DOSE-RESPONSE RELATIONSHIPS AT DIFFERENT EXPOSURE LEVELS – NECESSITY OF REEVALUATION IN ESTABLISHING NO-EFFECT LEVELS

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ABSTRACT

When criteria or standards of exposure to environmental chemicals or pollutants are being established, dose-effect or dose-response relationship is employed in order to determine no-effect levels of a substance.

PbB and ALA-U were determined in workers from three lead factories which have three different levels of air lead in the working environment. Dose-effect and dose-response relationships between PbB and ALA-U of these three different exposure populations indicated three different curves, although theoretically they should all have indicated an identical or similar curve where the no-effect level should be at a single dose level. The no-effect level observed for the highest exposure group was lower than that for the lowest exposure group. The difference is statistically explained for the most part by variations in the determinations of dose (PbB) as well as effect (ALA-U). However, another reason is most probably due to the fact that effect (ALA-U) remained at increased levels even after PbB levels decreased, although this was not actually demonstrated in this study.

In view of the results obtained in this study, it would seem that reexamination and reevaluation are required in establishing the criteria of no-effect levels for dose-response relationships of various environmental chemicals.

LD_{50} or ED_{50} determined in various animal species are widely used as an indicator of the toxicity of a chemical. In animal experiments a clear sigmoid-shaped curve indicating dose-response relationship is usually obtained. Recently this type of observation using a dose-response relationship curve has also been frequently applied in epidemiological studies on humans for deriving the no-effect level of a given chemical. However, in human epidemiological studies, study design cannot be planned as well as in animal experiments. First of all, a toxic chemical cannot generally be administered to humans in amounts so large as to cause toxic effects. Thus information on the *exact dose* of exposure cannot be obtained in both exposed and control populations, and the *dose* or grade of exposure is frequently represented by the concentration of a chemical in *indicator media*[7]. Furthermore, effects are very often found in control populations which

are not necessarily specific for the chemical concerned. Also, the concentration in indicator media may be determined long after an effect has developed when the concentration has decreased but the effect still remains. These conditions may serve as obstacles to an epidemiological study which aims to determine the toxicity of a chemical.

In recent studies[8,10] the present authors found that *no-effect levels* in dose--effect or dose-response relationships differed according to subject populations with different exposure levels. The present study was conducted to demonstrate these differences and to discuss theoretical reasons for the differences in three population groups with different grades of exposure. Since indicator media and early effects in lead exposure have been well documented[1,7,8,10] a model using lead studies will be demonstrated in this paper.

MATERIALS AND METHODS

Concentrations of lead in air of working environments were determined in three lead plants with clearly different grades of exposure. The lowest concentration of lead was in a rubber hose factory where 170 workers were employed (Factory A). The medium exposure group was composed of 154 lead workers from a storage battery plant (Factory B). The third plant, which showed the highest lead concentration in the air of workplace, was a lead reclaiming smelter (Factory C) in which 56 workers were examined.

Lead in blood (PbB) and delta-aminolevulinic acid in urine (ALAU) were determined in a total of 380 workers from the three factories. PbB was determined by atomic absorption spectrometry after wet ashing and MIBK extraction. ALAU was determined by the Mauzerall-Granic method[6].

RESULTS

Table 1 shows the means of PbB and ALAU and correlations between the two. Lead concentrations in air (PbA) in each factory are shown in the same table. As was expected, there are good correlations among PbA, PbB and ALAU. However, it should be noted that the higher the exposure to lead, the

TABLE 1
PbB, ALAU and PbA for each factory.

