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Correlation between Lead Content in Human Biological Fluids and the Use of Vitrified Earthenware Containers for Foods and Beverages

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ABSTRACT

Methods for the measurement in the aqueous phase and organic phase (MIBK) after extraction with APDC of lead by atomic absorption spectrometry in beverages and in the biological fluids of humans assumed to be poisoned were studied and applied. The detection limits obtained were 0·045 and 0·025 ppm. Correlations were obtained between the content of lead in beverages and the use of vitrified earthenware containers and between the content of lead in beverages and in the biological fluids of the consumers.

INTRODUCTION

Together with other heavy metals, lead is an important toxic element in modern industrialized society. The incidence of poisoning by this metal has increased, parallel to its use in man's activities.

The main source of poisoning by lead is its use in industry and the dumping of lead-rich residues. In this sense, the residues from the chemical,

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steel and iron industries, from pesticides, from the antidetonating components of gasolines and other emissions that disperse lead into the atmosphere, together with other residues that are transported to the soil and water, affect the flora and fauna of the planet and, eventually, man himself (Carbonell Martin & Nuñez Orgaz, 1986). Other sources which affect man directly are the misuse of substances containing lead, such as paints, varnishes, etc (Boeck, 1986). A further source of lead poisoning of relative importance in economically underdeveloped areas is the improper use of vitrified earthenware containers whose vitrification material is rich in the element. This latter factor was considered in this study.

The quality and tolerance limits for lead, together with those considered to be pathological, are highly variable, depending on the different legislations and the current state of epidemiological studies. In foods and drinking water they can range between 1 ppm (foods) and 0·02 ppm (drinking water). Depending on different authors, in biological fluids pathological limits are reported to vary between 0·07 and 0·40 ppm (in the blood of children and adults, respectively). The limit for the lead content in urine is set at 0·08 ppm (Dinsmoor Webb, 1974; Mareca Cortes, 1983; Wyngarden & Smith, 1985; Bryce-Smith & Ward, 1987; Davis & Svendsgaard, 1987; Harrison, 1986).

The aim of the present work was to quantify the levels of lead in wines, vinegars and food preserving liquids (i.e. liquids used in the traditional-style preservation of different vegetables (peppers, tomatoes, etc.) mainly composed of vinegar, salt and a little olive oil) stored in vitrified earthenware or other types of containers and in the biological fluids of persons belonging to presumedly poisoned families. To do so, a study was first made of the analytical methods applied to the determination of lead in the above-mentioned samples. The analytical data obtained were then correlated with different variables related to the nature of the samples and of the containers, thus deducing the main factors involved in lead poisoning in persons accustomed to consuming such foods and beverages.

MATERIALS AND METHODS

Reagents

Standard solutions of Pb^{2+} at 100 and 1000 ppm.
Ammonium pyrrolidine-dithiocarbamate (APDC). AR.
Methyl-isobutylketone (MIBK). AR.
Solutions of HNO_3, H_2SO_4 and $HClO_4$. AR.
Solutions of NH_3.

These solutions were purified by extraction when necessary.

Apparatus

Varian model 1475 atomic absorption flame spectrometer equipped with a deuterium lamp.
Lead hollow-cathode lamp.
Radiometer pH meter. Model 51.
'Apple Macintosh' P.C.

Sampling and treatment of samples

The samples of wine, vinegar and preserving liquids were taken from the containers used to store them. Blood samples were taken intravenously from members of families who consumed foods and beverages with a lead content higher than 0·40 ppm and were stored in heparinized test tubes. Urine samples were from 24 h samples (Blanco, 1986).

It was necessary to mineralize the organic material to avoid interferences in the atomic absorption measurements. The mineralization was assayed by the dry and wet methods with H_2SO_4—HNO_3 for wines, vinegars and preserving liquids and with HNO_3—$HClO_4$ for the biological fluids. The wet method was chosen owing to the drawbacks inherent to the dry method; this latter is long and tedious, requiring treatments that lengthen the time of analysis. Despite this, the results obtained with both analytical methods were similar.

The size of samples depended on their content in lead and the amount of samples available. Generally, for the samples of wine, vinegar, preserving liquids and urine, an initial amount of 25 ml was used; blood samples were of 3 ml. In all cases analysis was performed in triplicate.

Measurement procedures

Owing to the variety of samples analyzed and of their lead content it was necessary to use two different measuring methods (Pinta, 1975; Ministerio de Sanidad y Consumo, 1985).

Direct measurement in the aqueous phase

This was performed on the solution resulting from mineralization of the samples under suitable conditions for the analytical technique (see Table 1).

