Relationship between personal-sampled air lead and blood lead in low-lead-exposure workers in Japan to apply multiple regression models determining permissible air lead concentration

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Abstract
Objectives: We investigated the relationship between lead in air (Pb-A) measured by personal sampling and blood lead (Pb-B) in workers with relatively low lead exposure to estimate the permissible air concentration of lead corresponding to the biological tolerance value of Pb-B of 15 μg/dL.

Methods: We collected air samples at a lead-acid battery factory in Japan by personal sampling devices attached to 32 workers (19 males and 13 females) and measured Pb-A by a graphite furnace atomic absorption spectrophotometer in 2017-2020. In addition, we collected information on age, smoking habits, Pb-B, and urinary δ-aminolevulinic acid from the records of medical examinations for lead poisoning. Samples were collected two times from four workers, resulting in 36 data sets.

Results: Before analyses, we excluded four inappropriate data sets. The levels of Pb-A in the factory and Pb-B in the workers were almost under the current permissible limits. Multiple regression models showed significant correlations between Pb-B and Pb-A, and sex, and borderline significance between Pb-B and age. Based on them, we calculated Pb-A corresponding to Pb-B 15 μg/dL, and obtained similar values to the current occupational exposure limit (OEL) of 30 μg/m³, with slight variation between sex and age.

Conclusion: These results validate OEL, although supplementary conditions in terms of sex and age may be necessary.

KEYWORDS
air lead, blood lead, multiple regression, δ-aminolevulinic acid

1 | INTRODUCTION

Lead is a major metal that has been used in various industrial fields since ancient times. In previous times, many workers engaged in lead refining, printing, and storage battery manufacturing, and inhaled lead-containing dust leading to the effects of lead poisoning such as anemia, abdominal pain, and peripheral and central nerve disorder. In recent years in Japan, however, few workers are exposed to high levels of lead due to protective measures against lead...
Blood lead (Pb-B) levels are representative of the Biological Exposure Indices (BEI) for occupational lead exposure.\(^1\)\(^2\) In Japan, the current biological tolerance value of Pb-B is proposed by the Japan Society for Occupational Health as 15 µg/dL, which was amended in 2013 from 40 µg/dL that had been set in 1994.\(^3\)\(^4\)

The relationship between Pb-B and lead in air (Pb-A) has been studied since the 1970s to estimate the permissible air concentration of lead, but many of the studies involved workers with high exposure levels. King et al\(^5\) investigated workers at three lead factories and found that the slopes of the regression equations for Pb-A and Pb-B were 0.014-0.068, and the intercept was 30 to 46 µg/dL. Garside et al\(^6\) similarly obtained the regression equation \(Y = 0.0514X + 38.54\), but it was based on workers with Pb-B of 30 µg/dL or higher. Bishop and Hill\(^7\) obtained similar results, but the exposure levels of lead in workers were also high, without data around 15 µg/dL, the biological tolerance value of Pb-B of 15 µg/dL, based on the permissible air concentration of lead corresponding to the biological tolerance value of Pb-B, 15 µg/dL.

In recent years, Karita et al\(^8\) investigated workers in copper smelting plants in Japan, whose lead exposure levels were relatively low. They obtained the regression equation \(Y (\text{Pb-B}) = 10.34 \log X (\text{Pb-A}) - 0.64\), and estimated 32 µg/m\(^3\) as the permissible air concentration of lead corresponding to the biological tolerance value of Pb-B, 15 µg/dL. Based on the results, the occupational exposure limit (OEL) of lead was set at 30 µg/m\(^3\) by the Japan Society for Occupational Health in 2016.\(^9\) However, although the levels of Pb-B were relatively low, ranging from 8.9 to 25.7 µg/dL, the range of Pb-A in the workplace was marked, from 7 to 313 µg/m\(^3\), which were not obtained by personal sampling, but in the field. In short, these previous studies did not consider the biological tolerance value of Pb-B in Japan, or their exposure assessment of Pb-A was not precise because of the lack of personal sampling, leading to hesitation to apply them for the estimation of the permissible air concentration of lead in workers in Japan. Therefore, the purpose of this study was to estimate the permissible air concentration of lead corresponding to the biological tolerance value of Pb-B of 15 µg/dL, based on multiple regression models obtained from Pb-B of workers with relatively low lead exposure and Pb-A measured by their personal sampling.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

