

## Biological Monitoring of Environmental Lead on School Children Subsequent to the Use of Unleaded Gasoline (1998) in Malaysia

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### ABSTRACT

**Introduction:** A study on biological monitoring of lead on children was conducted when unleaded gasoline was widely used in 1998. The objectives were to monitor lead exposure using blood lead, urine  $\delta$ -aminolevulinic acid ( $\delta$ -ALA) and urine lead concentrations as biological indicators and to determine the relationship between these variables. **Methodology:** Two hundred and sixty-nine school children, 169 from an urban school of Kuala Lumpur and 100 from an industrial school in southern Malaysia were selected for the study. These were Malay children in the age range of 6 $\frac{1}{2}$  to 8 $\frac{1}{2}$  years old. Blood and urine lead concentrations were analyzed using the Graphite Furnace Atomic Absorption Spectrophotometer. Urine  $\delta$ -ALA was measured with Spectrophotometer UV/VIS. **Results:** The mean blood lead concentrations of the urban children (3.56  $\mu$ g/dl) and the industrial children (3.75  $\mu$ g/dl) were not significantly different ( $p=0.451$ ). The urine  $\delta$ -ALA (urban=9.606; industrial=6.965 mg/g creatinine) and urine lead (urban=2.625; industrial=4.548  $\mu$ g/g creatinine) of the urban children were significantly higher than the industrial children ( $p=0.014$ ;  $p<0.001$ ) respectively. No significant correlation was found between blood lead and urine  $\delta$ -ALA ( $r=0.058$ ;  $p=0.350$ ), blood lead and urine lead ( $r=0.044$ ;  $p=0.517$ ) as well as urine  $\delta$ -ALA and urine lead ( $r=0.097$ ;  $p=0.165$ ). Only 2% of the total children have blood lead > 10 mg/dl. About 78% of the urban children and 76% of the industrial children have urine  $\delta$ -ALA in a normal range (<0.6 mg/100ml) while 22% of the urban children and 24% of the industrial children were in the acceptable range (0.6 - 2.0 mg/100ml). All the children had normal urine lead concentrations (<8  $\mu$ g/100ml). **Conclusion:** These children were not highly exposed to lead as indicated by their blood lead, urine  $\delta$ -ALA and urine lead concentrations which were below the allowable standard in both study areas. This may be due to the total ban on leaded gasoline in the country since 1998 and as a result, the environmental lead exposure in these areas was quite low.

**Keywords:** Children's lead exposure, blood lead, urine lead, urine  $\delta$ -ALA

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## INTRODUCTION

Thirty years ago, measurement of blood and urine lead concentrations as well as urine  $\delta$ -ALA were the three most reliable indicators of lead exposure among workers<sup>[1]</sup>. Compared to the general population, blood lead concentrations and urine  $\delta$ -ALA were more frequently used as indicators for lead exposure among workers highly exposed to lead in their daily job activities as a screening process<sup>[1]</sup>. Urine  $\delta$ -ALA has been used as a simple and appropriate method for measurement of lead toxicity in the industries.<sup>[2]</sup>

There are multiple sources of exposure to lead from the environment as well as workplace. Environmental sources include mobile sources such as leaded gasoline combustion from motor vehicle exhaust; stationary sources would be smelters, lead mines, industrial emissions from processes that use lead. Occupational exposures include circuit board soldering activities in electronic industries, car batteries manufacturing, lead smelting and paint manufacturing activities. Other than these, the population, especially children, is exposed to lead from some consumer products such as paint, crayons, toys, etc. A research reported that the main source of lead in the atmosphere in Klang Valley is motor vehicles<sup>[3]</sup>. In Malaysia, blood lead is the indicator for lead exposure that is most frequently used among the general population<sup>[4,5]</sup> and workers<sup>[6,7,8]</sup> compared to the urine lead and urine  $\delta$ -ALA. In industries, the environmental monitoring is often supplemented with biological indicators in cases of high exposure among the workers.

