per cent had a Hb-value $\leq 12{\cdot}7$ g per cent (below the minimum "normal" range), and 27 per cent a value $\geq 14{\cdot}8$ g per cent. We classified the values for basophilia and coproporphyrinuria according to the levels of Hb, as shown in *Table 1*. Only workers with more than 6 months' exposure were included.

Table 1. Distribution of values for basophilia and coproporphyrinuria in 344 lead-workers arranged according to the Hb-level

	Hb-values (g%)	*Coproporphyrinuria values*	*Basophilia values* (per thousand) 0·0–0·5	0·6–0·9	1·0–1·5	⩾1·6	*Number of workers*	*% of these workers in each group*
Group I 73 workers	⩾14·8	1–2	43	0	1	0	44	60·2
		3–4	18	3	0	0	21	28·8
		⩾5	5	1	1	1	8	11·0
Number of workers			66	4	2	1	73	
% of these workers in Group I			90·4	5·5	2·7	1·4		
Group II 115 workers	13·8–14·7	1–2	50	2	1	0	53	46·1
		3–4	34	5	0	1	40	34·8
		⩾5	15	2	3	2	22	19·1
Number of workers			99	9	4	3	115	
% of these workers in Group II			86·1	7·8	3·5	2·6		
Group III 97 workers	12·8–13·7	1–2	25	2	1	0	28	28·9
		3–4	21	0	1	3	25	25·7
		⩾5	16	9	7	12	44	45·4
Number of workers			62	11	9	15	97	
% of these workers in Group III			63·9	11·4	9·3	15·4		
Group IV 59 workers	⩽12·7	1–2	4	0	0	0	4	6·8
		3–4	5	0	1	0	6	10·2
		⩾5	3	5	7	34	49	83·0
Number of workers			12	5	8	34	59	
% of these workers in Group IV			20·3	8·5	13·6	57·7		

If we accept a value of 4 as M.A.L. for coproporphyrinuria, then, according to *Table 1*

11 per cent of the workers with Hb $\geqslant 14{\cdot}8$ per cent,
19·1 per cent of those with Hb 13·8–14·7 g per cent,
45·4 per cent of those with Hb 12·8–13·7 g per cent,
and 83 per cent of those with Hb $\leqslant 12{\cdot}7$ g per cent

would have been compelled to discontinue their exposure.

From this it will be evident that such an M.A.L. for coproporphyrinuria would create too sensitive a criterion.

It would be attractive to attempt the same analysis with a higher scale value, *e.g.*, 5 or 6, but because of the variability between individuals with values ranging from 5 up to 7 the reliability of this method does not seem to be sufficiently guaranteed.

The percentage of the persons with a basophilia $\geqslant 1{\cdot}0/1000$ shows a very sharp increase in the last Hb-group. According to the table, 24·7 per cent of the Hb-values 12·8–13·7 g per cent and 71·3 per cent of the values $\leqslant 12{\cdot}7$ g per cent would have been prevented if the M.A.L. for basophilia had been accepted as 1·0/1000. In reality this criterion would result in higher percentages than indicated above, because *Table 1* included workers of the following types:

(a) those with very long exposure, in which cases the basophilia tends to fall;

(b) those with low Hb-values, not due to reaction to lead;

(c) those who were convalescing after poisoning and therefore already exhibited decreased basophilia.

From the investigation of 344 male workers it can be concluded that:

(a) applying an *M.A.L. for basophilia of* 1·0/1000 prevents a substantial decrease in a Hb;

(b) the level of coproporphyrin excretion, determined according to Donath's method, is too sensitive a criterion to be applied as an M.A.L.

These conclusions are valid for the evaluation of the state of health of *individuals*.

ESTABLISHMENT OF AN M.A.L. FOR GROUPS OF WORKERS

So far we have only dealt with individuals, but it also proved to be possible to establish an *M.A.L. for groups of workers*. We investigated several occupational groups; workers in pigment factories, accumulator factories, lead burners (chemical industries, shipyard), painters, printers, *etc.* Comparing the frequency-distribution of the values for coproporphyrinuria and haemoglobin, we found in groups of more than 20 workers a consistent relationship between:

(a) the average coproporphyrin excretion $\left(\frac{\Sigma \text{ porphyrin values}}{n}\right)$ and the percentage of Hb-values $\leqslant 13{\cdot}7$ g per cent;

(b) the percentage of coproporphyrinuria values $\geqslant 2$ and of the Hb-values $\leqslant 14{\cdot}7$ g per cent (where n = number of workers).