The target factory of the present study was a lead-acid battery factory. The factory manufactured lead-acid batteries for automobiles and industrial use, and lead oxide dust and fumes were generated in the factory. There were seven workplaces in the factory and categorized into five: battery assembly, laundry, battery case molding, battery inspection and packaging, and casting. During the period from September 2017 to March 2020, after obtaining informed consent, 32 workers (19 males and 13 females) engaged in their work while wearing a personal sampler near the mouth or nose for measuring Pb-A. These personal samplings were performed within 1-2 weeks before or after a medical examination for lead poisoning, and we used their Pb-B and urinary δ-aminolevulinic acid (ALA-U) data for analysis. Pb-B and ALA-U levels in the workers were measured every 6 months as medical examinations for lead poisoning. We also collected information on age and smoking habits from the results of such medical examinations. One male and three female workers participated two times in the personal samplings, whose intervals were at least 6 months, resulting in 20 male data sets from 19 male workers and 16 female data sets from 13 female workers. Therefore, the total number of data sets was 36. The workers did not wear dust masks because the lead concentrations in the air of the workplaces were not so high that enough to require them.

### 2.2 | Sampling and measurement of Pb-A levels

A personal sampling device for Pb-A measurement consisted of a mini pump (MP-W5P type, SIBATA SCIENTIFIC TECHNOLOGY LTD., Saitama, Japan), a personal sample holder (PS-33 type, SIBATA SCIENTIFIC TECHNOLOGY LTD., Saitama, Japan), and a fluorine-resin-treated glass fiber filter (TF98, φ25, SIBATA SCIENTIFIC TECHNOLOGY LTD., Saitama, Japan). The filter holder was attached to the collar of the worker, with a flow rate of 2.5 L/minute for about eight working hours. The sampling periods were 3-10 consecutive days per person, set according to the numbers of days that covered all the work performed by the worker. Based on “Working Environment Measurement Guideline for Metals,” the collected samples were heated and dissolved in a solution diluted with 60% nitric acid, and then quantitatively analyzed using an ICP emission spectroscopic analyzer (iCAP7400 Duo, Thermo Fisher Scientific, Tokyo, Japan) at a wavelength of 220.353 nm. A linear calibration curve was prepared by the matrix matching method and concentrations of lead were quantified. From the measured values, the 8-hour Time Weighted Average (TWA) of each day was calculated, and the geometric means (GMs) of the TWAs of all days were taken as the individual Pb-A. The limit of quantification was 0.17 µg/m\(^3\) for Pb-A.
2.3 Measurement of Pb-B and ALA-U levels

Pb-B levels were measured using a graphite furnace atomic absorption spectrophotometer (ZA3700, ZA3000, Hitachi High-Tech Science Corporation, Tokyo, Japan) with a wavelength of 283.3 nm. The analysis was performed using the methods of Subramanian et al.11 with modifications. A peripheral blood sample was diluted with ultrapure water containing Triton X-100 as a surfactant and diaminonitromethane phosphate as an interference inhibitor for the analysis. A linear calibration curve was prepared by the matrix matching method and quantified.

ALA-U levels were measured by HPLC (LaChrom Elite L-2000 Series, Hitachi High-Tech Corporation). The analysis was performed by modifying the methods of Okayama et al.12 and Endo et al.13 Reaction solution A (acetylacetone/ethanol/sodium chloride/ultrapure water) and reaction solution B (formaldehyde/ultrapure water) were added to urine and heated in boiling water to make ALA a fluorescent derivative for the analysis. In the liquid chromatograph, the mobile phase (methanol/acetic acid/ultrapure water) and ODS column (TSK-gel ODS-80Ts, 4.6 × 150 mm, 5-μm particles, Tosoh) were separated, and the fluorescence detector (excitation wavelength 363 nm, measurement wavelength 463 nm) was used for detection. A linear calibration curve was prepared by the absolute calibration curve method and quantified. ALA-U was corrected with a urine-specific gravity of 1.020.

