Determination of diethyllead in the urine by flameless atomic absorption spectrometry

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ABSTRACT A method for the determination of diethyllead in urine by flameless atomic absorption spectrometry after chelation with glyoxal-bis (2-hydroxyanil) and extraction of the formed complex with methyl isobutyl ketone is described. The method is specific in relation to both triethyllead and inorganic lead. The limit of detection was 3.2 µg Pb/l and the relative standard deviation in the concentration range 20-100 µg Pb/l was 0.076.

Despite the high toxicity of tetraethyllead (Et₄Pb) it is still used in many countries as an antiknock agent in petrol. The material is metabolised to triethyllead (Et₃Pb⁺⁺), diethyllead (Et₂Pb⁺⁺), and inorganic lead, all of which are excreted in the urine.⁴ In the case of acute intoxication with Et₂Pb, Et₂Pb⁺⁺ is the main metabolite excreted in the urine.³ Excretion of this metabolite in the urine of workers exposed to Et₄Pb has yet to be investigated.

The sensitivity of colorimetric methods⁴⁻⁶ for the determination of Et₂Pb⁺⁺ is too low to be applied to the evaluation of occupational exposure. Yamauchi et al proposed a method for the determination of the metabolites of Et₄Pb by hydride generation flameless atomic absorption spectrometry (AAS) in a quartz cell.⁷ This very sensitive method requires a system of hydride generation which is not commercially available and limits its applicability in the average laboratory.

This paper describes a method for the determination of Et₂Pb⁺⁺ by the use of simple flameless AAS. The method consists in the selective separation of Et₂Pb⁺⁺ from other metabolites by a specific reaction with glyoxal-bis (2 hydroxyanil (GHA)) and extraction of the formed complex with methyl isobutyl ketone (MIBK). During the elaboration of this method some earlier data such as the conditions of selective extraction of particular metabolic forms⁷ and the formation of the complex of Et₂Pb⁺⁺ with GHA⁸ have been used together with data from our own investigation in which the parameters of formation and stability of the Et₂Pb⁺⁺/GHA complexes in the urine were established (Z. Turlakiewicz and J. Chmielnicka, unpublished data).

Materials and methods

APPARATUS

A Beckman model 1248 atomic absorption spectrophotometer with a Massman 1268 graphite cell and deuterium lamp to compensate for non-specific absorption were used. The measurements were made at a wavelength of 281 nm. The following cell temperatures were used:

- Stage I: drying 150°-40 s
- Stage II: mineralisation 650°-30 s
- Stage III: atomisation 2500°-7.5 s
- Stage IV: cleaning tube 3100°-2 s

REAGENTS

Diethyllead chloride (Et₂PbCl₂) was synthesised by the method of Heap et al.* The identity of the obtained compound was confirmed by elementary analysis (anal calc for Et₂PbCl₂: C—14.29% H—2.9%; Cl—21.09%. Found: C—14.3%; H—3.26%; Cl—19.6%) and by mass spectroscopy (fig 1).

Standard solution: 32.4 mg of the synthesised compound was dissolved in 100 ml of distilled water.

Working standard solutions: 100 and 10 times dilution of the standard solution.

Triethyllead chloride (Et₃PbCl) — The compound was synthesised by the method of Heap and Saunders* and purified in an alcohol-water solution. The identity of the obtained compound was confirmed by elementary analysis (anal calc for Et₃PbCl: C—21.86%; H—4.55%; Cl—10.75%. Found: C—
Fig 1  Mass spectrogram of Et₂PbCl and Et₃PbCl₂.

22·1%; H—4·8%; Cl—10·65%) and by mass spectroscopy (Fig 1).

Inorganic lead standard Pb(NO₃)₂—Standard for atomic absorption spectrophotometry, Wzormat, Poland.

Standard solution: 1 ml—1·00 mg of Pb⁺².

Working solutions: 100 times dilution of the standard solution.

Perchloric acid—HClO₄ 60% pa, Hopkin and Williams.

Isopropyl acetone (methyl isobutyl ketone) MIBK—pa, Loba Chemie.

Glyoxal-bis (2-hydroxyanil) GHA—pa, Roanal, Hungary (0·24 g of GHA in 100 ml of methanol mg).

The solution was allowed to stand in the dark at room temperature for 24 hours before use.

Sodium hydroxide—NaOH—40% pa, Polish Reagents, Gliwice.

Sodium chloride—NaCl, pa, Polish Reagents, Gliwice.

The standard solutions were diluted directly before use to the required concentrations.

Procedure

Forty millilitres of urine were measured into a centrifuge tube and 8 ml of 60% HClO₄, 15 g of NaCl, and 20 ml of MIBK added. The contents were shaken vigorously for 20 minutes and centrifuged at 3000 rpm for 20 minutes.

The water layer, containing Et₂Pb⁺² and inorganic lead, was adjusted to pH 9·8 with 40% NaOH. Then, after the addition of 4 ml GHA solution and 10 ml of MIBK, the mixture was shaken for 10 minutes and centrifuged at 3000 rpm for 10 minutes. After separation, 20 μl of the MIBK layer containing Et₂Pb⁺²—GHA complex was injected into the graphite cell.

Results

DETECTION, ACCURACY, SPECIFICITY, RECOVERY RATES

In the range of concentrations from 20 μg Pb/l to 100 μg Pb/l there is a linear relation between the concentration of Et₂Pb⁺² and absorbance.

