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Environmental Lead Exposure in the European Kestrel (*Falco tinnunculus*) from Southeastern Spain: The Influence of Leaded Gasoline Regulations

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Lead is a widely spread heavy metal, distributed throughout all ecosystems and present in all living beings. In spite of this, no biological function is recognized for it. Although the study of heavy metals and their influence on animal health is important, additional information about both environmental contamination and risk assessment can be made available using animals as biomonitors (NCR, 1991). It is well known that lead concentrations in bones point to chronic exposure as lead uptake by bone is rapid whereas loss is slow; and lead residues in soft tissues such as liver and kidneys indicate recent exposure (Anderson and Havera, 1985, Scheuhammer, 1987, García-Fernández et al. 1997). This paper presents the results of research on lead-tissue levels in the European kestrel (*Falco tinnunculus*) as a useful tool in evaluating environmental contamination and its evolution in time throughout different areas of southeastern Spain.

The purpose of this study was to evaluate the consequences of leaded gasoline restrictions on environmental exposure to lead in the European kestrel (*Falco tinnunculus*) sampled in the Mediterranean area of the region of Murcia (southeastern Spain) far from potential sources of metal contamination. Liver, kidney, brain and bone samples from adult European kestrels were obtained over two periods, both before and after restrictions were placed on leaded fuel, 1995-97 and 2001, respectively.

MATERIALS AND METHODS

Soft tissue (liver, kidney and brain) and bone samples were taken from a total of 40 European kestrels (*Falco tinnunculus*) inhabiting different areas of southeastern Spain (Figure 1). Twenty kestrels were sampled before leaded gasoline regulations were applied, in the period between 1995 and 1997; another 20 kestrels were sampled, after these regulations were approved, in 2001. Kestrels were grouped according to the natural conditions and anthropogenic influence of the zone with respect to lead. The first group, called the "*rural area*", was formed by kestrels from a wide agricultural zone around small villages, far from intense road traffic; kestrels grouped into the "*city area*" were selected from a zone close to Murcia city, which has approximately 400,000 inhabitants, and where road traffic is very intense; and finally, the third group, called the "*mining area*", was formed by kestrels from an important, ancient mining area (La Unión) dedicated

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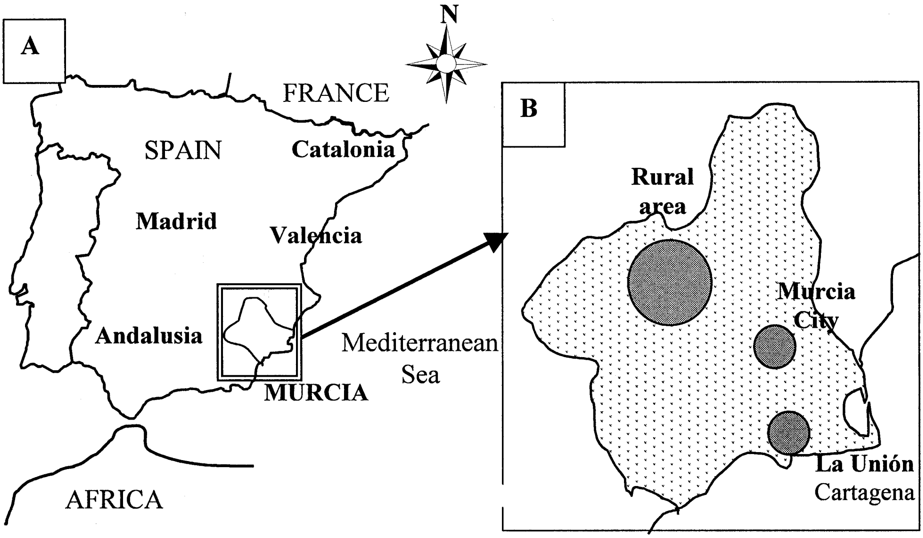

Figure 1. Maps showing the geographical location of Murcia Province, the areas studied and influencing areas. *Scale A = 1:16,800,000.*

to the extraction of Ag, Zn and mainly Pb. Mining activity ceased definitively in 1991.