Pb-B levels were measured at the Special Reference Laboratory Inc (SRL, Tokyo, Japan, which is a nationwide clinical laboratory). ALA-U levels were measured at Keio University but were measured at SRL only in the fall of 2018. The limits of quantification were 1.0 μg/dL for Pb-B and 0.1 mg/L for ALA-U.

2.4 Statistical analysis

The means of age, Pb-B, ALA-U, and Pb-A in the subgroups divided by sex or smoking habits were compared by Student’s t test. Smoking ratios in the subgroups divided by sex were compared by calculating the chi-square value and phi coefficient by the chi-square test. Pearson’s correlation coefficients between age, Pb-B, ALA-U, and Pb-A were calculated and simple regression analyses of Pb-A and Pb-B were performed. Outliers were judged by the Smirnov-Grubbs test. Multiple regression analyses were performed for data sets, using Pb-B as a dependent variable and other factors, such as Pb-A, age, sex, and smoking habit, as independent variables. Coefficients of determination (R²s) of the multiple regression models were calculated to judge their fitness, whose significance was tested by analysis of variance (ANOVA). Variance inflation factors (VIFs) were confirmed for the diagnosis of multicollinearity. Statistical analyses were performed using IBM SPSS Statistics V25 (SPSS Japan) along with Excel 2016 for simple calculations. The significance level was set to <5% two-tailed.

3 RESULTS

3.1 Subjects

Table 1 shows Pb-A levels in total data sets and subgroups divided by workplaces. Data on the control Pb-A were obtained from the same company’s non-lead-exposed office. In Laundry, Pb-A in Plant B (mean 12.00 μg/m³) was higher than that in Plant A (mean 5.56 μg/m³), probably because more clothes were handled in Plant B than in Plant A. The assembly and casting workplaces, which were prone to lead dust and fumes, had relatively high Pb-A levels (mean 6.79 μg/m³, 10.55 μg/m³). In addition, Pb-A was relatively high in laundry workplaces where lead dust was indirectly brought in (mean 9.59 μg/m³). Lead was also detected in controls, although Pb-A levels were very low (mean 0.68 μg/m³).

From the 36 data sets, we excluded four from subsequent analyses: a data set from one male included an extraordinarily high ALA-U, which suggested that he had been in an abnormal condition derived from unknown factors other than lead, and three data sets were from females in their 50s, who must have been in postmenopause, which may cause elevated Pb-B levels due to the intrinsic release of lead from bone stores14-17 (Table S1).

Table 2 shows the characteristics of the study data sets of workers, divided by sex and smoking habits. There were no significant differences in age, Pb-B, or Pb-A between sex or smoking habits, while ALA-U was significantly higher in smokers than non-smokers. The means of Pb-B in the total and subgroups were all lower than the biological tolerance value (15 μg/dL), but there were three data sets over it. However, all ALA-U data were lower than the biologically acceptable value of ALA-U, 5 mg/L.3 All Pb-A data were also lower than OEL of lead, 30 μg/m³.

Table 3 shows the age-classified numbers of smokers and Pb-B levels divided by sex. The smoking rate in males was higher than in females, but the difference was not significant (χ² = 0.622, P = .430, ϕ = 0.139). Although statistical analysis was impossible due to the small numbers, there was no obvious relationship between age and Pb-B, or age-classified smoking rates and Pb-B in either male or female data sets.
3.2 Relationships among age, Pb-B, ALA-U, and Pb-A

Next, we examined the relationship between Pb-A and Pb-B in the workers. Figure 1 shows scatter diagrams and regression lines between Pb-A and Pb-B in the total and sex-divided data sets. While the data were scattered widely in the scatter diagram in the total data sets, dividing them by sex led to distributions in a relatively high range of Pb-B in males and in a low range of Pb-B in females. However, the primary regression equations in total, males, and females were not significant. Actually, the matrix of correlation coefficients shows that those between Pb-A and Pb-B in males and females were higher than that in the total data sets, although not significant (Table 4). Only the correlation coefficient between age and Pb-A in females was significant, and that between age and Pb-B in males was relatively high, although not significant. The correlation coefficients between Pb-B and ALA-U were relatively low.
3.3 | Linear multiple regression models predicting Pb-B

