RESEARCH REPORT - No. 0801 - FY 2008-2010

Title of research project	A study on lead exposure and its health risks in children in Japan
Research project number	0801
Research period	FY 2008–2010
Name of principal research investigator (PI)	Dr Jun Yoshinaga, Associate Professor, Graduate School of Frontier Sciences, The University of Tokyo

Abstract

In Japan, there are very little data on blood lead levels in children, and little information is available about lead intake or its sources, leading to inadequate risk assessments and management for lead in children. To collect necessary data on lead exposure in children in Japan, in cooperation with pediatricians in three regions of Japan (i.e. Tokyo, Shizuoka, and Osaka), we measured blood lead levels in about 300 children (aged 1 to 15 years) over 3 years and obtained reference values for blood lead level in children.

Measurement of lead levels in 352 blood samples of children gave the geometric mean blood lead level of 1.07μg/dl, which was among the lowest in the world. Furthermore, the lead-related health risk i.e. decline in cognitive function in children estimated from the measured distribution is considered negligible. It was identified that variation factors of the blood lead levels in children were age, geographical location, and passive smoking.

The low blood lead levels obtained from the measurement were consistent with extremely low levels of estimated lead exposure (i.e. in a range of 2.4 - 13μg/day, and 5.4μg/day in average) for 11 out of all the children whose blood was sampled. The sources of lead investigated from environmental samples and precise lead isotope ratio measurement of blood with regards to these subjects allows us to estimate the contribution from food, house dust and soil approximately in equal proportions respectively.

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I. Introduction

Epidemiological studies conducted in the world since the 1980s revealed that lead, even at relatively low levels, is harmful to the development of cognitive functions in children. To address this fact, blood lead level in children has been monitored around the world, however, still there are little data on blood lead levels in children in Japan. Moreover, little information is available on the children's lead intake and its source.

The primary objective of this study is to overcome the scarcity of basic data on lead exposure in children in Japan, in cooperation with pediatricians in three regions of Japan (i.e. Tokyo, Shizuoka and Osaka), we measured blood lead levels in about 300 children (aged 1 to 15 years) for three years to obtain reference values for blood lead level in Japanese children.

In addition, for some subject children (about 20 persons), their blood sample were collected, lead intake and its sources were investigated through food including duplicate food samples and other food sampling via analysis of lead level and isotope ratios (207Pb/206Pb, 208Pb/206Pb) in each medium.

This study is designed to obtain fundamental data as required to assess the health risks of lead exposed in children. It is indisputable that data on blood lead level are fundamental to the risk assessment.

Through this study, we intended to identify possible routes of lead exposure involving duplicate food and drinks, soil, house dust, indoor air and other factors revealed the contribution of food to daily lead intake as well as the relative relationships between food and other lead sources; thus information was obtained on the contribution of foods to total lead intake. In Europe and the United States, there are reports of significant contribution of soil, house dust and other non-dietary sources to lead intake in children; however, from the standpoint of risk management, it is important to verify whether these findings apply to Japan, a country with different lifestyles.

For the implementation structure of this study, the chief researcher Dr Yoshinaga directed the overall research and conducted blood lead measurement, while co-researchers were responsible for specific parts of the study. Namely, Dr Kaji (Shizuoka City) established the pediatrician network and analyzed pediatric data, Dr Watanabe (The University of Tokyo), jointly with Dr Yoshinaga, participated in measurement of lead in blood and environmental samples, while Dr Tanaka (National Institute for Environmental Studies) estimated exposure sources based on lead isotope ratio measurement.

II. Methods

1. Blood lead levels and health risks in children in Japan

1) Basic considerations for blood lead analysis

We conduct a basic study to achieve highly reliable analysis of blood lead levels in children. Particularly, we pay attention to lead contamination in instruments and vessels during blood drawing, and select equipment designed to maintain least contamination level. Moreover, we considered acid digestion requirements for preprocessing of blood samples. Conditions for lead level analysis were optimized using ICP-mass spectrometry. Based on the mentioned considerations, we established optimal conditions for blood drawing and analysis, to verify the reliability of analysis using blood certified reference materials.

2) Analysis of blood lead levels in children and estimation of their health risks

Reference values for blood lead level in children in Japan are obtained via the mentioned analysis methods applied to blood samples of children aged 1 to 15 years, taken by a group of 8 collaborating pediatric

physicians in three regions of Japan (i.e. Tokyo, Shizuoka and Osaka). In addition to investigating variation in blood lead levels associated with age, sex and residency, we assessed the present health risks of lead exposure in children in Japan based on the obtained distribution of blood lead level.

2. Estimation of daily lead exposures to children and their exposure sources

1) Basic considerations for analysis of lead in environmental samples

Soil, indoor and outdoor dust, and food may act as potential sources of lead exposure in children; we consider preprocessing requirements for measurement of lead level in these environmental media, and to optimize the measurement conditions using ICP-mass spectrometry. Reliability of the analysis methods is verified using various certified reference materials.

2) Estimation of lead exposure level based on lead measurement in environmental samples

The mentioned environmental samples obtained from the homes of the subjects are analyzed using the methods described in (1) 1) above, preprocessing of blood samples and measurement of lead levels. By multiplying the lead concentration in each obtained environmental samples by the corresponding intake rates, the lead exposure from each medium was calculated, thus summing all the medium, the total daily lead intake was estimated.

3. Analysis of lead exposure sources in children using stable isotope analysis

1) Basic considerations for stable isotope analysis of lead in blood and environmental samples

Soil, indoor and outdoor dust, and food may act as potential sources of lead exposure in children and children's blood; to allow accurate measurement of stable isotope ratios ($^{206}Pb/^{204}Pb$, $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$) of lead in these environmental media by using MC (Multiple-Collector)-ICPMS, we optimize conditions for sample preprocessing and MC-ICPMS measurement.

2) Exposure source analysis based on measurement of lead isotope ratios in blood and environmental samples

The methods described in (3) 1, preprocessing of blood samples and measurement of lead stable isotope ratio a, are applied to analysis of blood samples of the subjects and environmental samples taken from the homes of the subject children; thus obtained results are analyzed concurrently with lead levels obtained in this research(2) "Estimation of daily lead exposure and its breakdown in children", and the sources of lead exposure in the children are analyzed by linear programming and other methods.

III. Considerations of ethical aspects

In this study, collecting blood samples from children and collecting home environment samples from some of the subject children's home, were explained to children's caretakers from collaborators (pediatricians) both orally and in writing; children whose caretaker voluntarily agreed to their participation and submitted written consent were subjects of this study.

Particularly, blood samples were not only taken for the purposes of this study but sampled from subject child at the time of blood sampling for diagnosis and other routine examination; extra blood from 1- 2 mL was obtained from the child for this study. All samples are handled with ID numbers, and only the collaborators can match the ID numbers and names of the subject children. This study is approved by an ethical committee which is responsible for ethical review processes at institutions (i.e. The University of Tokyo and National Institute for Environmental Studies) with which the chief researcher and co-researchers are affiliated and, as far as possible, at hospitals and other institutions with which the collaborators are affiliated.

IV. Results

Research in FY 2008

1. Summary

Methodology for blood lead measurement in children is established in the first half of FY 2008. To measure blood lead level, we aim to collect blood samples from 50 subjects through three Japanese hospitals in the second half of FY 2008. From FY 2009, we aim to collect blood samples from over 100 subjects per year, totaling to 300 subjects in three years. Environmental samples including duplicate food and other lead exposure media are aimed to be collected from five households; as soon as the sampling is completed, lead is analyzed, results of the analysis are obtained within the fiscal year, and the daily intake is estimated. Establishment of the preprocessing method for lead isotope ratio analysis in blood is the main objective for FY 2008; measured values of actual lead isotope ratios are to be obtained, even though it could be as few as about five cases.

2. Methods

Evaluation of lead contamination from blood drawing equipment

We checked and purchased needles and syringes (from two manufacturers) used normally in the hospitals with which the collaborators were affiliated, and conducted dissolution tests using purified water. In addition, the same tests were conducted to select blood collection tubes for dispensing and storage of blood in this study. From the result, the amount of contamination was 0.06 ng (0.006 ng from needle/syringe + 0.05ng from blood collection tube). We confirmed that contamination would not interfere with implementation of the study since it was below 0.3% of estimated amount of lead in blood samples.

Establishment of blood digestion method and lead measurement requirement

In order to prevent contamination during course of digestion, we adopted high-pressure thermal decomposition using a closed vessel for blood digestion, and investigated ratio of nitric acid, used for digestion, and blood and other parameters including digestion time (of blood). Thus we found that adequate results are obtained when 0.5 mL blood + 0.5mL nitric acid are taken in a 4.5 or 7 ml volume in a capped Teflon vessel, at 140°C for 4 hours. However, the rise of internal pressure caused some problems such as deformation of the digestion vessel. Lead level in digestion solution was measured using an ICP mass spectrometer. 0.5 mL blood was digested and diluted to 15 g; as the internal standard to final concentration of Bi (1 ng/g), and measurement was done with the integration time of 0.3 sec.

Reliability evaluation of lead analysis values

One whole blood certified reference material (Seronorm^{TM} Trace Element Whole Blood 1) was repeatedly digested and measured by the mentioned method; This reference material has two values: the certified reference value (range 2.62-2.90 μg/dl) and our measured value (2.72 ± 0.05 μg/dl , n = 6) , and those values demonstrated the validity as well as accuracy of our blood Pb analysis. The lower limit of our detection of measurement was 0.003 μg/dl, demonstrating high sensitivity. Furthermore, blood lead analysis was cross-checked by two laboratories.

Establishment of study structure

A total of eight pediatricians from three regions (i.e. Tokyo, Shizuoka and Osaka) served as collaborators, and with the cooperation of all the doctors, the study structure was established; blood samples obtained from the children were sent to The University of Tokyo for lead analysis. Ethical reviews were carried out at every pediatrics department and at institutions with which the chief researcher and co-researchers are affiliated, and approvals were acquired (completed by November 2008). [Kaji, Yoshinaga]

Measurement of blood lead level

In October-November 2008, soon after the approval procedures were completed, the collaborators started blood sampling, and the total of 44 samples were collected as of 23 January 2009 (Tokyo: 23, Osaka: 16, Shizuoka: 5). 37 out of 44 samples collected were analyzed for lead level using the mentioned method.