The data are given in *Table 2.*

Table 2. Relation of coproporphyrin excretion and Hb-values in several occupational groups

Occupational group	*Number of persons*	*Average coproporphyrinuria*	% of Hb – $\leqslant$13·7 g%	% of copr. $\geqslant$2	% of Hb – $\leqslant$14·7 g%
Non-exposed workers	117	1·4	4	(26)	(26)
Printers	53	1·5	23	40	57
Workers exposed to < 0·05 mg Pb/m³	30	1·8	33	50	70
Department of pigment-factory producing lead-free pigments	27	2·1	33	71	71
Technical staff of pigment-factories	61	2·3	24	63	67
Painters	50	2·4	27	80	59
Workers exposed to 0·05–0·1 mg Pb/m³	47	2·6	17	77	64
Lead-burners (shipyard)	44	2·7	46	73	90
Wet production-departments of pigment factories	28	3·2	39	89	75
Workers exposed to 0·1–0·15 mg Pb/m³	45	3·3	47	82	78
Lead-burners (chemical industry)	36	4·4	47	92	84
Workers exposed to >0·15 mg Pb/m³	38	4·7	74	100	95
Small pigment factories	29	4·8	69	97	97
White lead factory	32	4·9	75	79	97
Dry milling departments of pigment factory	44	4·9	76	98	93
Small accumulator factories	21	5·0	62	95	81

Statistical calculation of the regression-equations and correlation-coefficients yields the following results:

$y = 15{\cdot}5x - 6{\cdot}9$

$r = 0{\cdot}91^{***}$ ($P < 0{\cdot}001$)

y = percentage with Hb $\leqslant 13{\cdot}7$ g per cent

$$x = \frac{\Sigma \text{ porphyrin values}}{n}$$

$y = 0{\cdot}5x + 37{\cdot}3$

$r = 0{\cdot}67^{**}$ ($P < 0{\cdot}01$)

y = percentage with Hb $\leqslant 14{\cdot}7$ g per cent

x = percentage with coproporphyrinuria $\geqslant 2$

From the frequency-distribution of the values for coproporphyrin excretion it is, therefore, possible to estimate the extent of disturbance of haemopoiesis. The most important indicator for Hb-values in a group is the percentage of Hb-values $\leqslant 13{\cdot}7$ per cent, *i.e.*, the low values. This percentage shows a very high correlation with the average coproporphyrinuria. Determination of the frequency-distribution of the coproporphyrinuria in an occupational group is a quick and easy procedure; about 50 persons can be examined within one hour.

The observed frequency-distribution of coproporphyrinuria therefore indicates in a *group* of workers:

(a) the presence of a lead problem;

(b) the severity of the lead problem.

In our investigations the fact that 25–30 per cent of the Hb-values were $\leqslant 13\cdot7$ g per cent in the non-skilled workers in lead-processing industries did not necessarily indicate a decrease in haemoglobin due to lead. Therefore *an average coproporphyrin excretion of 2–2·5*, determined by Donath's method, may be considered as the *maximum allowable limit in a group of workers* ($n > 20$).

It should be possible to establish similar relations between the frequency-distributions of the basophilia and haemoglobin, but this would not be of any value, because, if the basophilia is examined, the haemoglobin can be examined as well at the same time.

RELATION BETWEEN LEAD CONCENTRATION, DURATION OF EXPOSURE AND REACTION OF THE ORGANISM

Finally, we tried to establish the relation between the lead concentration in air (c) and the duration of exposure (t) on the one hand, and the reaction of the organism on the other hand. The values of c and t were determined by the Section for Industrial Hygiene of the Institute for Health Technique T.N.O.; the reaction of the organism was assessed by the Department o Occupational Medicine of the Netherlands Institute for Preventive Medicine. The lead-intake of groups of workers was therefore *independently* assessed by technical hygiene methods and by medical methods. In his paper, Dr Hartogensis will deal more extensively with the technical hygiene aspect of this investigation. In this paper only some relevant points from the medical examination will be presented.

The investigation took place in 2 pigment factories of the same size (81 and 80 workers respectively), manufacturing lead and lead-free pigments. The workers were classified according to average exposure, *i.e.*, during their whole working time, t, as estimated by Dr Hartogensis. As no correlation could be established between either haemoglobin, basophilia, reticulocytosis or coproporphyrinuria with t, there was no objection against classifying the workers into classes according to c, disregarding t, as shown in *Table 3*.

