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The Relevance of Arguments for Excluding ALAD from the Recommended Biological Limit Values in Occupational Exposure to Inorganic Lead (WHO 1980)*

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Summary. Data on 394 simultaneous measurements of blood lead (PbB) and biological indicators of effect are considered with regard to dose-effect and dose-response relationships, as well as the association between the biological indicators of effect themselves. The indicators are delta-aminolevulinic acid dehydratase (ALAD) and zinc-protoporphyrin (ZPP) in blood, delta-aminolevulinic acid (ALAU) and coproporphyrin (CPU) in 24-h urine specimens. The specimens were taken from periodically controlled male workers "moderately" to "excessively" exposed to inorganic lead. In addition, data are presented on the spontaneous recovery of biological indicators in 14 male workers examined immediately after, approximately 4.5 months and 10 months after cessation of lead exposure. Highly significant ($P < 0.001$) correlations were found between all of the indicators examined, with the following order of agreement with regard to PbB: ALAD > ZPP > ALAU > CPU. Comparative advantages of ALAD in typical (variable) occupational exposure conditions were found to include: (a) the highest sensitivity at both low and relatively high lead exposure levels, (b) better reflection of biologically active lead as opposed to PbB (particularly compared to ALAU and CPU), (c) higher specificity compared to other indicators of lead effect, and (d) generally higher reliability with regard to both biologically and methodologically induced variations. The data obtained undoubtedly demonstrate that urinary indicators ALAU and CPU are not sensitive enough for the recommended health-based occupational exposure limit, as defined by relatively low PbB concentration (WHO 1980). Despite possible theoretical considerations resulting in recommendations for ZPP and ALAU but excluding ALAD (WHO 1980), practical implications seem to be

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far more in favour of ALAD, which permits a maximal safety margin in preventing "adverse" effects in the entire work population because of its sensitivity and absence of time-lag with regard to lead exposure.

Key words: ALAD – Occupational lead exposure – Biological limit values – PbB – Indicators of lead effect

The depression of erythrocyte delta-aminolevulinic acid dehydratase (ALAD) activity is almost[1] unanimously considered the most sensitive indicator of early lead effect (WHO 1977; Zielhuis 1977). ALAD already has a highly significant correlation with blood lead (PbB) in the very low PbB range, and the methodology is simple and standardized (Berlin and Schaller 1974) coupled with relatively high precision. Despite these advantages, this biological indicator of increased lead absorption has failed to receive adequate attention in keeping with its relative advantages over other parameters relevant in the control of occupational lead exposure. In the recent report by the WHO Study Group on recommended health-based limits in occupational exposure to inorganic lead (WHO 1980) surprisingly less attention was paid to ALAD, as compared to the previous report of another expert group dealing with the same subject (Zielhuis 1977). At the same time, the recommended exposure limit defined by PbB was lowered (from 600 µg/l to 400 µg/l for males, and from 400 µg/l to 300 µg/l for females in the reproductive age range) to a level almost undetectable by any other sensitive biological or electrophysiological parameter characteristic for lead induced effect. Taking into account the concept of protecting the individual, not a "group", it is hardly credible that any other indicator of effect, apart from ALAD, could be considered sensitive enough to provide a safety margin for the entire work population in such a low PbB range. However, the alternative health-based recommendations for erythrocytic protoporphyrin (PP) and urinary delta-aminolevulinic acid (ALAU)—"since PbB measurements are impracticable in some countries" (WHO 1980)—appear to have practically no margin of safety at all in variable lead exposure. This is obvious if one considers the apparently more typical dose-response data in the previous report (Zielhuis 1977), which indicate the relatively lower sensitivity of different parameters (including PP and ALAU). This is also obvious if one allows for the possibility of underestimation of recent excessive lead absorption, when using PP or zinc-protoporphyrin (ZPP), due to the time-lag between the increase of lead absorption and the appearance of the equivalent ("steady-state") ZPP or PP levels in the peripheral blood (Beritić et al. 1977, 1978a; Telišman 1979).

It is generally agreed (Nordberg 1976; WHO 1977, 1980; Zielhuis 1977) that the PbB level mainly reflects current (recent) lead exposure. Therefore, PbB might give relatively poor information on biologically active lead in the organism (particularly in variable external exposure conditions typical for occupational lead exposure), which is more relevant when assessing a health hazard. Zielhuis (1977) concluded

1 Though not clear, there are only two known exceptions in favour of erythrocyte protoporphyrin (PP): (1) one is in the tabulation of data but not the text of Hernberg (1976), also cited by Nordberg (1976); (2) the other is on p. 50 but not on p. 53 of WHO (1980). Both exceptions are probably because PP was considered a "relevant" and ALAD a "non-relevant" effect

that in addition to blood lead, *simultaneous measurement* of at least one indicator of effect should be performed for evaluation of health significance. In agreement with *this concept* and the fact that after 10 years' experience with ALAD we found it to be the most reliable and sensitive indicator of increased lead absorption in investigations dealing with widely different lead exposure conditions (acute and chronic, low level and high level, variable and relatively stable), more evidence on the comparative advantages of ALAD over other biological indicators will be presented.