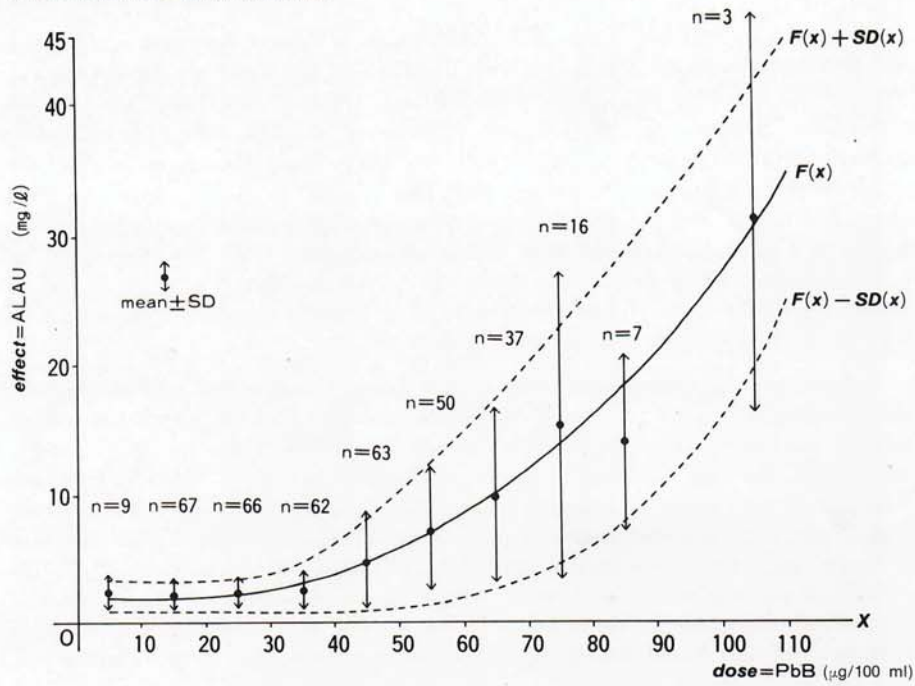| | No. of samples | PbB (μg/100 ml) | | ALAU (mg/l) | | r(PbB, ALAU) | PbA(μg/m^3) |
		Mean	S.D.	Mean	S.D.		
Factory A	170	23.8	10.8	2.3	1.3	0.235**	0– 60
Factory B	154	48.0	14.9	6.1	5.1	0.513**	50– 150
Factory C	56	61.1	16.0	11.3	10.1	0.626**	150–1890

**: $P < 0.01$

FIG. 1 – Dose (PbB) – effect (ALAU) relationship of lead workers. F(x): theoretical dose--effect relationship. S. D. (x): theoretical standard deviation of F(x).
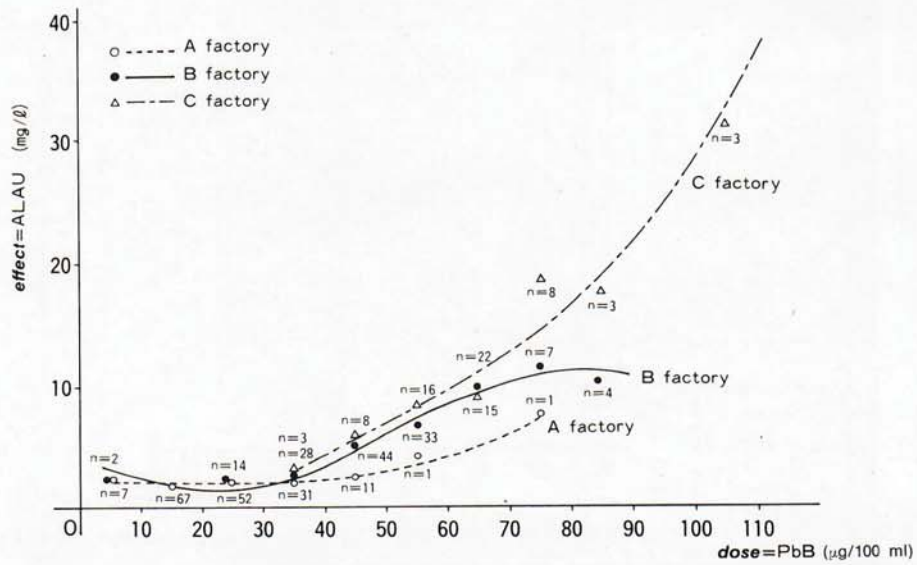

FIG. 2 – Theoretical dose (PbB) – effect (ALAU) relationships of lead workers in 3 factories.

greater the standard deviations. The relationship is shown in Figure 1, which is the polynominal regression curve with its standard deviation of the association between PbB (dose) and ALA values (effect). Data of the three factories are combined. Similar regression curves have been already observed by many authors including ourselves. Figure 2 shows the polynominal regression curves of dose-effect relationship between PbB and ALAU in each factory. As seen from this figure, ALAU values in the factories with greater lead exposure are higher at a given level of PbB than in the factories with lower lead exposure. In particular, the differences in the means of ALAU between Factories A and B were statistically significant in the samples ranging from 30 μg to 40 μg/100 ml as well as in PbB samples 40 μg $-$ 50 μg/100 ml.

