Comparison of Colorimetric and HPLC Methods for Determination of δ -Aminolevulinic Acid in Urine with Reference to Dose-Response Relationship in Occupational Exposure to Lead

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Abstract: Both traditional colorimetry and recently developed HPLC-fluorometry have been in use for determination of δ -aminolevulinic acid in urine (ALA-U), an effect marker of occupational exposure to lead (Pb). The present study was initiated to compare the values by the two methods on an epidemiology basis among workers occupationally exposed to lead (Pb), to estimate quantitatively the colorimetry-associated increment over the values by the HPLC method, to evaluate ALA-U determination in occupational health service for Pb-exposed workers, and to identify a critical Pb-B to induce an elevation in ALA-U. For this purpose, blood and urine samples were collected from three groups of Pb-exposed workers (both men and women in combination, including smokers) and analyzed for Pb in blood (Pb-B; measured in all subjects) and ALA-U (by colorimetry or HPLC), i.e., Group 1 (164 subjects with urinalysis by the two methods), Group 2 (2,923 subjects by colorimetry), and Group 3 (2,540 subjects by HPLC). ALA-U when measured by colorimetry was higher than the values by HPLC, and that the mean difference on a group basis was 1.4 mg/l (in a range of 1.1 to 1.8 mg/l), irrespective of Pb-B levels. It was also found that the increase in ALA-U was small when Pb-B was relatively low (e.g., $\leq 40 \mu\text{g}/100 \text{ ml}$), and that the increase on a group basis in response to an increase in Pb-B from 5 to $40 \mu\text{g}/100 \text{ ml}$ was as small as $\leq 0.6 \text{ mg/l}$. Thus, ALA-U appeared to be not a sensitive marker of Pb effects at low Pb-B levels. ALA-U however increased substantially with a point of inflection at the Pb-B level of about $17\text{--}34 \mu\text{g}/100 \text{ ml}$. Thus it was concluded that ALA-U as measured by colorimetry is greater than ALA-U by HPLC by 1.4 mg/l on average irrespective of intensity of Pb-exposure, which may induce bias in evaluation of health effect, and that ALA-U levels will increase when Pb-B is in excess of $17\text{--}34 \mu\text{g}/100 \text{ ml}$.

Key words: δ -Aminolevulinic acid in urine, Biological monitoring, Colorimetry, HPLC-fluorometry, Lead in blood, Occupational lead exposure

Introduction

δ -Aminolevulinic acid level in urine (ALA-U) has been used as an effect marker of occupational exposure to lead (Pb) in combination with blood lead (Pb-B) as a marker of

exposure, and current regulation in Japan¹⁾ requests that workers occupationally exposed to Pb be examined for these markers (together with protoporphyrin in erythrocytes, when necessary) twice a year. In practice, colorimetric methods^{2,3)} had been used for years for ALA-U determination, and more specific HPLC-fluorometry methods (to be called HPLC methods in short) were developed recently^{4–8)}. The

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regulation¹⁾ does not specify the method for determination, and the two methods are still in use in parallel in occupational health practice⁹⁾. Furthermore, colorimetry results are still in use even in scientific publications^{10–13)}. It is therefore of practical importance to clarify the compatibility of the two methods with regard to the results of determination, and whether the difference in the values obtained affects the evaluation of the health effects in association with exposure to Pb. Accordingly, Tomokuni *et al.*⁶⁾ made comparative evaluation of the two methods in a factory survey and showed that the color-generating agent reacts with physiological urine components in addition to the target analyte of ALA and thus gives greater value than ALA-specific HPLC, and that the difference cannot be ignored especially when ALA-U levels are low.

The present study was initiated to compare the values of the two methods on an epidemiological scale, to estimate quantitatively the colorimetry-induced increment over the values by the HPLC method, to evaluate ALA-U determination in occupational health service for Pb-exposed workers, and to identify a critical Pb-B level above which ALA-U will increase.

