British Journal of Industrial Medicine 1982;**39**:157-160

Assessment of the body burden of chelatable lead: a model and its application to lead workers

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ABSTRACT A hypothetical model was introduced to estimate the body burden of chelatable lead from the mobilisation yield of lead by calcium disodium ethylenediamine tetra-acetate (CaEDTA). It was estimated that, on average, 14 and 19% of the body burden was mobilised into the urine during the 24 hours after an injection of 53·4 μmol (20 mg) and 107 μmol (40 mg) CaEDTA per kg bodyweight, respectively. The body burden of chelatable lead ranged from 4 μmol (0·8 mg) to 120 μmol (24·9 mg) (mean 37 μmol (7·7 mg)) in lead workers with blood lead concentrations of 0·3-2·9 μmol/kg (6-60 μg/100 g) (mean 1·4 μmol/kg (29 μg/100 g)). There were linear relationships between blood lead concentrations and body burden of chelatable lead on a log scale.

In assessing the dose-response relationships of lead the dose may be estimated indirectly from the blood lead (BPb) and urinary lead concentrations, and the mobilisation yield of lead by calcium disodium ethylenediamine tetra-acetate (MPb).[1] There has been, however, no evidence that any of the indicators is correlated with lead concentrations in critical organs.[2] The blood lead concentration is considered to be the most reliable indicator of recent exposure, and to be particularly useful for epidemiological studies.[2] MPb, on the other hand, may serve as a "chemical biopsy" of the concentration of lead in critical organs,[2] and be a sensitive indicator of excess lead absorption.[3] The following evidence also suggests that there is some difference in the biological significance of BPb and MPb: (1) there is a curvilinear relationship between BPb and MPb[4 5]; (2) age does not correlate significantly with BPb in "healthy" adults under "normal" environmental conditions[6 7] whereas MPb does[8]; and (3) there are different diminution rates between BPb and MPb after cessation of occupational exposure.[9]

Teisinger *et al*[10] attempted to quantify the body burden of chelatable lead in rabbits. The point on which special emphasis should be placed in their study was that the MPb amounted, on average, to 8·3% of the body burden of chelatable lead, regardless of the period after termination of lead exposure.

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Received 2 February 1981
Accepted 29 May 1981

An attempt has been made to introduce a model for estimating the body burden of chelatable lead from the mobilisation yield of lead by calcium disodium ethylenediamine tetra-acetate (CaEDTA) in lead workers.[11] In the present paper the model is simplified so that only two days of CaEDTA injection are needed for the estimation. The percentage of the body burden of chelatable lead that is mobilised into the urine by CaEDTA is then estimated, and some estimate of the actual size of the body burden in lead workers is made. In addition, BPb is related to the body burden thus estimated.

Model for estimation of body burden of chelatable lead

The relationship between MPb and the body burden of chelatable lead is assumed to be as follows: $MPb = k A$ .....(1), where A is the body burden of chelatable lead just before CaEDTA injection, and k is a constant. The linear relationship between MPb and body burden of chelatable lead has been confirmed in rabbits.[10]

In man at least 90% of CaEDTA was excreted in the urine by seven hours after the injection[12]; and lead was mostly mobilised into urine during the first 24 hours after CaEDTA injection.[13] When the CaEDTA injection is repeated on two successive days, the mobilisation yield of lead on the first day [MPb(1)] is kA. The size of chelatable lead is then reduced from A to A—kA + αA—that is, $(1 - k + \alpha)$ A, where αA is the quantity of chelatable lead converted from non-chelatable lead during the 24 hours

after the CaEDTA injection. Hence the mobilisation yield on the second day [MPb(2)] is k (1 − k + α) A; and the chelatable lead is reduced to (1 − k + α)²A.
That is,
MPb(1) = k A .....(2),
and
MPb(2) = k(1 − k + α)A .....(3).
Dividing equation (3) by equation (2), an equation for the value of k is derived:

$$k = 1 - \frac{MPb(2)}{MPb(1)} + \alpha \quad \text{.....(4).}$$

The biological half time of non-chelatable lead should be very long since this form of lead is assumed to be firmly bound to the bone matrix.[10] Therefore, the daily conversion rate from non-chelatable lead to chelatable lead is almost negligible and so is the value of α. Hence equation (4) may be now written:

$$k = 1 - \frac{MPb(2)}{MPb(1)} \quad \text{.....(5).}$$

The value of A can be calculated by rearranging equation (2):

$$A = \frac{MPb(1)}{k} \quad \text{.....(6).}$$

The values of k and A are calculated from equations (5) and (6) when MPb(1) and MPb(2) are measured.

Estimation of the body burden of chelatable lead in lead workers

The nature of the procedure was explained to all the subjects and this study was carried out with their informed consent. After measurements of BPb, CaEDTA was injected into eight male lead workers in a daily dosage of 53·4 μmol (20 mg) per kg bodyweight in 250 ml of 5% glucose solution for one hour for two successive days (study 1). Urinary lead excretion was measured on both 24-hour urinary samples. The adequacy of urinary sampling was checked by measurements of urinary creatinine and specific gravity together with notification by the subjects. The subjects were free from occupational lead exposure during the period of the urinary collection. None had ever suffered from renal disease, and results of urine analysis for albumin was negative in all cases.

Blood and urinary lead concentrations were measured by atomic absorption spectrophotometry (Perkin-Elmer 403) after wet ashing, chelation by ammonium pyrrolidine dithiocarbamate and extraction to water-saturated methylisobutylketone.[8] When 53·4 μmol (20 mg) CaEDTA per kg bodyweight was injected into 25 lead-unexposed male Japanese with BPbs 0·2-1·0 μmol/kg (4-21 μg/100 g) (mean 0·6 μmol/kg (12 μg/100 g)), MPb averaged 0·46 μmol/24 h (95 μg/24 h) with lower and upper 95% confidence limits of 0·16 and 0·86 μmol/24 h (34 and 179 μg/24 h).[8] The reproducibility of analysis of urinary lead was 2·9% when expresssed as a coefficient of variation.

Estimated k and A values are shown in table 1 together with BPb, MPb(1), MPb(2); the age and occupation of each subject are also shown in this table. It is estimated that about 14% of the body burden of chelatable lead is mobilised into the urine by the injection of 53·4 μmol CaEDTA per kg bodyweight. This estimation is similar to that found in rabbits.[10] Further studies of the k value for other animal species would disclose whether the similarity is truly biological.

Figure 1 shows a linear relationship between BPb and the body burden of chelatable lead on a log scale. The BPb of 2·9 μmol/kg (60 μg/100 g) corresponds to a body burden of 87 μmol (18 mg) on the regression line; and the BPb of 0·5 μmol/kg (10 μg/100 g), to a body burden of 6 μmol (1·2 mg). These

Table 1 *Estimated values of A and k from study 1: dose of CaEDTA = 53·4 μmol (20 mg) per kg bodyweight*

Subject			*BPb* (*μmol/kg*)	*MPb(1)* (*μmol/24 h*)	*MPb(2)* (*μmol/24 h*)	*A* (*μmol*)	*k*
No	*Age (y)*	*Occupation (y)*					
1	32	Lead smelter (4)	2·9	14·20	12·07	95	0·15
2	64	Lead founder (33)	2·9	16·83	14·43	120	0·14
3	57	Lead smelter (28)	1·5	3·19	2·80	27	0·12
4	59	Welder (26)	0·9	1·63	1·45	15	0·11
5	60	Paint maker (1)	0·8	2·64	2·00	11	0·24
6	68	Type founder (21)	0·5	1·00	0·84	6	0·16
7	43	Stereotype founder (24)	1·7	2·16	1·93	20	0·11
8	44	Enameller (20)	0·3	0·29	0·27	4	0·07
Mean	53		1·4	5·24	4·47	37	0·14

BPb = Blood lead concentration (1 μmol/kg = 21 μg/100 g).
MPb(1) and MPb(2) = Mobilisation yield of lead by CaEDTA on the first day and the second day, respectively (1 μmol/24 h = 207 μg/24 h).
A = Body burden of chelatable lead just before the first CaEDTA injection (1 μmol = 207 μg).
k = Proportion of MPb(1) to A.

