Concentration of Lead in Saliva of Affected Primary School Children

Arweena Iskandar, Siti Nor Ain Seri Masran, and Ahmad Razali Ishak

Abstract—The use of a noninvasive (i.e. saliva) approach to measure heavy metals are seen as a reliable method of investigating heavy elements profile in humans. The main objective of this study is to study the concentration of lead in saliva of affected primary school children. This cross-sectional study was carried out at two different primary schools in Sarawak and specimens were taken from children aged between 7 - 12 years old (n=50). A survey form consisting demographic questions adapted from previous journals was also used to obtain other information. All null hypotheses of this study were rejected since the p-values showed there have a significant association ($p<0.05$) for lead concentration between exposed and non-exposed school children while except that for the health risk assessment, where the hazard index (HI) of less than 1 (indicating low risk). There was a mean difference of lead concentration in exposed and non-exposed primary school children. There was also a significant difference for Primary 1, Primary 2 and Primary 5 standard children.

Index Terms—Lead (Pb), lead concentration, saliva, hazard index (HI).

I. INTRODUCTION

Pollution itself can be defined as the introduction of contaminants into the environment that cause harm or discomfort to humans or other living organisms, or that damage the environment, which can come in the form of chemical substances, or energy such as noise, heat or light. Pollutants can be naturally occurring substances or energies, but are considered contaminants when in excess of natural levels [1]. The major form of pollutions is air, water, noise, light, soil or land, radioactive, thermal, and also visual pollution. Pollutants, the elements of pollution, can be either foreign substances/energies or naturally occurring pollutants above safe limits. Components and quantity of dust deposited in roofs at schools could provide an indirect measurement of air pollution integrated over varying time periods.

Hair and saliva has become one of the most valuable and effective tools in analyzing trace elements status in human [6]. It becomes a tool which can help to facilitate the identification of any abnormalities be it toxic or deficiency. At present, the use of trace elements concentrations in plasma or serum is widely accepted as norm. The use of multi-element analysis to assess an individual’s nutritional health or predisposition to disease has been controversial, but more studies which correlate concentrations of essential elements in parameters like high levels in hair to deficiencies in tissue and body level, has made it more acceptable due to excessive exposure, and serves as a useful diagnostic index of toxic element [7]. Experts have also now agreed that diagnosis and prevention of disease using saliva assay is possible due to steady progress made over years. Prompting more institutions, laboratories and medical practitioners to be involved in various researches using saliva, thereby making it more acceptable and due to the experience and easy way of collection, and lack of pains during collection as observed in the use of blood. The noninvasive technique is now been considered a useful tool in determining trace elements and heavy metals [8].

The principles and methods of risk assessment for non-carcinogenic chemicals are similar in different parts of the world, it is striking that approaches for risk assessment of carcinogenic chemicals vary greatly. There are not only marked differences between countries, but even within a...
country different approaches are applied or advocated by various regulatory agencies, committees and scientists in the field of risk assessment. Risk assessment for non-carcinogens is rather consistent and pretty well established partly because of the long history and better understanding of the nature of toxic effects in comparison with carcinogens and a high degree of consensus and confidence by both scientists and the general public on methods used and their outcome [9], [10].

The present study aims in determining the concentration of lead in saliva of affected primary school children, and to study the different level of exposure for each primary standards with the concentration of lead in saliva of affected students as well as estimating the health risk and hazard index of lead exposure towards the exposed primary school children.

II. LITERATURE REVIEW

A. Lead and Its Toxicity

Lead occurs in the environment in a wide range of physical and chemical forms which greatly influence its behavior and its effect on the ecosystem. Most of the lead in the environment is in the inorganic form as salts, oxides or hydroxides [11]. In addition, lead is also present in the organic form. Alkyl lead is used on a large scale as an anti-knock agent in petrol, although most of the lead reaching the environment from car exhausts has been converted to inorganic lead. The size of the particles containing the lead significantly influences both the aerial transport and distribution and its effect on the ecosystem [12], [13].