Materials and Methods

Populations studied

Three groups of workers (including both men and women; Group 1-A, Group 2-A and Group 3-A) agreed to participate in the study by offering blood and spot urine samples for determination of Pb-B and ALA-U; a majority of the subjects were engaged in the production of lead storage batteries, and some served in second smelters, soldering lines or production/handling of lead powder. Both men and women were taken together, because the difference of the two sexes in Pb-B and ALA-U relationship was minute¹²⁾. Smokers were included as the direct effect of smoking on ALA-U appears not quite clear; e.g., in a previous study, no effect of smoking was detected among Pb-unexposed subjects whereas an increase was observed in Pb-exposed workers¹⁴⁾. ALA-U was determined both by colorimetry and HPLC (as to be described below) for Group 1-A subjects (164 cases), whereas the urine samples from Group 2-A (2,923 cases) were analyzed by colorimetry, and those from Group 3-A (2,540 cases) were by HPLC, respectively. Those with Pb-B below the limit of detection (for the value of the limit, see below) were excluded because all measured values should be quantitatively evaluated.

In a subsequent step, the cases with $\leq 40 \mu\text{g Pb}/100 \text{ ml blood}$ ¹²⁾ were selected out of Groups 1-A, 2-A and 3-A, in

order to examine if the difference between ALA-U by colorimetry and ALA-U by HPLC was due to cases with high Pb-B. The selection gave Groups 1-B (145 cases), 2-B (2,852 cases) and 3-B (2,475 cases), respectively.

Methods of determination of lead in blood and δ -aminolevulinic acid in urine

The collected samples were kept frozen until analyzed. Pb-B was measured by graphite furnace atomic absorption spectrometry as previously described; the limit of detection in practice was $5 \mu\text{g Pb}/100 \text{ ml blood}$ ^{12, 15)}. ALA-U was measured by two methods, i.e., by colorimetry after Tomokuni and Ichiba³⁾, and by HPLC after Okayama *et al.*⁵⁾. Under the study conditions, the limit of detection was 0.1 mg ALA/l urine for both methods. The recovery (%) and the coefficient of variation (%; $N=6$) were 100.3% and 1.6% for Pb-B, 98.2% and 5.8% for ALA-U by colorimetry, and 98.5% and 5.0% for ALA-U by HPLC. Specific gravity (SG or sg) was measured by refractometry as a measure of correction for urine density. In practice, SG was expressed in terms of Factor G¹⁶⁾, which is defined as

$$\text{Factor G} = (\text{SG} - 1.000) \times 1,000.$$

The ALA-U values were described as observed (ALA- U_{ob}) and also as corrected for a specific gravity of 1.016 (ALA- U_{sg}) after Rainsford and Lloyd Davies¹⁷⁾. No measurement was made for urinary creatinine concentration.

Statistical analysis

A preliminary analysis showed that both Pb-B and ALA-U (irrespective of urine density correction) distributed log-normally so that the distribution was expressed in terms of a geometric mean (GM) and a geometric standard deviation (GSD). Nevertheless, our experiences¹²⁾ showed that the relation of Pb-B and ALA-U (without logarithmic conversion) fits best with the 3rd degree regression, and the non-converted values were also employed when considered appropriate. StatView Version 5 was employed for conducting, e.g., paired and unpaired *t*-test, chi-square test, Kolmogorov-Smirnov test, and Wilcoxon test. Comparisons of slopes and intercepts, and calculation of *p* values for correlation coefficients were conducted after Ichihara¹⁸⁾.

Results

Distribution of Pb-B and ALA-U in the three groups of workers

GM and GSD values for Pb-B and ALA-U in Groups 1-A, 2-A and 3-A are summarized in Table 1 as basic parameters

Table 1. Levels of lead in blood and δ -aminolevulinic acid in urine in the three groups

Group ^a (No. of cases)	Statistical parameters	Pb-B	Factor G ^b	ALA-U _{ob} ^c			ALA-U _{sg} ^c		
				Colorimetry (mg/l)	HPLC (mg/l)	p ^d	Colorimetry (mg/l)	HPLC (mg/l)	p ^d
1-A (n=164)	M ^e SD ^e	18.1 2.172	19.8 6.2	2.8 1.924	1.4 2.245	<0.01	2.4 1.597	1.2 1.909	<0.01
1-B (n=145)	M ^e SD ^e	15.7 2.025	19.7 6.4	2.5 1.729	1.2 1.804	<0.01	2.2 1.344	1.0 1.408	<0.01
	p ^f	>0.05	>0.05	>0.05	<0.05		<0.05	<0.01	
2-A (n=2923)	M ^e SD ^e	7.0 1.918	21.5 6.7	2.84 1.741			2.24 1.441		
3-A (n=2540)	M ^e SD ^e	7.2 1.959	21.1 7.0		1.26 1.894			1.02 1.523	
	p ^g	>0.05	<0.05		<0.01			<0.01	
2-B (n=2852)	M ^e SD ^e	6.7 1.784	21.5 6.6	2.79 1.703			2.21 1.392		
3-B (n=2475)	M ^e SD ^e	6.9 1.816	21.1 7.0		1.21 1.784			0.99 1.389	
	p ^g	>0.05	<0.05		<0.01			<0.01	