Tsuchiya and co-workers[11] reported a mean ALAU value with a standard deviation of 2.0 mg/l $\pm$ 1.0 mg/l in policemen who had no abnormally high exposure to lead. Thus the highest "normal" standard value of $\overline{X} + 3$ S.D. should be 5.0 mg/l and the values exceeding 5.0 mg/l were defined as "abnormal" or "positive response", and the "positive response" of ALAU at each PbB level was calculated. Figure 3 shows the dose-effect (see the left ordinate) and the dose-response (the right ordinate) curves between PbB and ALAU of all workers in the three factories. As can be seen from this figure, the dose-response curves follow a sigmoid shape. The dose-response relationships between PbB and ALAU according to factories are shown in Figure 4. Again it is
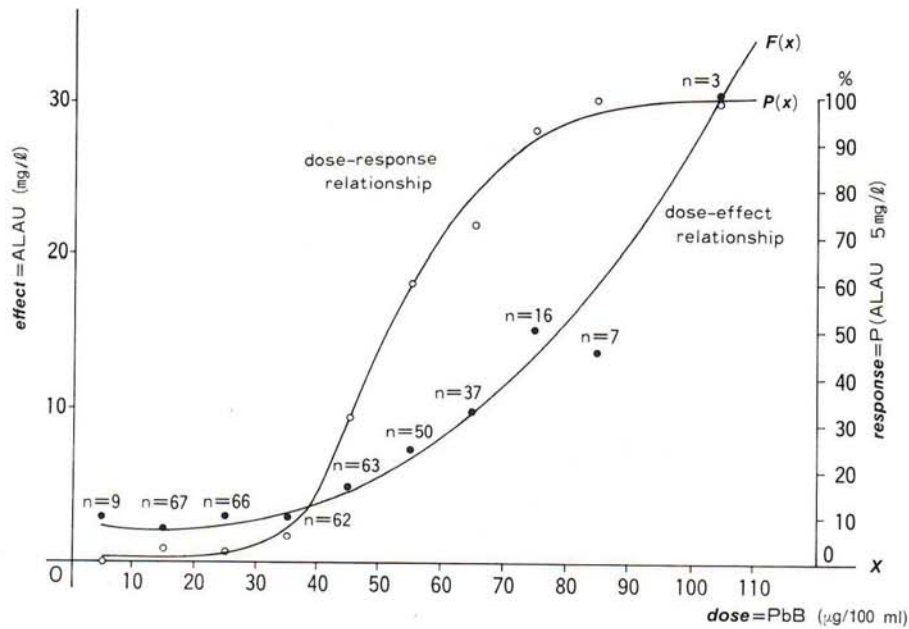

FIG. 3 $-$ Dose (PbB) $-$ response [P(ALAU $>$ 5 mg/100 ml)] relationship and dose-effect (ALAU) relationship in lead workers. P(x): theoretical dose-response relationship; F(x): theoretical dose-effect relationship; Open circles: observed response; Solid circles: observed effect.
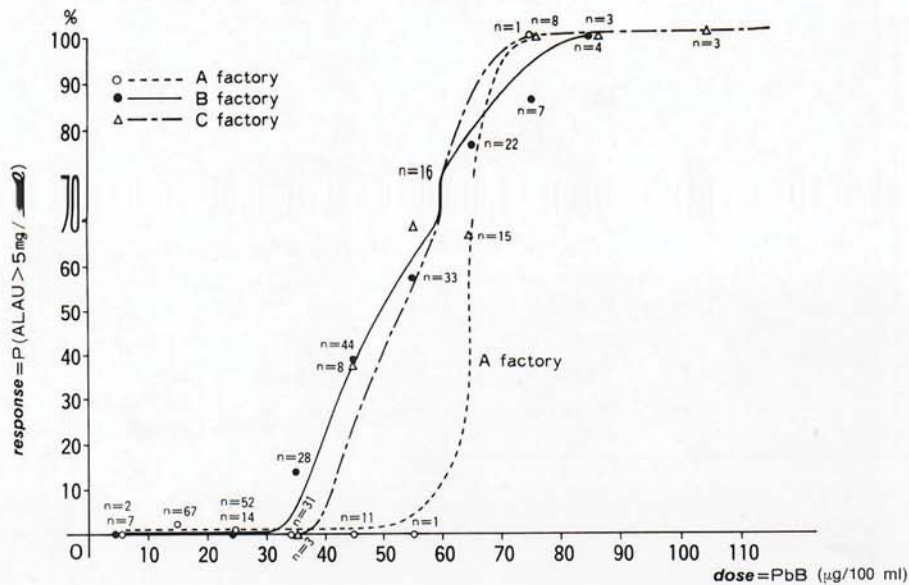
FIG. 4 – Theoretical dose (PbB) – response [P(ALAU) > 5 mg/l] relationships of lead workers in 3 factories.

