Epidemiological Assessment of Significance for Hair Minerals Measured by PIXE Method

Shohei Kuroda, Todd Saunders, Tsuyoshi Nakamura, Kouichirou Sera, and Yoshiaki Nose

***Abstract*— Many metabolic disorders, such as atopic dermatitis, are suspected to be associated with alterations in the concentration of some trace elements caused by the intake of bio-accumulated toxic foods. The U.S. Environmental Protection Agency suggests that human hair be considered an important indicator for worldwide biological monitoring of toxic trace elements. In the epidemiological risk assessment of trace elements accumulated in the human body, it is crucial to be able to estimate the body burden of these toxic elements for each individual. However, the quantitative assessment of this burden for infants is hardly possible through questionnaires or even with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), since the amount of hair available from them may only be a few strands. Only the particle induced X-ray emission (PIXE) method seems readily applicable in this situation. Since risk estimates may be biased if an uncertainty in PIXE measurements is simply ignored, we have studied the statistical nature of some PIXE measurements in healthy mothers and infants. This paper develops a method of assessing the usefulness of the PIXE measurements for use in epidemiological risk assessment. The method is described and illustrated using PIXE measurements of the hair samples of 842 pairs of mothers and infants at the national one-month and 10-month medical checkup.**

***Index Terms*— PIXE, inter-intra variance ratio, risk assessment, detection probability**

I. Introduction

Environmental contamination is suspected to be a cause of atopic dermatitis as well as some other allergies, though no definite evidence has been obtained yet. In 2001, the Agency for Toxic Substances and Disease Registry (ATSDR) reviewed the current state of hair analysis [1]. The goal of the panel was to determine the overall utility of hair analysis as a tool to evaluate exposure at hazardous waste sites. The principal lesson learned from the meeting was that for most substances, data are insufficient to predict health effects from the concentration of a substance in hair. One of the significant problems is that a verification method for accuracy of measurements has not been well developed.

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The objective of this study is to develop a statistical method to assess the reliability of the estimates of hair mineral concentrations measured by the PIXE method. PIXE measurements of the hair samples of 842 pairs of mothers and infants at the national one-month and 10-month medical checkup are completed. The PIXE measurements will be linked with the clinical data for a cause and effect analysis of atopic dermatitis. A preliminary investigation on the reliability and effective utilization of those measurements that are crucial for epidemiological use is being reported here.

II. Reproducibility for Epidemiological Risk Analysis

A. Epidemiological Reproducibility of Measurements

The reproducibility of PIXE measurements is a great concern from the risk assessment point of view. In general, PIXE measurements for a specific mineral in a hair sample depend not only on the amount of the mineral in the sample but also environmental and machine factors in the PIXE measurement process. The reliability of the PIXE measurement is low when the amount of mineral is close to its physical detection limit. There is another crucial factor that may seriously affect the reliability of PIXE measurements. Hair samples obtained from one person were sent to several labs with widely variable results [2]. Since ICP-AES, the method used by them, is a standard established method, the results seem to indicate highly variable mineral amounts for hair sampled from virtually identical places. ATSDR is also concerned about the variations in hair sample scalp location. To assess the variability of mineral amounts due to sampling, we obtained PIXE measurements from two sets of hair strands sampled from virtually identical places for each of 208 babies. Since the hairs of the two sets were

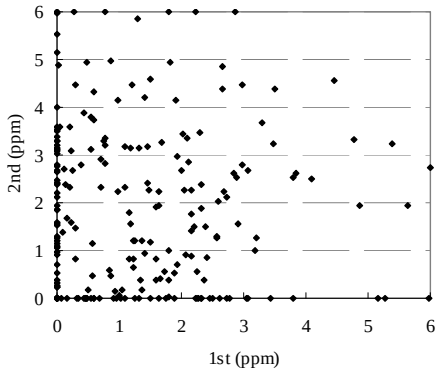

Fig. 1: Scatter plot of 1st and 2nd Hg measurements.