^a ALA-U was measured by both colorimetry and HPLC in Group 1, by colorimetry only in Group 2, and by HPLC only in Group 3. Groups 1-A, 2-A and 3-A include all cases (i.e., those with $\geq 5 \mu\text{g}/100 \text{ ml}$ Pb-B) whereas Groups 1-B, 2-B and 3-B are those with relatively low Pb-B (i.e., >5 to $40 \mu\text{g}/100 \text{ ml}$ Pb-B). ^b For the definition of factor G, see the Materials and Methods section. ^c ALA-U_{ob} and ALA-U_{sg} are ALA-U as observed and as corrected for a specific gravity of 1.016, respectively. ^d p for the difference between the colorimetry values and HPLC values. ^e Arithmetic mean and arithmetic standard deviation for factor G, and geometric mean and geometric standard deviation for Pb-B and ALA-U. ^f p for the difference between 1-A and 1-B. ^g p for the difference between 2-A and 3-A, or 2-B and 3-B (irrespective of the methods).

related to the intensity of Pb exposure in the three groups. Pb-B values were in ranges of 5–79 $\mu\text{g}/100 \text{ ml}$, 5–85 $\mu\text{g}/100 \text{ ml}$ and 5–106 $\mu\text{g}/100 \text{ ml}$ in Groups 1-A, 2-A and 3-A, respectively. Nevertheless, the GM Pb-B of 18 $\mu\text{g}/100 \text{ ml}$ for Group 1-A appeared to suggest that the average Pb exposure was more intense in Group 1 than in other two groups (Pb-B; 7 $\mu\text{g}/100 \text{ ml}$). ALA-U did not differ apparently. Further comparison of ALA-U as measured by colorimetry with that by HPLC showed that the two values differed significantly ($p < 0.01$) in Group 1-A, as well as between Group 2-A (by colorimetry) and Group 3-A (by HPLC), irrespective of correction for urine density.

Effects of Pb-B levels on colorimetry/HPLC ratio

Possibilities were examined if the ratio of the colorimetry value divided by the HPLC value vary as a function of Pb-B. For this purpose, the 164 cases in the Group 1-A were employed because all of Pb-B, ALA by colorimetry and ALA by HPLC were available. When the ratios of colorimetry results over the HPLC results (the COL/HPLC ratio) were plotted against Pb-B, the scatter diagram (Fig. 1) showed that the ratios distributed in a wide range (up to >5) when

Fig. 1. The relation of the ratio of the colorimetry result over the HPLC result (the COL/HPLC ratio) with Pb-B.

164 cases (Group 1-A in Table 2) are depicted to show that the ratio tend to decrease in pace with increasing Pb-B.

Pb-B was $\leq 5 \mu\text{g}/100 \text{ ml}$, and gradually decreased and converged (being irrespective of urine density correction) toward a ratio of 1 as a function of increasing Pb-B; it was especially so when Pb-B was $\geq 50 \mu\text{g}/100 \text{ ml}$. The trend

Table 2. Cubic regression analysis to find points of inflexion in the regression curves

Group ^a	No. of Cases	Methods of analysis	Correction for urine density	Regression curve parameters ^b					Pb-B ^c ($\mu\text{g}/100\text{ ml}$)
				α	β	γ	δ	r	
Group 1-A	164	Colorimetric	Observed	1.589	0.265	-0.013	0.00018	0.85	33.8
			SG-corrected	1.371	0.184	-0.009	0.00013	0.90	31.0
Group 1-A	164	HPLC	Observed	-0.021	0.247	-0.012	0.00017	0.91	32.6
			SG-corrected	0.094	0.171	-0.008	0.00012	0.93	23.2
Group 2-A	2923	Colorimetric	Observed	2.520	0.161	-0.009	0.00015	0.65	25.0
			SG-corrected	1.694	0.156	-0.009	0.00013	0.78	34.2
Group 3-A	2540	HPLC	Observed	0.916	0.126	-0.008	0.00014	0.88	26.1
			SG-corrected	0.876	0.041	-0.003	0.00007	0.92	16.6

^aFor groups, see Table 1. ^bThe parameters so that $y = \alpha + \beta x + \gamma x^2 + \delta x^3$, where x is Pb-B ($\mu\text{g}/100\text{ ml}$), and y is ALA-U (mg/l) as measured by the colorimetry or HPLC methods (for details, see the Materials and Methods section), and corrected for urine density (i.e., observed as corrected for none, or SG-corrected for the values corrected for a specific gravity of 1.016). r is a correlation coefficient. ^cPb-B at the point of inflexion.