clear that the response at a given value of PbB is higher in the high lead exposure group than in the low lead exposure group. If a PbB value of, for example, 50 μg/100 ml is taken, only a few per cent show response in Factory A while in Factories B and C 40 to 50 per cent show response. However, because of an insufficient number of samples, the difference between Factories B and C is not distinct.

EVALUATION AND COMMENTS

As clearly observed, the regression curves of dose-effect or dose-response relationships differ according to the grade of lead exposure; in other words, ALAU excretions are higher among workers exposed to greater amounts of lead than among those exposed to lower amounts of lead at the same PbB level. The reason for this difference was examined as explained below and the following conclusions were drawn: 1) the existence of artificial variation caused by measurement errors of both *dose* and *effect*, and 2) effects in higher exposure are altered not only qualitatively but quantitatively as well.

When "dose" is plotted on the abscissa and "response" on the ordinate and $x = f(X)$ and $y = g(Y)$ are properly converted, a positive linear regression should be obtained. Theoretically, regression curves of dose-response relationship obtained from a high exposure group and a low exposure group are expected to show similar inclines or slopes and fall on the same line. If there are no measurement errors, two probable ellipses of dose-response relationships obtained from both high and low exposure groups should show purely biological
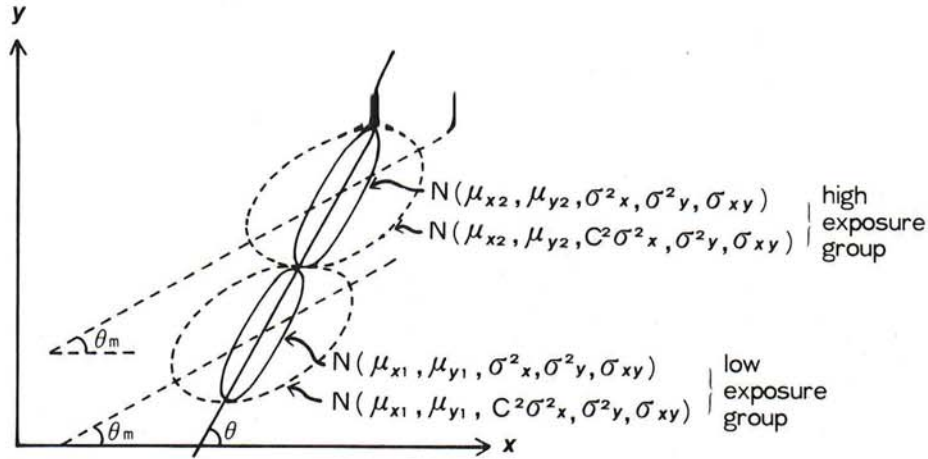
FIG. 5 – Theoretical and observed probable ellipses and their major axes for high and low exposure groups. Solid line: theoretical, dotted line: observed; x = f (dose); y = g (effect) or y = g (response); $C^2 > 1$.

variations, as indicated in Figure 5. When the means of the low exposure group (x) are expressed as μx_1 and μy_1, and those of the high exposure group (y) as μx_2 and μy_2, and the variance and the covariance of x, y are expressed as $\sigma^2 x$, $\sigma^2 y$ and σxy, the distributions of the low exposure group and the high exposure group are represented by two variable normal distributions as N (μx_1, μy_1, σx^2, σy^2, σxy) and N (μx_2, μy_2, $\sigma^2 x$, $\sigma^2 y$, σxy) respectively.