Lead is abundant in the earth's crust and can be mobilized by natural weathering. However, compared with the anthropogenic inputs the contribution from natural sources tends to be insignificant, except in certain localized areas. It has been estimated that the world-wide natural mobilization due to weathering of mineral deposits and through gaseous emissions during volcanic activity is approximately 210 000 tons per year. This compares with a world-wide annual consumption in 1980 of about 5.2 million tons [14]. Man releases lead into the environment during mining, smelting and refining of the metal and of other ores containing lead, and also during production, use, recycling and disposal of lead-based products and the burning of coal [4].

B. Non-Invasive Sampling Methodology

Saliva analysis provides a profile of the biologically active compounds at cellular levels and is therefore a representation of what is clinically relevant. Blood analysis provides a profile of the compounds as they travel through circulation, mostly bound to protein. Saliva analysis enables one to predict, diagnose or prevent many health problems and diseases [15]. In human body, the heavy elements, such as lead, comprises of less than 0.01% of the body weight. The routes of exposure are usually through ingestion, inhalation, or absorption through skin contact [16].

C. Health Risk Assessment of Lead

Toxic responses in infants and children can differ markedly from those seen in adults, both in severity and in the nature of the adverse effect. During the growth and maturation process there is an evolution of membranes, including receptors, in infants and children as they approach adulthood. These changes represent a potential for a very different environment for chemical and drug interactions with receptors. Reference [17] shows an example of differences in drug-receptor interactions between children and adults are provided by the paradoxical responses to phenobarbital and Ritalin in children versus adults. Phenobarbital, a sedative in adults, produces hyperactivity in children, whereas Ritalin, which is used as an antihyperactive agent in children, produces an opposite effect in adults.

The explanation for these widely differing responses in children and adults is believed to reside in differences in receptor-drug interactions [18], [19]. Infants and children differ from adults in their exposures both qualitatively and quantitatively, in part because they eat more food, drink more water, and breathe more air per unit of body weight than adults do. For example, the air intake of a resting infant is twice that of an adult under the same conditions, and the activity patterns of children further increase their exposure to pollutants. Because children are typically engaged in more physical activity, play close to the ground, and engage in characteristic hand-to-mouth behavior, they are exposed to higher levels of toxicants such as pesticides, radon, and particulate matter. The micro- and macro environments for infants and children change through development. Additionally, these environments may vary by demographic or cultural group, and these differences may influence exposure [20], [21].

D. Health Effects of Lead

Lead has been demonstrated to be toxic to a wide variety of organs in both humans and experimental animals. The organ systems that have been shown to be most sensitive to low-level exposures of lead are such [22], [23]:

- Nervous System: Changes in Neurotransmitter levels
- Biochemistry: Impairment of Vitamin D metabolism
- Reproductive System: Irregular estrus and decreased sexual hormone levels
- Immune System: Impaired lymphocyte function and impaired antibody formation
- Gastrointestinal: “Colic” (severe cramping and nausea; characteristic of high-dose poisoning)
- Reproductive: Decreased gestation duration and decreased growth rate in offspring

III. METHODOLOGY

A. Study Area

The study was carried out between April and June 2012, at one selected primary school - SK Tabuan, which is exposed to industrial area at Pending, Kuching, Sarawak. Another primary school in the opposite condition of the subjected schools will be the control group - SK Dato Mohd Musa, Kota Samarahan. Both of the study areas are illustrated in Fig. 1 and Fig. 2 below:
B. Study Protocol

A study protocol will be applied for consent to carry on with the study by the Institutional Review Board and Ethic Committee of the Faculty of Health Sciences, Universiti Teknologi MARA, Puncak Alam.

C. Sampling and Sample Preparation

Sampling methods is adapted from ref. [3], [8], [16] and [24] with minor modification. The specimen will be taken from children aged between 7 - 12 years old. 50 primary children will be randomly selected from each of the selected school. A survey form consisting demographic questions adapted from previous journals will be used to obtain other information [3], [24].

D. Data Collection Techniques

1) Questionnaires

The questionnaires were adapted from Guidelines for Blood Lead Screening and Lead Risk Assessment, and Lead Exposure Risk Assessment Questionnaire for Children with minor modifications [3], [24]. All respondents were to answer the questionnaires with the help of parents and teachers (for reference purposes). The data includes about demographic profile, other possible source of exposure, and the sign and symptoms experienced by the respondents.

