

# 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 (Et<sub>4</sub>Pb) it is still used in many countries as an antiknock agent in petrol. The material is metabolised to triethyllead (Et<sub>3</sub>Pb<sup>+</sup>), diethyllead (Et<sub>2</sub>Pb<sup>2+</sup>), and inorganic lead, all of which are excreted in the urine.<sup>1-3</sup> In the case of acute intoxication with Et<sub>4</sub>Pb, Et<sub>2</sub>Pb<sup>2+</sup> is the main metabolite excreted in the urine.<sup>3</sup> Excretion of this metabolite in the urine of workers exposed to Et<sub>4</sub>Pb has yet to be investigated.

The sensitivity of colorimetric methods<sup>4-6</sup> for the determination of Et<sub>2</sub>Pb<sup>2+</sup> 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 Et<sub>4</sub>Pb by hydride generation flameless atomic absorption spectrometry (AAS) in a quartz cell.<sup>7</sup> 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 Et<sub>2</sub>Pb<sup>2+</sup> by the use of simple flameless AAS. The method consists in the selective separation of Et<sub>2</sub>Pb<sup>2+</sup> 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 forms<sup>7</sup> and the formation of the complex of Et<sub>2</sub>Pb<sup>2+</sup> with GHA<sup>6</sup> have been used together with data from our own investigation in which the parameters of formation and stability of the Et<sub>2</sub>Pb<sup>2+</sup>/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* (Et<sub>2</sub>PbCl<sub>2</sub>) was synthesised by the method of Heap *et al*.<sup>8</sup> The identity of the obtained compound was confirmed by elementary analysis (anal calc for Et<sub>2</sub>PbCl<sub>2</sub>: 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* (Et<sub>3</sub>PbCl)-The compound was synthesised by the method of Heap and Saunders<sup>9</sup> and purified in an alcohol water solution. The identity of the obtained compound was confirmed by elementary analysis (anal cal for Et<sub>3</sub>PbCl: C-21.86%; H-4.55%; Cl-10.75%. Found: C-

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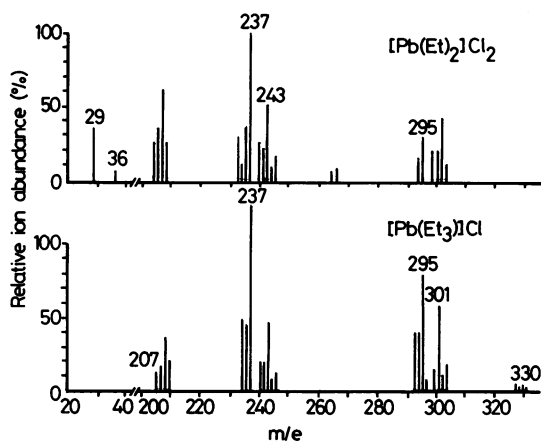


Fig 1 Mass spectrogram of  $\text{Et}_2\text{PbCl}$  and  $\text{Et}_2\text{PbCl}_2$

22.1%; H—4.8%; Cl—10.65%) and by mass spectroscopy (fig 1).

**Inorganic lead standard  $\text{Pb}(\text{NO}_3)_2$** —Standard for atomic absorption spectrophotometry, Wzormat, Poland.

Standard solution: 1 ml—1.00 mg of  $\text{Pb}^{+2}$ .

Working solutions: 100 times dilution of the standard solution.

**Perchloric acid**— $\text{HClO}_4$ , 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 rg). The solution was allowed to stand in the dark at room temperature for 24 hours before use.

**Sodium hydroxide**— $\text{NaOH}$ —40% pa, Polish Reagents, Gliwice.

**Sodium chloride**— $\text{NaCl}$ , 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%  $\text{HClO}_4$ , 15 g of  $\text{NaCl}$ ,

Table 1 Recovery rates and relative standard deviation for  $\text{Et}_2\text{Pb}^{+2}$  in urine at various concentrations

Concentration ( $\mu\text{g Pb/l}$ )	No	Recovery rate	$s_r$
20	10	90.4%	0.081
50	10	92.1%	0.034
100	10	97.0%	0.034

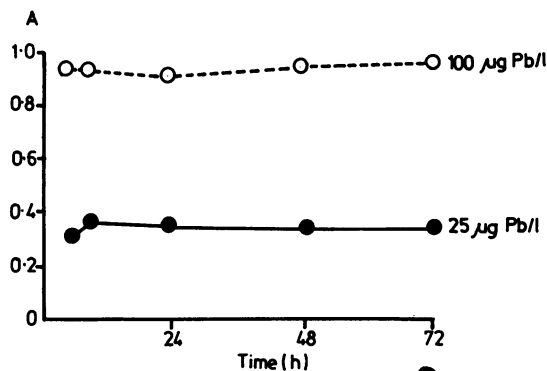


Fig 2 Stability of  $\text{GHA-Et}_2\text{Pb}$  complex in methyl isobutyl ketone. A = Average value of complex absorbance ( $n = 3$ ) in relation to the absorbance of standard solution of  $\text{Pb}^{+2}$  at a concentration of  $100 \mu\text{g Pb}^{+2}/\text{l}$ .

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  $\text{Et}_2\text{Pb}^{+2}$  and inorganic lead, was adjusted to pH 9.8 with 40%  $\text{NaOH}$ . 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  $\mu\text{l}$  of the MIBK layer containing  $\text{Et}_2\text{Pb}^{+2}$ —GHA complex was injected into the graphite cell.