Fig. 2. Fitness of Pb-B vs. ALA-U relation to the 3rd degree regression.

The relation of Pb-B and ALA-U (by the HPLC method) with Pb-B is shown. The curve in the figure is the result of the 3rd degree regression, the equation of which is $Y = 0.876 + 0.041X - 0.003X^2 + 0.00007X^3$ ($r=0.92$, $n=2,540$), where X is Pb-B ($\mu\text{g}/100\text{ ml}$) and Y is ALA-U (mg/l) by HPLC as corrected for a specific gravity of 1.016.

suggests that the difference in colorimetry and HPLC values is substantial when Pb-B is low but may be almost negligible when Pb-B is high, e.g., $\geq 50\text{ }\mu\text{g}/100\text{ ml}$. Further comparison of the colorimetry values with the HPLC values disclosed that colorimetry values were greater than the HPLC values especially when ALA-U (by HPLC) was e.g. $< 5\text{ mg/l}$, but the two values in pair were closer to each other when ALA-U values were greater.

Application of the 3rd degree regression and estimation of the Pb-B above which ALA-U would increase substantially

Following our previous experiences¹²⁾, the 3rd degree regression was applied to the three sets of data (Groups 1-

A, 2-A and 3-A) to find the Pb-B levels, above which ALA-U would increase substantially. The application (Table 2) showed that the data fit well with the 3rd degree regression with $r=0.65$ to 0.92 ($p<0.01$ for all cases). A typical scatter diagram of Pb-B and ALA-U (by the HPLC method) of 2,540 cases in Group 3-A (Table 2) is shown together with the 3rd degree regression curve in Fig. 2.

Selection of cases with low Pb-B

The removal of cases with higher Pb-B (i.e., $> 40\text{ }\mu\text{g}/100\text{ ml}$), i.e., selection of Groups 1-B, 2-B and 3-B, tended to reduce GM Pb-B values in Group 1- (i.e., from $18.1\text{ }\mu\text{g}/100\text{ ml}$ in Group 1-A to $15.7\text{ }\mu\text{g}/100\text{ ml}$ in Group 1-B, although p was greater than 0.05 ; Table 1), but GM Pb-B did not differ between Group 2-B and Group 3-B ($p>0.05$). Significant difference ($p<0.01$) was retained between colorimetric and HPLC values in Group 1-B, and also in Group 2-B and Group 3-B irrespective of correction for urine density.

Eight sets of data, i.e., 145 cases (in Group 1-B) of Pb-B and ALA-U_{ob} (both by colorimetry and by HPLC), 145 cases (in Group 1-B) of Pb-B and ALA-U_{sg} (by both methods), 2,852 cases (in Group 2-B) of Pb-B and either ALA-U_{ob} or ALA-U_{sg} (by colorimetry), and 2,475 cases (in Group 3-B) of Pb-B and either ALA-U_{ob} or ALA-U_{sg} (by HPLC), were subjected to linear regression analysis (Table 3). The correlation coefficients were statistically significant ($p<0.01$) in most cases (i.e., except for ALA-U by colorimetry in Group 1-B for which p was >0.05 irrespective of urine density correction), partly because the number of cases examined was large in Groups 2-B and 3-B. Colorimetry tended to give smaller coefficients ($p \doteq 0.07$ by Wilcoxon test) with larger p values than HPLC. When slopes and intercepts

Table 3. Linear regression lines in the Pb-B range of >5 to 40 $\mu\text{g}/100\text{ ml}$

