



Predatory Bird
Monitoring Scheme
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Lead (Pb) concentrations in predatory bird livers 2010 and 2011: a Predatory Bird Monitoring Scheme (PBMS) report

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Executive Summary

The Predatory Bird Monitoring Scheme (PBMS; <http://pbms.ceh.ac.uk/>) is the umbrella project that encompasses the Centre for Ecology & Hydrology's National Capability contaminant monitoring and surveillance work on avian predators. By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife.

Lead (Pb) is a highly toxic metal that acts as a non-specific poison affecting all body systems and has no known biological requirement. Sources of Pb in the environment include lead mining, the refining and smelting of lead and other metals, the manufacture and use of alkyl lead fuel additives, and the use of lead ammunition.

The present study is the first two years of a PBMS monitoring programme to quantify the scale of exposure to [and associated risk from] Pb in predatory birds. The aim is to quantify the extent of exposure to lead [as assessed from liver residues] in two predatory bird species, the red kite (*Mivus milvus*) and the sparrowhawk (*Accipiter nisus*). The red kite is a scavenger and, as such, is particularly at risk from consumption of Pb ammunition in unretrieved game. Sparrowhawks prey predominantly upon live passerine birds that are unlikely to be shot in the UK; likely sources of exposure are diffuse Pb contamination although some individuals may also be exposed to Pb particles ingested by their prey. We also examined the liver Pb isotope ratios in to explore whether they can be used to ascribe likely sources of any Pb detected in the birds.

Red kites had significantly higher Pb concentration than those measured in sparrowhawks but the majority of sparrowhawks and all the red kites had liver Pb concentrations below those thought to cause clinical and sub-clinical adverse effects in Falconiforme species. There was overlap in the liver Pb isotope ratios of red kites and sparrowhawks yet there was evidence of separation between the two species. There was also evidence of overlap with the isotope signature for coal and for Pb shot but the isotope signatures in the bird livers were distinct from that of petrol Pb. The Pb isotope pattern observed in the red kites and sparrowhawks in the current study may reflect the fact that liver Pb concentrations were low in the small sample of birds that were analysed and may have been a result of exposure to low-level, diffuse contamination.

1. Introduction

1.1 Background to the PBMS

The Predatory Bird Monitoring Scheme (PBMS; <http://pbms.ceh.ac.uk/>) is the umbrella project that encompasses the Centre for Ecology & Hydrology's long-term contaminant monitoring and surveillance work on avian predators. The PBMS is a component of CEH's National Capability activities.

By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and, in particular, to vertebrate wildlife. Our monitoring provides the scientific evidence needed to determine how chemical risk varies over time and space. This may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change. Our monitoring also allows us to assess whether detected contaminants are likely to be associated with adverse effects on individuals and their populations.

Overall, the PBMS provides a scientific evidence base to inform regulatory decisions about sustainable use of chemicals (for example, the *EU Directive on the Sustainable Use of Pesticides, Biocides Directive 98/8EC, UNEP Convention on migratory species: Minimizing the risk of poisoning to migratory birds; UNEP/CMS 10.26*). In addition, the outcomes from the monitoring work are used to assess whether mitigation of exposure is needed and what measures might be effective. Monitoring also provides information by which the success of mitigation measures can be evaluated.

Currently, the PBMS has two key objectives:

- (i) to detect temporal and spatial variation in exposure, assimilation and risk for selected pesticides and pollutants of current concern in sentinel UK predatory bird species and in species of high conservation value
- (ii) in conjunction with allied studies, to elucidate the fundamental processes and factors that govern food-chain transfer and assimilation of contaminants by top predators.

Further details about the PBMS, copies of previous reports, and copies of (or links to) published scientific papers based on the work of the PBMS can be found on the [PBMS website](#).

1.2 Lead (Pb) and predatory birds

Lead (Pb) is a highly toxic metal that acts as a non-specific poison affecting all body systems and has no known biological requirement (Franson and Pain, 2011). The major sources of lead in the environment arise from lead mining, the refining and smelting of lead and other metals,

and the manufacture and use of alkyl lead fuel additives (WHO, 1989), although the use of these fuel additives has been banned in the European Union since 2000². Natural releases of Pb into the environment arise from volcanic emissions, geochemical weathering and emissions from sea spray. The consequence of these anthropogenic and natural releases is that Pb is ubiquitous in air, water and soil.