Measurement in the organic phase

An aliquot of mineralized sample (from 3–10 ml, according to the type of sample) was adjusted to pH 7 with NH_3; 1 ml of a 1% aqueous solution of

TABLE 1
Instrumental Parameters

Method	*Air flow (litres/min)*	*C_2H_2 flow (litres/min)*	*Observation height (mm)*	*I mA*
Direct measure	12	1·7	12·5	5
Extract, org. phase	12	1·2	10·0	5

APDC and 3 or 5 ml of MIBK were added. The mixture was shaken for 2 min, left to stand for at least 30 min and the lead content was measured in the organic phase at 217 nm, using a deuterium lamp and suitable instrumental conditions (Table 1). Standard solutions were subjected to the same treatment.

For both methods a study was conducted to optimize the instrumental parameters: gas flow, observation height and intensity of the lamp in relation to the absorbance signal. In the extraction method, a study was also carried out on the variables affecting extraction: pH, stirring time, and standing time.

RESULTS AND DISCUSSION

Analytical methods

The optimal instrumental parameters found are shown in Table 1. From the data obtained in the extraction procedure it was seen that the extraction yield is very close to 100% at a pH above 5, for a stirring time greater than 90 s and a standing time of more than 30 min. A pH value of 7 was chosen for the extraction step.

Recovery values were close to 100% for all the methods, with a variation ranging between 97·1 and 101·2%. The detection limit, ($x_b + 2s/m, n = 6$) was 0·045 ppm with the direct measurement, and 0·025 ppm for the method of measurement in the organic phase after extraction. It was observed that the organic phase measurement method is slightly more sensitive; in any case, the detection limits are sufficient for the purposes of this work.

Precision (relative standard deviation) ranged between 2% and 3·5% for the method of direct measurement and between 3% and 3·5% for measurement in the organic phase (Table 2).

The intervals of the calibrations corresponding to both methods were calculated (0·0–6·0 ppm and 0·0–0·40 ppm of lead, respectively); the results obtained for the determination in the aqueous phase fitted the straight line $A = 0{\cdot}053\,C(ppm) + 0{\cdot}005$, with a correlation coefficient of 0·999. For the

TABLE 2
Analytical Characteristics of the Methods Employed

Method	*Recovery (%)*	*Detection limit (ppm)*	*Precision (RSD%)*
A-1	101·2	0·044	1·9
A-2	98·0	0·046	3·6
A-3	98·3	0·050	—[a]
B-1	99·9	0·020	2·9
B-2	97·1	0·028	3·5

A—Direct measurement method in the aqueous phase: (1) standards, (2) foods and beverages, and (3) biological fluids.
B—Extraction method and measurement of the organic phase: (1) standards, (2) foods and beverages.
[a] Not determined owing to practical difficulties of obtaining sufficient amounts of the same sample.

determination in MIBK the values fitted $A = 0{\cdot}100\,\mathrm{C(ppm)} + 0{\cdot}001$, the correlation coefficient also being 0·999.

Comparison of methods

Both analytical methods were applied to 129 samples of beverages (wines, vinegar and preserving liquids), 22 samples of blood and 22 samples of urine.

The results obtained from the application of both methods to the foods and beverages were compared by fitting the data to a straight line with a correlation coefficient of 0·996, obtaining an F value of 2·75, while the theoretical F value for a confidence level of 95% was 9·51. From both sets of data together with the value of the slope, 1·08, a good correlation between both procedures could be deduced.

The methods applied to the blood samples also fitted a straight line, with a correlation coefficient of 0·967, an F value of 17·3 and a theoretical F value of 19·4, which showed that the correlation was not entirely significant; however, the slope value of 0·958 showed that the methods were relatively comparable.

The data from urine samples were not processed because most of them were close to the detection limit of the methods.

STATISTICAL TREATMENT OF THE RESULTS

Treatment of the results was performed in two ways:

(a) Performing a distribution of relative frequencies between the lead content in the beverages and the nature of the samples and containers.

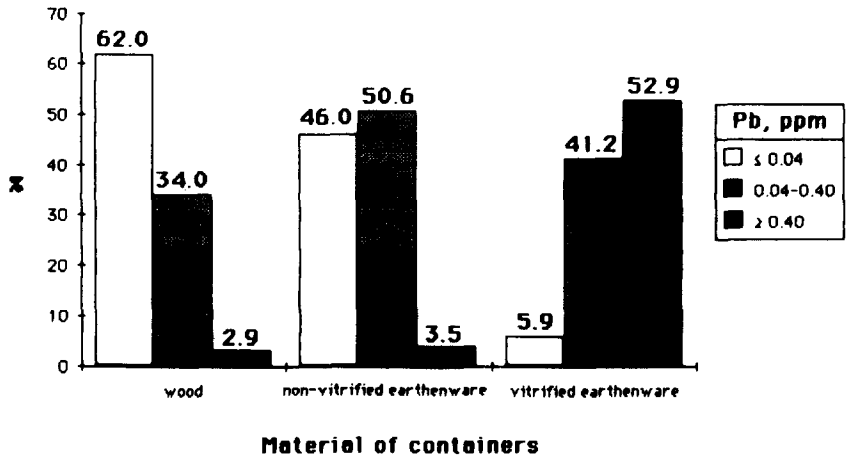

Fig. 1. Frequency histogram of the level of lead in the products contained in different containers.