The aim of this paper is to stress the well-known fact, often hidden in theoretical generalisations, that the "average" response might have widely different limits when the conditions differ and that the so-called "steady-state condition" cannot always be expected in environmental health practice, and particularly not in occupational health practice (WHO 1977, p. 133). Data derived from experimental studies on animals or carefully selected (homogenous) population groups under strictly controlled (stable) exposure conditions can be misleading to those less well acquainted with the pharmacokinetic and pharmacodynamic characteristics of inorganic lead in human organism. On the other hand, diversity is the rule; therefore, in epidemiologic studies attention should be focused on dissimilarity and not on similarity (Zielhuis 1978). The main danger of undesirable effects due to occupational lead exposure might come from both undetected past over-exposure and recent incidental excessive exposure episodes, while the highest internal exposure levels are not reflected the same way in different biological indicators with regard to the time-related changes in intensity and duration of external lead exposure. The data selected for presentation in this paper might explain why, in cases of highly variable or undefined exposure conditions, the whole spectra of biological indicators should occasionally be applied in order to obtain a true picture of the dynamic changes in individual exposure levels.

Subjects and Methods

Data are presented for the period 1977–1980 on 394 simultaneous measurements of biological indicators of increased lead absorption: PbB, ALAD, ZPP, ALAU and CPU in male workers (average age 39 years, range 26–63 years) periodically controlled for lead exposure in our department (T. Beritić, Head, Department for Occupational Diseases). The population group examined consisted of workers mainly long-term (average duration 16 years, range 4–32 years) "moderately" to "excessively" exposed to inorganic lead in a crystal glass factory, a ceramics factory and in the manufacture of lead articles. The group was selected to represent the relationship between different biological indicators in typical (variable) occupational exposure conditions. In addition to this, data are shown on the spontaneous recovery (no chelation therapy) of biological indicators in 14 male workers following 0 and approximately 4.5 and 10 months after cessation of lead exposure.

Heparinized venous blood samples and 24-h urine specimens (average diuresis 1205 ml, range 910–1620 ml) were used for analysis. All determinations were performed in duplicates within 24 h of specimen collection, and the mean values were used for the calculations. PbB was determined according to the modified ET-AAS method (Fernandez 1975) verified by British QCS and CEC QC programmes, CV $\leq$ 5%; ALAD by the standard European method (Berlin and Schaller 1974), CV $\leq$ 2%; ZPP by means of Bell-Aviv hematofluorometer (Blumberg et al. 1977) calibrated in mmol ZPP/mol Hb, CV $\leq$ 5%; ALAU by means of the Bio-Rad Lab. test (Davis and Andelman 1967), CV $\leq$ 4%; CPU by the modified spectrofluorometric method (Weber and Valić 1957),

CV ≤ 5%. The established "normal" values of biological indicators are ($\bar{x} \pm$ SD): PbB 185 ± 77 μg/l, ALAD 43.5 ± 8.7 European units, ZPP 0.22 ± 0.05 mmol/mol Hb, ALAU 3.40 ± 1.26 mg/d, CPU 67.5 ± 25.0 μg/d. Normal limit values (NLV) are defined as $\bar{x} \pm 2$ SD.

The results of simultaneous determination of biological indicators were considered with regard to blood lead by means of dose-effect and dose-response relationships, as well as indirectly by the association between the biological indicators of effect themselves. Statistical evaluation included correlation and regression analyses for the semi-logarithmic dose-effect relationships, and correlation analyses for the logarithmic relationships between indicators of effect. The "validity" (i.e. "sensitivity" + "specificity") of indicators of effect in predicting PbB values is calculated according to MacMahon and Pugh (1970), as recommended by Zielhuis and Verberk (1974). Due to a skewed distribution and a wide scatter of data, the results on spontaneous recovery of the biological indicators in different periods following cessation of lead exposure are presented by group median and range values.