Based on the results above, linear multiple regression analyses were performed to investigate the relationship between Pb-B as a dependent variable and Pb-A with other factors as independent variables. Table 5 shows a summary of multiple regression models predicting Pb-B in total data sets. In model 1, Pb-A, age, sex, and smoking habits including a constant term were used as independent variables. Among them, Pb-A and sex showed significant correlations with Pb-B, and age showed a borderline significance. All VIFs were <10.0, meaning no problem with multicollinearity, and $R^2$ of the model was significant, indicating its utility to predict Pb-B. Model 2, where smoking habits were omitted from the independent variables because of their low $\beta$, also showed significant correlations of Pb-B with Pb-A and sex. All VIFs were <10.0, meaning no problem with multicollinearity, and
6 of 9 | ONO AND HORIGUCHI

**DISCUSSION**

In the present study, we investigated the relationship of Pb-B and Pb-A in workers with relatively low lead exposure in a lead-acid battery factory in Japan, using data on Pb-A measured by a personal sampler. By performing multiple regression analysis, we obtained multiple regression models to predict Pb-B from Pb-A along with age, sex, and smoking habits.

The average Pb-A level in the total data sets of workers was 6.65 μg/m³, ranging from 1.61 to 17.74 μg/m³, which were all above Pb-A in the control workplaces (0.68 μg/m³) and all below OEL of 30 μg/m³ for lead in the air. The average Pb-B based on them was 10.2 μg/dL, ranging from 3.1 to 18.0 μg/dL, which were all over the previously reported GM of Pb-B (2.2 μg/dL) in workers before engaging in work involving exposure to lead and below the biological tolerance value of Pb-B (15 μg/dL), except for three data sets. These results indicate that the targeted subjects were suitable for the purpose of this study, to aim at workers with low lead exposure. Indeed, the targeted lead workplaces were not designated as the area necessary for protective measures against lead exposure based on the results of measurement of airborne lead concentrations, and the observed low Pb-A levels assured the occupational safety. Therefore, the workers were not required to wear dust masks, which enabled us to observe the unhindered relationship between Pb-A and Pb-B in them. However, there were three data sets of workers whose Pb-B levels were above the biological tolerance value, although the levels were only slightly excessive. We do not know the reason for this, but they were all male and two had smoking habits, and levels may be affected by other factors such as lead-contaminated food or fluctuation of lead exposure in the workplace.

In previous studies, primary linear regression equations between Pb-B and Pb-A were derived in lead-exposed workers, and several values of Pb-A corresponding to the biological tolerance value of Pb-B were proposed (Table S2). In most cases, the investigated workers were exposed to high
levels of lead, which allowed the drawing of suitable linear regression lines because of the wide ranges of Pb-B and Pb-A. In this study, however, the lead exposure levels were relatively low, making it difficult to identify a significant correlation between Pb-B and Pb-A (the correlation coefficient was 0.167; the regression equation was $Y = 0.156X + 9.135, P = .361$). Therefore, we performed multiple regression analyses, including possible confounding factors as independent variables, such as age, sex, and smoking habits, which were obtained from the results of medical examinations for lead poisoning.

We excluded four data sets from the total of 36 to construct multiple regression models. The value of ALA-U in the excluded male data set was extremely high in consideration of its Pb-B level, and statistically judged as an outlier by the Smirnov-Grubbs test as well (Table S1). In general, ALA-U levels are affected by various factors other than lead, such as acute intermittent porphyria (AIP) that increases ALA-U, and barbiturates can affect ALA-U. We do not know the reason for the high ALA-U (4.84 mg/L), but it may be beneficial to exclude outliers that can alter the relationship between Pb-A and Pb-B. In addition, we excluded three data sets from females in their 50s, two of whom showed Pb-B above the biological tolerance value. In general, the Pb-B levels of postmenopausal women may increase since the decrease in estrogen secretion induces enhanced bone metabolism, leading to the release of lead that is accumulated in bones to peripheral blood. Therefore, it may be reasonable to exclude postmenopausal women to observe the relationship between external exposure to lead and Pb-B.

