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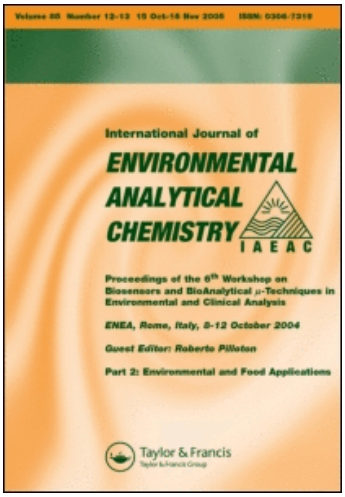

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LEAD DETERMINATION IN AVIAN BLOOD: APPLICATION TO A STUDY OF LEAD CONTAMINATION IN RAPTORS FROM FRANCE

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Marsh harriers and other raptors may become poisoned by ingesting lead gunshot in the flesh of their prey. Blood lead is a suitable indicator of exposure to lead. The method usually used in our laboratory for the analysis of human blood induced coagulation of avian blood. In this paper we report upon the optimization and quality control of a method to determine lead in avian blood samples. Samples were prepared using a diluent and matrix modifier. Analysis was carried out using graphite furnace AAS with a L'vov platform and Zeeman-effect background correction. The method was validated using international intercalibration comparisons of lead in human blood. The method proved to have a high degree of sensitivity, reproducibility and accuracy. The detection limit was low (0.004 ppm). The method used is discussed in relation to those previously used for lead analysis in avian blood.

KEY WORDS: lead, avain blood, *Circus aeruginosus*, graphite furnace AAS, matrix modifier.

INTRODUCTION

Anthropogenic inputs of lead into the environment through lead ammunition cause a very specific pollution problem. Ingestion of spent lead shot by waterfowl and other birds feeding in shot-over areas causes lead poisoning. This problem was first recognised over a century ago[1], and is today established as an important waterfowl mortality factor in many countries [2,3]. In addition, lead poisoning through the ingestion of game species with shot in their flesh, or lead poisoning casualties, has been identified in several raptor species in the USA, including the bald eagle *Haliaeetus leucocephalus*[4] and California condor *Gymnogyps californianus*[5]. Although interest has recently been stimulated concerning lead poisoning in waterfowl in Europe and elsewhere, very little published information is available relating to raptors. Consequently, a study was recently initiated in France to investigate this problem[6].

Blood lead is the most widely used index of lead exposure in humans, and is equally suitable in other animals[7,8]. Blood samples are easily obtained from live individuals and reflect present or very recent exposure, whereas samples of bone and other tissues are usually only available at post mortem, and bone samples reflect lifetime exposure. Feathers can be obtained from live birds, but lead in feathers has not been shown to be related to exposure to lead[9]. Although Pb in the skeletal system and in feathers is physiologically inert, Pb in the blood has identifiable toxic effects[10].

Probably as a consequence of differences in blood constituents, the method usually run in our laboratory for the analysis of human blood Pb[11] was inadequate for avian blood, since dilution of samples with an aqueous solution of 2,2′-diaminodiethylamine, nitric acid and 1-octanol induced coagulation of avian blood. In the present study, we propose a method involving Triton X-100 as a dilutent and including the use of a matrix modifier. Lead determination was then achieved by electrothermal atomic absorption spectrophotometry (AAS) using L'vov platform, pyrocoated graphite tubes, maximum power mode atomization, change over to an alternate gas in the graphite tube furnace and Zeeman-effect background correction [10,12,13]. This method was validated using international interlaboratory comparisons of lead in human blood. The method was subsequently used for the determination of blood Pb in 94 blood samples from wild marsh harriers *Circus aeruginosus* trapped in France.

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METHODS

Sample collection

Wild marsh harriers were trapped over the winters of 1990 and 1991 using clap traps baited with mammalian lung tissue (bovine/ovine) from two sites in France, Charente-Maritime and the Camargue.

