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., ≤40 µg/100 ml), and that the increase on a group basis in response to an increase in Pb-B from 5 to 40 µg/100 ml was as small as ≤0.6 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 17–34 µg/100 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 17–34 µg/100 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 methods²,³) had been used for years for ALA-U determination, and more specific HPLC-fluorometry methods (to be called HPLC methods in short) were developed recently⁴–⁸). The
regulation\textsuperscript{11} does not specify the method for determination, and the two methods are still in use in parallel in occupational health practice\textsuperscript{9}. Furthermore, colorimetry results are still in use even in scientific publications\textsuperscript{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 \textit{et al.}\textsuperscript{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.

\section*{Materials and Methods}

\subsection*{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 minute\textsuperscript{12}. 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 workers\textsuperscript{14}. 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 $\leq 40 \mu g$ Pb /100 ml blood\textsuperscript{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.

\subsection*{Methods of determination of lead in blood and $\delta$-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 $\mu g$ Pb/100 ml blood\textsuperscript{12, 15}. ALA-U was measured by two methods, i.e., by colorimetry after Tomokuni and Ichiba\textsuperscript{3}, and by HPLC after Okayama \textit{et al.}\textsuperscript{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 G\textsuperscript{16}, which is defined as

\[ \text{Factor G} = (\text{SG} - 1.000) \times 1.000. \]

The ALA-U values were described as observed (ALA-U\textsubscript{ob}) and also as corrected for a specific gravity of 1.016 (ALA-U\textsubscript{sg}) after Rainsford and Lloyd Davies\textsuperscript{17}. No measurement was made for urinary creatinine concentration.

\subsection*{Statistical analysis}

A preliminary analysis showed that both Pb-B and ALA-U (irrespective of urine density correction) distributed lognormally so that the distribution was expressed in terms of a geometric mean (GM) and a geometric standard deviation (GSD). Nevertheless, our experiences\textsuperscript{12} 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 Ichihara\textsuperscript{18}.

\section*{Results}

\subsection*{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.
related to the intensity of Pb exposure in the three groups. Pb-B values were in ranges of 5–79 µg/100 ml, 5–85 µg/100 ml and 5–106 µg/100 ml in Groups 1-A, 2-A and 3-A, respectively. Nevertheless, the GM Pb-B of 18 µg/100 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 µg/100 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 Pb-B was ≤5 µg/100 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 ≥50 µg/100 ml. The trend

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Statistical parameters</th>
<th>Pb-B</th>
<th>Factor G</th>
<th>ALA-U&lt;sub&gt;c&lt;/sub&gt;</th>
<th>ALA-U&lt;sub&gt;g&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Colorimetry</td>
<td>HPLC</td>
</tr>
<tr>
<td>1-A</td>
<td>M&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18.1</td>
<td>19.8</td>
<td>2.8</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>SD&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.172</td>
<td>6.2</td>
<td>1.924</td>
<td>2.245</td>
</tr>
<tr>
<td>(n=164)</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>1-B</td>
<td>M&lt;sup&gt;e&lt;/sup&gt;</td>
<td>15.7</td>
<td>19.7</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>SD&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.025</td>
<td>6.4</td>
<td>1.729</td>
<td>1.804</td>
</tr>
<tr>
<td>(n=145)</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>2-A</td>
<td>M&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.0</td>
<td>21.5</td>
<td>2.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.918</td>
<td>6.7</td>
<td>1.741</td>
<td></td>
</tr>
<tr>
<td>(n=2923)</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3-A</td>
<td>M&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.2</td>
<td>21.1</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.959</td>
<td>7.0</td>
<td>1.894</td>
<td></td>
</tr>
<tr>
<td>(n=2540)</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2-B</td>
<td>M&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.7</td>
<td>21.5</td>
<td>2.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.784</td>
<td>6.6</td>
<td>1.703</td>
<td></td>
</tr>
<tr>
<td>(n=2852)</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3-B</td>
<td>M&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.9</td>
<td>21.1</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.816</td>
<td>7.0</td>
<td>1.784</td>
<td></td>
</tr>
<tr>
<td>(n=2475)</td>
<td></td>
<td></td>
<td></td>
<td>&gt;0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

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 ≥5 µg/100 ml Pb-B) whereas Groups 1-B, 2-B and 3-B are those with relatively low Pb-B (i.e., >5 to 40 µg/100 ml Pb-B). b For the definition of factor G, see the Materials and Methods section. c ALA-U<sub>c</sub> and ALA-U<sub>g</sub> 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).
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., ≥50 µg/100 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 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 experiences, 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 µg/100 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 µg/100 ml in Group 1-A to 15.7 µg/100 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-Uob (both by colorimetry and by HPLC), 145 cases (in Group 1-B) of Pb-B and ALA-Uob (by both methods), 2,852 cases (in Group 2-B) of Pb-B and either ALA-Uob or ALA-Usg (by colorimetry), and 2,475 cases (in Group 3-B) of Pb-B and either ALA-Uob or ALA-Usg (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=0.07 by Wilcoxon test) with larger p values than HPLC. When slopes and intercepts