The detection limit, determined according to the International Union of Pure and Applied Chemistry recommendations,¹⁰ was 3·2 μg Pb/l (blank measures, n = 11).

The relative standard deviation (sₑ) in the whole concentration range was 0·076. Table 1 shows the

<table>
<thead>
<tr>
<th>Compound added</th>
<th>No</th>
<th>Added quantity (μg Pb/l)</th>
<th>Detected quantity (μg Pb/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb⁺²</td>
<td>8</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Et₂Pb⁺²</td>
<td>8</td>
<td>125</td>
<td>Below detection limit</td>
</tr>
</tbody>
</table>
Determination of diethyllead in the urine by flameless atomic absorption spectrometry

![Graph showing stability of Et,Pb+2 in urine at different pH and temperature.](image)

Fig 3  Stability of Et,Pb+2 in urine at different pH and temperature. pH = 3 (○—○ +24°C; ○—○ +4°C). pH = 6 (□—□ +24°C; △—△ +4°C; ■—■ -18°C).

values of recovery rates and relative standard deviations for particular concentrations.

SAMPLE STABILITY
The complex of Et,Pb+2 with GHA was stable in MIBK at +4°C in the period ranging from four to 72 hours (fig 2). The observed stability of the complex permits the MIBK solutions to be kept till the next day for determination.

SPECIFICITY OF THE METHOD
The specificity of the method for the determination of Et,Pb+2 in relation to the other two metabolic products (triethyllead and inorganic lead) was tested by adding samples of the other metabolites to urine at much higher concentrations than those expected in occupationally exposed individuals. The results are shown in table 2.

STABILITY OF ET,Pb+2 SOLUTIONS IN URINE
Investigations were carried out by adding Et,Pb+2 at the concentration of 80 μg Pb/l to fresh urine and to urine adjusted to pH 3. The urine samples were stored at +24°C, +4°C, and at -18°C. The results are presented in fig 3.

DETERMINATION OF ET,Pb+2 IN THE URINE IN CASES OF ACUTE INTOXICATION WITH ET,Pb
Et,Pb+2 was determined in the urine of patients treated in hospital after drinking from 10 to 100 ml of Et,Pb. The results are shown in table 3.

The method was also used to determine Et,Pb+2 in the urine of several workers adding Et,Pb to petrol. Preliminary results of these investigations showed that Et,Pb+2 was excreted in the urine after occupational exposure to Et,Pb, although in much lower concentrations (15–25 μg/l) than in the case of acute intoxication (Z Turlakiewicz and J Chmielnicka, unpublished data).

Discussion
The proposed method for determining Et,Pb+2 uses a specific reaction to form a complex of Et,Pb+2 with GHA which allows the selective separation of this metabolite from the other metabolites of Et,Pb. The extract of MIBK containing a complex of Et,Pb+2 with GHA is injected directly into a graphite cell without additional re-extraction and hydrogenation. The complex of Et,Pb+2 with GHA in MIBK is stable and can be stored in glass stoppered vessels at +4°C for 72 hours. The method is specific both in relation to Et,Pb+ and inorganic lead, even when these metabolites are present in high concentrations.

The investigations of the stability of Et,Pb+2 in the urine showed that after adjusting the urine to pH 3 it can be stored for three days at +4°C.

The results of the determination of Et,Pb+2 in the urine by the proposed method in the cases of acute intoxication with Et,Pb are similar to those obtained by Yamamura et al who also observed that the excretion of Et,Pb+2 exceeded 50% of the total lead excreted in the urine. Our results, and those of Yamamura et al show that Et,Pb+2 is a specific indicator of acute Et,Pb intoxication.

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Table 3  Levels of Et,Pb+2 and total lead in urine in cases of acute intoxication with Et,Pb

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No of days after intoxication</th>
<th>μg of Pb/l</th>
<th>Total Pb</th>
<th>Et,Pb+2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man aged 19*</td>
<td>1*</td>
<td>403.2</td>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>Dose 100 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatal</td>
<td>4</td>
<td>442.2</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td>Woman aged 63</td>
<td>11*</td>
<td>6.8</td>
<td>0.172</td>
<td></td>
</tr>
<tr>
<td>Dose unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatal</td>
<td>30</td>
<td>0.952</td>
<td>0.294</td>
<td></td>
</tr>
<tr>
<td>Man aged 22</td>
<td>2</td>
<td>0.112</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>Dose 10 ml</td>
<td>7</td>
<td>0.090</td>
<td>0.065</td>
<td></td>
</tr>
<tr>
<td>Survived</td>
<td>10</td>
<td>0.100</td>
<td>0.090</td>
<td></td>
</tr>
</tbody>
</table>

*After EDTA.
†Total lead was determined by flameless AAS.
References


Correction

Do amines induce occupational asthma in workers manufacturing polyurethane foams? (November 1984)

The formula for 2,4-toluendiisocyanate was incorrectly given. The correct formula is given below.

\[
\begin{align*}
\text{NCO} & \quad \text{NCO} \\
\text{CH}_3 & \quad \text{2H}_2\text{O} \\
\text{2,4-toluendiisocyanate} & \quad \text{2CO}_2 \quad \text{2NH}_2 \\
\text{2,4-toluendiamine} & \quad \text{(from Candura, 1974)}
\end{align*}
\]
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