### Results

#### DETECTION, ACCURACY, SPECIFICITY, RECOVERY RATES

In the range of concentrations from 20  $\mu\text{g Pb/l}$  to 100  $\mu\text{g Pb/l}$  there is a linear relation between the concentration of  $\text{Et}_2\text{Pb}^{+2}$  and absorbance.

The detection limit, determined according to the International Union of Pure and Applied Chemistry recommendations,<sup>10</sup> was 3.2  $\mu\text{g Pb/l}$  (blank measures,  $n = 11$ ).

The relative standard deviation ( $s_r$ ) in the whole concentration range was 0.076. Table 1 shows the

Table 2 Specificity of the method for determination of  $\text{Et}_2\text{Pb}^{+2}$  in urine

Compound added	No	Added quantity ( $\mu\text{g Pb/l}$ )	Detected quantity ( $\mu\text{g Pb/l}$ )
$\text{Pb}^{+2}$	8	500	—
$\text{Et}_3\text{Pb}^{+2}$	8	125	Below detection limit

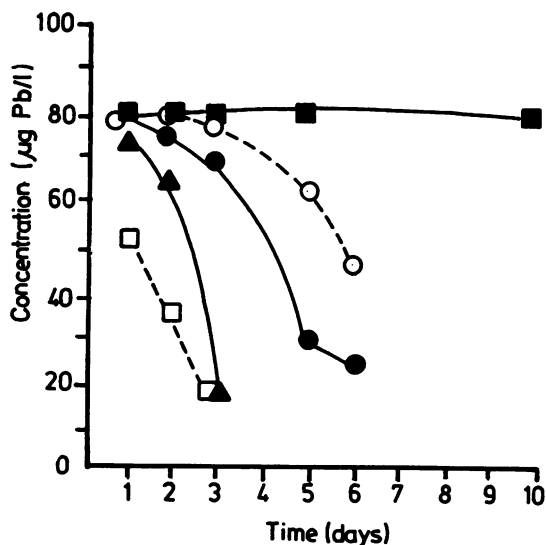


Fig 3 Stability of  $\text{Et}_2\text{Pb}^{+2}$  in urine at different pH and temperature. pH = 3 (●—● +24°C; ○—○ +4°C). pH = 6 (□—□ +24°C; ▲—▲ +4°C—; ■—■ -18°C).

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

#### SAMPLE STABILITY

The complex of  $\text{Et}_2\text{Pb}^{+2}$  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  $\text{Et}_2\text{Pb}^{+2}$  in relation to the two other metabolic products (triethyllead and inorganic lead) was tested

Table 3 Levels of  $\text{Et}_2\text{Pb}^{+2}$  and total lead in urine in cases of acute intoxication with  $\text{Et}_4\text{Pb}$

Subjects	No of days after intoxication	mg of Pb/l	
		Total Pb	$\text{Et}_2\text{Pb}^{+2}$
Man aged 19* Dose 100 ml	1*	403.2	100
Fatal	4	442.2	236
Woman aged 63 Dose unknown	11*	6.8	0.172
Fatal	30	0.952	0.294
Man aged 22 Dose 10 ml	2	0.112	0.075
Survived	7	0.090	0.065
	10	0.100	0.090

\*After EDTA.

†Total lead was determined by flameless AAS.

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 $\text{Et}_2\text{Pb}^{+2}$ SOLUTIONS IN URINE

Investigations were carried out by adding  $\text{Et}_2\text{Pb}^{+2}$  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 $\text{Et}_2\text{Pb}^{+2}$ IN THE URINE IN

CASES OF ACUTE INTOXICATION WITH  $\text{Et}_4\text{Pb}$   
 $\text{Et}_2\text{Pb}^{+2}$  was determined in the urine of patients treated in hospital after drinking from 10 to 100 ml of  $\text{Et}_4\text{Pb}$ . The results are shown in table 3.

The method was also used to determine  $\text{Et}_2\text{Pb}^{+2}$  in the urine of several workers adding  $\text{Et}_4\text{Pb}$  to petrol. Preliminary results of these investigations showed that  $\text{Et}_2\text{Pb}^{+2}$  was excreted in the urine after occupational exposure to  $\text{Et}_4\text{Pb}$ , 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  $\text{Et}_2\text{Pb}^{+2}$  uses a specific reaction to form a complex of  $\text{Et}_2\text{Pb}^{+2}$  with GHA which allows the selective separation of this metabolite from the other metabolites of  $\text{Et}_4\text{Pb}$ . The extract of MIBK containing a complex of  $\text{Et}_2\text{Pb}^{+2}$  with GHA is injected directly into a graphite cell without additional re-extraction and hydrogenation. The complex of  $\text{Et}_2\text{Pb}^{+2}$  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  $\text{Et}_3\text{Pb}^+$  and inorganic lead, even when these metabolites are present in high concentrations.

The investigations of the stability of  $\text{Et}_2\text{Pb}^{+2}$  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  $\text{Et}_2\text{Pb}^{+2}$  in the urine by the proposed method in the cases of acute intoxication with  $\text{Et}_4\text{Pb}$  are similar to those obtained by Yamamura *et al*<sup>3</sup> who also observed that the excretion of  $\text{Et}_2\text{Pb}^{+2}$  exceeded 50% of the total lead excreted in the urine. Our results, and those of Yamamura *et al*<sup>3</sup> show that  $\text{Et}_2\text{Pb}^{+2}$  is a specific indicator of acute  $\text{Et}_4\text{Pb}$  intoxication.

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