MC-ICPMS measurement

Standard solution was prepared with matrix elements such as Na and Fe Isotope ratio measurement using the lead standard solution added with matrix elements such as Na and Fe, which are the main components of human blood and food samples, revealed that lead must be separated and purified from basic components of blood and other samples for achieving accurate isotope ratio measurement.

Lead separation method

We attempted solid-phase extraction using iminodiacetic acid chelating resin and crown ether-based resin but did not obtain satisfactory results; hence we decided to apply the conventional method which is bromide complex followed by anion-exchange. Certified reference materials such as soil, blood, total diet and actual samples were digested in acid and deionized; thus recovery rate of lead ranged from 99.8 ± 7.6% (n = 18), which was considered to be satisfactory. In addition, extraction ratio of matrix elements in all samples was above 99%, which confirmed sufficient separation.

3. Results

Blood drawing devices were selected so as to prevent lead contamination of blood samples at the time of blood drawing by the collaborators. We established a method for acid digestion of blood using nitric acid and closed vessel and implemented requirements for measuring lead in digested blood samples.

The established methods for digestion and measurement (of lead?) using the measurement of certified reference material provide the validity and accuracy (of blood Pb analysis).In addition, after the ethical reviews were approved, starting from the end of FY2010, 44 blood samples were collected for lead measurement, and the following results were obtained (Table II-1, Fig. II-1).

Table II-1 Blood lead level in blood samples depending on regions

	Number of samples (males + females)	Age (range)	Lead level (range) μg/dl
Tokyo	19(m6+f6)*	6.5±5.5 (1-15)*	1.25±0.33 (0.82-2.56)
Osaka	16(m7+f9)	5.9±4.0 (2-15)	1.36±0.50 (0.71-2.17)
Shizuoka	2(m1+f1)	8,11	0.57, 0.87
Total	37		1.26±0.43

* As of the end of FY 2008, sex and age of 7 subjects were unknown

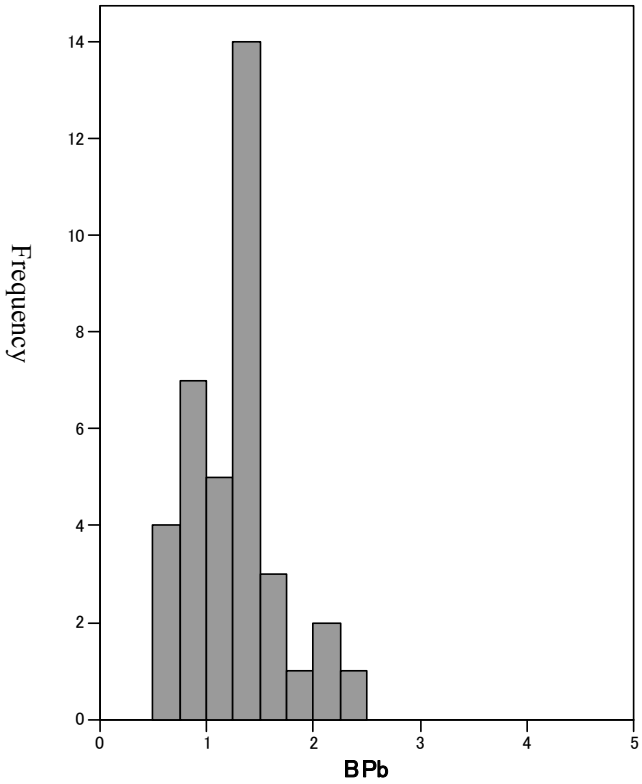

Fig. II-1. Histogram of blood lead level (unit: μg/dl)

Lead isotope ratio analysis for diet, house dust, soil and other environmental samples were conducted for source apportionment of contamination, and we found out that lead must be separated from the sample matrix. We confirmed that the bromide-complexation followed by anion-exchange method is optimal for lead separation in terms of separation efficiency, lead recovery rate, etc. All preparations for precise lead isotope ratio analysis for environmental samples collected beyond FY2009 were completed.

Research in FY 2009

1. Summary

The research for FY 2009 was conducted:

(1) To add blood lead level data of about 150 children by FY 2009 (Note: totals to about 200 subjects in FY 2008 – 2009);

(2) To aim at recruiting collaborating pediatricians from another region, one region from Kyushu or Tohoku, in addition to the three regions initially planned for blood sampling (Tokyo, Osaka, and Shizuoka);

(3) To aim to collect environmental samples of potential lead sources from ten homes;

(4) With regard to (3), to conduct lead analysis, and to estimate daily lead intake and intake and/or inhalation levels of lead from each source; and

(5) With regard to (3), to conduct lead stable isotopes ratio analysis for blood and environment samples collected from three homes.

2. Results

Lead analysis was carried out on blood samples taken from 76 subject children as well as 132 children's blood samples that had been taken and stored in Shizuoka Prefecture in 2005-2006. Including the samples of FY 2008, the mean level of 252 samples was 1.21μg/dl, among the lowest in the world. Blood lead level was slightly higher in children aged 0 to 3 years, showing a slight significant variation due to region. Sex and passive smoking were not influencing factors. Distribution of blood lead level in samples of children indicated that about 0.07% of all children have the levels above 4 μg/dl.

Among the blood-sampled children, families of ten subjects cooperated in taking environmental samples from their homes. The total daily lead exposure for three children, estimated from lead levels in duplicate food, soil, indoor and outdoor dust, etc. and intakes from each medium, was 2.5-5.0μg per day, which was rather low as compared to previous reports; food and indoor dust, respectively contributed to about half of the total exposure to lead, while contribution of soil was small. Comparison of lead isotope ratios in blood and environmental samples in two out of three children also confirmed that indoor dust contributed to over half of the total exposure to lead.

Lead stable isotope ratios in blood of two children were slightly different from those in duplicate food and showed isotope ratio distribution patterns close to indoor dust and soil. For one out of the two subject children, contributions of food, indoor dust and soil estimated by linear programming were19%, 26-50% and 31-54%, respectively While for the other subject children, contributions could not be found using linear programming because of some discrepancies observed in isotope ratios in blood and environmental samples; however, the ratios were very close to those of indoor dust. These results for isotope ratios also suggested a significant contribution of non-dietary oral exposure (soil, indoor dust and other sources) to lead exposure in children in Japan. Results for blood lead level in children analyzed in this fiscal year (n = 252) are presented in Table III-1 together with results for 44 samples obtained in FY 2008. Comparison with data from other countries also shows that lead exposure level in children in Japan is among the lowest in the world. The histogram is shown in Fig. III-1.

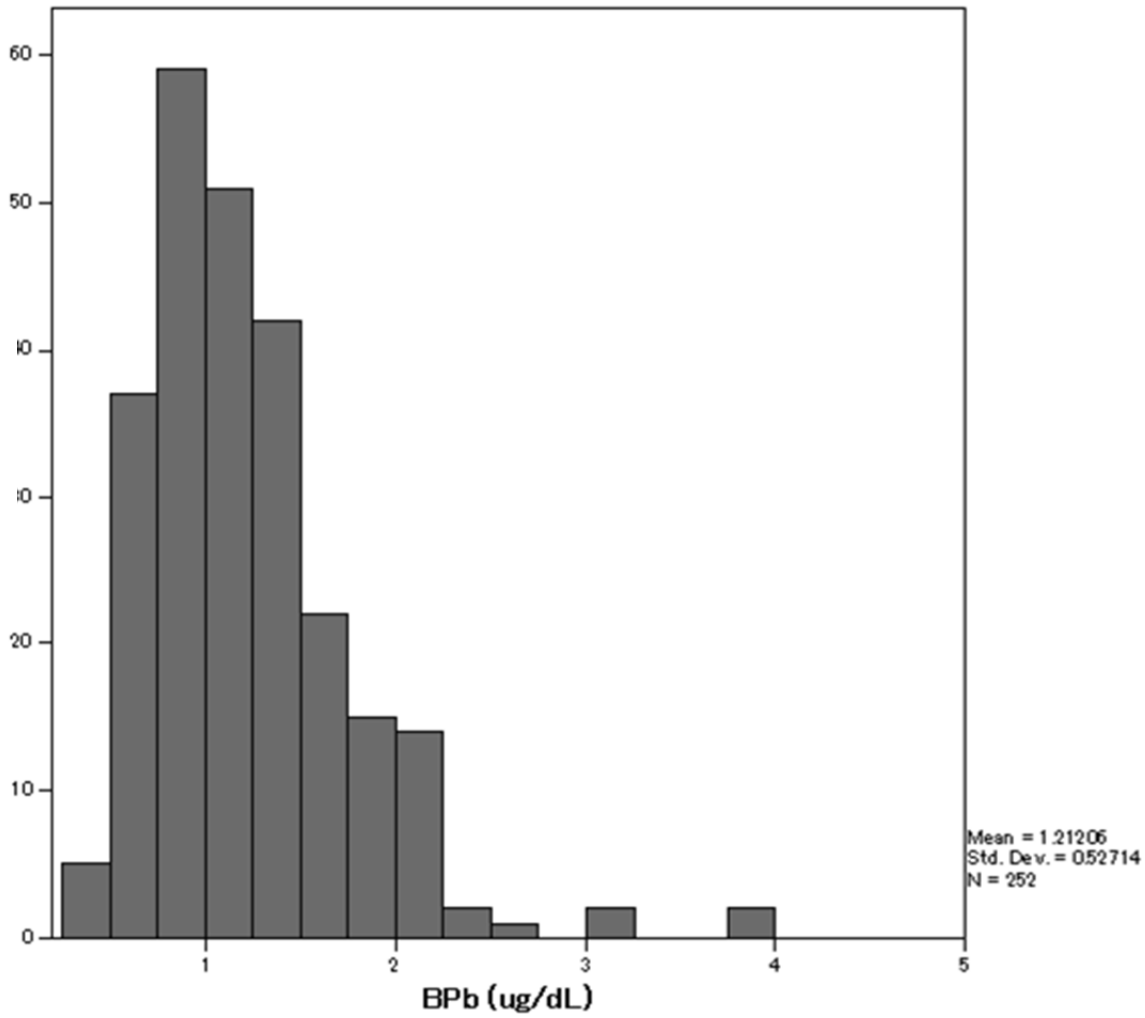

Fig. III-1. Histogram of blood lead level

Table III-1. Blood lead level in children in Japan

Region	Blood sampling period	N	Age	Blood lead level (μg/dl)	
				mean±SD	Median (min-max)
Tokyo	2008-2009	73	6.1±3.9	1.04±0.31	0.97(0.30-2.14)
Osaka	2008-2009	26	5.4±3.9	1.33±0.45	1.33(0.64-2.44)
Shizuoka	2008-2009	21	8.9±3.2	1.07±0.38	1.08(0.46-2.01)
	2005-2006	132	7.0±3.6	1.31±0.62	1.20(0.43-3.87)
Total		252	6.8±3.8	1.21±0.53	1.12(0.30-3.87)

Assuming that this distribution is representative of the population, the distribution was estimated by the geometric mean and geometric standard deviation, and about 0.07% of all children were estimated to have blood lead levels above 4 μg/dl. Since 18 million Japanese are aged 15 or under, the number of children with blood lead levels exceeding 4 μg/dl can be estimated at 13 thousand.