Blood samples (0.5–1.5 ml) were taken (under licence from the French Environment Ministry) via brachial venipuncture using 23 gauge needles into trace metal-free polycarbonate tubes (Tak Lab) using EDTA as an anticoagulent. Blood samples were agitated to prevent coagulation and frozen prior to analysis for lead.

Analytical method development

Principles of the method Blood was diluted with the surfactant Triton X-100, which caused complete lysis of the blood cells, minimized frothing, reduced the sample/graphite interfacial tension, improved the contact between sample and furnace walls and provided a homogeneous solution[12]. Matrix modification of the whole blood in the graphite furnace was performed by using ammonium hydrogen phosphate. At the ashing temperature permissible with this matrix modifier (see Table 1), the organic matter is removed without concurrent volatilization of Pb[10]. Air was introduced during the ash cycle to prevent residue buildup[13],

Table 1 Temperature programme for AAS (PE3030).

Step	*Furnace temperature (°C)*	*Time (s)*		*Internal gas flow (ml/min)*[a]
		Ramp	*Hold*	
Dry	90	5	30	300
Dry	130	30	20	300
Dry	200	20	10	300
Ash	500	5	10	300
Ash	500	5	20	50
Ash	900	10	10	300
Atomization	1800	0	5	Gas Stop
Clean	2650	1	5	300
Cooling	30	1	10	300

a – argon-U except during step 5 (air).

owing to its oxidation capacity. During atomization, the argon stream through the graphite tube was shut off, so that the free atoms remained in the radiation beam for several tenths of a second. Consequently, a considerably larger number of atoms are stimulated to absorption, thus allowing the use of very small sample amounts or the detection of very small trace amounts.

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Instrumentation A Perkin-Elmer Model 3030 spectrophotometer was used, in conjunction with a Model AS-60 autosampler. An electrodeless discharge lamp was used as the radiation source with a lamp current of 10 mA. The lead line used was 283.3 nm and the spectral slit width was 0.7 nm. Pyrocoated graphite furnaces equipped with L'vov platforms were chosen. Zeeman effect background correction was used. A specific temperature programme was developed for the spectrophotometer PE 3030 (Table 1).

Labware Polyethylene bottles were used to store all reagents. All labware was soaked in 10% hydrochloric acid, rinsed three times with deionized water (Milli-Q water system) and dried in a desiccator sheltered from atmospheric dusts.

Reagents A solution containing 1% Triton X-100 (Merck for scintillation grade) was prepared in ultrapure water. 20 g of ammonium hydrogen phosphate $NH_4H_2PO_4$ (Carlo Erba Analytical RPE) were dissolved in 1 litre of ultrapure water. To prepare the matrix modifier, 50 ml of the Triton solution and 15 ml of the ammonium hydrogen phosphate solution were made up to 100 ml with ultrapure water.

Standards were prepared using Merck lead standard solution containing $Pb(NO_3)_2$ (1.000 ± 0.002 g/l) in HNO_3 (0.5 mol/l). A 400 µg/l acidified stock solution was prepared and fresh standards were made up each day by serial dilution of the stock solution.

Mode of operation Blood samples were defrosted and inverted at least 20 times to ensure thorough mixing. 100 µl blood samples were added to 900 µl of matrix modifier in plastic cups adapted to the sample tray of the AS60. The solution was homogenized.

Table 2 Variation coefficients (%) within 4 runs, each including 6 determinations of lead in a blood sample X and added concentrations (concentrations based upon peak height).

Concentrations	*Variation coefficient*				
(ng Pb/ml)	*1*	*2*	*3*	*4*	*mean*
X	2.77	1.62	2.38	1.03	1.95
X+12.5	0.48	2.35	4.46	0.68	1.99
X+25	0.84	1.15	2.30	1.11	1.35
X+50	0.75	1.31	2.24	0.75	1.26
X+100	0.47	0.51	0.78	0.40	0.54

Lead was determined in this solution. The analysis was achieved according to the method of standard addition in an isomedium (which consisted of a pool of avian blood samples the lead concentrations of which have previously been determined to be low). Concentrations of 12.5, 25, 50 and 100 ng Pb/ml were added.