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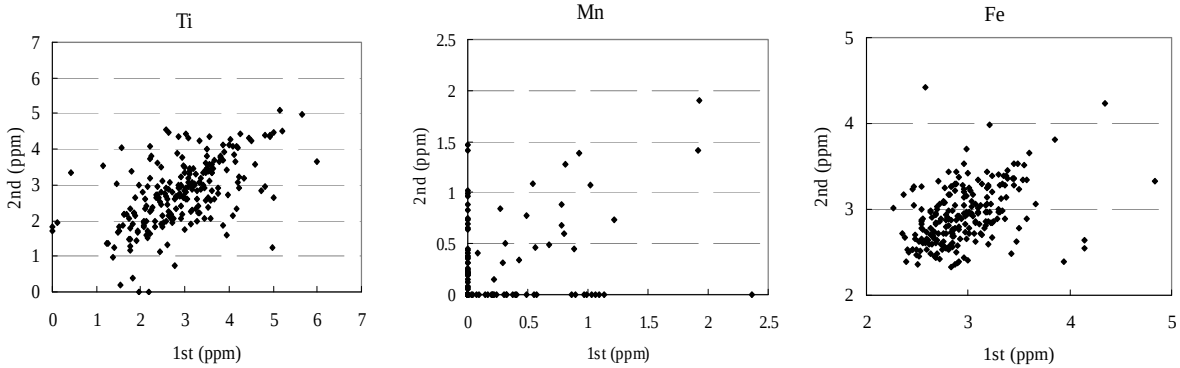
measured in a blind manner at 2 different times with about one month apart, we will refer to them as the first and the second measurements. The reason we chose babies will be explained in the DISCUSSION.

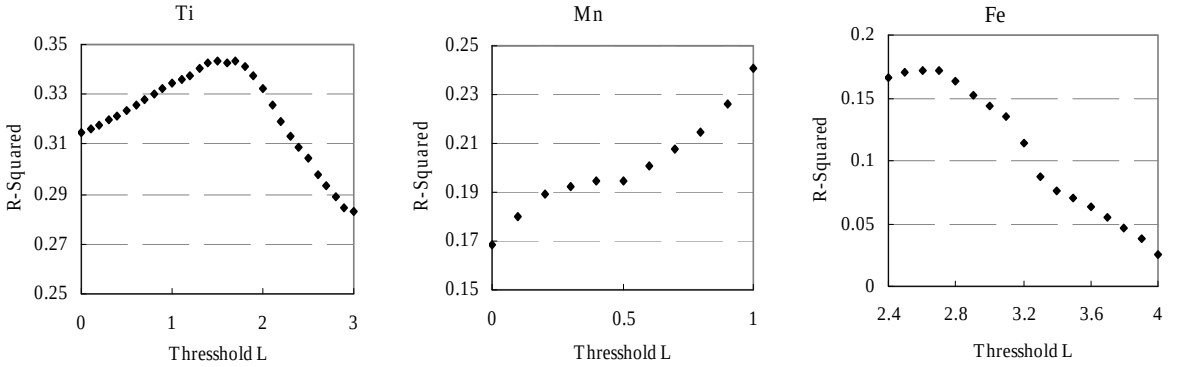
Here, we choose Hg to perform a detailed illustration on the reproducibility specific to PIXE measurements. The coefficient of determination, or square of the Pearson correlation coefficient, between the Hg measurements less than 6ppm was $R^2<0.01$ (Fig. 1). The result shows extremely poor reproducibility of Hg measurements less than 6ppm. This statistical analysis suggests Hg measurements of less than 6ppm were not very reliable. This finding gives insight into the results reviewed by [3]. They found statistically significant hair-mercury levels associated with child development only at greater than 6ppm. They may have failed to obtain significant associations for less than 6ppm, because of degraded statistical power due to less accuracy with those measurements [4].

The above results suggest that there should be a threshold of PIXE measurements in order to assure sound reproducibility, besides the physical detection limit and measurement error incidental to the PIXE method. This consideration prompted us to develop a method to determine the threshold value from an epidemiological point of view. Since the physical detection limit of the PIXE method is regarded as 1ppm irrespective of elements, measurements less than 1ppm were replaced by 1ppm followed by log transformation. Hereafter, "measurements" imply the log-transformed ones unless otherwise stated. The trace elements Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Sr, Nb, Mo, Hg and Pb were investigated.