Analysis of lead exposure was completed for three out of ten subjects whose families gave their consent for the home visit by a researcher for taking environmental samples. The results are given in Table III-2.

Table III-2. Daily lead exposure of 3 subjects

Subject ID	Sex	Age	Blood lead level (μg/dl)	Exposure source level (mg/kg)	Daily exposure (μg/day)
1	m	7	3.12	Outdoor dust 158	0.032
				Dupl. food 0.0017	2.3
				House dust 100	2.5
				Soil 15.6	0.13
				Total	5.0
2	f	8	1.1	Outdoor dust 284	0.042
				Dupl. food 0.0010	1.2
				House dust 43.5	1.1
				Soil 25.5	0.22
				Total	2.5
3	f	9	3.34	Outdoor dust 180	0.027
				Dupl. food 0.0024	2.8
				House dust 66.3	1.7
				Soil 26.2	0.22
				Total	4.7

Fig. III-2 shows lead isotope ratios measured by MC-ICPM. Available lead was extracted from duplicate food, soil and indoor dust; lead was separated and purified using bromide-complexation followed by anion-exchange or chelating resins. Blood lead was also separated and purified using bromide-complexation followed by anion-exchange or chelating resins.

Contributions of each medium estimated by linear programming, were 19, 26-50 and 31-54 for food, indoor dust and soil, respectively.

Data on environmental lead level and lead isotope ratio of three children showed that lead exposure in the children was < 5 μg/day, and that indoor dust and soil were the main sources of lead exposure. These results are vastly different from some previous reports.

Nakanishi et al. estimated lead exposure in children in Japan (average of 0-6-year olds) as 1.6 μg/kg/day based on publicly available data such as Total Diet Study (TDS) by the Ministry of Health, Labour and Welfare (MHLW), and lead levels in the atmosphere, tap water and soil. Since assumed body weight of these children is 16 kg (average weight of 0-6-year olds), the total daily lead exposure is about 26 μg/day. Specifically, food, drinking water, and soil/indoor dust are reported to contribute 82%, 9.2% and 8.9%, respectively. TDS by the MHLW, on which Nakanishi et al. base their study results are different from the methodology of the present study. TDS uses the market basket method, while the duplicate portion method is adopted in our research. In the duplicate portion method, sampling does not necessarily provide a 100% accurate result for all consumed food; hence one would expect somewhat lower values. This, however, can hardly explain the ten-time difference between 21 μg/day reported by Nakanishi et al. and 2.1 μg/day obtained in this study. The reason for the difference is not clear at the moment.

Results of isotope ratio analysis using multi-collector ICP-mass spectrometry

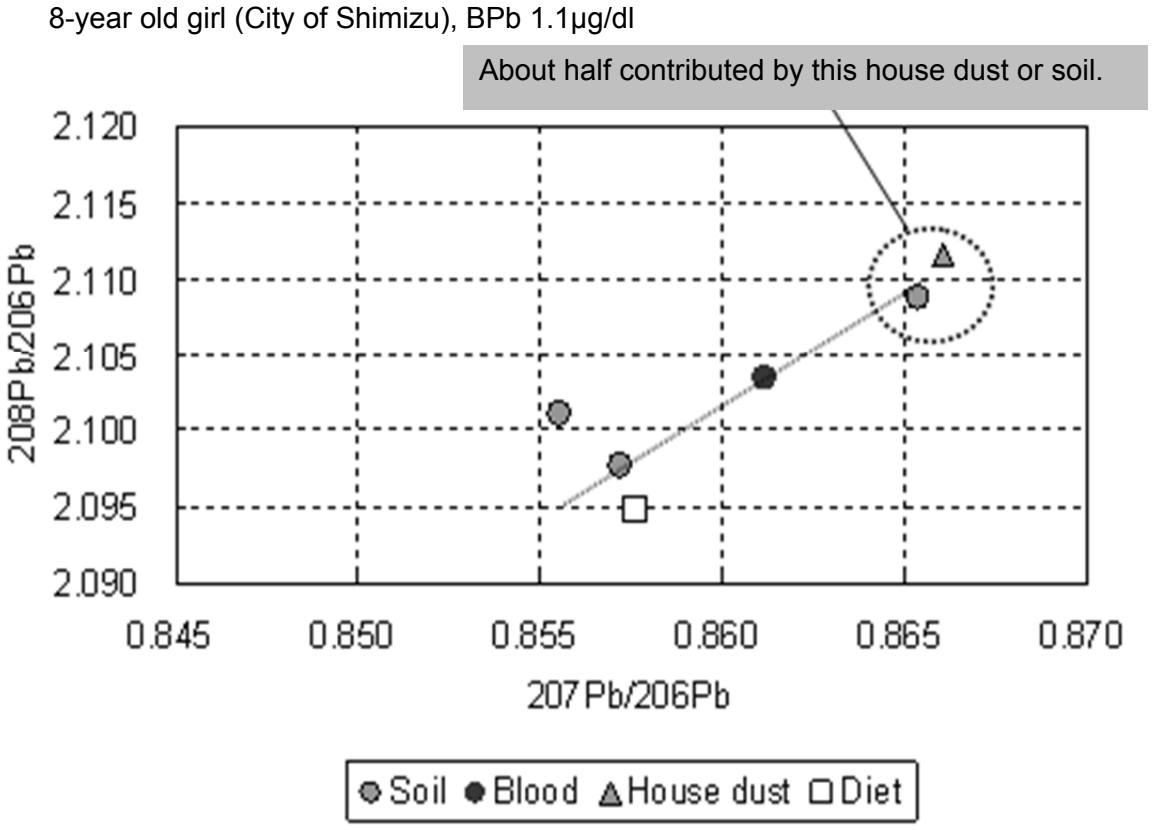

Fig. III-2. Stable isotope ratios of lead in blood and environmental media (8-year old girl)

Research in FY 2010

1. Summary

Children's blood sampling and blood lead analysis will be continued in FY 2010. The initial target of obtaining 300 blood samples would be achieved by collecting 48 more samples however, since the reliability may improve by increasing the sample size, we aim to collect large numbers of samples as possible. In FY 2010, we will arrange home visit with consent and continue the lead analysis and samplings on duplicate food, indoor dust as well as other samples, with regards to the research "Estimation of daily lead exposure and its breakdown in children in Japan". The samples from 10 homes have been already obtained, and the target for FY 2010 is to obtain ten more samples (a total of 20 homes for 3-year research period). With regards to the research "Analysis of lead exposure sources in children in Japan using stable isotope analysis", the measurement and analysis will be continued for FY 2010. Since samples from two homes have been analyzed to date, we set to analyze samples from 8 more homes as the target for FY 2010 (a total of ten homes for three-year research period).

2. Results

Blood lead levels and health risks in children

Lead analysis of 352 children's blood samples was completed by the end of FY 2010. The mean level was 1.07 μg/dl (geometric mean value), and estimation by distribution indicated that 0.075% of the children exceeded 4 μg/dl. Investigation of variation factors showed that significant factors were age, residency and parents' smoking.

Estimation of lead exposure level and measurement in environmental samples

A total of 14 homes were visited in FY 2009-2010 for the purpose of collecting samples of house dust and other environmental media. All samples scheduled for collection including duplicate food, house dust, outdoor dust and soil were obtained from 11 homes; these samples underwent acid digestion, and then lead levels were measured. The total daily intake of lead calculated from measured intake (food), estimated intake (atmosphere, soil, house dust) and levels was 5.4±4.0 μg/day (mean±SD) ; the respective contributions were 3.4±3.5 μg/day for food, 1.8±1.2 μg/day for house dust, 0.18±0.13 μg/day for soil, and 0.03±0.03 μg/day for atmosphere.

Analysis of lead exposure sources in children using stable isotope analysis

Samples from one out of the mentioned 11 homes could not be obtained due to the lack of the sample amount required for isotope analysis; for the remaining 10 homes, available lead was extracted from the environmental samples using simulated gastric fluid, lead was separated and refined using bromide complex followed by anion-exchange method (established in FY 2008), and lead isotope ratios ($^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$) were measured using MC-ICPMS. Blood lead was also measured in a similar way. These results combined with lead level data were used in linear programming to analyze contributions of each environmental sample to blood lead. As a result, solutions were obtained for four homes. All four homes showed nearly same respective contributions of house dust, food and soil; particularly, contribution of house dust was 50% for two homes and 30% for two homes. Solutions could not be obtained for the remaining 6 homes. This implies the presence of lead sources other than the samples, or poor representativeness of the samples in terms of lead levels and isotope ratios.

Estimation of daily lead exposures to children and their exposure sources

The geometric mean lead level in 352 blood samples collected with cooperation of pediatricians in Tokyo, Shizuoka and Osaka was 1.07 μg/dl, among the lowest in the world, as expected. The lead health risk (decline in cognitive function) in children estimated from the measured distribution proved negligibly low. Variation factors of the blood lead levels in children were age, geography, and passive smoking. The results of low blood lead levels were consistent with extremely low estimated lead exposure level (2.4~13 μg/day, average 5.4 μg/day) for 11 out of all the children whose blood was sampled. With regards to these subjects, the lead intake sources assumed from precise isotope ratio measurement were food, house dust and soil, with approximately in equal proportions respectively. .