The multiple regression models obtained indicate that Pb-B could be explained by Pb-A in low-level lead-exposed workers, and would be affected by sex, and probably by age as well, but not by smoking habits. In other words, the level of Pb-B in males was higher than that in females, and the Pb-B level may increase with aging, while smoking was not relevant to Pb-B. Bone lead levels increase with age in both males and females, and adult males have higher lead levels in bones than females. In addition, more lead is concentrated in the blood of males, because males have higher blood hematocrit levels than females, and the lead binds to red blood cells. The potential effects of sex and age on Pb-B can be explained by the above. It is widely believed that smokers have higher Pb-B than non-smokers because tobacco contains lead, which is inconsistent with the results of this study. It may be necessary to reconsider the close relationship between smoking habits and Pb-B, which may be just an incidental observation derived from some confounding factors.

Since smoking habit was not a significant variable, we used model 2 to calculate Pb-A corresponding to Pb-B 15 μg/dL by substituting sex and age. The following multiple regression equation was obtained. Workers’ predicted Pb-B is equal to $1.787 + 0.351 \times (\text{Pb-A}) + 0.091 \times \text{(age)} + 4.030 \times \text{(sex)}$, where Pb-A is measured in μg/m$^3$, age is measured in years, and sex is coded as 0 = female, 1 = male. Regarding the age, four patterns of 20, 40, and 60 years, and mean age were substituted, and the results are shown in Table S3. The calculated values of Pb-A at the mean ages were 16.8 μg/m$^3$ and 26.0 μg/m$^3$ in males and females, respectively, although they showed an age-dependent decrease, ranging from 10.6 μg/m$^3$ in males of 60 years to 32.5 μg/m$^3$ in females of 20 years.

When the current OEL in Japan was set at 30.0 μg/m$^3$, the research results of Ibiebele and Karita et al revealed that Pb-A levels corresponding to Pb-B 15 μg/dL were 22-25 and 32 μg/m$^3$, respectively (Table S2). The results of our study were similar to these previous ones despite the different methods of Pb-A measurement: using personal samplers in the former and field measurements in the latter. Because more reliable data on Pb-A can be obtained by personal sampling than the measurement in the field, the current OEL in Japan, 30.0 μg/m$^3$, has been further validated by the results of our study. However, it may be necessary to add supplementary conditions in terms of sex and age.

This study has some limitations. The data size of this study was relatively small compared with those of previous ones (Table S2). However, it was difficult to obtain data on Pb-A from workers by personal samplers, which are much more accurate and valuable than those by field measurements. We excluded data on female workers in their 50s, leaving the necessity of additional consideration of postmenopausal women. We calculated Pb-A corresponding to the current biological tolerance value of Pb-B 15 μg/dL, which is not an established value; for example, that level of Pb-B may show reproductive toxicity. In the future, it will be necessary to review the biological tolerance value of Pb-B as well as OEL corresponding to it.

### 5 CONCLUSIONS

We demonstrated that Pb-B of workers working in a lead-acid battery factory in Japan could be predicted from Pb-A, which was measured using personal samplers, by multiple regression models including sex and age as independent variables. Based on the models, we calculated Pb-A corresponding to Pb-B 15 μg/dL, and obtained values similar to the current OEL of 30 μg/m$^3$, with slight variation between sex and age. These results validate OEL, although supplementary conditions in terms of sex and age may be necessary.
DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

AO conceived the study, collected and analyzed the data, and wrote the manuscript. HH reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

DISCLOSURE

Approval of the research protocol: This study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the Kitasato University Medical Ethics Organization (No. B17-108).

Informed consent: Informed written consent was obtained from each participant before starting the study. Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

Conflicts of interest: AO is an employee of The Furukawa Battery Co., Ltd. No other author has reported a potential conflict of interest relevant to this article.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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