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

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Cases</th>
<th>Methods of analysis</th>
<th>Correction for urine density</th>
<th>Regression curve parameters</th>
<th>Pb-B&lt;sup&gt;c&lt;/sup&gt; (µg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1-A</td>
<td>164</td>
<td>Colorimetric</td>
<td>Observed</td>
<td>α = 1.589, β = 0.265, γ = -0.013, δ = 0.00018, r = 0.85</td>
<td>33.8</td>
</tr>
<tr>
<td>Group 1-A</td>
<td>164</td>
<td>HPLC</td>
<td>Observed</td>
<td>α = 1.371, β = 0.184, γ = -0.009, δ = 0.00013, r = 0.90</td>
<td>31.0</td>
</tr>
<tr>
<td>Group 2-A</td>
<td>2923</td>
<td>Colorimetric</td>
<td>Observed</td>
<td>α = -0.021, β = 0.247, γ = -0.012, δ = 0.00017, r = 0.91</td>
<td>32.6</td>
</tr>
<tr>
<td>Group 3-A</td>
<td>2540</td>
<td>HPLC</td>
<td>Observed</td>
<td>α = 0.094, β = 0.171, γ = -0.008, δ = 0.00012, r = 0.93</td>
<td>23.2</td>
</tr>
<tr>
<td>Group 1-A</td>
<td>164</td>
<td>HPLC</td>
<td>SG-corrected</td>
<td>α = 2.520, β = 0.161, γ = -0.009, δ = 0.00015, r = 0.65</td>
<td>25.0</td>
</tr>
<tr>
<td>Group 2-A</td>
<td>2923</td>
<td>Colorimetric</td>
<td>SG-corrected</td>
<td>α = 1.694, β = 0.156, γ = -0.009, δ = 0.00013, r = 0.78</td>
<td>34.2</td>
</tr>
<tr>
<td>Group 3-A</td>
<td>2540</td>
<td>HPLC</td>
<td>SG-corrected</td>
<td>α = 0.916, β = 0.126, γ = -0.008, δ = 0.00014, r = 0.88</td>
<td>26.1</td>
</tr>
</tbody>
</table>

For groups, see Table 1. The parameters so that y = α + βx + γx² + δx³, where x is Pb-B (µg/100 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. Pb-B at the point of inflexion.

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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 – 0.003X² + 0.00007X³ (r=0.92, n=2,540), where X is Pb-B (µg/100 ml) and Y is ALA-U (mg/l) by HPLC as corrected for a specific gravity of 1.016.
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 \((5 \text{ and } 40 \, \mu g/100 \, ml)\) 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 \(>40 \, \mu g/100 \, ml\) 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 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 \((n=2,852 \text{ and } 2,475, \text{ respectively})\). 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
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 ± 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-defined 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 µg Pb/100 ml), 2 (>20 to 40 µg Pb/100 ml) and 3 (>40 µg Pb/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 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.

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 ± 0.2 mg/l.

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 µg/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 µg/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 \( \beta, \gamma \) and \( \delta \), see Table 2 and footnotes) gave Pb-B in a range of 16.6 to 34.2 µg/100 ml (Table 2). A Pb-B of 28 µg/100 ml may be considered to be the critical Pb-B concentration as an average of 8 estimations, i.e., \([33.8 + 31.0 + 32.6 + 23.2 + 25.0 + 34.2 + 26.1 + 16.6]/8\) µg/100 ml. The average level is close but somewhat greater than what was found in the previous analysis (i.e., 23 µg/100 ml12)).

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

<table>
<thead>
<tr>
<th>Classification by Pb-B</th>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>73</td>
<td>0</td>
<td>0</td>
<td>73</td>
</tr>
<tr>
<td>Colorimetry</td>
<td>67</td>
<td>6</td>
<td>0</td>
<td>73</td>
</tr>
<tr>
<td>Colorimetry-M</td>
<td>72</td>
<td>1</td>
<td>0</td>
<td>73</td>
</tr>
<tr>
<td>P for diff.</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P for diff.</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Analysis was conducted with 164 cases in Group 1-A. a Classes 1, 2 and 3 are ≤20, >20 to 40, and >40 µg Pb/100 ml blood (Ministry of Labour1)). b Classes 1, 2 and 3 are ≤5, >5 to 10, and >10 mg ALA/l urine (Ministry of Labour1)). c Classification by modified criteria of ≤6.4 (=5+1.4), >6.4 to 11.4, and >11.4 mg ALA/l urine. d Comparison by chi-square test. e Comparison by chi-square test when the modified criteria were applied to the colorimetry values.
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.\(^9\) 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 Cramér\(^9\) 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, 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 Morita\(^24\) showed ALA-U 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 higher.\(^12\)

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 whole.\(^10\) 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\(^12, 13\), or even as high as ≥100 µg/100 ml\(^22\).

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