Blood lead levels and health risks in children

Histogram of blood lead level in the 352 samples is shown in Fig. IV-1. The geometric mean value was 1.07 μg/dL. Based on this distribution, we carried out Monte Carlo simulations to estimate the probability that blood lead exceeds 4 μg/dl (no-effect reference value according to the latest epidemiological studies) and 10 μg/dl (action level of the US Centers for Disease Control and Prevention); the levels were exceeded in 75 and 0 cases, respectively, among 100,000 trials. Thus, the current health risks of lead exposure in Japanese children were judged to be negligibly small. We used factorial analysis of variance (ANOVA) for statistical analysis to examine blood lead level in children. The blood lead level (log-transformed) showed significant negative correlation with the age of children (r = -0.193, p < 0.001). We did not find significant sex difference. Comparing the 3 regions of Tokyo, Osaka and Shizuoka, lead level in Tokyo was significantly lower than in Osaka and Shizuoka (Table IV-1). To adjust for regional age difference, were-examined regional difference using analysis of covariance (ANCOVA), but lead level in Tokyo was still low as compared to Osaka and Shizuoka. In addition, we compared the data by dividing the subject children into three groups depending on the family's smoking status, namely, (1) cohabiting family members are all non-smokers, (2) there are smokers but they do not smoke in front of the children, (3) there are smokers, and they smoke in front of the children. Analysis of covariance (ANCOVA) applied to the three groups did not show significant variations. However, the mean age was different among the three groups (group (3) included older children); thus, we conducted covariance analysis that revealed significantly high blood lead level in children of group (3) as shown in Fig. IV-2.

We compared blood lead level of children by smoking status and regional difference (Table IV-1). Thus we found out that fewer families belong to group (3) in Tokyo than in Osaka or Shizuoka, though the difference is not statistically significant. Therefore, the observed regional difference in blood lead level might be related to the regional difference in passive smoking status.

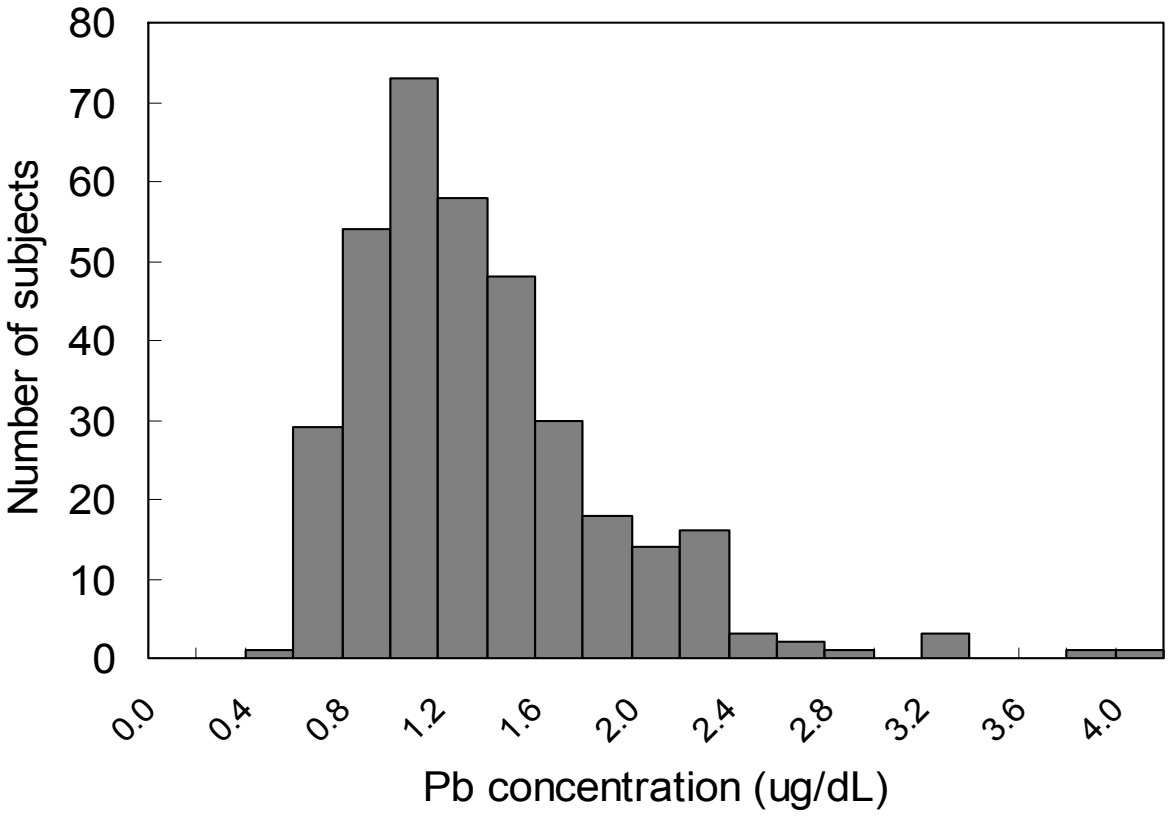

Fig. IV-1. Histogram of blood lead level (n = 352)

Table IV-1. Interregional comparison in blood lead level

	N	Age (years) **	Geometric mean blood lead level (μg/dl) **
Tokyo	131	6.2±3.8*	0.940 (1.42)#
Osaka	34	4.8±3.6	1.24 (1.41)
Shizuoka	187	7.3±3.6*#	1.15 (1.56)
Total	352	6.6±3.8	1.07 (1.51)

** Variation in age and blood lead concentrations due to the regions was significant (ANOVA, $p < 0.001$)

Significantly different from other mean values (Scheffe's multiple comparison, $p < 0.05$)

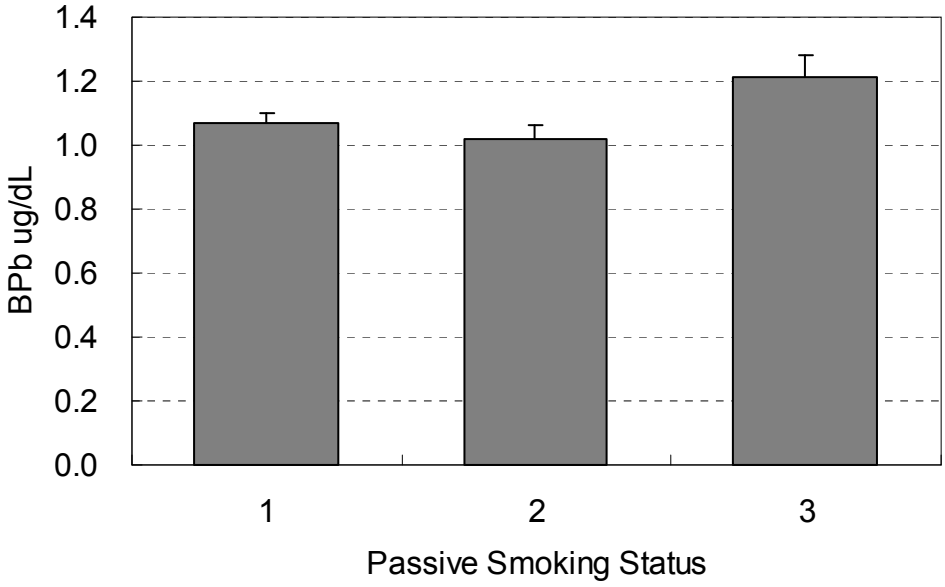

Fig. IV-2. Passive smoking status and blood lead levels in children

1: no smoking family member, 2: smoking family member present but does not smoke in the presence of child, 3: smoking family member present and smokes in the presence of child. Mean values were age-corrected by covariance analysis. There is significant difference between 1 and 3, 2 and 3. Estimation of daily lead exposure and its sources

With regards to the children blood-sampled in Shizuoka during FY 2009-2010, 14 families gave their consent for taking duplicate food and other environmental samplings (potential lead sources) after hearing the explanation from collaborators and (pediatricians). Researchers visited all these homes and took samples of food (1-day duplicate portions), house dust (vacuum cleaner waste), outdoor dust (deposited on outside window frames), surrounding soil, cigarette (in case that there were cohabiting smokers), etc.

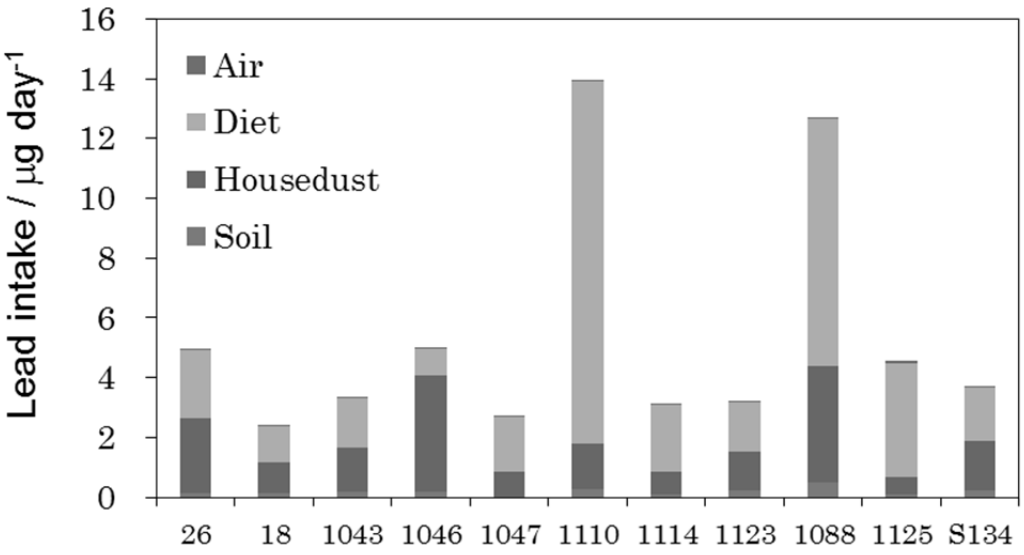

Fig. IV-2. Estimated daily lead intake and its exposure sources for each subject

After acid digestion of the samples, lead levels were measured using ICPMS, and the daily lead intake from each medium and total daily lead intake, which is the sum of the lead intake from each medium, were calculated for each subject from measured daily intake (food), estimated intake (house dust, atmosphere, soil) and levels of lead (Fig. IV-2). The amount of daily lead intake ranged rather widely from 2.4 to 13.9 μg/day. The mean value of 5.4 μg/day was low as compared to the value estimated by Nakanishi et al (30 μg/day). The main lead sources were food and house dust, with average shares of 58% (range: 18-86%) and 38% (range: 11-78%) respectively. Nakanishi et al. report the food contributes to above 80% of total lead intake, while the present study indicates greater contribution of house dust. On the other hand, this study agrees with the study of Nakanishi et al. that the atmosphere and soil do not contribute substantially to lead intake.