There were also researchers who used urine  $\delta$ -ALA as an indicator of exposure to lead among workers and children<sup>[2,5,9]</sup>. However, no similar study has been conducted in Malaysia on the relationship between blood lead concentration and urine  $\delta$ -ALA as well as urine lead concentrations especially after the wide use of unleaded gasoline. The objective of this study is to determine lead exposure using blood lead concentrations, urine  $\delta$ -ALA, as well as urine lead as biological indicators and to determine the relationship between these indicators.

## METHODOLOGY

This cross-sectional study was conducted at the Pasir Gudang Industrial Area in southern Peninsular Malaysia and in the city of Kuala Lumpur. These areas were chosen because of the assumptions that environmental lead would come mostly from the gasoline combustion of automobiles in the high traffic areas of the urban areas and from the industrial stack as a result of processes in the metallurgical industries. Reference is made to the Department of Environment Annual Report<sup>[10]</sup> and a local study<sup>[11]</sup> that the atmospheric lead in these areas is higher than in the rural or suburban areas. The respondents in this study were 269 school children from the industrial (100) and urban (169) areas; they were in the age range of 6 1/2 to 8 1/2 years old (Year 1 and 2). The name lists were obtained from the class teachers. Sampling was stratified by proportion according to class and sex using the table of random number<sup>[12]</sup>. Only the Malay students were selected because this is a part of a bigger study in which intelligent quotient (IQ) measurements were made and the IQ test battery was only in the Malay language.

Questionnaires were used to collect the background information from each respondent. The blood samples were collected using a method carried out in a Port Pirie study in

Australia<sup>[13]</sup>. Blood lead concentrations were measured using the GBC 908AA Model of the Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS). Modifications were carried out on the temperature programme of the blood analysis method by 'GBC Applications'<sup>[14]</sup> which was then tested against Lyphocheck reference blood lead samples. Lyphocheck test was used as a quality control measure during blood lead analysis.

The urine lead concentration was measured using the HITACHI Graphite Furnace Atomic Absorption Spectrophotometer; Model Z-5000 Series Polarized Zeeman<sup>[15]</sup>. The urine samples were preserved according to a modified method<sup>[16]</sup>. The Spectrophotometer UNICAM 5675 UV/VIS was used to measure the concentration of urine  $\delta$ -ALA<sup>[17]</sup>.

## RESULTS

The average age of the respondents in this study was 7 years and 6 months in the urban area and 7 years and 3 months in the industrial area. All the respondents were Malay children (Table 1). The parents from both the areas had only lower secondary school education. The education level was directly related to the total household income. Results show that the mean household income of the respondents' families was RM 1864.96 (urban) and RM 1893.23 (industrial).

**Table 1.** Background data of respondents

Location (area)	Total (N)	Sex		Age (mean)
		Male (n)	Female(n)	
Urban	169	89	80	7.50 years
Industrial	100	48	52	7.25 years

In the urban area, most of the respondents' fathers were either professionals or worked in sales, whereas in the industrial area, the fathers were mostly production and transport operators as well as labourers, since this is the largest industrial city in southern Malaysia. Almost half of the respondents' mothers in both the study locations were housewives. However, there were also some mothers in the urban area who were either professionals or worked in the clerical or technical fields.

Based on the Kolmogorov Smirnov Normality Test, the frequency distribution that was obtained for blood lead concentration was not normal ( $p < 0.05$ ). Therefore, the blood lead concentration was transformed to log based 10 in order to obtain a normal distribution. The blood lead concentrations ranged from 0.13 to 14.72  $\mu\text{g}/\text{dl}$ . Table 2 shows that the arithmetic mean for blood lead concentrations for all the respondents was 3.692  $\mu\text{g}/\text{dl}$ .