2) Saliva collection

For collection of the saliva, the participant first rinsed the mouth thoroughly with distilled water [25]. A minimum volume of 5.0mL mixed saliva was collected from the learners by direct collection. The participant will be given a sampling tube. The minimum sample required will be 3 ml. The samples will be checked for food and blood or nasal discharge contamination and contaminated samples were discarded. The bottle will be capped, placed in a zip lock bag, and then frozen and stored in a freezer at 0°C to -4°C until analysis using the AAS was performed [8], [16].

E. Determination of Lead in Saliva Samples

The determination of lead in saliva uses the photometric method in analyzing the digested samples using an atomic absorption spectrophotometer. Prior to sample preparation, the saliva samples were defrosted and allowed to equilibrate to room temperature before being rechecked for any traces of contaminants. Five L of saliva was then measured into a beaker and 20mL of 2% nitric acid (HNO₃) was added. This solution was filtered with Whatman no. 42 filter paper into a volumetric flask and diluted to a final volume of 100mL with distilled water (AAS) [8], [16].

F. Estimation of Health Risk Assessment and Hazard Index of Exposed Study Group

Calculation for Carcinogenic Risk for Lead

\[
LADD = \frac{CXIRXEFXD}{WXAT}
\]

where:

- ADD = Average daily dose (mg/kg day)
- C = Predicted mean concentration of lead (mg/m³)
- IR = Ingestion/inhalation rate (m³/day) = 20m³/day
- EF = Exposure frequency (days/year) = 350 days/year
- ED = Exposure duration (year) = 6 (child), 30 or 40 (adult) years
- WB = Body weight (kg) = 70 kg
- AT = Averaging time (days), for chronic non-carcinogenic effect, days/year = 25,550 days

Calculation for Hazard Index

\[
HI = \frac{ADD}{RfD / ADI}
\]

where:

- HI = Hazard index
- ADD = Average daily dose
- RfD = Reference dose or ADI Acceptable daily intake RfD for Pb= 0.004 mg/kg

G. Data Processing and Statistical Analysis

Statistical analysis will be perform using “SPSS Ver. 17 for Windows” to evaluate the data. All values will be expressed as mean ± SD. Differences will be considered statistically significant when p<0.05. ANOVA, Independent T-Test and Pearson’s Chi-square statistical analysis will be employed to analyze the data statistically.
IV. RESULT AND DISCUSSIONS

A. Demographic Data of Respondents

1) Age distribution between exposed and non-exposed primary school children

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exposed primary school children n (%)</th>
<th>Non-exposed primary school children n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 years old</td>
<td>12 (24)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>8 years old</td>
<td>11 (22)</td>
<td>10 (20)</td>
</tr>
<tr>
<td>9 years old</td>
<td>10 (20)</td>
<td>14 (28)</td>
</tr>
<tr>
<td>10 years old</td>
<td>8 (16)</td>
<td>11 (22)</td>
</tr>
<tr>
<td>11 years old</td>
<td>9 (18)</td>
<td>7 (14)</td>
</tr>
</tbody>
</table>

Based on table I above, the percentage of age for age range of 7 years old is 24% for exposed (SK Tabuan, Pending, Kuching, Sarawak) and 16% for non-exposed primary school children (SK Dato Mohd Musa, Kota Samarahan, Sarawak). For age range of 8 years old is 22% for exposed and 20% for non-exposed; for 9 years old age range is 20% for exposed and 28% for non-exposed; for 10 years old age range is 16% for exposed and 22% for non-exposed; and for age range of 11 years old consists of 18% from exposed primary school children and 14% of non-exposed primary school children.

2) Gender Distribution between Exposed and Non-exposed Primary School Children

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exposed primary school children n (%)</th>
<th>Non-exposed primary school children n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>25 (50)</td>
<td>23 (46)</td>
</tr>
<tr>
<td>Female</td>
<td>25 (50)</td>
<td>27 (54)</td>
</tr>
</tbody>
</table>

Table II shows that percentages of male and female respondents were equally same at exposed primary school children (SK Tabuan, Pending, Kuching, Sarawak). On the other hand, for the non-exposed primary school children (SK Dato Mohd Musa, Kota Samarahan, Sarawak) consists of 23 (46%) male and 27 (54%) female.