Group ^a	No. of cases	Correction for urine density	Method ^b	Regression line ^c parameters				P for diff. in		Yd when Xe is		
				α	β	r	P for r	Slope	Inter-cept	X=5	X=40	Diff. in Y ^f
1-B	145	None (observed)	Colorimetry	2.853	-0.002	-0.02	>0.05	>0.05	<0.01	2.8	2.8	-0.1
1-B	145	None (observed)	HPLC	1.060	0.017	0.28	<0.01			1.1	1.8	0.6
1-B	145	SG-corrected	Colorimetry	2.218	0.001	0.02	>0.05	<0.01	<0.01	2.2	2.3	0.0
1-B	145	SG-corrected	HPLC	0.797	0.016	0.50	<0.01			0.9	1.4	0.6
2-B	2852	None (observed)	Colorimetry	3.054	0.013	0.06	<0.01	>0.05	<0.01	3.1	3.6	0.5
3-B	2475	None (observed)	HPLC	1.285	0.014	0.15	<0.01			1.4	1.8	0.5
2-B	2852	SG-corrected	Colorimetry	2.247	0.008	0.09	<0.01	>0.05	<0.01	2.3	2.6	0.3
3-B	2475	SG-corrected	HPLC	0.941	0.011	0.26	<0.01			1.0	1.4	0.4

^aFor information on groups, see under Table 1. ^bThe method of determination for ALA-U. ^c $Y = \alpha + \beta X$, in which Y is ALA-U_{ob} or ALA-U_{sg} (mg/l), and X is Pb-B ($\mu\text{g}/100\text{ ml}$). ^dY is ALA-U_{ob} or ALA-U_{sg} (mg/l). ^eX is Pb-B ($\mu\text{g}/100\text{ ml}$). ^fDisagreement from simple subtraction may be induced by rounding of figures.

Table 4. Estimation of the difference induced by colorimetry and HPLC

Group ^a	No. of cases	Correction for urine density	Method ^b	ALA-U (mg/l) ^b						Diff. ^e (mg/l) in intercept (α)
				P by K-S ^c	P by χ^2	AM	Diff. in AM	GM	Diff. ^e in GM	
1-B	145	None (observed)	Colorimetry	>0.05	>0.05	2.82	1.42	2.48	1.28	1.79
1-B	145	None (observed)	HPLC	>0.05	>0.05	1.40		1.20		
1-B	145	SG-corrected	Colorimetry	>0.05	>0.05	2.24	1.14	2.15	1.11	1.42
1-B	145	SG-corrected	HPLC	>0.05	>0.05	1.11		1.05		
2-B	2852	None (observed)	Colorimetry	>0.05	<0.01	3.16	1.75	2.79	1.58	1.77
3-B	2475	None (observed)	HPLC	>0.05	<0.01	1.41		1.21		
2-B	2852	SG-corrected	Colorimetry	>0.05	<0.01	2.32	1.28	2.21	1.22	1.31
3-B	2475	SG-corrected	HPLC	>0.05	<0.01	1.04		0.99		

^aFor information on groups, see under Table 1. ^bALA-U measured by the methods as described and corrected for urine density also as described in the table. ^cp value for the difference in the distribution pattern from a normal distribution as examined by Kolmogorov-Smirnov test. ^dp value for the difference in the distribution pattern from a normal distribution as examined by chi-square test. ^e α 's are cited from Table 3; disagreement from simple subtraction may be due to rounding of figures.

were compared between the colorimetry values and HPLC values, it was observed that the intercepts differed significantly ($p < 0.01$ in all cases) from each other. The slopes however did not differ ($p > 0.05$) except for the pairs of regression lines (colorimetry and HPLC) when ALA-U values in the Group 1-B (with a small number of 145 cases) were corrected for SG.

It should also be noted that the slopes were very shallow in all cases. Thus, the increments in ALA-U values when Pb-B was lowest or highest in the range studied (i.e., 5 and 40 $\mu\text{g}/100\text{ ml}$) were generally small, i.e., from -0.1 to 0.6 mg/l (the right-most column in Table 3).