The Centre for Disease Control<sup>[18]</sup> categorizes blood lead concentrations as normal if it does not exceed the limit of 10  $\text{mg}/\text{dl}$ . Almost all the respondents (98.1%) had a normal blood lead concentration and only 1.9% showed concentrations higher than the normal limit. Based on the study location, there were 2 respondents (1.2%) in the urban area and 3 respondents (3.0%) in the industrial area with blood lead concentrations that were  $> 10 \mu\text{g}/\text{dl}$ .

**Table 2.** Comparisons of blood lead concentrations, urine  $\delta$ -ALA and urine lead concentrations between the study areas

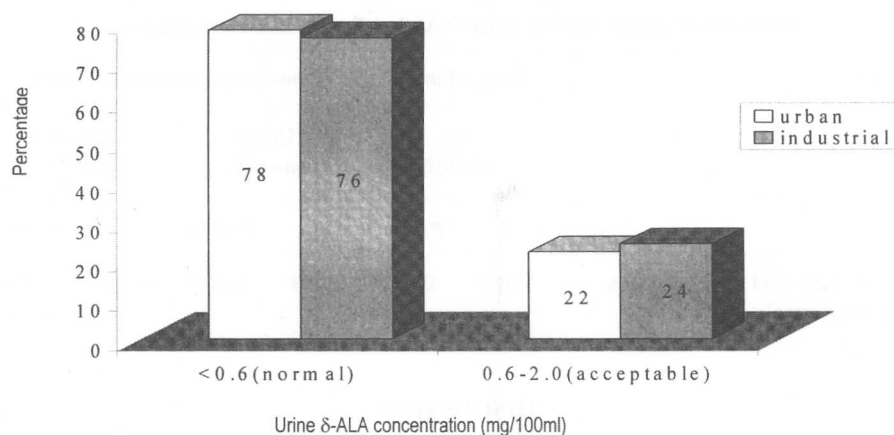
Variables	Urban (n = 164)	Industrial (n = 98)	t value	p value
	Mean $\pm$ SD	Mean $\pm$ SD		
Blood lead concentrations ( $\mu\text{g}/\text{d}\ell$ )	3.56 $\pm$ 1.95	3.75 $\pm$ 2.16	-0.076	0.451
Log <sub>10</sub> of blood lead concentrations ( $\mu\text{g}/\text{d}\ell$ )	0.489 $\pm$ 0.245	0.489 $\pm$ 0.309	-0.002	0.998
Urine $\delta$ -ALA concentrations (mg/g creatinine)	9.606 $\pm$ 8.655	6.965 $\pm$ 8.209	2.466	0.014
Log <sub>10</sub> of urine $\delta$ -ALA concentrations (mg/g creatinine)	0.853 $\pm$ 0.338	0.681 $\pm$ 0.347	3.916	<0.001
Urine lead concentrations (mg/g creatinine)	2.625 $\pm$ 1.272	4.548 $\pm$ 4.729	-3.384	<0.001
Log <sub>10</sub> urine lead concentrations (mg/g creatinine)	0.160 $\pm$ 0.520	0.452 $\pm$ 0.468	-4.294	<0.001

The mean concentration of blood lead in the urban children was 3.56 mg/dl and in the industrial children was 3.75 g/dl. The difference in the means blood lead concentration between the two groups of children (urban and industrial) was not significant.

Based on the Kolmogorov-Smirnov Normality Test, results showed that the concentration of urine  $\delta$ -ALA was not normally distributed ( $p < 0.05$ ). The  $\delta$ -ALA values were later transformed to log based 10 to obtain a normal distribution. The arithmetic mean of the urine  $\delta$ -ALA concentration was 0.458 mg/100mL and 0.465 mg/100mL for the urban and industrial areas respectively. The average urine  $\delta$ -ALA concentration that was obtained in this study was 0.461 mg/100ml. Statistically, there was no significant difference in the mean concentration of log urine  $\delta$ -ALA between the urban and industrial children. However, the same test showed a significant difference between the two groups, when the concentration of log urine  $\delta$ -ALA was measured in mg/g creatinine (Table 2).