The first histogram obtained (Fig. 1) correlates the material of the containers and the lead contents of the products contained in them. It is seen that wood and non-vitrified earthenware show the greatest frequency for the lowest levels of lead, while the vitrified earthenware has the highest frequency for the highest lead contents. From this one may infer, as expected, the relationship between the vitrified earthenware and beverages and food contamination.

The second histogram (Fig. 2) relates the frequency of the lead content

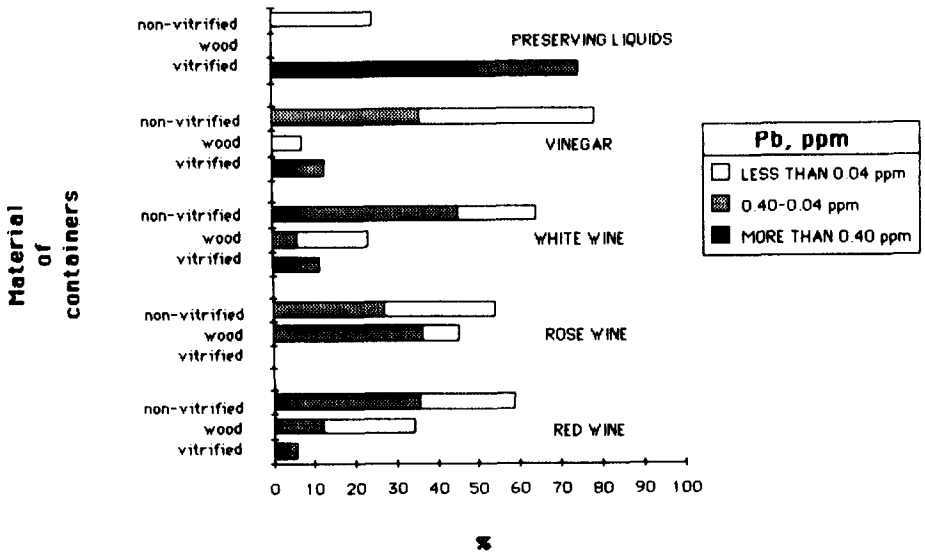

Fig. 2. Frequency histogram of the level of lead in different products and containers.

TABLE 3
Content of Lead (ppm) in the Contaminated Foods and Beverages and in the Human Biological Fluids of the Consumers

Samples	*V-3*	*V-6*	*V-8*	*V-28*	*P-1*	*P-3*	*P-4*	*P-9*	*P-10*	*P-17*	*P-20*	*P-22*	*E-1*	*E-2*	*E-9*
Foods and beverages	0·16	1·10	0·20	3·10	0·17	0·15	1·80	0·16	11·0 3·60 2·80	11·0 3·60	0·20 0·08	0·14	0·13	0·38	0·23
Blood	0·16	0·16	0·14	0·30[a] 0·16[b]	0·66	0·09	0·34[a] 0·25[b]	0·20[a] 0·18[b]	0·80[a] 0·45[b]	0·50[a] 0·20[b]	0·30[a] 0·19[b]	0·50[a] 0·15[b]	0·15	0·20	0·19
Urine	0·04	0·04	nd	0·06[a] nd[b]	0·04	0·05	0·05[a] 0·04[b]	0·03[a] 0·04[b]	0·06[a] 0·03[b]	0·10[a] 0·07[b]	0·08[a] 0·06[b]	0·04[a] 0·05[b]	0·03	0·04	0·04

Note: nd = not detectable.
[a,b] See explanation in the text.

with the material of the containers and the products contained in them. A correlation is seen between the frequency of products in vitrified earthenware and the frequency of products with more than 0·40 ppm of lead, with a correlation coefficient of 0·999, from which one may infer the correlation between both parameters.

(b) Performing simple and multiple regression analysis and obtaining the subsequent simple, partial and multiple coefficients of correlation.

From this analysis variable coefficients were obtained, although they do indicate a high correlation between the lead content, the vinegar product and the material of the vitrified earthenware container with a multiple coefficient of correlation of 0·973, which shows that they are the most important factors in food contamination by lead under the conditions of the present study.

The correlation coefficients between the lead content of the contaminated foods and that of the biological fluids of the families consuming the foods were between 0·24 and 0·75 (Table 3). (In the Table the term 'sample' is used to refer to a single family group. Each column includes both the analyses performed on persons from the same family (*a* and *b* indicate different individuals) and the analyses of the foods and beverages consumed by those persons. Only the data relating to individuals whose food contains significant amounts of lead are included.) This points, as expected, to a certain correlation between the lead content in foods and in the biological fluids of the consumer families. The correlation shows that the data do not exactly fit a straight line since the lead content in blood is affected by other factors apart from the level of lead to which the consumer is exposed; however, there is a trend or relationship between exposure to the element and its level in blood.

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