The solid lines in Figure 5 show the major axes with probable ellipses of both the low and high exposure groups, provided there is no measurement error. The slope of the major axes[5] is

$$\tan \theta = \frac{-(\sigma^2 x - \sigma^2 y) + \sqrt{(\sigma^2 x - \sigma^2 y)^2 + 4\sigma^2 xy}}{2\sigma xy} = \frac{\mu y_2 - \mu y_1}{\mu x_2 - \mu x_1}$$

Now, if the measurement error ($\sigma \varepsilon^2$) of "dose" is added, the total variation is $\sigma x^2 + \sigma \varepsilon^2$, $\sigma^2 x + \sigma^2 \varepsilon = C^2 \sigma^2 x$ ($C^2 > 1$). The two variable normal distributions are expressed as N (μx_1, μy_1, $C^2 \sigma^2 x$, $\sigma^2 y$, σxy) for the low exposure group and N (μx_2, μy_2, $C^2 \sigma^2 x$, $\sigma^2 y$, σxy) for the high exposure group. This is shown by the dotted lines in Figure 5. The slope of the major axes, in this case, is

$$\tan \theta m = \frac{-(C^2 \sigma^2 x - \sigma^2 y) + \sqrt{(C^2 \sigma^2 x - \sigma^2 y)^2 + 4\sigma^2 xy}}{2\sigma xy}$$

The grade of variation of tan θm due to measurement error is differentiated by C^2,

$$\frac{d \tan \theta m}{dC^2} = \frac{\sigma^2 x}{2\sigma xy} \left(\frac{C^2 \sigma^2 x - \sigma^2 y}{\sqrt{(C^2 \sigma^2 x - \sigma^2 y)^2 + 4\sigma^2 xy}} - 1 \right)$$

Since the association between x and y is positive correlation and $\sigma xy > 0$, $\dfrac{d \tan \theta m}{dC^2}$ $\therefore$ $\tan \theta > \tan \theta m$ and $\theta > \theta m$. Thus, if "dose" is accompanied by meameasurement error, the slope of the major axis of the probable ellipse becomes less steep. Since the slopes of the two major axes from the low and high exposure groups are equal and pass the average of each ellipse $(\mu x_1, \mu y_1)$, $(\mu x_2, \mu y_2)$, they become parallel, as shown in Figure 5. Furthermore, the slopes of the regression coefficients of the two groups are $\beta = \sigma xy/\sigma^2 x$ when there is no measurement error, and $\beta m = \sigma xy/C^2\sigma^2 x = \beta/C^2$ when "dose" is accompanied by measurement error. The correlation coefficient without measurement error is $\rho = \sigma xy/\sqrt{\sigma^2 x \times \sigma^2 y}$ and that accompanied by measurement error, $\rho m = = \sigma xy/\sqrt{C^2\sigma^2 x \sigma^2 y} = \rho/(C)$. Since $C^2 > 1$, both the regressions and correlation coefficients become lower with greater measurement error.

According to the above theoretical or mathematical explanation, it is clear that the theoretical elongated probable ellipse with pure biological variation becomes rounded due to measurement error which is practically unavoidable.

The second point which was raised earlier in this paper, i.e., that effects in higher exposure are altered not only qualitatively but quantitatively as well, should also be taken into consideration. Sakurai and co-workers[8] have shown that a decrease in ALAD activity remains for some time after a decrease in PbB in lead exposed workers. Means and standard deviations of ALAU by PbB levels are shown in Table 2. The average ALAU of Factory A (low exposure) indicates the lowest value and Factory C the highest for both the $30-40$ µg/100 ml and $40-50$ µg/100 ml categories. In higher exposures to chemicals, effects may remain for some time after cessation of exposure or decrease of the chemical in index media. The time lapsed differs of course according to the chemical in question. However, it is rather difficult to measure the duration of effects due to various chemicals after exposure at the present time.

TABLE 2
Means of ALAU by PbB levels.

| ALAU (mg/l) / PbB (µg/100 ml)	Factory A			Factory B			Factory C			Total		
	mean	S.D.	n	mean	S.D.	n	mean	S.D.	n	mean	S.D.	n
$30 \sim 40$	2.2*a	1.0	31	3.1*a	1.9	28	3.4	0.9	3	2.6	1.5	62
$40 \sim 50$	2.7*a	1.0	11	5.2*a	3.8	44	5.9	5.6	8	4.9	3.8	63
Total $(30 \sim 50)$	2.3**a**b	1.0	42	4.4**a	3.3	72	5.2**b	4.8	11	3.8	3.1	125

* : P < 0.05
** : P < 0.01
a : between Factories A and B
b : between Factories A and C