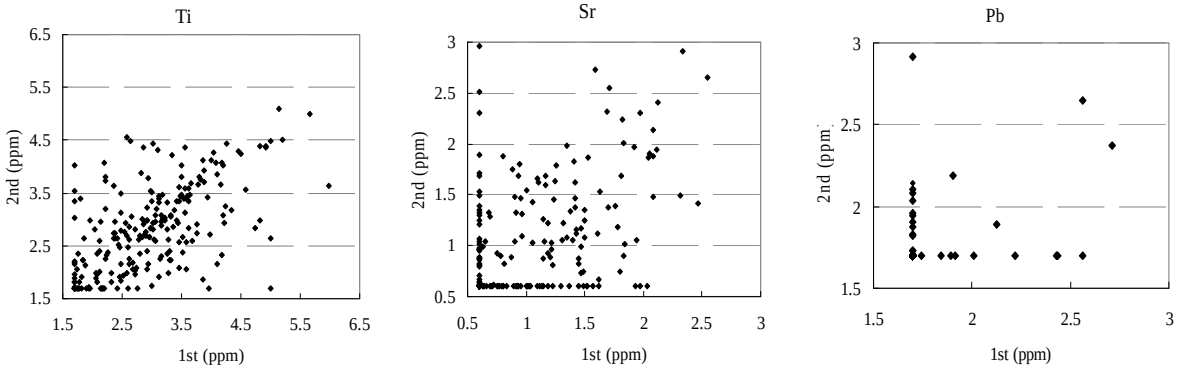
In the first place, the coefficient of determination, R^2, between the first and the second measurements was obtained for each element. For some of them, R^2 was nearly 0 like Hg, suggesting poor reproducibility of those elements. Even if we include such elements in an epidemiological risk analysis,



(a) Scatter Plot of 1st and 2nd measurements



(b) Graph of $R^2(L)$



(c) Scatter Plot of x* and y*

Fig 2: Iliustration for the Epidemiological Significance Limit

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those elements will not demonstrate statistically significant associations between the elements and the diseases of interest due to the large measurement errors [4]. Thus, we decided not to use those elements in the future risk analysis. Those elements are V, Cr, Co, Ni, As, Se, Rb, Nb, Mo and Hg.

B. Epidemiological Significance Limit (ESL)

Statistically examining the rest of the elements revealed that some of the elements showed a common characteristic; that is, when both the 1st and 2nd measurements are larger than a threshold value, there is good agreement between them. We define the characteristics as follows. Suppose we have a sample of *n* subjects with two hair specimens from each subject and those specimens are measured for mineral amounts by PIXE method. Let $S=\{(x_1, y_1),\ldots\ldots, (x_n, y_n)\}$ denote the PIXE measurements for a specific element for a subject. To attain better reproducibility of measurements, we will consider transforming x ,y to x*=Max(x, L), y*=Max(y, L) for an appropriate real number L. In other words, measurements smaller than L are replaced by L. Let $R^2(L)$ denote the coefficient of determination between x* and y*. Let *Max* and *Min* denote the maximum and the minimum of $\{x_1,\ldots\ldots,x_n, y_1,\ldots\ldots,y_n\}$, respectively.

Definition We call L* the epidemiological significance limit when $R^2(L)$ takes a local maximum at L*. When $R^2(L)$ is monotone decreasing as L increases, define L*=*Min*. On the other hand, when $R^2(L)$ is monotone increasing as L increases, define L*=*Max*.

When L* is obtained as a value attaining a local maximum, x* and y* should be used for a risk analysis, instead of x and y. On the other hand, L*= *Min* indicates that all measurements x's and y's may be used for it. Whilst L*= *Max*, indicating the element itself should not be used. We illustrate a method of determining and merit of the ESL taking Ti, Mn, and Fe as examples. Fig. 2(a) shows the scatter plot for x and y, and Fig. 2(b) the graph of $R^2(L)$. As for Mn in Fig. 2(a), there are number of cases on the coordinate axes, showing poor reproducibility. Fig. 2(b) shows that ESL=1.7 for Ti, but L*= *Max* for Mn and L*= *Min* for Fe. In fact, even if Mn is used in an epidemiological risk analysis, it may cause only confusion and not be of any help in establishing a cause-effect relationship.

Similar analysis revealed that ESL=0.6 for Sr, ESL=1.5 for Pb, L*=*Min* for Cu and Zn, and L*=*Max* for Br and Ga. Fig. 2(c) shows the scatter plot of x* and y* for Ti, Sr and Pb. The results suggest using Ti, Fe, Cu, Zn, Br and Sr for future risk analysis, since the other elements are subject to substantial measurement errors and lack of reproducibility due to combined effects of the sampling errors and the physical measurement errors.

Table. I Mathematical symbols and notations.