Results

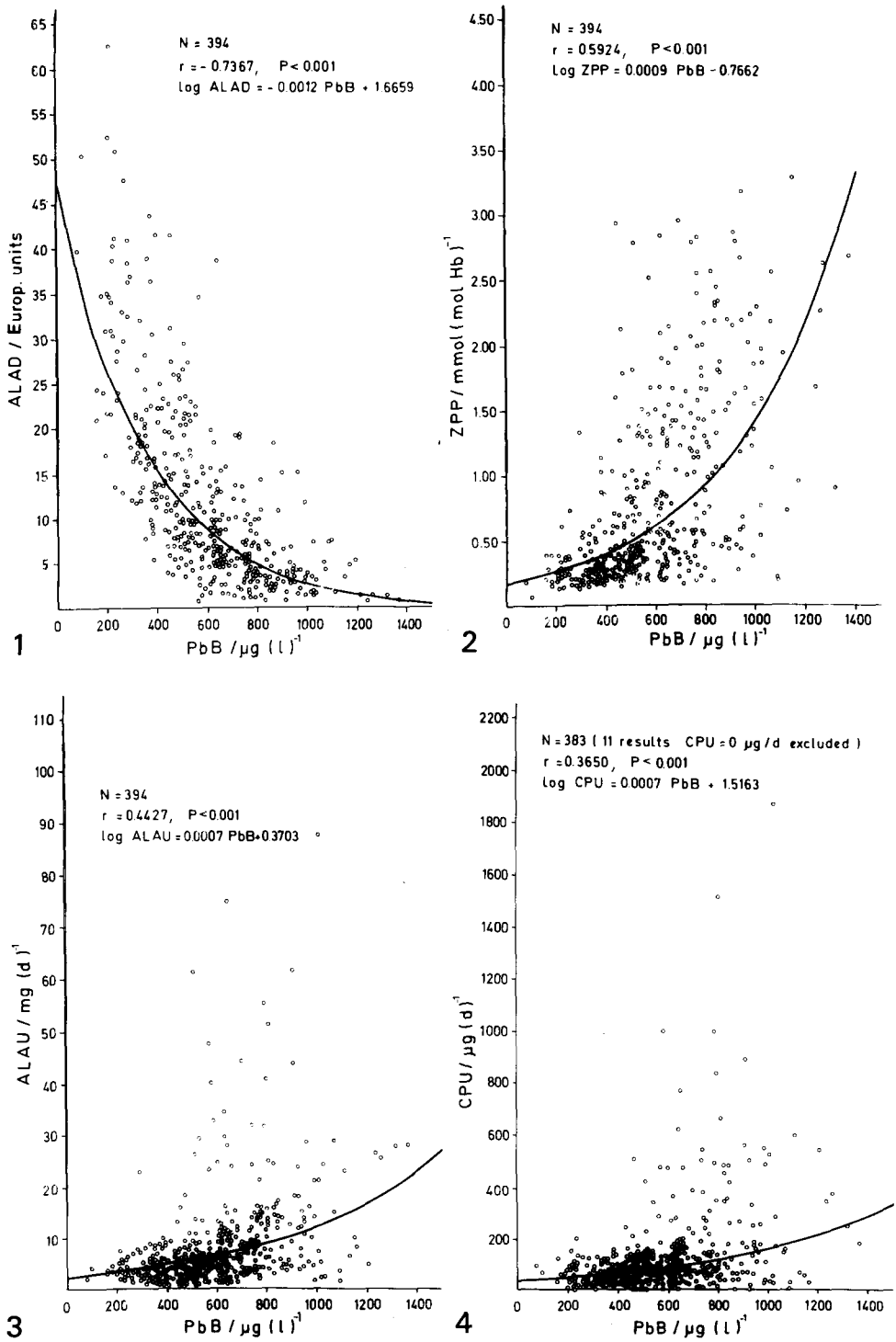
The dose-effect relationships between PbB and ALAD, ZPP, ALAU, and CPU, respectively, together with the corresponding correlation coefficients *(r)*, levels of significance *(P)* and equations obtained by regression analyses for the semi-logarithmic relationships, i.e. $\log y = ax + b$, are presented in Figs. 1–4.

Table 1 shows the decreasing order of relative agreement between the biological indicators examined, considered directly/indirectly with regard to PbB, indicated by the values of the correlation coefficients.

The dose-response relationships between PbB and ALAD, ZPP, ALAU, and CPU, respectively, are presented in Figs. 5–8. These results are shown as the percentage response of a total number of subjects indicating PbB level in 100 μg/l segments (i.e. PbB of 50–149, 150–249, 250–349 μg/l, etc.), and were not statistically evaluated further for better insight into the variations in the specific PbB segment. PbB levels < 550 μg/l and > 750 μg/l were mainly due to the recent variations of long-term established average lead exposure (≥ 8 years' follow-up, ≥ twice a year) for the population examined. It should be pointed out that, in most workers, the actual values of biological indicators showed considerable improvement and lower lead exposure level as compared to the past.

The "validity" ("sensitivity" + "specificity") of ALAD, ZPP, ALAU, and CPU in predicting the actual PbB levels is presented in Table 2. However, in the authors' opinion, the "validity" of PbB per se in predicting the "true" situation, i.e. the internal lead dose which caused the effect, should be considered with caution since it might be influenced by certain relevant factors (e.g. time elapsed from the change of exposure level, variable versus "stable" exposure, acute versus chronic exposure, integrated versus recent exposure level). The validity of PbB may be very high in predicting *recent external exposure* level, but in *variable* exposure it might not be of high validity in predicting the actual internal *lead dose at the site(s) of effect.* Particular attention should be paid to the fact that the *actual* levels of lead in *blood* cannot be regarded as a "true" situation and a "cause" of the actual ZPP (or PP) levels in the peripheral blood, since these are causally related to the average lead dose in the *bone marrow* during the *preceding 3–4 months.*

The data on spontaneous recovery of the simultaneously measured biological indicators in 14 male lead workers, examined immediately after, approximately 4.5