Quantitative estimation of the difference in ALA-U as measured by colorimetry and HPLC

Deletion of cases with Pb-B >40 $\mu\text{g}/100\text{ ml}$ from Groups

1-A, 2-A and 3-A (i.e., the selection of Groups 1-B, 2-B and 3-B) resulted in the removal of those with high ALA-U. Thus, the distributions of ALA-U no longer differed from that of a normal distribution irrespective of the method of determination or correction for urine density when examined by Kolmogorov-Smirnov test ($p > 0.05$; in the middle of Table 4). When examined by chi-square test, however, the difference from normality was significant in Groups 2 and 3 ($p < 0.01$; Table 4) possibly because a large number of cases were examined ($n = 2,852$ and $2,475$, respectively). The observation indicated that the tailing in the distribution toward high ALA-U values might be substantial. In contrast, the difference was insignificant ($p > 0.05$) for Group 1-B with a smaller number of cases ($n = 145$). Accordingly, both normal and log-normal distributions were assumed, and both AM and GM values were calculated. The differences in AM or

Table 5. Classification of cases in Groups 1-A, 2-A and 3-A in accordance with regulation-defined categories

Classification by ALA-U ^b	Classification by Pb-B ^a															
	Class 1				Class 2				Class 3				Sum			
	Class 1	Class 2	Class 3	Sum	Class 1	Class 2	Class 3	Sum	Class 1	Class 2	Class 3	Sum	Class 1	Class 2	Class 3	Sum
HPLC	73	0	0	73	72	0	0	72	9	5	5	19	154	5	5	164
Colorimetry	67	6	0	73	68	4	0	72	9	7	3	19	144	17	3	164
Colorimetry-M ^c	72	1	0	73	71	1	0	72	10	6	3	19	153	8	3	164
P for diff. ^d	<0.05				<0.05				>0.10				<0.05			
P for diff. ^e	>0.05				>0.05				>0.05				>0.05			

Analysis was conducted with 164 cases in Group 1-A. ^aClasses 1, 2 and 3 are ≤ 20 , >20 to 40, and >40 $\mu\text{g Pb}/100$ ml blood (Ministry of Labour¹⁾).

^bClasses 1, 2 and 3 are ≤ 5 , >5 to 10, and >10 mg ALA/l urine (Ministry of Labour¹⁾). ^cClassification by modified criteria of ≤ 6.4 ($=5+1.4$), >6.4 to 11.4, and >11.4 mg ALA/l urine. ^dComparison by chi-square test. ^eComparison by chi-square test when the modified criteria were applied to the colorimetry values.

GM between the colorimetry and HPLC values tended to be smaller ($p=0.07$ by Wilcoxon test) for GM (1.1 to 1.6 mg/l) than for AM (1.1 to 1.8 mg/l) as expected.

Even simpler would be the comparison of α (the intercepts) because the two regression lines in comparisons ran in parallel in most cases (Table 3). The differences in α values ranged from 1.3 to 1.8 mg/l. The 12 values thus obtained were in a range of 1.1 to 1.8 mg/l with a grand AM $\pm$ ASD of 1.4 ± 0.2 mg/l.

Effects of adjustment for the difference between colorimetry and HPLC values on classification

It was of practical interest to know if the adjustment of the regulation-defined¹⁾ classification criteria for the difference of 1.4 mg/l (between the colorimetry values and the HPLC values) can reduce the difference in the distribution among classes. The 164 cases in Group 1 were first classified (Table 5) in terms of Pb-B to Classes 1 (≤ 20 $\mu\text{g}/100$ ml), 2 (>20 to 40 $\mu\text{g}/100$ ml) and 3 (>40 $\mu\text{g}/100$ ml), and then by classes for ALA-U of ≤ 5 mg/l for Class 1, >5 to 10 mg/l for Class 2 and >10 mg/l for Class 3 (classification by Ministry of Labour, Japan¹⁾). It was evident that the classification results of colorimetry values were significantly different from that of HPLC values ($p<0.05$ irrespective of Pb-B classes except for Pb-B Class 3). In the following step, the original criteria for ALA-U classification were modified to ≤ 6.4 mg/l, >6.4 to 11.4 mg/l and >11.4 mg/l, respectively, by the addition of 1.4 mg/l. When colorimetry values were grouped in accordance with modified classes (i.e., 6.4 mg/l, >6.4 to 11.4 mg/l and >11.4 mg/l for Classes 1, 2 and 3), however, no significant difference ($p>0.05$) persisted in classification distribution between the colorimetry values and the HPLC values.

Discussion

The present analysis disclosed that ALA-U when measured by the colorimetric method were higher than the values by HPLC, and that the ratio between the two values in pair will converge to 1 with greater ALA-U, in confirmation with the observation by Tomokuni *et al.*⁶⁾. It was also made clear quantitatively that the difference on a group basis ranged from 1.1 to 1.8 mg/l with an average of 1.4 mg/l, irrespective of Pb-B levels. No efforts were made in the present study, however, to identify the urine components that cross-react with the color-generating reagent in the colorimetry.