For ethical reasons, only kestrels incapable of recuperating were selected and tissue samples were obtained via necropsy following euthanasia. Soft tissues and bone samples were refrigerated and immediately sent to the Laboratory of Toxicology where they were stored in sterilized flasks at –40°C until being analyzed. Anodic Stripping Voltammetry (ASV) analysis methods have been used to determine lead concentrations following our method (García-Fernández et al. 1995). This involves the wet digestion of samples (0.2 g of tissue approximately) using 0.5 ml of a mixture of acids (nitric/perchloric/sulfuric, 8/8/1). The sample was then submitted to a progressive thermal treatment. A voltammeter with a VA-646 processor and a VA-647 workstation (Metrohm, Switzerland) was used for the analytical determination of lead. An analytical lead standard (Sigma, St. Louis, Mo) has been used as a reference and a detection limit of 0.005 ppm was obtained. The repeatability for lead, determined by analyzing 10 identical samples of reconstituted lyophilized blood (European Union Reference Standards) CRM195, was 96.5 ± 1.2%. To calculate the percentage recovery, we processed 10 identical samples, which had been spiked with known amounts of the analytical lead standard. The mean for recoveries was 101.9%.

Data were grouped according to sampling areas and periods. They were expressed as a mean ± standard deviation, standard error of the mean, and minimum and maximum values. Kruskal-Wallis and Mann-Whitney non-parametric tests for independent samples were used to examine differences among zones and between sampling periods, respectively. All statements of significance were based on a 0.05 level of probability.

RESULTS AND DISCUSSION

In previous studies, the age and size of the birds (García-Fernández et al. 1997), as well as dietary characteristics and sampling areas (García-Fernández et al. 1995) were assessed in order to define their influence on the lead-body burden. Individuals were rigorously selected and therefore differences in weight and age were minimal.

In both sampling periods, comparisons of mean total concentrations among sampling areas showed significant differences: *Mining* > *City* > *Rural*; except in bones and brains when kestrels from rural and city areas were compared. These results confirm the well-known close relationship between human activities and lead exposure.

We agree that levels in raptors are primarily a consequence of the consumption of prey containing lead-shot pellets in their gizzards or containing high levels of lead biologically incorporated in their viscera (Guitart et al. 1999, Mateo et al. 2001). However some authors have also suggested that hepatic lead concentrations in several species, including raptors, could be in part due to environmental lead exposure as a consequence of liberation via leaded fuel combustion (Gretz and Bayle, 1983, Pain and Amiard-Triquet, 1993, García-Fernández, et al. 1995).

Since 1998 several regulations were approved in the European Union (EU) and Spain, progressively restricting and finally banning the use of leaded gasoline in vehicles (Directive 98/79/CE, Royal Decree 785/2001). The complete prohibition of leaded gasoline in Spain has been effective since August 1, 2001. Also, the Spanish Government established another law subsidizing the renovation of old cars in their entirety in order to reduce lead and sulfur emissions (Law 39/1997). In order to assess the influence of leaded fuel on lead exposure in raptors we compared the internal tissue levels of this metal in kestrels sampled over two periods of time, both before and after the restriction on leaded fuel, respectively.

Taking into account both factors (sampling time and areas), statistical differences between years were only found in the brains and livers of kestrels from rural and city areas (Table 1). In a similar study using goats' blood as a biomonitor we did not observe statistical differences between sampling years in the rural area (Garcia-Fernàndez et al., 2003). Our results with kestrels from the "*rural area*" could probably be explained by the greater size of the sampling zone studied (Figure 1), which includes small cities and some secondary roads. In the *city area* (Murcia city) statistical differences between years could be explained by the aforementioned laws.