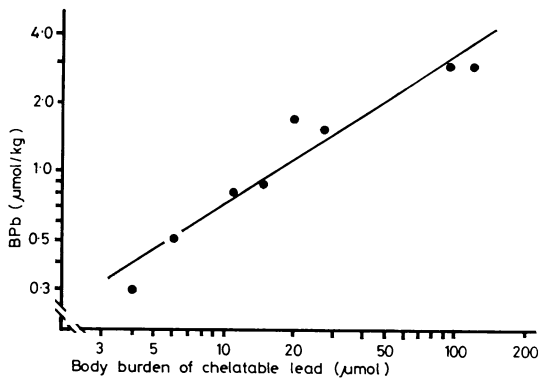

Fig 1 *Relationship between blood lead (BPb) and body burden of chelatable lead (A). Study 1: the regression equation, significance level for the regression coefficient (p) and correlation coefficient (R) are log BPb = 0·650 log A − 0·798 (BPb = 0·159 A $^{0.650}$), p < 0·001 and R = 0·966, respectively.*

values for the body burden of "chelatable" lead are extremely small when compared with the reported values of the body burdens of "total" lead; 8166 μmol (1692 mg) in a lead worker[14] and 404 μmol (83·7 mg) in healthy Japanese man.[15]

The regression equation of BPb on body burden of chelatable lead (fig 1) suggests that as the body burden increases the incremental rise in BPb decreases progressively. The same evidence has been given by the relationship between BPb and MPb in children and adolescents[4] and in lead workers.[5]

Table 2 shows estimated k and A values when the dose of CaEDTA was increased to 107 μmol (40 mg) per kg bodyweight (study 2). In this case the first six of the 13 subjects were the same as those examined by 53·4 μmol CaEDTA per kg bodyweight (study 1): the time interval between the two studies was over two weeks. There is also a linear relationship between BPb and the body burden of chelatable lead on a log scale (fig 2). The difference in the k value between the two studies is significant when all the subjects in tables 1 and 2 are compared by the Wilcoxon rank sum test ($p < 0·05$): but the difference is not significant in a paired comparison for the paired six subjects (Wilcoxon signed rank test: $p > 0·05$), probably due to the small sample size.

Comments on validity and reproducibility of the estimation

The introduction of the k value in this study is based on two assumptions: (1) the proportion of MPb to the body burden of chelatable lead in lead workers is constant regardless of the size of the chelatable lead pair, as in rabbits,[10] and (2) non-chelatable lead is converted to chelatable lead in negligibly small quantities during the 24 hours after the CaEDTA injection.

The validity of the first assumption may be tested by examining the correlations between the k and A values. When tested under the null hypothesis using Spearman's rank correlation coefficient, the correlation is not significant in either of the two studies ($r_S = 0·137$ and 0·280 for studies 1 and 2, respectively $p > 0·05$). The first assumption, therefore, could not be discarded.

With regard to the second assumption, there is, so far, no evidence against it. The half time of MPb diminution is extremely long in lead workers and in rabbits after the termination of lead exposure. [9 10] For instance, the half time of diminution was 103 and 174 weeks in two lead workers when 53·4 μmol CaEDTA per kg bodyweight was injected once a week for 3·5 years.[9] Hence the diminution rate of MPb (and also chelatable lead) is assumed to be 0·7 and 0·4% a week, respectively. In other words, the quantity of chelatable lead is reduced from A to 0·993A or 0·996A during a week after a single injection of CaEDTA. On the other hand, after the

Table 2 *Estimated values of A and k from study 2: dose of CaEDTA = 107 μmol (40 mg) per kg bodyweight*