Lead exposure based on the concentration of urine  $\delta$ -ALA is categorized into four biological index limits;<sup>[19]</sup> a urine  $\delta$ -ALA concentration of less than 0.6 mg/100mL is classified as normal, 0.6 to 2.0 mg/100mL as acceptable, 2.1 to 4.0 mg/100mL as excessive and above 4.0 mg/100mL is classified as dangerous. The distribution of the children's urine  $\delta$ -ALA is shown in Figure 1.

The analysis of urine lead concentration showed a distribution that was not normal. The urine lead concentration was therefore transformed to log base 10 to obtain a normal



**Figure 1.** Category of urine  $\delta$ -ALA concentrations in study areas

distribution. Results show that the concentration of urine lead among respondents in the industrial area ( $0.306 \mu\text{g/dl}$ ) was significantly higher than those in the urban area ( $0.125 \mu\text{g/dl}$ ). Results in Table 2 also show that the urine lead concentration for both the areas did not exceed the recommended limit of  $8 \mu\text{g/dl}$ <sup>[20]</sup>.

The Pearson Correlation showed no significant correlation between the  $\log_{10}$  of blood lead concentration and the  $\log_{10}$  of urine  $\delta$ -ALA concentration (both in  $\text{mg}/100\text{m}\ell$  and  $\text{mg}/\text{g}$  creatinine) for both the urban and industrial area (Table 3). The correlation test shows that there is no significant correlation between the  $\log_{10}$  of blood lead concentration and  $\log_{10}$  of urine lead concentration (Table 4).

**Table 3.** Correlation between blood lead concentration and urine  $\delta$ -ALA concentrations

Variables	Log <sub>10</sub> of Blood Lead Concentrations					
	All (n=262)		Urban (n=164)		Industrial (n=98)	
	r	p value	r	p value	r	p value
Log <sub>10</sub> of urine $\delta$ -ALA concentrations (mg/100mℓ)	0.051	0.413	0.014	0.863	0.103	0.313
Log <sub>10</sub> of urine $\delta$ -ALA concentrations (mg/g creatinine)	0.058	0.350	0.093	0.238	0.016	0.878
Log <sub>10</sub> of urine lead concentrations (mg/100mℓ)	0.104	0.122	0.170	0.053	0.052	0.623
Log <sub>10</sub> of urine lead concentrations (mg/g creatinine)	0.044	0.517	0.204	0.020*	-0.151	0.151

\* Significant at  $p < 0.05$

**Table 4.** Correlation between urine  $\delta$ -ALA and urine lead concentrations

Variable	Log <sub>10</sub> of urine $\delta$ -ALA concentrations (mg/g creatinine)					
	All (n=262)		Urban (n=164)		Industrial (n=98)	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
Log <sub>10</sub> of urine lead concentrations (mg/g creatinine)	0.097	0.165	0.038	0.690	0.149	0.155

## DISCUSSION

The mean blood lead concentration for the urban and industrial children was 3.69  $\mu\text{g}/\text{dl}$ . This value for the urban children is lower compared to a previous study that was conducted in Malaysia in 1997, which reported a mean of 4.30  $\mu\text{g}/\text{dl}$ .<sup>[6]</sup> The result that was obtained was also lower compared to some other research findings<sup>[21, 22, 23]</sup>.

The lower blood lead concentrations could be due to a lower concentration of lead in the atmosphere, which is a direct result of reduced lead content (from 0.40 g/L to 0.15 g/L) in gasoline, in the early 1990s<sup>[10]</sup> and the total ban of leaded gasoline in 1998. The same trend of blood lead reduction in the US population was also observed after the use of unleaded gasoline as reported in the National Health and Nutrition Examination Survey in the US (NHANES III)<sup>[21]</sup>.

Results show that 77.1% of the urine  $\delta$ -ALA concentration of the respondents is in the normal range and 22.9% of the respondents is in the acceptable range. The mean urine  $\delta$ -ALA of 0.46 mg/100ml is higher than that found by a local study<sup>[24]</sup> (0.73 mg/100ml) which was conducted among children with high blood lead when leaded gasoline was widely used. In another study,<sup>[9]</sup> 32 to 42% of the 1038 children aged 7 to 12 years old were in the acceptable range.