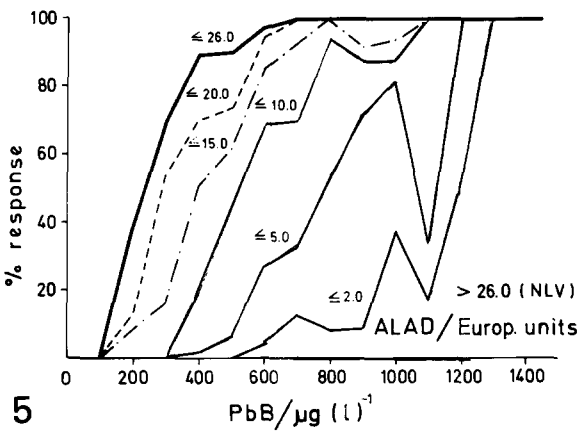


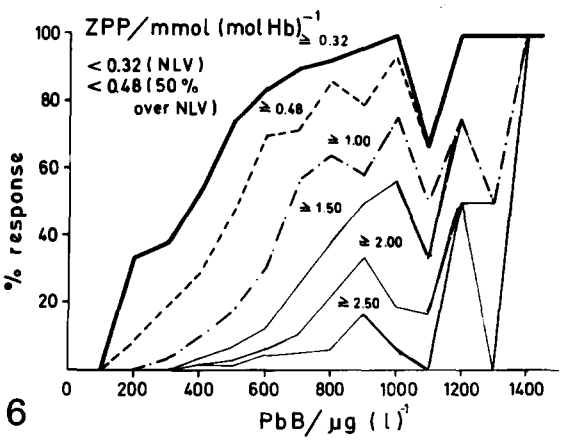
Figs. 1–4. Dose-effect relationships between *PbB* and *ALAD*, *ZPP*, *ALAU*, and *CPU*, respectively

Table 1. Order of relative agreement between biological indicators, considered directly/indirectly with regard to PbB

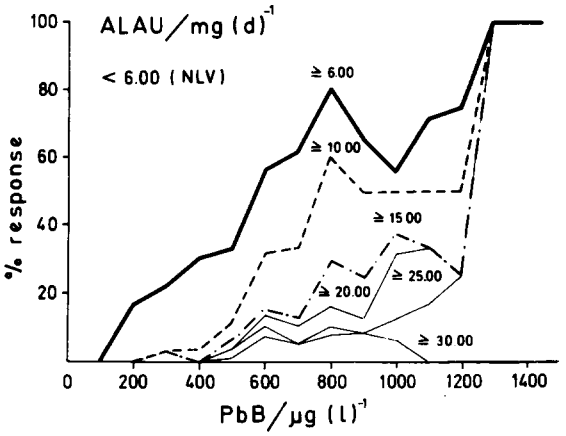
Relationship between biological indicators	Correlation coefficient[a] *(r)*
Log ALAD/log ZPP	−0.742
Log ALAD/*lin PbB*	−0.737
Log ALAU/log CPU	0.696
Log ZPP/log ALAU	0.600
Log ZPP/*lin PbB*	0.592
Log ALAD/log ALAU	−0.557
Log ALAD/log CPU	−0.523
Log ZPP/log CPU	0.489
Log ALAU/*lin PbB*	0.443
Log CPU/*lin PbB*	0.365

[a] $P < 0.001$ for *all* the relationships examined





Figs. 5–8. Dose-response relationships between *PbB* and *ALAD, ZPP, ALAU,* and *CPU,* respectively



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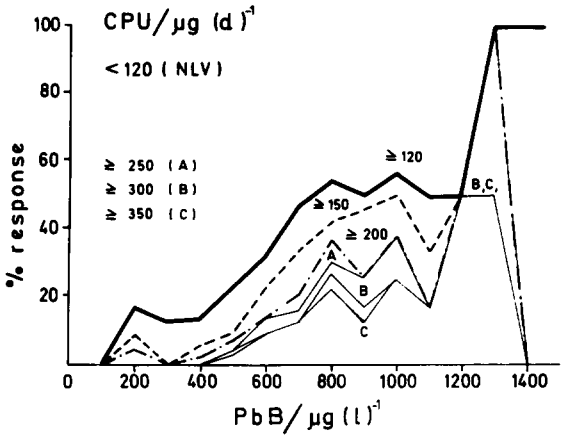

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months and 10 months after cessation of a recent excessive lead exposure (due to lead leakage in the ceramics factory), are presented in Table 3 and Fig. 9.

Discussion

According to the dose-effect data (Figs. 1–4) it is obvious that in the range of PbB levels up to 700 µg/l, which is the range relevant for the *control* of occupational lead exposure, ALAD is the most sensitive biological indicator of effect. According to the negative semi-logarithmic relationship, the change of ALAD per unit of increase in PbB is far more pronounced compared to other indicators of effect which, according to their positive semi-logarithmic relationship, are more sensitive in the higher PbB range.