Analysis of lead exposure sources in children using stable isotope analysis

Lead that may be absorbed by human body (available lead) was extracted from the environmental samples, which were in the same way as those used in the lead analysis of the previous research item, using simulated gastric fluid, then, separated and purified from the environmental sample matrix using bromide complexation followed by anion-exchange method; then lead isotope ratios ($^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$) were measured using MC-ICPMS. In addition, isotope ratios of blood lead in the children were measured in the same way after acid digestion of blood. Among the 11 subjects listed in Table IV-2, this analysis was applied to 10 subjects with samples sufficient for isotope ratio measurement.

In addition to Fig. III-2 of FY 2009, three cases are shown in Fig. IV-3~5.

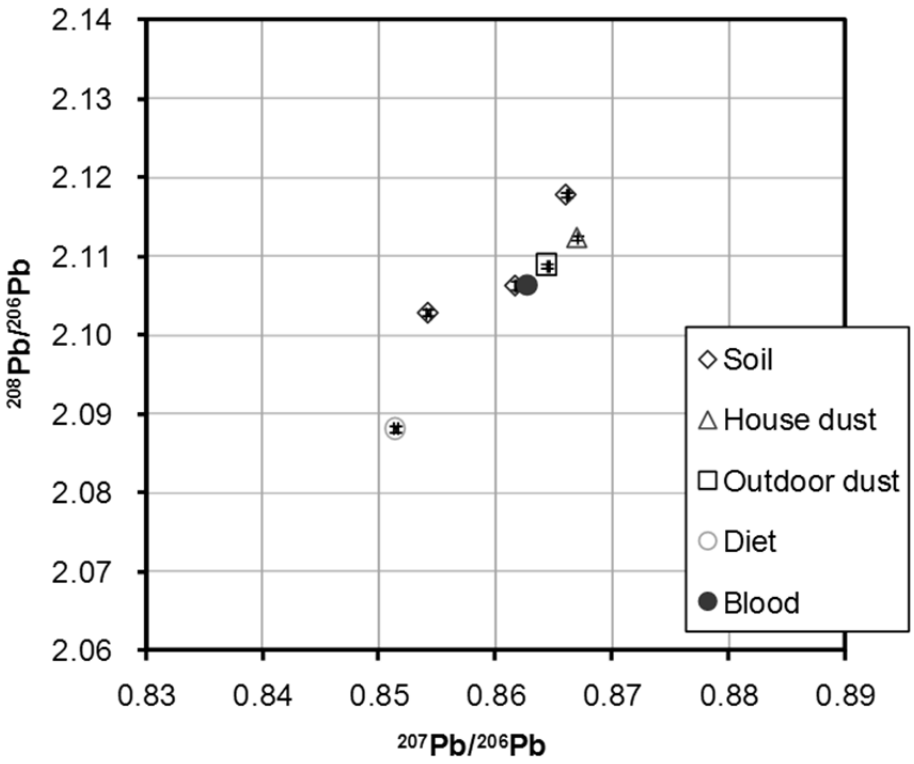

Fig. IV-3. Case of 7-year old girl (blood lead level: 1.5 μg/dl)

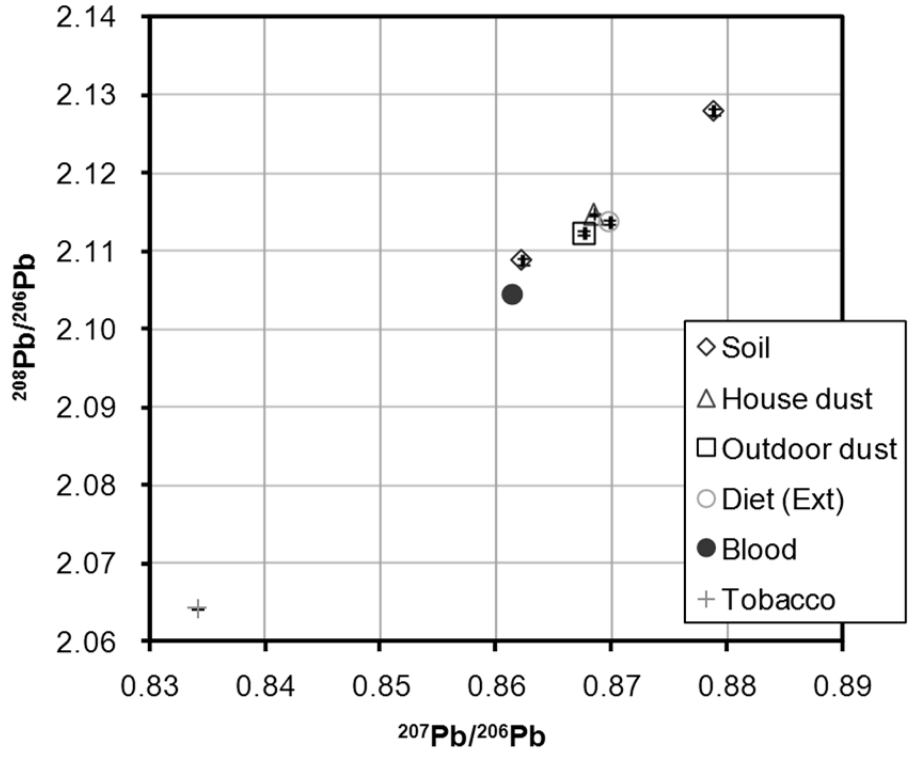

Fig. IV-4. Case of 11-year old girl (blood lead level: 1.3 μg/dL)

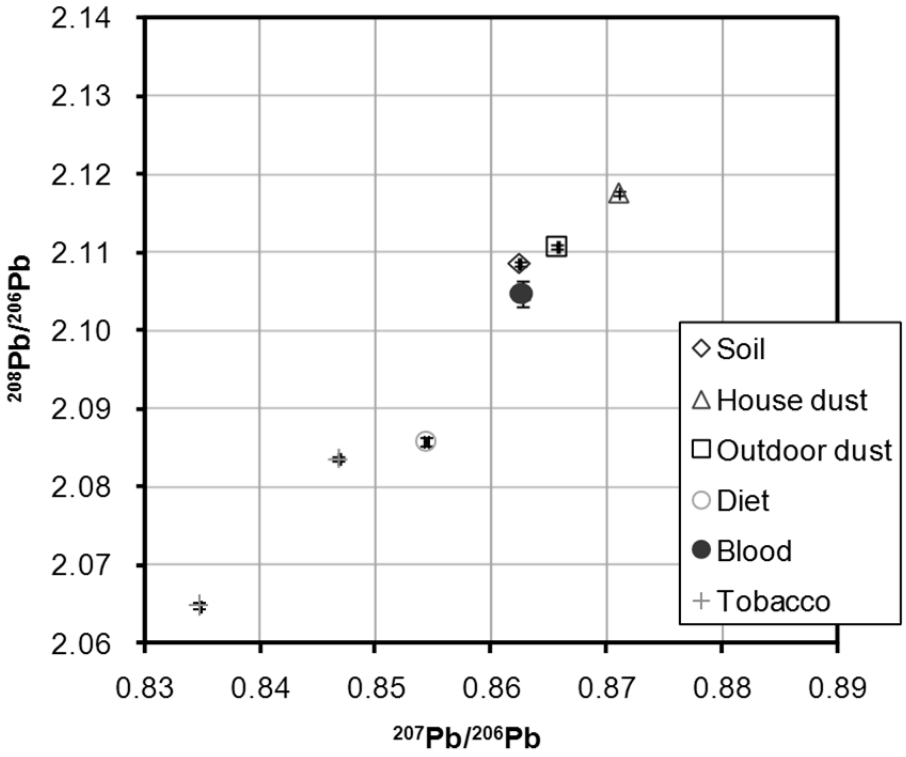

Fig. IV-5. Case of 7-year old boy (blood lead level: 0.46 μg/dl)

In case of a 7-year old girl in Fig. IV-3, lead isotope ratios of blood were distributed between those of food, soil, house dust etc. Lead sources estimated by linear programming were 18% for food, 26-34% for school-yard soil, 0-9% for home-yard soil, and 42-57% for house dust; demonstrating large contributions of house dust and soil to lead sources. Fig. IV-4 pertains to a 11-year old girl; in this case, lead isotope ratios of blood were lower than those of environmental samples, except for cigarette smoke. According to questionnaire survey, no family members smoked in the presence of the child; solution could not be obtained by linear programming, when cigarette smoke was assumed not to contribute to lead intake. Fig. IV-5 shows a case of a 7-year old boy. Similar to that of Fig. IV-3, lead isotope ratios of blood were distributed between those of the environmental samples, while linear programming estimated that food, soil and house dust contributed by 30%, 32-48% and 22-38%, respectively (in this case, as well, family members said that they did not smoke in the presence of the child; hence, isotope ratios in cigarette smoke were not included in linear programming). Contributions of different lead sources in four subjects, for which linear programming solutions were obtained, are given in Table IV-2. As may be seen from the table, contributions of house dust and soil were large as compared with the data on lead intake sources listed in Risk Assessment Report by the National Institute of Advanced Industrial Science and Technology.

Table IV-2. Contributions of lead intake sources estimated by linear programming

Subject ID	18	1043	1046	1047
Sex, age	Girl, 8 years old	Girl, 7 years old	Boy, 7 years old	Girl, 9 years old
Intake source, contribution (%)	Food, 19% Soil, 34-39% House dust, 42-47%	Food, 18% Soil (home), 0-9% Soil (school), 26-34% House dust, 42-57%	Food, 30% Soil, 32-48% House dust, 22-38%	Food, 38% Soil, 32-33% House dust, 29-30%

Linear programming solutions could not be obtained for 6 out of the 10 subjects, as in the case shown in Fig. IV-4. This may be indicative of existence of unidentified lead intake sources other than the samples, and poor representativeness of the samples in terms of lead levels and isotope ratios, etc.