The concentration of urine lead among industrial children was significantly higher than among the urban children. There is no significant correlation between the log<sub>10</sub> of blood lead concentration and log<sub>10</sub> of urine  $\delta$ -ALA concentration (both in mg/dl and mg/g) for both the urban and industrial area.

Contrary to the results<sup>[24, 25]</sup> which reported that there was a significant relationship between urine lead concentrations and urine  $\delta$ -ALA concentrations, results of this study did not show any significant correlation between the two variables. A significant relationship between blood lead concentrations and urine  $\delta$ -ALA concentrations occurs if the blood lead concentration is high at approximately 40  $\mu\text{g}/\text{dl}$ . It is also recommended that the use of urine  $\delta$ -ALA concentrations is a useful indicator if the exposure to lead is at a high level, but at low levels, the urine  $\delta$ -ALA concentrations may be useful as an indicator for blood synthesis activity at molecular level only<sup>[26]</sup>.

The urine  $\delta$ -ALA concentration may be used in the screening of high lead exposure in a community who live near a lead smelter or workers who deal with lead in their daily job activities. However, urine  $\delta$ -ALA concentration measurement is useful if the blood lead concentration cannot be done<sup>[9]</sup>. A previous local study<sup>[24]</sup> showed that blood lead concentrations can be used to explain a high percentage of the variability in the urinary  $\delta$ -ALA. This study was conducted when leaded gasoline was widely used and the children had higher mean blood lead (9.05  $\mu\text{g}/\text{dl}$ ) than the children in this study. Urinary  $\delta$ -ALA can be used in the screening of lead exposure among high risk populations such as children who live near lead mining areas or smelters or lead based industries. These need to be monitored frequently.

The correlation test shows that there is no significant correlation between the  $\log_{10}$  of blood lead concentrations and  $\log_{10}$  of urine lead concentrations. Urine lead measurement may be useful in monitoring the lead exposure among workers, along with other indicators. It is usually used as a form of medical supervision among workers exposed to high level of lead. However, due to the fluctuation in specific gravity of the urine and also possible contamination of the sample, a spot sample is not a reliable indicator to describe the levels of lead exposure, as in this study. In addition, urine lead is also not a useful indicator for body burden as it only describes the excreted lead<sup>[27]</sup>. It is found that the relationship between two blood lead and urine lead samples depend on the intensity of lead exposure, which can be determined through blood lead. The relationship between these two parameters show a significant relationship if the blood lead concentrations exceed 50  $\mu\text{g}/\text{dl}$ <sup>[28]</sup>.

A study by Shimbo *et al.*<sup>[29]</sup> on the blood and urine lead isotopes among adults and children, concluded that the use of urine lead is not suitable to predict the blood lead concentration, especially if the blood lead concentration is less than 100  $\mu\text{g}/\text{dl}$ . The study also found that there was little reliability in using urine lead concentration instead of blood lead as a biological indicator of lead exposure in the environment.

### CONCLUSION

The results of this study show that the blood lead concentrations of almost all the children was lower than 10  $\text{mg}/\text{dl}$ . The majority of the urine  $\delta$ -ALA and urine lead concentrations obtained in this study also showed a normal range, based on the biological index limit classification, even though these indicators are not reliable for low lead exposure. Therefore, results show that the children in this study are not at risk of lead exposure. The results indicate that blood lead is the best biological indicator of lead exposure and urine  $\delta$ -ALA can be used for screening purposes when exposure is high.

### ACKNOWLEDGEMENT

Research funded by the Ministry of Science, Technology and Environment, Malaysia (Grant no. IRPA 06-02-02-0023) and Universiti Putra Malaysia under Short-term Fund 1998-99 and Graduate Fellowship Award. Written permission was obtained from the children's parents or legal guardians on their participation in the study.

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