The biological reasoning for including the relationships between indicators of effect themselves is based on the assumption that *both* PbB and the indicators of effect are *dependent* variables of the "true" *internal lead dose* and *biologically active*

Table 2. The "validity" ("sensitivity" + "specificity") of biological indicators of effect in predicting PbB levels

PbB ≥ 400 μg/l:

	se	sp	se + sp		se	sp	se + sp
ALAD/European units:				*ZPP/mmol(mol Hb)$^{-1}$:*			
< 26.0	0.97	0.37	1.34	> 0.32	0.83	0.62	1.45
< 20.0	0.90	0.55	1.45	> 0.48	0.66	0.83	**1.49**
< 15.0	0.82	0.78	**1.60**	> 1.00	0.40	0.97	1.37
< 10.0	0.66	0.93	1.59	> 1.50	0.23	1.00	1.23
< 5.0	0.33	1.00	1.33	> 2.00	0.12	1.00	1.12
ALAU/mg(d)$^{-1}$:				*CPU/μg(d)$^{-1}$:*			
> 6.00	0.54	0.78	**1.32**	> 120	0.38	0.88	**1.26**
> 10.00	0.33	0.98	1.31	> 150	0.28	0.97	1.25
> 15.00	0.17	0.99	1.16	> 200	0.19	0.99	1.18
> 20.00	0.12	0.99	1.11	> 250	0.16	1.00	1.16
> 25.00	0.09	1.00	1.09	> 300	0.13	1.00	1.13

PbB ≥ 600 μg/l:

	se	sp	se + sp		se	sp	se + sp
ALAD/European units:				*ZPP/mmol(mol Hb)$^{-1}$:*			
< 26.0	0.99	0.20	1.19	> 0.32	0.87	0.42	1.29
< 20.0	0.99	0.37	1.36	> 0.48	0.78	0.66	**1.44**
< 15.0	0.94	0.54	1.48	> 1.00	0.54	0.88	1.42
< 10.0	0.82	0.73	**1.55**	> 1.50	0.33	0.96	1.29
< 5.0	0.48	0.94	1.42	> 2.00	0.18	0.98	1.16
ALAU/mg(d)$^{-1}$:				*CPU/μg(d)$^{-1}$:*			
> 6.00	0.68	0.71	**1.39**	> 120	0.48	0.82	**1.30**
> 10.00	0.47	0.92	**1.39**	> 150	0.38	0.92	**1.30**
> 15.00	0.24	0.95	1.19	> 200	0.26	0.95	1.21
> 20.00	0.17	0.96	1.13	> 250	0.24	0.97	1.21
> 25.00	0.11	0.97	1.08	> 300	0.18	0.98	1.16

PbB ≥ 800 μg/l:

	se	sp	se + sp		se	sp	se + sp
ALAD/European units:				*ZPP/mmol(mol Hb)$^{-1}$:*			
< 26.0	1.00	0.13	1.13	> 0.32	0.94	0.32	1.26
< 20.0	1.00	0.24	1.24	> 0.48	0.86	0.52	1.38
< 15.0	0.96	0.37	1.33	> 1.00	0.68	0.76	**1.44**
< 10.0	0.90	0.55	1.45	> 1.50	0.51	0.89	1.40
< 5.0	0.72	0.85	**1.57**	> 2.00	0.30	0.95	1.25

Table 2 (continued)

ALAU/mg(d)$^{-1}$:				*CPU/µg(d)$^{-1}$:*			
> 6.00	0.70	0.58	1.28	> 120	0.56	0.73	1.29
> 10.00	0.58	0.80	**1.38**	> 150	0.51	0.84	**1.35**
> 15.00	0.35	0.91	1.26	> 200	0.37	0.90	1.27
> 20.00	0.25	0.93	1.18	> 250	0.37	0.93	1.30
> 25.00	0.15	0.95	1.10	> 300	0.28	0.94	1.22
PbB ≥ 1000 µg/l:							
	se	sp	se + sp		se	sp	se + sp
ALAD/European units:				*ZPP/mmol(mol Hb)$^{-1}$:*			
< 26.0	1.00	0.11	1.11	> 0.32	0.90	0.28	1.18
< 20.0	1.00	0.21	1.21	> 0.48	0.90	0.47	1.37
< 15.0	1.00	0.33	1.33	> 1.00	0.65	0.70	1.35
< 10.0	1.00	0.50	1.50	> 1.50	0.60	0.84	**1.44**
< 5.0	0.75	0.77	**1.52**	> 2.00	0.35	0.92	1.27
ALAU/mg(d)$^{-1}$:				*CPU/µg(d)$^{-1}$:*			
> 6.00	0.70	0.54	1.24	> 120	0.60	0.70	1.30
> 10.00	0.55	0.75	1.30	> 150	0.55	0.80	**1.35**
> 15.00	0.45	0.88	1.33	> 200	0.40	0.86	1.26
> 20.00	0.45	0.92	**1.37**	> 250	0.40	0.89	1.29
> 25.00	0.30	0.95	1.25	> 300	0.30	0.91	1.21

Table 3. Spontaneous recovery of biological indicators in 14 male workers following cessation of lead exposure. Group median and range values

	Time elapsed after cessation of lead exposure/day		
	A	B	C
Biological indicator	0	135	294
	0	124 - 152	255 - 341
PbB/µg(l)$^{-1}$	860	690	460
	660 - 1280	410 - 960	360 - 530
ALAD/European units	0.9	8.1	10.8
	0.6 - 3.8	2.9 - 22.4	5.8 - 34.0
ZPP/mmol(mol Hb)$^{-1}$	1.37	1.30	0.42
	0.62 - 1.72	0.55 - 2.71	0.34 - 0.95
ALAU/mg(d)$^{-1}$	50.8	6.5	4.3
	17.4 - 109.5	2.4 - 39.1	3.1 - 13.6
CPU/µg(d)$^{-1}$	959	82	37
	322 - 1775	13 - 355	0 - 109