In confirmation with previous observation¹²⁾, it was also made clear that ALA-U may not reach the level over 5 mg/l as far as Pb-B stays not very high, e.g., ≤ 40 $\mu\text{g}/100$ ml (Fig. 1). The increase in ALA-U on a group basis was as small as ≤ 0.6 mg/l despite the increase in Pb-B from 5 to 40 $\mu\text{g}/100$ ml (Table 3). Thus, ALA-U as observed or after correction for a specific gravity appeared to be not sensitive as a marker of Pb effects even if measured by the more ALA-specific HPLC method. In this sense, the role of ALA-U in the health examination system for Pb-exposed workers deserves discussion.

In the present study, calculation to find the points of inflexion as Pb-B in the differential equation of $dy/dx = \beta + 2\gamma x + 3\delta x^2 = 0$ (where $x=\text{Pb-B}$; for β , γ and δ , see Table 2 and footnotes) gave Pb-B in a range of 16.6 to 34.2 $\mu\text{g}/100$ ml (Table 2). A Pb-B of 28 $\mu\text{g}/100$ ml may be considered to be the critical Pb-B concentration as an average of 8 estimations, i.e., $[(33.8 + 31.0 + 32.6 + 23.2 + 25.0 + 34.2 + 26.1 + 16.6)/8]$ $\mu\text{g}/100$ ml. The average level is close but somewhat greater than what was found in the previous analysis (i.e., 23 $\mu\text{g}/100$ ml¹²⁾).

There are several studies on Pb-exposed factory workers (combined with controls in some cases) on the critical Pb-B to induce substantial increase in ALA-U. Thus, Murata *et al.*¹⁹⁾ recently observed by the bench-mark dose analysis that 10 μg Pb/100 ml blood is a critical level to induce an increase in ALA-U. Historically however, Selander and Cramér²⁰⁾ gave 40 μg /100 ml as the Pb-B level above which ALA-U showed a sharp increase. Similarly, Tola *et al.*²¹⁾ showed that ALA-U was higher in when Pb-B was ≥ 40 μg /100 ml as compared with the ALA-U levels for 35 μg Pb/100 ml blood. Lauwerys *et al.*²²⁾ reported a dose (Pb-B)-response (ALA-U) relationship in which ALA-U_{cr} tended to increase in the Pb-B range of ≤ 40 μg /100 ml. In recent studies, Tomokuni *et al.*^{6, 7, 23)} have shown that ALA-U increased when Pb-B was about 40 μg /100 ml or higher. Sakai and Morita²⁴⁾ showed ALA in plasma increased sharply when Pb-B was in excess of ca. 40 μg /100 ml; it has been known that ALA in plasma correlates closely with ALA-U^{8, 24-26)}. According to Makino *et al.*²⁷⁾, ALA-U tended to increase at ≥ 22.4 μg Pb/100 ml blood and a sharp increase in ALA-U was found at ≥ 35.5 μg Pb/100 ml blood. This study group also showed that the proportion of those with elevated ALA-U (>10 mg/l by colorimetry) increased when Pb-B was around 40 μg /100 ml or higher¹²⁾.

The over-all evaluation was therefore such that a substantial increase in ALA-U may take place with Pb-B of 17 to 40 μg /100 ml blood, which suggests that ALA-U as an effect marker of Pb-B exposure will be meaningful at relatively high Pb exposure levels. It should be known that, in such a relatively high Pb-B range, in turn, the difference in the ALA-U values between colorimetry and HPLC is no longer substantial (Fig. 2).

Thus, it may be plausible on a group basis to apply urinalysis for ALA as an effect marker when Pb-B is relatively high (e.g., 17–40 μg /100 ml blood), whereas Pb-B can be employed as a common indicator of Pb-exposure not only for workers occupationally exposed to Pb^{28, 29)}, but for general populations as a whole³⁰⁾. This evaluation however does not exclude the need of ALA-U determination on an individual basis in workers with lower Pb-B, because Pb-B and ALA-U are the most convenient markers of exposure and health effect in the prevention of Pb poisoning. It should be added that, even in modern communities, cases with high Pb exposure may take place in which Pb-B is well in excess of 40 μg /100 ml^{12, 31)}, or even as high as ≥ 100 μg /100 ml³²⁾.

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