Overall results and conclusion obtained through FY 2008 - 2010

1. Summary

352 blood samples of children in Japan were collected with cooperation of pediatricians in Tokyo, Shizuoka and Osaka, and lead levels were measured. The geometric mean lead level in blood of children was 1.07 μg/dl, which was among the lowest in the world, as expected. The lead health risk (decline in cognitive function) in children estimated from the measured distribution proved negligibly low. Variation factors of the blood lead levels in children were age, geography, and passive smoking. The results of low blood lead levels were consistent with extremely low estimated lead exposure level (2.4~13 μg/day, average 5.4 μg/day) for 11 out of all the children whose blood was sampled. With regards to these subjects, the lead intake sources estimated from environmental samples and precise lead isotope ratio measurement of blood were food, house dust and soil, with approximately in equal proportions respectively.

2. Results

The present research intended to acquire information on blood lead levels in children in Japan, as well as lead intake and its sources, to be used as the basis for the assessment of lead health risks in children. In this study, blood samples were obtained from children in Tokyo, Shizuoka and Osaka with help of collaborators (pediatricians); the samples were preprocessed and put to lead level analysis at The University of Tokyo. In addition, daily lead intake and its sources were estimated using samples of potential sources of lead intake (duplicate food, house dust, soil, outdoor dust, etc.) obtained during home visits to blood-sampled children, as well as lead level analysis; furthermore, sources of blood lead (intake sources) were estimated through precise lead isotope ratio analysis in the samples (performed at the National Institute for Environmental Studies).

Since blood lead level can be determined in a trace level of the order of μg/dl, first, it was necessary to set up conditions which would allow highly reliable measurement of blood lead. Thus we checked contamination levels of needles and syringes used for blood drawing (0.006 ng on average). Moreover, blood collection tubes with acceptable low-contamination were selected for blood collection and storage through actual measurement (0.05 ng per blood collection tube). The total contamination was 0.06 ng at its highest, which was sufficiently low as compared to expected lead content in blood (20 ng/2 mL). We optimized parameters for acid digestion of blood samples, conditions for ICPMS measurement of lead level in digested blood and other conditions. Moreover, our evaluation of the validity and accuracy of the blood lead analysis by using blood certified reference material (Sero^{R}, Norway) and cross-checking between laboratories demonstrated the high reliability of our analysis, .(Yoshinaga and Watanabe).

Eight collaborators in Tokyo, Shizuoka, Osaka and the co-researcher Kaji obtained a total of 352 blood samples from children (age: 1-14 years old). The measured lead levels are shown as a histogram in Fig. V-1, and regional comparison is given in Table V-1.

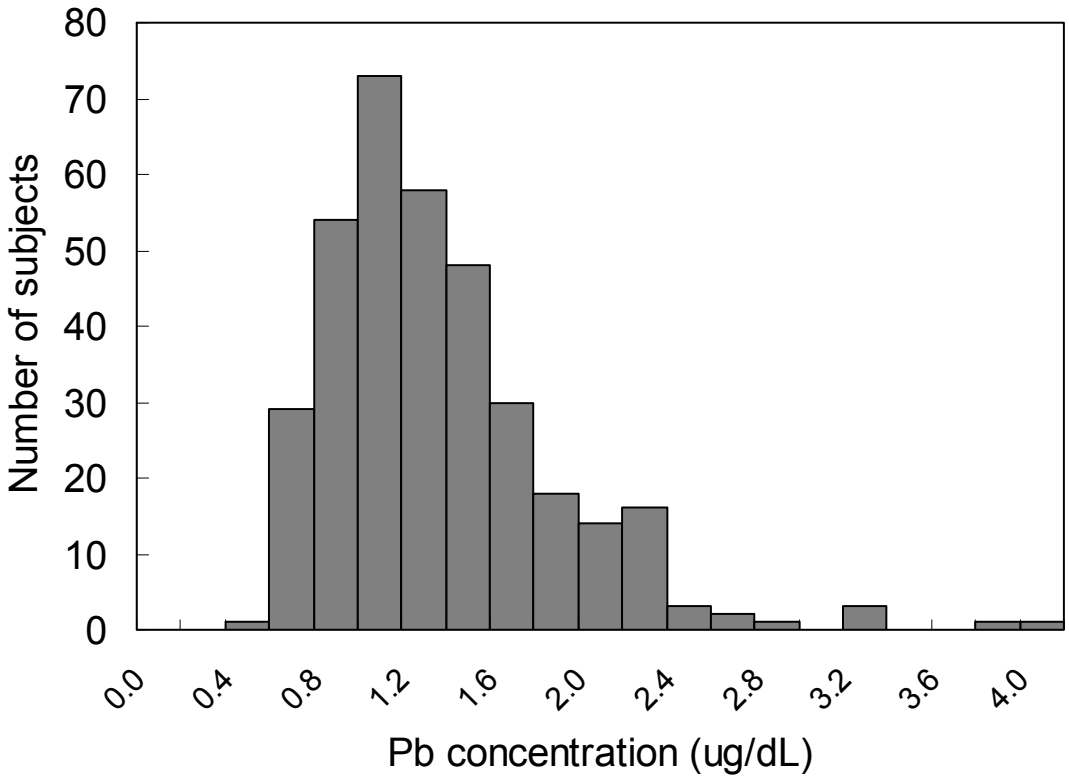

Fig. IV-1. Histogram of blood lead level (n = 352)

Table V-1. Interregional comparison in blood lead level

	N	Age (years) **	Geometric mean blood lead level (μg/dl) **
Tokyo	131	6.2±3.8*	0.940 (1.42)#
Osaka	34	4.8±3.6	1.24 (1.41)
Shizuoka	187	7.3±3.6*#	1.15 (1.56)
Total	352	6.6±3.8	1.07 (1.51)

** Variation in age and blood lead concentrations due to regions was significant (ANOVA, p , 0.001)

Significantly different from other mean values (Scheffe's multiple comparison, $p < 0.05$)

The geometric mean value of all 352 subjects was 1.07 μg/dl, among the lowest in the world (Table V-2).

Table V-2. International comparison of blood lead level in children

	Number of samples	Sampling period	Age	Geometric mean μg/dL	Reference
Japan	352	2005-2010	1-14	1.07	This research
Canada	910	2008-2009	6-11	0.90	Bushnik et al.
USA	968	2005-2006	1-5	1.43	US CDC
	934		6-11	1.02	
Germany	1560	2003-2006	3-14	1.63	Kolossa-Gehring et al.
Korea	667	2008	8-11	1.9*	Cho et al.
South Africa	429	2002-2003	5-11	6.4*	Mathee et al.
China	94,778	2000-	0-14	8.07*	He et al.
India	754	2002	<12	8.36	Nichani et al.

Based on the distribution of blood lead level in Fig. V-1, we carried out 100,000 random trials of estimated blood lead level in children in Japan to check whether it exceeded the action level set by US CDC (10 μg/dl) or no adverse effect level on children's cognitive function based on the latest epidemiological studies (4 μg/dl). As a result, no case exceeded the CDC action level, and 75 cases exceeded the level of 4 μg/dl (0.075%). Thus, estimation based on the distribution of blood lead levels obtained in this study suggests that health risks of lead intake in children in Japan are negligibly low.

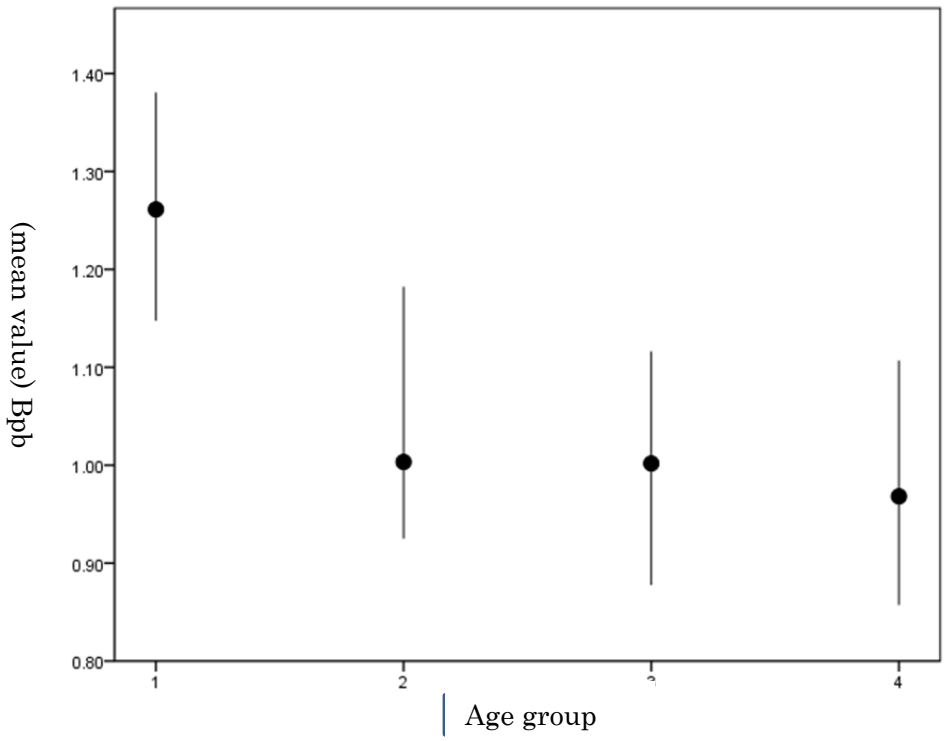

Fig. V-2. Blood lead level by age groups (median value)

1: 1-3 years old, 2: 4-6 years old, 3: 7-9 years old, 4: >10years old

We identified several variation factors of blood lead levels in children. For example, significant negative correlation (r = -0.193, $p < 0.001$) was observed between age and blood lead level. Previous studies conducted in various countries indicated that blood lead level was the highest in 2-year olds, and then followed by decrease thereafter; this is consistent with the results of our research suggesting that the level was high in the age group of 1-3 years old, and then remained almost unchanged (Fig. V-3).