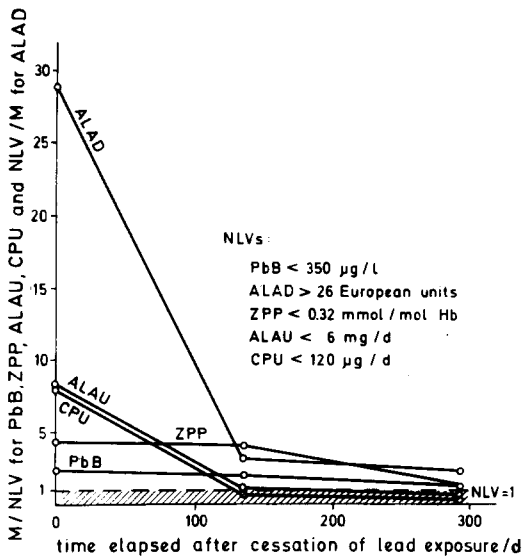

Fig. 9. Spontaneous recovery of biological indicators in 14 male workers following cessation of lead exposure. Group median *(M)* values, expressed as a relative decrease *(ALAD)* or increase *(PbB, ZPP, ALAU, CPU)* of the corresponding normal limit values *(NLVs)*

lead deposit, which is the "cause" of effect. Namely, PbB is also an indirect assessment (although it is the most *specific,* it is not necessarily the most *sensitive*) of the lead dose in a *critical organ,* i.e. bone marrow. The main focus was on the degree of association (correlation) between different biological indicators (Table 1), although more valuable information can be obtained from the scatter diagrams (Figs. 1–4) showing widely different assessment of lead exposure level by the use of different biological indicators.

The results obtained by the statistical evaluation indicate a highly significant ($P < 0.001$) correlation between all of the parameters examined, with decreasing order of agreement, as shown in Table 1. The different levels of agreement between various biological indicators can be explained by various *dynamic patterns* (time-lag and/or recovery) with regard to the time-dependent changes in intensity of external lead exposure (Beritić et al. 1977, 1978a, b), as well as by the fact that the *recent* and the *integrated* (long-term) exposures do not appear to be reflected by the same relative magnitude in different biological indicators (Telišman 1979; Telišman and Prpić-Majić 1979). However, both biologically and methodologically induced variations may also contribute to a certain extent, which is evident in urinary indicators. Despite the lowest correlation coefficients obtained with regard to both PbB and ALAD, a relatively high agreement is obtained between ALAU and CPU themselves, indicating that poor correlations with blood indicators might be influenced by the higher biological variability in excretion of urinary metabolites. This supports the belief that urinary indicators may often be inferior to blood indicators (Zielhuis 1978).

In agreement with our previous observations in various lead exposure conditions it is obvious that with regard to PbB, which is considered the primary criterion in assessing internal lead dose and for defining lead exposure limit value, ALAD yields a considerably higher correlation coefficient than any other indicator examined. The fact that it yields an even better correlation with ZPP (or PP), which

is considered the best available indicator of biologically active lead (Zielhuis 1977), confirms our previous data indicating that both ALAD and PP can distinguish between different *integrated* lead exposure levels better than PbB. Therefore, they might give highly significant correlation with lead-induced health effect (ALAD even better than PP, with respect to blood hemoglobin concentration), while at the same time only a trend, but no significant correlation, existed with PbB (Telišman and Prpić-Majić 1979). Other authors also reported similar observations on better agreement between lead-induced health effects and ZPP (Valciukas et al. 1978; Grandjean and Lintrup 1978), as well as between biologically active (chelatable) lead and both PP and ALAD (Alessio et al. 1980), as compared to PbB.

When considered with regard to the NLVs of biological indicators of effect, the dose-response relationships presented in Figs. 5–8 indicate that approximately 50% of the workers examined already had lower than "normal" ALAD values at a level of PbB = 250 μg/l and increased ZPP values already at PbB = 350 μg/l. However, approximately 50% had higher than "normal" ALAU values, which were only at the level of PbB = 600 μg/l, and particularly CPU with about 50% of higher than "normal" values only at a level of PbB = 750 μg/l. In the population studied (mostly long-term, excessively exposed workers) the corresponding values of ALAD, equivalent to the same PbB level, are *lower* than within "normal" population. This fact supports our previous findings that ALAD activity (as well as ZPP or PP concentration) seems to give better indication of the *integrated* lead exposure and biologically active lead in organism, as compared to blood lead. Similar findings of a lower ALAD activity in lead workers, compared to the corresponding ALAD values equivalent to the same PbB level in "normal" population, can also be observed from the data of Sakurai et al. (1974). The present results on ALAU and CPU indicate that the sensitivity of urinary indicators is by far inadequate for the recommended occupational lead exposure limit for male workers, which is defined by PbB at a level of 400 μg/l (WHO 1980). This level might be underestimated, i.e. regarded as "normal" (according to the NLVs as defined by $\bar{x} + 2\,SD$), in 70% of workers when using ALAU and in 87% of workers when using CPU (compared to 11% for ALAD and 47% for ZPP). Moreover, some of the individual data (scatter diagrams) indicate that there might be a certain time-lag between the *recent* increase of lead absorption, as indicated by both PbB and ALAD but not by ZPP, and the appearance of the equivalent ("steady-state") levels of ALAU and CPU. Since blood sampling is always carried out away from the work area and carefully controlled for contamination, there is practically no possibility that lead has an influence on PbB and ALAD level in vitro.