The above facts and theoretical proof that dose-response relationship curves vary according to observation groups with different grades of exposure will thus affect the establishment of a no-effect level or screening level. Figure 6 explains schematically this situation where the difference between high and low exposure groups is not very great. As shown in the figure, the probable ellipses are very close to each other, but the difference in the screening levels derived from the low and high exposure groups is great and very distinct. In this case, a great portion of the low exposure group will belong to the affected group if the screening level of the high exposure group is used. In fact, taking an example from data shown in Figure 4, the screening level of PbB derived from the lowest exposure group (Factory A) exists around 50 μg/100 ml whereas another screening level from the higher exposure groups (Factory B or C) is lower than 40 μg/100 ml. This example corresponds to a smaller difference in exposure level, as shown in Figure 6.
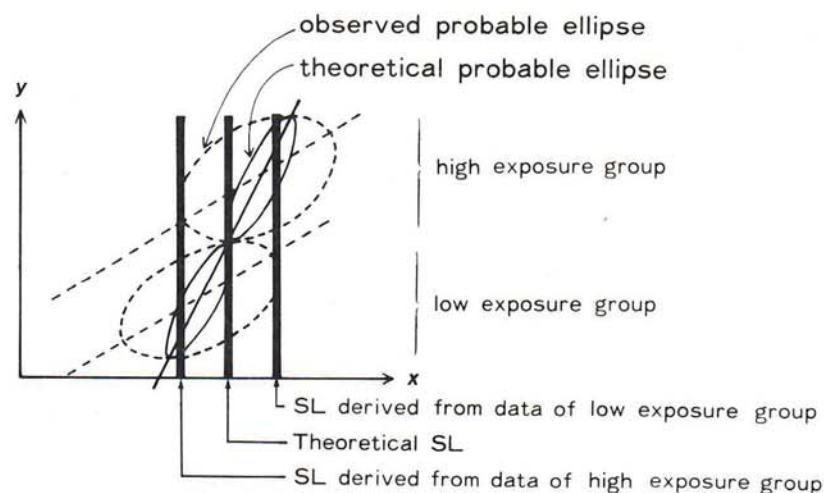
FIG. 6 – Screening levels derived from data of high and low exposure groups.

If the difference in exposure is very great, the difference in the screening levels becomes smaller. Thus, in this case, the screening level derived from the high exposure group seems to be valid. However, even in this case, as shown by Minamata disease[3,4], the figures of dose from people with the disease and a control group overlap. The average and standard deviation of brain mercury concentrations of 15 fatal cases of Minamata disease were 8.41 μg/g and 7.15 μg/g respectively, with a range of 0.09 – 24.8 μg/g. Those in the kidney in 14

fatal cases were 51.0 µg/g and 41.4 µg/g with a range of 3.11 – 106.0 µg/g. In a control group, the average mercury concentration in the brain of eight cases was 0.32 µg/g with a standard deviation of 0.51 µg/g and a range of 0.05 – 1.54 µg/g. Those in the kidney were 2.38 µg (average), 3.49 µg/g (standard deviation) and 0.25 – 10.7 µg/g (range). When mercury concentrations in the organs are taken as "dose" for Minamata disease victims and non-Minamata disease controls where there is a distinct difference in response, portions of the two probable ellipses overlap.

For cadmium, a critical concentration (200 µg/g) in the renal cortex which may cause a critical change in the kidneys has been proposed[2]. On the other hand, the cadmium concentrations in the renal cortex of 326 autopsy cases of sudden death in Tokyo without known high exposure to cadmium were reported to be 46.2 µg/g on an average, with a standard deviation of 36.3 g and a high of 202 µg/g[9]. The report calculated a value of the upper three standard deviations as 256 µg/g for a logarithmic normal distribution. The value of 256 µg/g exceeds well the aforementioned critical concentration of 200 µg/g, which seems to be conservative when the reasoning for this level by the authors is closely examined. However, as mentioned a number of times, the application of this critical concentration derived from high exposure groups cannot be applied indiscriminately to the general population. If a screening level derived from a high exposure group is applied to a much lower exposure group, the "false positive" probability will be greater than the true situation. On the other hand, if a screening level derived from a lower exposure is applied to a higher exposure group, it will result in greater "false negative" probability than expected.

CONCLUSION

In three groups of workers with different exposures to lead from three factories, dose-effect and dose-response relationships between PbB and ALAU were closely examined. It was clearly demonstrated that no-effect level or no--response level changed according to the grade of lead exposure. No-effect level derived from a higher exposure group was shown to be lower than that derived from a lower exposure group. This difference was caused by measurement errors, particularly of "dose", and it was shown that according to measurement error both probable ellipses and their major axes differed from those which were expected. Thus the screening levels determined by different exposure groups will result in different figures, and careful evaluation is required in establishing the screening or no-effect level of a given chemical.

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