Subject			*BPb* (*μmol/kg*)	*MPb(1)* (*μmol/24 h*)	*MPb(2)* (*μmol/24 h*)	*A* (*μmol*)	*k*
No	*Age (y)*	*Occupation (y)*					
1	32	Lead smelter (4)	2·8	15·18	10·21	46	0·33
2*	64	Lead founder (33)	1·8	5·14	4·51	43	0·12
3	57	Lead smelter (28)	1·3	3·40	2·88	23	0·15
4	59	Welder (26)	1·0	1·78	1·44	9	0·19
5	60	Paint maker (1)	0·8	1·74	1·48	11	0·15
6	68	Type founder (21)	0·5	1·08	0·95	9	0·12
7	36	Battery maker (18)	2·8	5·91	5·12	45	0·13
8	38	Stereotype founder (19)	2·0	3·01	2·32	13	0·23
9	21	Plastic worker (3)	2·3	14·83	11·12	59	0·25
10	51	Lead mill worker (3)	1·9	4·02	3·22	20	0·20
11	42	Lead burner (21)	1·8	5·14	4·51	43	0·12
12	29	Battery maker (10)	1·3	4·81	3·36	16	0·30
13	67	Dye maker (2)	0·4	0·40	0·33	2	0·17
Mean	48		1·6	5·36	4·06	25	0·19

*CaEDTA was injected nine times for two months between studies 1 and 2. Abbreviations as in table 1.

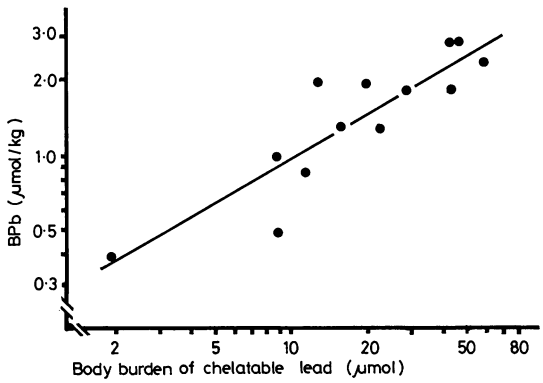

Fig 2 *Relationship between blood lead (BPb) and body burden of chelatable lead (A). Study 2: the regression equation, significance level for the regression coefficient (p) and correlation coefficient (R) are log BPb = 0·590 log A − 0·609 (BPb = 0·246 $A^{0.590}$), $p < 0.01$ and R = 0·883, respectively.*

single injection, the chelatable lead is reduced from A to $(1 - k + \alpha)A$ for 24 hours, $(1 + \alpha - \beta)(1 - k + \alpha)A$ for two days, $(1 + \alpha - \beta)^2(1 - k + \alpha)A$ for three days, and $(1 + \alpha - \beta)^6(1 - k + \alpha)A$ for seven days: β is the proportion of spontaneous 24-hour urinary excretion to chelatable lead and is assumed to be, on average, 0·012 in our preliminary study.[11] Therefore,

$(1 + \alpha - \beta)^6(1 - k + \alpha)A = 0.993A$ or $0.996A$.

This equation is rearranged by substituting 0·012 and 0·14 for β and k, respectively, as follows:

$(\alpha + 0.998)^6(\alpha + 0.86)A = 0.993A$ or $0.996A$.

Only one value of α can be obtained from this equation. That is,

$\alpha = 0.022$.

The conversion rate thus assumed, therefore, is not too large by comparison with the value of k. The detail of the estimation will be reported in another paper. The reproducibility of the estimation of the value for k was assessed by calculating k values five times in a 59-year-old retired male lead worker, whose BPb was 0·8 μmol/kg (17 μg/100 g) at the start of the examination. CaEDTA was injected in a daily dosage of 53·4 μmol per kg bodyweight in 250 ml of 5% glucose solution for two successive days with an interval of three weeks. The k values obtained were 0·22, 0·18, 0·23, 0·28, and 0·30 (average 0·24).

We thank Dr R Chizuka, Mr S Yanagihara, Miss Y Kunugi, and Mrs S Nakahira for their technical help and Professor T Suzuki for his valuable suggestions.

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