The data obtained indicate that the recommended alternative exposure limit expressed by ZPP, i.e. up to 50% increase *over* NLV for ZPP (WHO 1980), may not be sufficiently sensitive, since a PbB of 400 μg/l can be underestimated in 72% of male workers.

It is necessary to explain the response data obtained for the NLVs of ALAD, ZPP, ALAU and CPU. It appears that the no-response PbB levels for these indicators are "exactly" the same (corresponding to PbB of approximately 100 μg/l), which disagrees with data published by other authors. It should be pointed out that the response data, as presented here, are not strictly comparable to the data in most of the literature, which are usually presented after being statistically evaluated by

fitting into the "average" sigmoid curve. However, the "average" no-effect PbB levels obtained for the NLVs of these indicators (Figs. 1–4) are widely different (corresponding to PbB of 209 μg/l for ALAD, 302 μg/l for ZPP, 583 μg/l for ALAU, and 804 μg/l for CPU). At least part of this "disagreement" can then be attributed to the wide scatter of data on different biological indicators due to highly variable lead exposure. Consequently, the result of a wider scatter of different indicators is that the response curve becomes flatter, yielding a lower 0% response PbB level and a higher 100% response PbB level, while the 50% response PbB level remains the same. This effect is even more pronounced for the less sensitive indicators of effect, i.e. those showing a lower slope with increasing PbB levels (e.g. CPU). According to Tsuchiya et al. (1979), and in agreement with our previous data (Telišman and Prpić-Majić 1979), it appears that both the no-effect and no-response levels can also be influenced by the specific *extent* of exposure under examination, yielding lower no-effect and no-response PbB levels in the population exposed to greater amounts of lead compared to those exposed to smaller amounts. The relatively lower no-response level for ZPP (to a lesser extent for ALAD and even less for ALAU and CPU) can be explained by the generally high level of *integrated* (long-term) lead exposure in the population studied, as well as by the lower actual lead exposure compared to the past. Furthermore, the lower no-response levels for both ALAU and CPU might also be explained by the possible influence of alcohol consumption, although the personal interviews indicated drinking habits similar to those of our general ("normal") population. One should take into account, however, the fact that "average" alcohol consumption is relatively high in our population and that personal interviews on drinking habits may not always yield reliable information.

In agreement with data on correlation analyses between PbB and biological indicators of effect, the data on calculating "validity" (Table 2) indicate the following order: ALAD > ZPP > ALAU > CPU, covering the PbB range under examination (i.e. PbB of ≥ 400 μg/l, ≥ 600 μg/l, ≥ 800 μg/l, and ≥ 1000 μg/l). However, the "validity" (as well as the correlation coefficients) of all indicators of effect is generally lower in comparison with population groups under less variable lead exposure. It should be stressed that the above results on calculating "validity" should be considered with some reservation, since they represent only the capability of the indicators of effect to predict the actual PbB level, but not exactly the "true" situation—i.e. the actual *internal lead dose at the site(s) of effect.* For example, the relatively low "specificity" for the defined level of ALAD, which in fact is equivalent to the given level of long-term average lead exposure[2] (resulting in poor "validity" of ALAD) might well be due to the low "sensitivity" of PbB (and therefore the poor "validity" of PbB) in reflecting biologically active lead deposit, which is relevant in assessing health significance. In the authors' opinion, the sensitive biological indicators of effect (ZPP or PP, and ALAD) are much more valuable if considered as providing *additional* information, apart from predicting the actual PbB level, since they appear to reflect better the intensity and duration of *integrated* (long-term) lead exposure, as compared to PbB.