3) Symptom experience between exposed and non-exposed primary school children

![Symptoms Experienced by the Respondents](image)

Fig. 3. Symptoms experienced by the respondents

Fig. 3 above represents symptoms experienced by the respondents from both primary school - SK Tabuan, Pending, Sarawak and SK Dato Mohd. Musa, Kota Samarahan, Sarawak. The Pearson’s chi-square statistics for the symptoms are: headache is 2.94; heavy headed is 1.05; fatigue is 0.51; drowsiness is 1.33; dizziness is 6.14; cough is 3.05; stuffy nose is 2.21; and eye irritation is 7.85, with the degree of freedom (df) for all symptoms is 1.

Based on the p-value, the symptoms of headache (p=0.09), Dizziness (p=0.01), cough (p=0.09) and eye irritation (p=0.005) experienced by the respondents is less than 0.05. Thus, there is a significance difference for exposed and non-exposed primary school children for the four (4) symptoms experienced by them.

The result also shows that 38 (76%) of the respondents in exposed primary school experienced this symptoms compared to the non-exposed primary school. However, the headache be caused by other factors too, such as stress, health conditions, and others. The same conclusion can be made with respondents whom experienced the symptoms of dizziness [exposed - 19 (38%); non-exposed - 8 (16%)]. The next symptom that has p-value less than 0.05 is cough.

B. Levels of Lead (Pb) Concentration in Exposed and Non-Exposed Primary School

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exposed</th>
<th>Non-exposed</th>
<th>95% CI (Mean Diff.)</th>
<th>t-stats (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead Level</td>
<td>2.20</td>
<td>0.90</td>
<td>0.92, 1.68 (1.30)</td>
<td>6.78 (74)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

According to Table IV above, the level of TSP and PM\textsubscript{10} at both places is below the permissible standard. However, at the exposed primary school, the level of TSP and PM\textsubscript{10} is higher compared to non-exposed primary school. Thus, this might cause the students to suffer from coughing. As an addition, the road construction is also in progress, which may contribute to particulate matter release.

The last symptom reported by the respondents is the eye irritation. Eye irritation will some how related to the particulate matter present in the environment. Since the both of the school is not using chalk as a teaching tool, the dusts present due to the industrial activities (i.e. cement manufacturing factory, plywood manufacturing, etc) will worsen the situation and may cause eye irritation especially towards the exposed primary school.
statistically significant, which is 1.30 and the standardized difference, \( t = 6.78 \). The two-tailed p-value of the test is 0.001, which is less than 0.05. The 95% CI for mean difference is [0.92, 1.68].

The mean value for lead concentration (Pb) in exposed primary school children is 2.2 with a standard deviation 1.20 and the mean value for non-exposed primary school children is 0.90 with standard deviation 0.63. Since the p-value is less than 0.05, there is a difference in mean lead levels between the exposed and non-exposed primary school children. The lead levels in exposed primary school children are higher than the lead levels in non-exposed primary school children. Based on the statistically analysis, it can be assumed that the difference is between 0.92 and 1.68.

The similar study by other authors also shows the same result, whereby the levels of Lead in saliva of the study group were high (1.07±1.31\( \mu \)g/L), when compared to the standard values of <1.0\( \mu \)g/L [16]. Apart from that, the current study also justifies the findings of other researchers who have shown the advantages of analyzing substances using samples obtained by non-invasive methods. On the other hand, previous study also justifies that, Lead may be exposed to the population nearby through three routes of exposure: soil ingestion (4.12E±1.94E), dermal contact (1.46E±6.88E) and through air inhalation (1.35E±1.64E) [6]. It is well established that ingestion (i.e. food consumption) is the main route of human exposure to most organic pollutants and metals [26].

Through the literature review, it can be justify that the possible reason for the higher levels of lead in exposed primary school children is due to the environmental exposure of lead through particulate matters released by the nearby industries. However, the possibility of other confounding factors (i.e. lifestyle, dietary, lead in paint, lead from other sources, etc) might be the cause of lead exposure. This is stated in the limitations of the study.