Moreover, there was a regional difference in blood lead levels in children. As shown in Table V-1., the mean blood lead level in Tokyo was lower than that in Osaka and Shizuoka. There was also a regional difference in the mean age; however, lead level remained lower in Tokyo even though the age difference was adjusted by means of covariance analysis.

In addition, based on the questionnaire survey from the families of the children whose blood was sampled, we examined the relationship between blood lead levels and passive smoking status. The subjects were divided into three groups: ‘cohabiting family members are all non-smokers’, ‘there are smokers but they do not smoke in the presence of the children’ and ‘there are smokers, and they smoke in the presence of the children’; comparison among the groups did not show significant fluctuations (ANOVA, P = 0.09). However, the groups differed significantly in the average age (the average age was high in the group of ‘smoking family members present and smokes in the presence of child ’); thus we applied ANOVA. Fig. V-4 shows age-adjusted mean blood lead level according to passive smoking status.

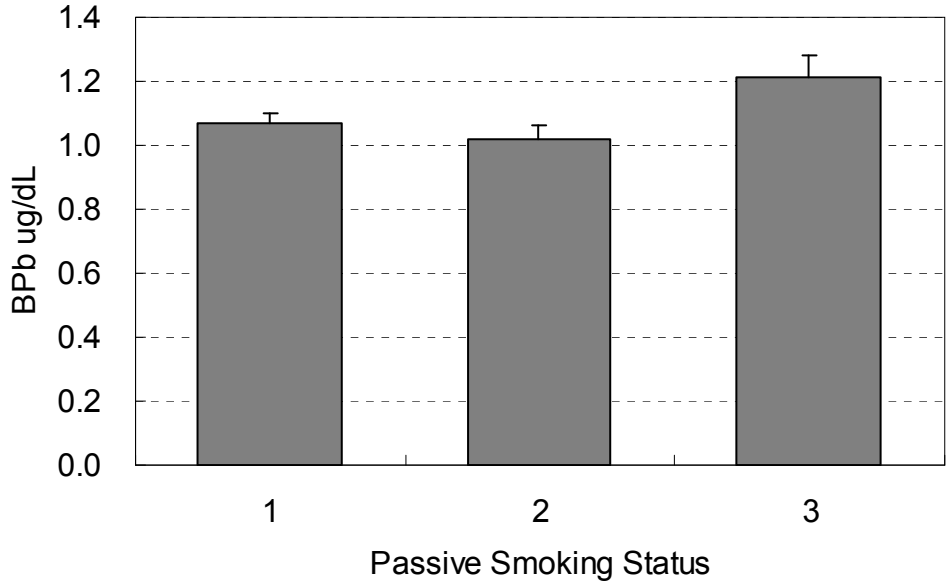

Fig. V-3. Passive smoking status and blood lead levels in children

1: no smoking family member 2: smoking family member present but does not smoke in the presence of child 3: smoking family member present and smokes in the presence of child. Mean values were age-corrected by covariance analysis. There is significant difference between 1 and 3, 2 and 3.

The diagram indicates that blood lead level is higher by about 10% in children whose family members smoke in the presence of them, that is, the children exposed to passive smoking. It is common knowledge that cigarette contains lead but it is not clear whether these results are caused by direct effect of inhaling the lead contained in smoke, or by effects of some confounding factors.

Research collaborators in Shizuoka recruited families of the blood-sampled children to cooperate in a survey of lead intake sources. Investigators from The University of Tokyo visited 14 homes of the families who agreed to cooperate and sampled potential sources of lead such as food (duplicate portions), soil at home and school, house dust (vacuum cleaner waste), outdoor dust, tap water and cigarette (in case of inhabiting smokers). In addition, the caretakers were asked to fill in a questionnaire about the children's living conditions and family situation.

Thus obtained environmental samples were put to acid digestion, and then lead level was measured using ICPMS. Weight of food intake was measured (duplicate portion weight), while ventilation ratio, soil ingestion and house dust ingestion were set to their respective default values with reference to existing literature, and total daily lead intake was estimated as $\sum[concentration]\times[intake]$. Among 14 homes visited, samples were insufficient for 3 homes (for example, vacuum cleaner waste was provided but duplicate portions were not); hence lead intake was estimated only for 11 subjects. The results are presented in Table V-3.

Table V-3. Daily lead intake (total and its sources) for every subject (μg per day)

Subject ID	Food	Soil	House dust	Atmosphere	Total
18	1.2	0.12	1.0	0.042	2.4
26	2.3	0.13	2.5	0.027	5.0
134	1.8	0.22	1.7	0.027	3.7
1043	1.6	0.21	1.5	0.002	3.3
1046	0.88	0.18	3.9	0.015	5.0
1047	1.8	0	0.84	0.045	2.7
1088	8.3	0.50	3.9	0.017	12.7
1110	12.0	0.25	1.6	0.009	13.9
1114	2.2	0.074	0.79	0.007	3.1
1123	1.7	0.22	1.3	0.012	3.2
1125	3.8	0.08	0.60	0.093	4.6
Average	3.4	0.18	1.8	0.027	5.4

The total daily lead intake in children was spread in the range of 2.4~13.9 μg/day, the mean±SD being 5.4±4.0 μg/day. Respective shares were 3.4 μg/day for food, 0.18 μg/day for soil, 1.8 μg/day for house dust, and 0.027 μg/day for atmosphere; on average, food and house dust accounted for about 63% and 33%, respectively, thus amounting together to 95%. Contribution of soil was estimated as small as 3%.

Nakanishi et al. estimate daily lead intake of 6-year-old children as 1.5μg/kg/day from oral intake and 0.015μg/kg/day from inhalation. With the body weight of a 6-year-old child being 24 kg as assumed by Nakanishi et al., the total daily lead intake is 36 μg/day. The result of the present study (5.4 μg/day) is 1/7 of the estimate by Nakanishi et al.; this difference can be related to the difference in intake from all foods (and tap water). The daily lead intake of is about 10 times lower than 33 μg/day estimated by Nakanishi et al.. Whereas 7-day long duplicate portion survey of 33 Japanese nursery school toddlers conducted by Aung et al. (2006) produced the result of reported the daily lead intake as 4.3 μg/day. TDS conducted by the Ministry of Health, Labour and Welfare, to which Nakanishi et al. refer for lead intake data, is based on the market basket method, and the difference in methodology might have caused the difference in results.

The mentioned estimation of the sources, intakes of lead from all sources except for food, were not measured but substituted by default values; hence uncertainties were involved. Thus we used lead stable isotope ratios ($^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$) as an additional index to check whether the blood lead in subject children originated from some environmental sources. However, since lead isotope ratios of environmental samples were not significantly different from each other, accurate isotope ratio measurement was required; thus we examined suitability of multiple-collector ICPMS (MS-ICPMS) for high-precision isotope ratio measurement. As a result, we found that the accuracy of lead isotope ratio measurement was deteriorated by matrices (aluminum, iron, calcium, potassium, etc.) existing in environmental samples. On the other hand, we confirmed that very high accuracy in measurement ($2\sigma = 0.02\%$) is feasible provided that lead had been separated and purified from the matrix. The method established for lead separation and purification is shown in Table V-5.

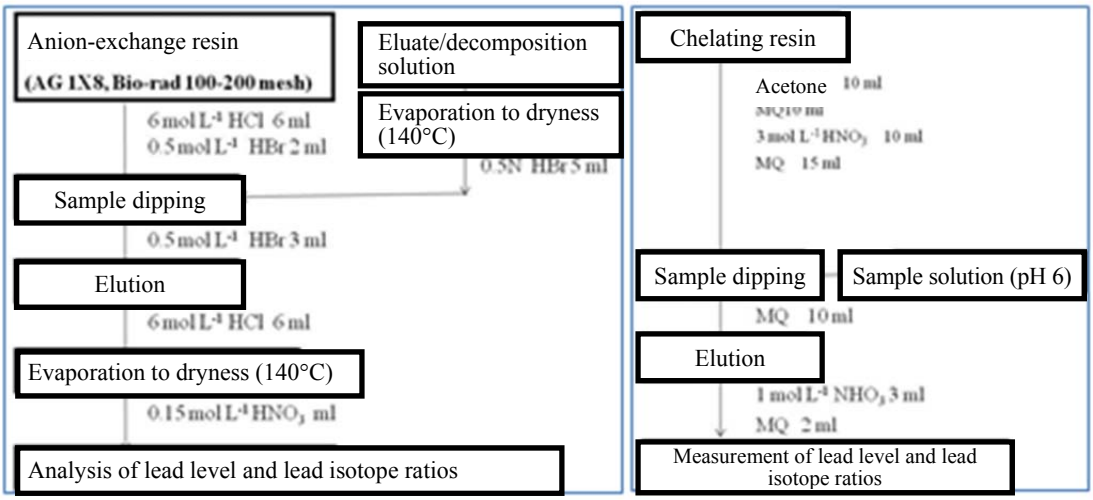

Fig. V-4. Method of separation and purification of lead from blood and environmental samples

This separation method was applied to environmental samples obtained at homes of the mentioned 11 subject children as well as to blood samples of the subjects, and lead isotope ratios were measured by MC-ICPMS. Samples from ten out of 11 subjects were processed because in one subject, the sample was insufficient for isotope ratio measurement. In this research, lead absorbed in human body (i.e. lead in blood and environmental samples) had to be compared in order to measure lead isotope ratios. For this purpose, we analyzed only the lead extracted from environmental samples using simulated gastric fluid (0.04 mol/L glycine aqueous solution, pH 1.5).

Three cases are illustrated in Fig. V-6~8. In the diagrams, $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ are plotted on the horizontal and vertical axes, respectively, while error bars denote show measurement errors of MC-ICPMS.