2 ALAD = 20.0 European units for PbB = 400 μg/l, obtained in non-occupationally exposed subjects

Data are presented in Table 3 on the spontaneous recovery of biological indicators in 14 male workers examined immediately after cessation of recent overexposure to lead (period A), following approximately 4.5 months (period B), and approximately 10 months (period C). As shown in Fig. 9, the data may serve as a relative indication of how clearly the individual indicators separate "normal" and increased integrated lead absorption. It is evident that by far the best indication of increased lead absorption in period A was found for ALAD. It should be pointed out that in the present group of workers the value of ALAD gives an even better indication of actual increased integrated lead exposure and biologically active lead than that of ZPP. This is because most of the individual ZPP values were "too low", due to the well-known time-lag of equivalent ZPP levels in peripheral blood with respect to the *recent* increase of lead exposure level. In period B the group median value of ZPP was only slightly decreased (more than half of the individual ZPP values in this period were even higher than in period A), while ALAU and CPU already approached the range of "normal" values. In period C ALAD activity was still well below the NLV, while ALAU and CPU were practically in the middle of the "normal" range. The present data on ALAU and CPU support our previous findings on the relatively quick normalization of the urinary indicators after cessation of lead exposure (Beritić et al. 1978b) and support the impression that these indicators provide poor information on integrated lead exposure and biologically active lead. This is in agreement with the data of Alessio et al. (1980) who found that the ALAU levels do not reflect biologically active (chelatable) lead in subjects with past lead exposure (about 1 year after cessation of lead exposure) and therefore cannot be used for assessment of biologically active lead deposit. It should be pointed out, however, that again the value of ALAD, in all three periods examined (A, B, C), indicated a slightly higher degree of inhibition than the equivalent value for the same PbB level of the population with relatively lower integrated lead exposure (e.g. short-term lead exposure). As we have shown previously in male subjects currently exposed to lead (Telišman and Prpić-Majić 1979), the dose-effect relationship between ALAD and PbB (as well as between PP and PbB) seems to be different in population groups with different levels of integrated (long-term) lead exposure. For the same PbB value the corresponding decrease of ALAD activity (and the increase of PP concentration) is considerably more pronounced in individuals with higher integrated lead exposure (absorption). In agreement with the present data on relative capability of distinguishing abnormal lead absorption (with regard to NLVs) but contrary to the general opinion that ALAD is not sensitive enough at high levels of lead exposure, our previous results have shown that ALAD is as sensitive as PP and considerably more sensitive than PbB in distinguishing groups with relatively *high* integrated lead exposure levels (group median PbB of 659 µg/l and 894 µg/l, respectively).

The comparative advantages of ALAD with respect to other biological indicators of effect (ZPP or PP, ALAU, CPU) seem to include relatively higher specificity for increased lead absorption than previously suspected. Our recent data obtained in an experimental study in man (Prpić-Majić et al. 1981) indicate the possibility that the influence of ethanol (EtOH) on transient inhibition of ALAD in vivo is not the influence of EtOH per se, but mainly the influence of ethanol-induced change between the "stable" and the "mobile" (i.e. biologically active) lead

fractions of the total body lead pool. With regard to the application of ALAD as a biological indicator of lead exposure, the observed influence of EtOH on ALAD activity (Moore et al. 1970; Krasner et al. 1974) is generally interpreted as an "interference" which decreases the specificity of ALAD for lead. However, since the influence of EtOH on ALAD appears to be mediated through *lead,* this "interference" may in fact be in favour of the specificity of ALAD for lead. Some of the possible interfering factors that decrease the specificity for lead of other biological indicators of effect are the influence of impaired iron metabolism on ZPP and PP (Lamola and Yamane 1974; Piomelli 1977); erythropoietic protoporphyria on PP (Lamola et al. 1975); bilirubin and carboxyhemoglobin on ZPP (Karačić et al. 1980; Telišman et al. 1980); liver disease on ALAU and CPU, acute alcoholism on CPU, and various porphyrias on both ALAU and CPU (Baloh 1974). When one considers all published interferences (including those for ALAD), the *relative* specificity of ALAD for lead seems to be even more pronounced.

The comparative advantages of ALAD should be regarded with respect to two of the three main objectives for application in occupational health practice (Zielhuis 1977, 1978; Telišman 1979):

1) Screening the work population without known risk of lead exposure (to identify population segments with increased risk)
2) Periodical control of population groups with increased risk due to known lead exposure (to prevent the development of adverse effects)

For both purposes, ALAD can be regarded as relatively superior to PbB from the methodological point of view as well as the capability of better reflecting biologically active lead, which is relevant in assessing a health hazard. It is superior to ZPP or PP in identifying recent variations of lead exposure level (approximately 120 days of time-lag for the equivalent "steady state" ZPP and PP levels), and is more sensitive in the low PbB range. ALAD is particularly superior to ALAU and CPU because of better correlation with PbB and far better sensitivity in the lower PbB range. As previously mentioned, ALAD seems to be superior to other indicators of effect with regard to specificity of reflecting increased lead absorption. It should be pointed out, however, that because of its specificity blood lead should always be considered the primary (but *not* the only) criterion, particularly in circumstances where *reliable* blood lead analyses can be expected.

In conclusion, it appears that the arguments for including health-based recommendations for ZPP or PP and ALAU, but excluding ALAD, in the recent WHO document (WHO 1980) were mainly based on theoretical considerations. However, practical implications seem to be far more in favour of ALAD, which permits a maximal safety margin in preventing "adverse" effects (equivalent to the recommended, relatively low lead exposure limit) in the entire work population. Even from the theoretical point of view "a certain degree of ALAD depression" might be considered equally important as "a certain degree of ALA accumulation" (resulting in increased excretion of ALAU), due to the fact that both might be equally relevant/non-relevant with regard to health significance, and to the fact that the former is the cause of the latter. If one considers the application of biological indicators for a specified concept called "biological monitoring", i.e. as the measurement or estimation of exposure and not of health effects (Zielhuis 1978), the afore-mentioned relative advantages of ALAD (the highest sensitivity,

specificity and correlation with internal lead dose estimated by PbB level) over other indicators of effect (ZPP or PP, ALAU, CPU) are even more pronounced.

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