**TABLE V: Statistical Analysis of Lead Comparison for Each Primary Standard**

<table>
<thead>
<tr>
<th>Primary Standard</th>
<th>No. of students</th>
<th>Mean ± sd</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary 1</td>
<td>20</td>
<td>2.59 ± 0.82</td>
<td>0.001 [p&lt;0.05]</td>
</tr>
<tr>
<td>Primary 2</td>
<td>21</td>
<td>1.88 ± 0.66</td>
<td></td>
</tr>
<tr>
<td>Primary 3</td>
<td>24</td>
<td>0.89 ± 0.80</td>
<td></td>
</tr>
<tr>
<td>Primary 4</td>
<td>18</td>
<td>0.82 ± 0.67</td>
<td></td>
</tr>
<tr>
<td>Primary 5</td>
<td>17</td>
<td>1.07 ± 0.98</td>
<td></td>
</tr>
</tbody>
</table>

With regards to Table V, the p-value for Levene’s test for equality of variance is 0.002, which is less than 0.05. The p-value of the test is 0.001, which is less than 0.05. Hence, at least one pair of means differ significantly. Post-hoc test were carried out to determine which pair differs significantly.

**TABLE VI: Determination of The Significant Pair by Using Post-Hoc Test**

<table>
<thead>
<tr>
<th>Primary Standard</th>
<th>Number of students</th>
<th>Mean ± sd</th>
<th>F-stats*(df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary 1</td>
<td>20</td>
<td>2.79 ± 0.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary 2</td>
<td>21</td>
<td>2.18 ± 0.76</td>
<td>6.46 (4, 45)</td>
<td>0.001 [p&lt;0.05]</td>
</tr>
<tr>
<td>Primary 5</td>
<td>17</td>
<td>1.04 ± 1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a One-Way ANOVA test*

The result of post-hoc test in Table VI, reflects that the mean difference for Primary 1, Primary 2 and Primary 5 is -0.98, the p-value is 0.001 (<0.05) and the 95% confidence interval for the mean differences does not contain the value of 0. This shows that Primary 1, Primary 2 and Primary 5 are significantly different.

The mean concentration for Primary 1 and Primary 2 are higher compared to primary 5. Children are susceptible to the effects of lead [27]. Indeed, child appears to be more sensitive to lead than adults in all areas except kidney function. The most critical sphere for children is the potential for damage to their intellectual and behavioral development. The three key factors are: young children behave in ways that increase their exposure; children absorb more lead for a given exposure than do adults and developing organisms are inherently, more susceptible than mature ones.

Children also engage in “oral exploration” of their environment, placing non-food objects in their mouths frequently. This normal behavior can lead to ingestion of lead-contaminated soil and dust. Children also have a higher inhalation rate, relative to their body mass, than do adults. This leads to greater exposure to airborne contaminants [19].

C. Estimation of the Health Risk and Hazard Index for Exposed Primary School Children.

1) Calculation for non-carcinogenic effect of lead

\[
ADD = \frac{2.2 \times 20 \times 250 \times 9}{27.42 \times 365 (1 \text{ year})} = 2.47 \text{ mg/kg.day}
\]

2) Calculation for carcinogenic effect of lead

\[
LADD = \frac{2.2 \times 20 \times 250 \times 9}{27.42 \times 25550 (70 \text{ years})} = 0.14 \text{ mg/kg.day}
\]

3) Hazard index calculation for lead exposure

\[
HI = \frac{0.14}{0.004} = 0.03
\]

Referring to calculation 1 and 2, the assumed ADD value for non-carcinogenic risk assessment is 2.47/kg.day while for LADD calculation, the assumed value is 0.14 mg/kg.day. ADD value given is the assumption of the concentration of lead being exposed to the school children everyday for one year, while the LADD value is the assumption of the concentration of lead being exposed to the school children over 25,220 days (70 years), in which is the averaging number of days the children is exposed.