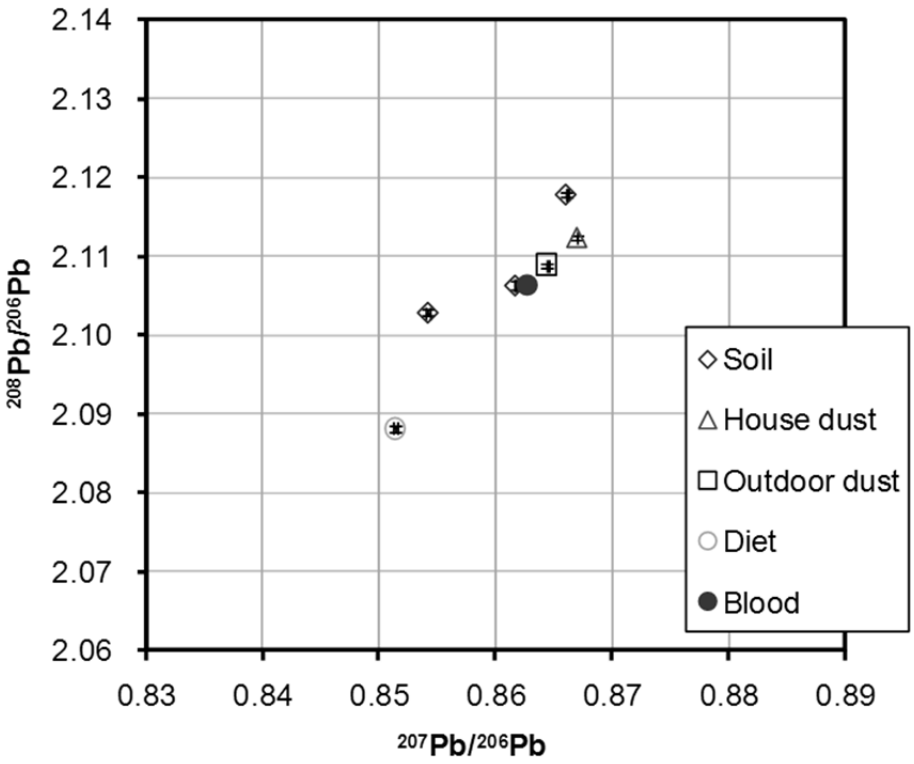

Fig. V-5. Case of 7-year old girl (blood lead level: 1.5 μg/dl)

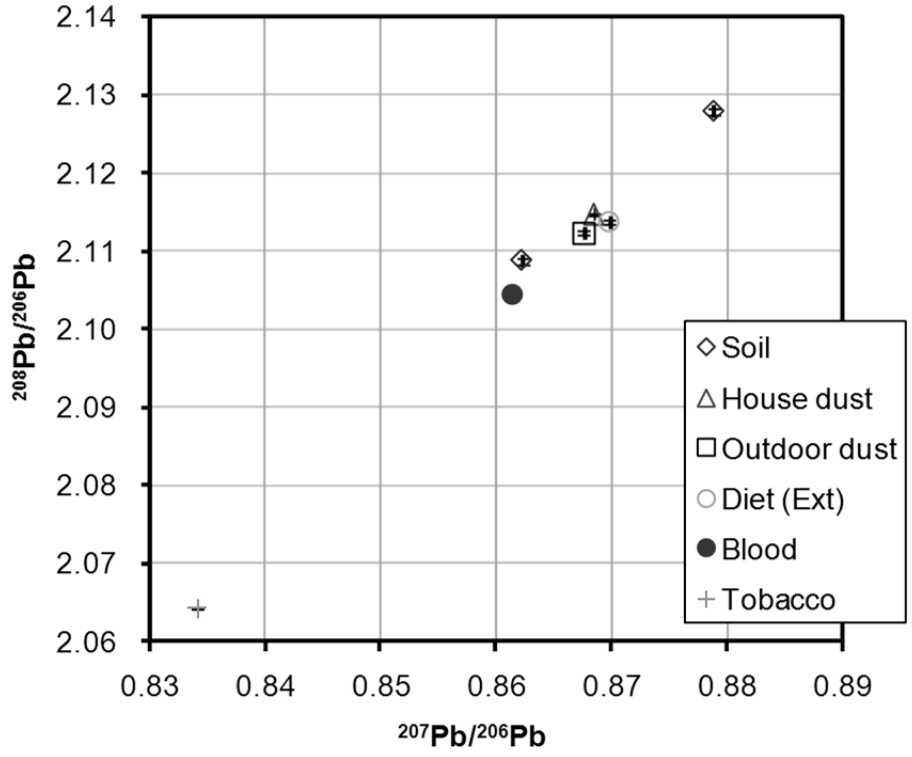

Fig. V-6. Case of 11-year old girl (blood lead level: 1.3 μg/dl)

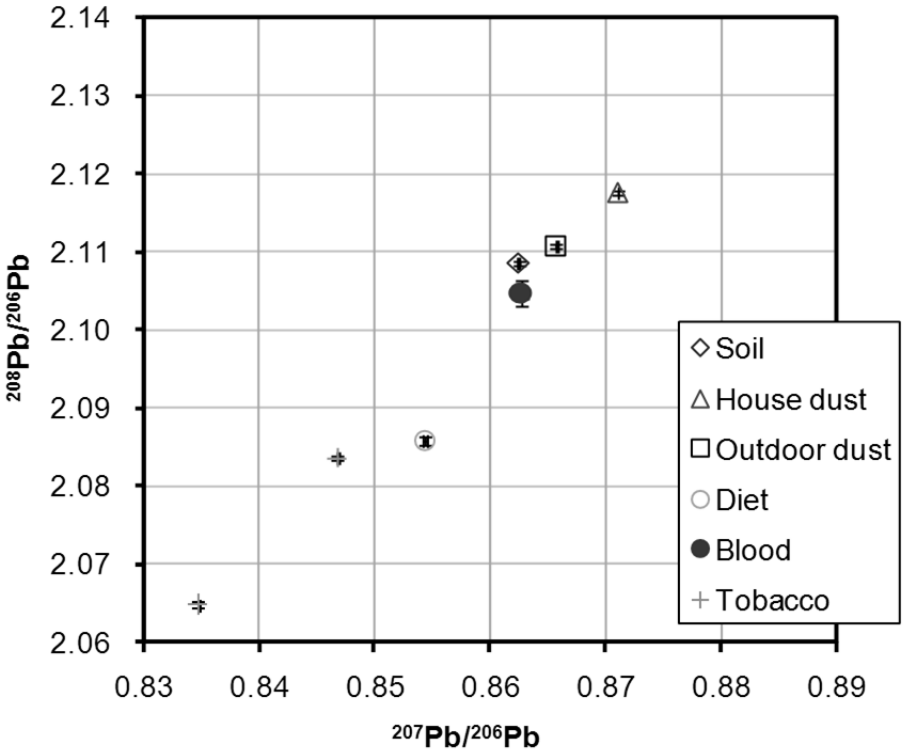

Fig. V-7. Case of 7-year old boy (blood lead level: 0.46 μg/dl)

In case of a 7-year old girl in Fig. V-6, lead isotope ratios of blood were distributed between the lead isotope ratios in food, soil, house dust etc. Lead exposure sources estimated by linear programming were 18% for food, 26-34% for school-yard soil, 0-9% for home-yard soil, and 42-57% for house dust; demonstrating large contributions of house dust and soil to lead intake. Fig. V-7 pertains to a 11-year old girl; in this case, lead isotope ratios of blood were lower than those of environmental samples, except for cigarette smoke. According to the questionnaire survey, no family members smoked in the presence of the child; solution could not be obtained by linear programming, when we assumed that cigarette smoke did not contribute to lead intake.

Fig. V-8 shows a case of 7-year old boy. Similar to that of Fig. V-6, lead isotope ratios of blood were distributed between those of the environmental samples, while linear programming estimated that food, soil and house dust contributed by 30%, 32-48% and 22-38%, respectively (in this case, as well, family members said that they did not smoke in the presence of the child; hence, isotope ratios in cigarette smoke were not included in linear programming).

Contributions of different lead sources in four subjects, for which linear programming solutions were obtained, are given in Table V-4. Based on the estimation of lead level in the environmental samples (Table V-3), contributions of food and house dust amounted to about 90% of the total daily lead intake, while lead isotopic ratios indicated larger contributions of house dust and soil. The cause of this uncertainty may arise from the calculations of daily lead intake used in Table V-3, which default values were used rather than measured values for soil and house dust ingestion.

Table V-4. Contribution of lead intake sources estimated by linear programming

Subject ID	18	1043	1046	1047
Sex, age	Girl, 8 years old	Girl, 7 years old	Boy, 7 years old	Girl, 9 years old
Intake source, contribution (%)	Food, 19% Soil, 34-39% House dust, 42-47%	Food, 18% Soil (home), 0-9% Soil (school), 26-34% House dust, 42-57%	Food, 30% Soil, 32-48% House dust, 22-38%	Food, 38% Soil, 32-33% House dust, 29-30%

Based on these four cases, the estimation of the contribution of house dust, soil and other non-dietary oral sources to lead intake in children were greater than that of food.

Linear programming solutions were not obtained for six out of the ten subjects, as in the case shown in Fig. V-7. This implies the followings: the existence of unidentified lead sources other than the samples; poor representativeness of the samples in terms of lead levels and isotope ratios; low reliability of data provided from the questionnaire survey, etc.

Apart from the analysis of lead intake sources based on lead level in environmental samples (Table V-3) and lead isotope ratios (Table V-4), there is a possibility that cigarette smoke also contributed to lead intake; e.g., about 10% to blood lead level via passive smoking as shown in Table V-3. From the above, it may be assumed that non-dietary oral sources such as house dust, soil as well as cigarette smoke contribute to lead intake in children, in addition to food (and tap water). Thus, reduction of such sources is an effective measure to reduce lead intake in children in Japan in future. Lead exposure from house dust and soil can be controlled by simple measures such as hand-washing. Another simple and efficient measure is to refrain from smoking in the presence of the children. The information provided in this study can contribute to the risk management (of health risks due to lead exposure in children).

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Future issue

1) This research has clarified the outline of blood lead levels in children in Japan. However regional differences in blood lead level were found, and those differences should be confirmed by (sampling as well as analysis)

including more regions. Geographic factors may provide a clue to the lead sources. in Japanese children.

2) This research revealed that precise analysis of lead isotope in children's blood and home environmental samples can be an effective measure for source apportionment of lead. In future, there is a need to generalize findings of this study to the larger population of children by applying this analysis method to more children.

When collecting blood samples from children, understanding and cooperation from their caretakers, children themselves as well as pediatricians were required; however, obtaining cooperation for collecting home environmental samples were more difficult than that of blood samples.

A future plan to implement a full-scale survey is required for the precise identification of lead source in children in Japan.