However, the statistical significance of mean comparisons between sampling periods failed in the *mining* zone (Table 1). We must note that mining and smelting activities in this zone ceased in 1991, several years before the first group of samples were collected. Many attempts to remediate the zone have been initiated by the Local Government. However, this zone is filled with mineral residue deposits originating from the mining process. Wind and rainwater mobilize metal deposits and these are incorporated into the atmosphere, soil and waters of the zone. These facts would explain the higher lead-tissue concentrations in both periods of time with respect to the other zones. Similar results were found in the previous study using goats' blood (Garcia-Fernandez et al. 2003).

Table 1. Lead (µg/kg) in soft tissues (w.w.) and bone (d.w.) of European kestrel (*Falco tinnunculus*) sampled in different areas of southeastern Spain in 1995/97 and 2001.

Year	Area	N	Liver[a]	Kidney	Brain	Bone
1995--1997	Rural	7	152.6 ± 23.4* 119 – 178	206.7 ± 64.7 100 – 271	47.3 ± 12.5* 34 – 69	1365.1 ± 359.5 950 – 2100
	City	7	214.1 ± 30.8* 158 – 247	291.9 ± 47.2 240 – 350	65.7 ± 16.9* 40 – 90	1368.6 ± 312.1 980 – 1850
	Mining	6	344.8 ± 126.8 270 – 600	488.3 ± 82.8 340 – 560	95.7 ± 9.9 85 – 113	2438.3 ± 642.8 1580 – 3200
	Total	**20**	**231.8 ± 105.7 119 – 600**	**321.0 ± 133.4 100 – 560**	**66.5 ± 24.1 34 – 113**	**1688.3 ± 658.9 950 – 3200**
2001	Rural	6	95.2 ± 32.0* 47 – 130	155.7 ± 44.7 81-220	28.3 ± 10.5* 13 – 37	993.3 ± 420.9 640 – 1600
	City	7	168.0 ± 18.5* 146 – 200	232.4 ± 51.2 120 – 270	41.3 ± 9.0* 32 – 53	1199.3 ± 287.8 734 – 1600
	Mining	7	348.4 ± 95.7 230 – 450	400.3 ± 63.6 310 – 473	90.1 ± 19.4 65 – 120	1845.7 ± 908.51 1100 – 3840
	Total	**20**	**218.3 ± 121.6 47 – 450**	**278.2 ± 124.3 81 – 473**	**59.7 ± 31.7 13 – 120**	**1363.8 ± 687.3 640 – 3840**

*Statistical differences at level 0.01 between sampling years.
[a]Mean ± Standard Deviation; Minimum-Maximum

Liver and brain were the most sensitive tissues for demonstrating differences between sampling years as well as for zones. Data on brain-lead levels among kestrels is scarce in literature, but they are numerous for liver. In all kestrels analyzed, the mean hepatic-lead concentrations were 225.1 ± 112.7 µg/kg w.w. (47-600 µg/kg). These results were similar to levels found in American kestrels dosed with 10 ppm of lead over five months, and lower than for those that ingested 50 ppm (Franson et al. 1983). Moreover, liver concentrations were similar to those described in raptors exposed to contamination from vehicle exhaust fumes (Gretz and Bayle, 1983; Pain and Amiard-Triquet, 1993) and also similar to those in nestling American kestrels living near a mining and smelting zone (Henny et al. 1994). In studies on American kestrels exposed to dietary lead the hepatic concentrations in the control group ranged between 10 and 100 µg/kg (w.w.) (Franson et al. 1983, Stendell 1980, Custer et al. 1984), similar to the concentrations found in our kestrels sampled in the rural area in 2001.

In conclusion, governmental regulations restricting the use of leaded gasoline since 1998 and the subsidized renewal of old cars initiated in 1997 in Spain have diminished environmental contamination with respect to lead in rural areas influenced by moderated road traffic and in important city areas. In the mining zone, similar results in both periods of time (1995-2001) suggest that the leaded gasoline restrictions applied by Spanish and European laws have not as yet improved the environmental health status of this highly contaminated zone. Finally, liver appears to be the best tissue for biomonitoring lead exposure in kestrels.

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