Reference [3] indicates that, there was no reference dose for lead. Thus, hazard index for lead cannot be calculated accordingly to respective standard. However, tolerable daily intake for lead is 0.0036 mg/kg-day. Thus, the Hazard Index (HI) for lead was compared to the tolerable daily intake from Health Canada [28]. As a result, the HI value being assumed is 0.03, which is less than 1. The HI value that is less than 1 poses no concern on the carcinogenic effect of, since the
exposure is low. However, precautions still needs to be taken in order to prevent prolong exposure and increasement in the concentration of lead being exposed. This is because, the value given is only assumption, which does not take into account the actual weight and height of the students. Thus, the actual exposure rate may vary. The exposure concentration of lead being used in this calculation is using the mean concentration, and therefore, may vary for each student.

V. RECOMMENDATIONS

The study shows that lead is present in the saliva of both exposed and non-exposed primary school children. However, for both study area, the levels of lead does not exceed the reference dose for lead in the body which is 0.004 mg/kg. However, few recommendations are given to reduce the exposure:

A. Housekeeping

1) Wet mop and wet wipe hard surfaces and windows using soap and water regularly. This will help to reduce the possibility of inhaling particulate matters which may not only carry dust and lead, but also other components, as well as heavy metals.

2) For the exposed primary school children, it is advisable not to vacuum hard surfaces suspected of lead contamination. This activity may scatter dust. If vacuuming is done, "hepa-vac" or use other comparably sensitive filters. Since the school is reported to be dusty by the teachers and students (through questionnaires and direct interviews given), it is advisable for the school to use the vacuums, instead of using normal broom. However, this will need to take into consideration of the budget and other expenses of enforcing this methodology.

3) Practice good personal hygiene (i.e. washing the hands and faces before eating, etc) to minimize exposure through ingestion. As being discussed in previous chapter, children usually likes to stick their hand in their mouth. Thus, the contaminated fingers may introduce lead into the body. The same practice is also advised for the teachers and also the rest of the staffs at the school, since they are also exposed.

B. Enclosed Classroom

Air ventilation system is crucial to ensure the quality of air inhaled. Since the classrooms are using the open-air classroom system, it is advisable for the school to re-consider in the enforcement of air-conditioned, enclosed classroom to reduce the exposure rate which may be cause through inhalation or ingestion. Thus, if enclosed classroom is being used, the inhalation of the contaminants can be reduced. However, this recommendation is also subjected to the budget and extra expenses.

C. Nutrition

Make sure children eat regular nutritious meals. Lead is more easily absorbed into the body on an empty stomach and malnutrition. Thus, it is advisable for the children’s diet to contain plenty of iron and calcium. Examples of foods high in iron are: liver, fortified cereal, cooked beans, spinach, and raisins. Examples of foods high in calcium are: milk, yogurt, cheese, and cooked greens. Parents and teachers should act together in ensuring that the children received enough nutrition.

D. Monitoring Program and Further Study to be Conducted

1) Do regular monitoring program (i.e. air monitoring emphasizing on lead in air, etc) to monitor the ambient air status. As an addition, the analysis of heavy metal (i.e. lead, cadmium, etc) can be carried out to monitor the levels of the heavy metals in air, since there is no such monitoring or study being carried out at the subjected study area.

2) Further study on the respected study title can be conducted with few improvements to enhance the design of the study itself and to obtain more findings and relationship of the exposure to the children’s health.

VI. CONCLUSIONS

From the findings, it can be concluded that the objective of this study is achieved, whereby the findings are:

- The levels of lead in saliva of the exposed study group are higher compared to the non-exposed study group. Thus, the alternative hypothesis is accepted, because the p-value is less than 0.05.
- The study also found that there are significance difference of lead concentration between exposed and non-exposed primary school children as the p-value is less than 0.05. However, both study groups were not exceeding the acceptable limit.
- There is a significant different for Primary 1, Primary 2 and Primary 5 students.
- The estimated health risk assessments is low, with the assumed hazard index value is 0.03. Since the value is less than 1, thus there is no concern on carcinogenic risk of lead exposure towards the primary school children. However, the value given is only for general assumption. Thus, extensive study should be carried out to determine the actual risk of exposure.

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REFERENCES


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