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RESULTS

Analytical method

Sensitivity The detection limit was calculated according to the recommendations of the IUPAC[14]. This is equal to the concentration which corresponds to 3 times the standard deviation established from a series of 8 results obtained with a blank. The detection limit for lead obtained with the method here proposed was 0.43 ug Pb/100 ml (0.02μmol Pb/l).

Reproducibility A sample, X, and added Pb concentrations (X + 12.5, ... X + 100 ng Pb/ml) were prepared and analysed within different runs on different days. To compare the reproducibility within a run, six readings were taken for X and each of the added concentrations. When the calculation of concentrations was based upon peak height, the variation coefficient within a run varied from 1.62 to 2.77 % for X. Classically, it decreased with increasing concentrations and varied from 0.40 to 0.78 % for X + 100 ng Pb/ml (Table 2).

The inter-run comparison based upon four different determinations of X on different days and using different furnaces gave the following results: mean concentration, 7.38 μg Pb/100 ml (0.35 μmol Pb/l); standard deviation, 0.62 μg Pb/100ml; variation coefficient, 8.4%.

Accuracy The method was used in international interlaboratory comparisons organized by the Centre de Toxicologie du Quebec[15,16]. Three samples of human blood were analyzed by seventy laboratories. Our results are shown in Table 3.

Table 3 Interlaboratory comparisons (70 labs) of blood lead concentrations in three samples of human blood.

*target value (μmol Pb/l)**	*our value (μmol Pb/l)*
4.10	4.02
1.80	1.78
0.36	0.31

*Target values from international intercalibration comparisons.[15,16]

Raptor blood samples

Blood samples were taken from 94 wild marsh harriers trapped at the two sites. Blood lead concentrations ranged from 2.9 – 284.0 μg/dl (0.14–13.7 umol Pb/l; Figure 1). Blood lead concentrations in raptors are considered as elevated when they exceed 30 μg/dl (1.45 μmol/l) and indicative of clinical poisoning when greater than 60 μg/dl (2.9 μmol/l). These values are fairly conservative, and some authors consider blood lead concentrations of over 20 μg/dl (0.96 μmol/l) to be elevated in raptors[5,17]. These values have been defined in relation to

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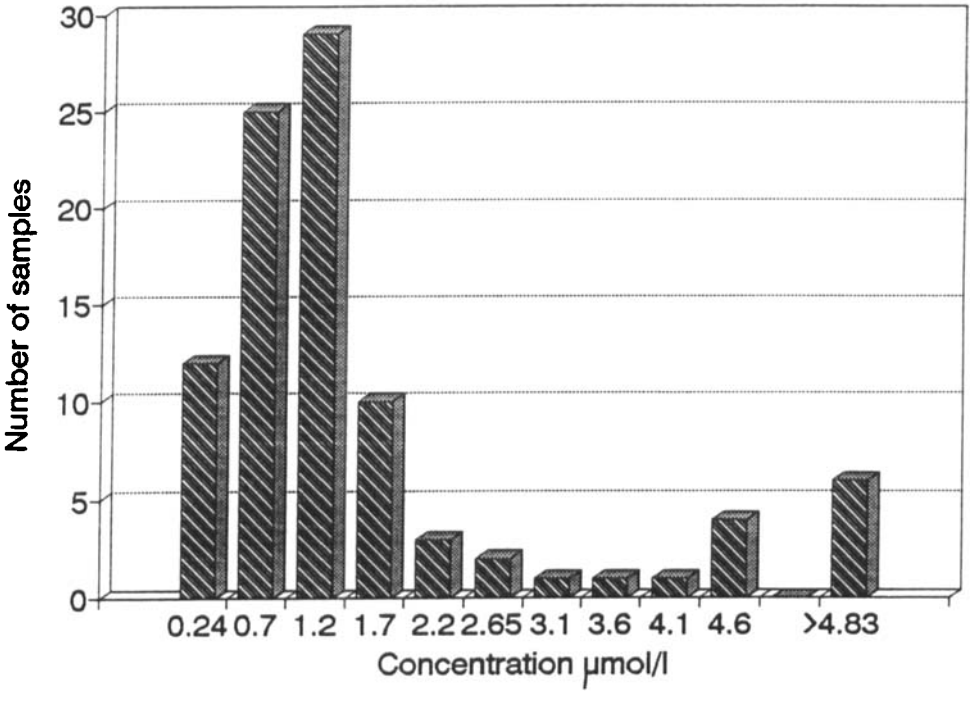