Notation	Definition	Estimate
Overall mean:	$\bar{\mu}=\sum_{i=1}^{n}\mu_i/n$	$\bar{\bar{X}}=\sum_{i=1}^{n}\bar{X}_i/n$
Inter-Individual Variance :	$\Lambda=\sum_{i=1}^{n}(\mu_i-\bar{\mu})^2/n$	$T^2=2\sum_{i=1}^{n}(\bar{X}_i-\bar{\bar{X}})^2/(n-1)$
Intra-Individual Variance :	σ^2	$S^2=\sum_{i=1}^{n}(X_{i1}-X_{i2})^2/2n$
Inter-Intra Variance Ratio :	Λ/σ^2	$F=T^2/S^2$ (Fisher's *F*) .

III. INTER-INTRA VARIANCE RATIO

The quantitative assessment of the intra-individual variances related to inter-individual variances is important in risk analysis. Thus we define the inter-intra variance ratio (IIVR) as the ratio of the inter-individual variance to the intra-individual variance. In this study, the intra-individual variance is defined as the variance of the difference between two measurements obtained from two different hair samples from the same individual. The inter-individual variance is defined as the variance of the mean of the two measurements. The larger the IIVR, the higher the ability to discriminate among individuals, and therefore the higher the ability to discriminate risks associated with individuals.

Suppose *n* subjects were sampled and two hair specimens from each were measured. Let X_{i1}, X_{i2} be the measurements and $\bar{X}_i=(X_{i1}+X_{i2})/2$ for i=1,…,n. We assume that X_{i1} and X_{i2} are normally distributed as $N(\mu_i, \sigma^2)$. Table. I denotes mathematical symbols and notations used in this study.

Theorem $E[F]=1+2(\Lambda/\sigma^2)$. Proof is straightforward.

This theorem indicates there is a linear relationship between the IIVR and the *F* statistics used in Analysis of Variance. Since *F* is available with any statistical software, we will use *F*, instead of IIVR, to compare the discrimination ability. *F*-value was obtained from our data and is shown in Table II (a). To compare the results, we also obtained the *F* value of SBP (systolic blood pressure), DBP (diastolic blood pressure) and Chol (cholesterol) using the Framingham cohort data [5], which is shown in Table II (b).

The *F* values of Cu and Zn in both mothers and children are much larger than those of SBP, DBP and Chol, indicating that those minerals have higher discrimination ability than SBP, DBP and Chol.

IV. DISCUSSION

Table. II F-value

Mineral	Mothers	Infants
sample	39	230
Ti	4.4	3.5
Fe	4.3	2.4
Cu	11.1	17.4
Zn	7.6	11.8
Br	3.4	3.3
Sr	3.8	2.9

(a) Trace elements

	Male	Female
SBP same day	7.5	6.0
DBP same day	4.1	4.9
SBP*	4.6	3.8
DBP*	3.4	3.9
Chol*	8.5	7.0

*denotes two years apart

(b) Medical tests

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The US EPA considers scalp hair as a suitable biological sample for estimating the body burden of trace elements [6]. Hair incorporates various elements from the blood at a relatively constant rate. After formation, the hair is separated from the body's internal metabolism; therefore, its composition reflects the concentration of elements in blood at the time of formation. PIXE experiment provides an excellent measurement method that enables the measurement of the amount of trace elements from only a few strands of hairs. PIXE presents not only measurements but also approximate standard error (SE) associated with the measurement based on physical considerations. Thus, to avoid false positive, it is recommended that a PIXE measurement be used only when the measurement is two or three times larger than its estimated standard error [7]. Since SE depends on a number of factors involved in the PIXE measurement process, it is not always very reliable [1]. Besides measurement errors due to the physical aspects of PIXE method, there seemed to be a completely different type of errors, namely sampling errors due to the variations in hair sample scalp location and in the part of hair strand, since hair grows about one centimeter a month and the different parts of a hair may contain different amounts of minerals [1]. Epidemiological analysis is concerned about the combined effects of those errors.

This study examined the kinds of errors in PIXE measurements by statistically analyzing two independent measurements obtained from different hairs of the same person. The results indicate that only Ti, Fe, Cu, Zn, Br and Sr have sufficient reproducibility for epidemiological risk analysis. But the results may be different with a different data set, since the reproducibility depends on the distribution of minerals in hair samples. Since the food and living conditions were far less restricted for mothers than for infants, it was speculated that the mineral amounts in different hairs of each infant would fluctuate less. Base on this consideration, we dealt with only infants in Section 3, since our major concern in the section was the reproducibility of PIXE experiments rather than sampling errors.

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