Statistical calculation yielded the following correlation-coefficients (r):

	Factory *A*	Factory *B*
c and haemoglobin	$r = -0\cdot24$*	$r = -0\cdot51$***
c and coproporphyinuria	$r = 0\cdot51$***	$r = 0\cdot65$***
c and basophilia	$r = 0\cdot35$**	$r = 0\cdot44$***
c and reticulocytosis	$r = 0\cdot25$*	$r = 0\cdot32$**

The classification into different C-classes according to the technical data was more feasible for factory B than for factory A. Group C_2-factory A had a higher average exposure than group C_2-factory B. These facts partly explain the higher correlation-coefficients in factory B. The exposure for C_2-B could be estimated to be $<0\cdot1$ mg Pb/m^3, the exposure for C_2-A $<0\cdot15$ mg Pb/m^3. The laboratory data for both C_2-groups differ

Table 3. Median and range for the laboratory data in relation to average exposure for two factories *A* and *B*

		Factory A	Factory B
Class C_1 No (or hardly any) exposure to lead	*n*	5	15
	Haemoglobin (g%)	14·4 (13·4–15·2)	14·1 (13·6–16·0)
	Coproporphyrinuria (Donath)	1 (1–2)	1 (1–3)
	Basophilia (/1000)	0·0 (0·0–0·2)	0·1 (0·0–0·4)
	Reticulocytosis (/1000)	3·0 (1·0–4·9)	4·6 (1·0–8·3)
Class C_2 Exposure to 0·05–0·15 mg Pb/m³	*n*	47	51
	Haemoglobin (g%)	13·7 (10·9–15·7)	14·4 (10·9–16·8)
	Coproporphyrinuria (Donath)	3 (1–7)	2 (1–5)
	Basophilia (/1000)	0·3 (0·0–3·2)	0·1 (0·0–1·2)
	Reticulocytosis (/1000)	6·0 (1·2–21·2)	4·6 (0·8–13·5)
Class C_3 Exposure to 0·15–0·65 mg Pb/m³	*n*	19	11
	Haemoglobin (g%)	13·5 (11·2–15·0)	12·8 (11·2–14·4)
	Coproporphyrinuria (Donath)	5 (2–7)	5 (2–6)
	Basophilia (/1000)	0·5 (0·0–3·0)	1·1 (0·0–2·7)
	Reticulocytosis (/1000)	7·0 (1·5–16·6)	10·6 (0·7–19·6)
Class C_4 Exposure to >0·65 mg Pb/m³	*n*	6	3
	Haemoglobin (g%)	13·0 (10·6–15·0)	11·5 (9·6–13·1)
	Coproporphyrinuria (Donath)	5·5 (4–6)	5 (5–6)
	Basophilia (/1000)	2·6 (0·0–4·9)	1·2 (0·1–7·1)
	Reticulocytosis (/1000)	7·8 (3·7–18·8)	11·8 (2·0–28·5)

significantly ($P < 0{\cdot}05$). The groups from *Table 3* may be arranged according to decreasing haemoglobin (*i.e.*, increasing functional disturbance): $C_1\text{-}A = C_1\text{-}B \rightarrow C_2\text{-}B \rightarrow C_2\text{-}A \rightarrow C_3\text{-}A = C_3\text{-}B \rightarrow C_4\text{-}A = C_4\text{-}B$. Group C_2-*B* differs from C_1-*A* and C_1-*B* only in a slightly elevated coproporphyrin excretion; the haemoglobin generally has not decreased in contrast to group C_2-*A*. The M.A.C. should therefore be somewhere between the average exposure for group C_2-*B* and C_2-*A*, *i.e.*, <0·15 mg Pb/m³. Dr Hartogensis will deal more extensively with this side of the problem.

From these data the frequency-distributions for coproporphyrinuria and other laboratory data could be established in relation with air concentrations. These " standard frequency distributions " can be used to estimate the value of *c* for other occupational groups. The frequency-distribution of the coproporphyrin excretion is most useful for this purpose because it may easily be assessed and the correlation with air-concentration is a high one. A frequency-distribution with an average of 2–2·5 will correspond with the M.A.C. value.

From this investigation it may be concluded that the M.A.C. for inorganic lead should not be raised to 0·2 mg Pb/m³ as proposed by the American Conference of Governmental Industrial Hygienists (1957), in any case not in industries with a moderate personal hygiene. The *M.A.C. should rather be lowered to about 0·1 mg Pb/m³* if prevention of significant disturbance in haemopoiesis is aimed at.

Note added in proof

These investigations have been extended to over 500 workers; the results are essentially the same. A full report is given in: R. L. Zielhuis " De industriële loodintoxicatie in Nederland (Industrial Lead Poisoning in the Netherlands) ", *Thesis*, Leiden (1959). The significance of coproporphyrinuria for the screening of groups of workers has been discussed in *Brit. J. Ind. Med.*, **18**, 58 (1961); the maximum allowable limits will be discussed in *Zentr. Arbeitsmed. u. Arbeitsschutz* (in press).

References

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