Figure 1 Distribution of blood lead concentrations in marsh harriers. Concentrations on X axis represent centres of class ranges, i.e., 3.85–4.35(4.1); 4.35–4.85(4.6); etc. Concentrations of over 1.45 μmol/l are elevated, and of over 2.9 μmol/l indicative of clinical poisoning.

concentrations found in: (a) wild and captive birds, unexposed to any known source of lead, (b) wild and captive birds that have been exposed to shot or other forms of lead, (c) wild and captive birds in which clinical effects were observed under experimental conditions.[18]. 31% of all birds had over 30 μg/dl blood lead and 14% of all birds had concentrations indicative of clinical poisoning (> 60 μg/dl; Figure 1). Results of blood lead concentrations in these birds will be presented in detail elsewhere.[18].

DISCUSSION AND CONCLUSIONS

Methods of blood lead analysis have become far more sophisticated over the last decade, allowing for the accurate determination of very low blood lead concentrations. The improvement in techniques is illustrated by the decrease in detection limits reported in the literature on lead concentrations in raptor blood. Ten years ago, Franson *et al.*[19] reported detection limits of 0.3–0.6 ppm when analysing blood from American kestrels, *Falco sparverius*. Samples were ashed and dissolved in acid (HCI and HNO_3) prior to AAS analysis. More recently, Wiemeyer *et al.*[5] reported blood lead concentrations from wild California Condors. Although the authors did not present detection limits, they assigned 0.025 ppm to samples with no detectable amounts of lead, which suggests that this approximated the detection limit. Pattee *et al.*[20] reported a detection limit of 0.01 ppm when analysing golden eagle, *Aquila chrysaetos* blood for lead. These and other studies have generally reported 85–90% recovery rates from spiked samples. However, in most publications on lead concentrations in bird blood, very few methodological details are given, and there is rarely any indication of whether or not quality control standards, such as international intercalibration exercises have been used by the analytical laboratories concerned.

The analytical method developed for our study overcomes the problem of coagulation and non-homogeneity of whole avian blood samples that occurred when using the method previously run in our laboratory for human blood lead[11]. The method developed was tested on human blood samples and found to have a high degree of sensitivity, reproducibility and accuracy. The detection limit (0.004 ppm) was far lower than reported for previous studies (see above), and analytical accuracy was high, our method giving results of 86%, 99% and 98% (range of 0.36–4.10 umol/l) of lead concentrations in target samples from international intercalibration exercises (Table 3).

It is important that methodological data are included in papers relating to lead contamination of birds and other animals, as detection limits and accuracy can significantly influence the interpretation of, and conclusions drawn from environmental data.

Marsh harriers scavenge considerably in the winter at the study sites, and frequently take sick and injured waterfowl, waders and mammals. Many of these may be hunting casualties carrying lead shot in their flesh. The birds were not exposed to industrial sources, or any other obvious sources of lead in the capture areas, but lead gunshot were found in 12% of regurgitated marsh harrier pellets collected below night roosts at one of the trapping sites (Charente-Maritime) during the winter of 1991/1992 (ref. 18). It is highly likely that the high blood Pb concentrations in marsh harriers in this study resulted from the ingestion of lead gunshot.

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Lead poisoning in raptors merits further investigation in Europe and elsewhere. It is hoped that the analytical method proposed here for the analysis of lead in avian blood (and also human blood) may be of use in future investigations into this problem.

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