The main route of exposure to lead in vertebrate wildlife is through inhalation and ingestion. Once Pb is absorbed into the blood stream, it is distributed among body tissues, primarily the blood, liver, kidney and bone (Franson and Pain, 2011). Exposure to even low levels of Pb can result in some measurable physiological effects, including inhibition of delta-amino levulinic acid dehydratase (ALAD) activity (WHO, 1989). Exposure to higher concentrations can adversely affect the central nervous system, peripheral nerves, kidney, haematopoietic system, reproduction, juvenile growth and, ultimately, survival.

The concentrations of Pb in the wider environment are not generally thought to directly affect the survival of most wildlife (Franson and Pain, 2011). However, significant numbers of wild birds may be exposed to large amounts of lead through the ingestion of spent lead from ammunition (shotgun pellets and bullets). Ingestion of fragmented lead ammunition is also a cause of dietary exposure and mortality in scavenging birds (Pain et al., 2009, Green et al., 2009). Ingestion can occur when birds feeding in hunting areas consume lead shot, mistaking them presumably for grit or food particles. Raptors, owls, and other scavenging birds can ingest Pb from ammunition lodged in the carcasses of wildlife that have been killed but remain unretrieved or that are shot but survive. All raptors that feed on game species, whether as predators or scavengers, are likely to be exposed to shot, especially if they feed in areas where game species are hunted (Pain et al., 1995).



The risk of Pb ingestion by waterfowl is well recognized and has been mitigated against by banning shooting with Pb shot over water bodies. However, there is currently no restriction on the use of Pb shot and ammunition in terrestrial systems, and this has led to concerns over the potential risks of ingesting Pb for both humans and wildlife (Pain et al., 1995, 2005, 2007, 2009, 2010).

As far as we are aware, there is no current long-term monitoring of Pb concentrations in predatory birds in Britain. The present report encompasses the first two years of a PBMS monitoring programme to quantify the scale of exposure to [and associated risk from] Pb in predatory birds. The aim is to quantify the extent of exposure to lead [as assessed from liver

² the only exception is aviation fuel for piston engine-powered aircraft

residues] in two predatory bird species, the red kite (*Mivus milvus*) and the sparrowhawk (*Accipiter nisus*). The red kite is a scavenger and, as such, is particularly at risk from consumption of Pb ammunition in unretrieved game. It is therefore the focus for our monitoring and the sentinel species for assessing risk from Pb ammunition and shot. Sparrowhawks feed predominantly upon live passerines that are unlikely to be shot in the UK; likely sources of exposure are diffuse Pb contamination although some individuals may also be exposed to Pb particles ingested by their prey. We hypothesise that sparrowhawks are likely to be suitable environmental sentinels of any changes in diffuse Pb contamination and act as a “control species” against which spatial and temporal variation in Pb concentrations in red kites can be assessed, although some exposure to lead ammunition may occur as sparrowhawks occasionally scavenge for food or prey upon wood pigeons that have been shot. We also predict that, compared with sparrowhawks, red kites will have a greater exposure to Pb because of the risk of ingesting Pb ammunition and shot.

In addition to measuring total Pb concentration in the livers of red kites and sparrowhawks, the stable isotope ratios for Pb were also determined to explore whether they can be used to ascribe likely sources of the Pb detected in the birds. Data on isotope ratios are presented in brief in this report and will be reported more fully later when a sufficient dataset has been acquired to permit statistical evaluation.

2. Methods

During 2011 dead sparrowhawk and red kite carcasses were collected as part of the Predatory Bird Monitoring Scheme and the Disease Risk Analysis and Health Surveillance Programme operated by the Centre for Ecology & Hydrology and the Institute of Zoology, respectively. In the case of both schemes members of the public submit dead birds that they find for examination. As part of the examination the age and sex of each bird was determined. Various tissue samples, including liver, were excised and stored at -20°C prior to analysis.

A total of 30 sparrowhawk and 18 red kite livers were analyzed for total Pb concentrations. The birds had died in 2011 from a variety of causes. A 1g wet weight sample of the liver was digested in 10ml of 70% ultrapure nitric acid (Baker, Ultrex II) in a microwave digestion system at 200°C for 15 minutes. The digested samples were then made up to an initial digest volume of 25ml using ultrapure water (Millipore, MilliQ). Samples were further diluted ten-fold using ultrapure water immediately prior to analysis by inductively couple plasma mass spectrometry (ICPMS) using a Perkin Elmer DRCII ICPMS. Lead concentrations were measured using the ICPMS operating under standard conditions. The moisture content of the sample was determined by drying a 0.5g sub-sample at 70°C for a minimum of 24 hours. Dry weight concentrations were calculated based upon the wet weight of the analysed sample and the moisture content of the sub-sample.

The instrumental limit of detection (LoD) for Pb (0.06 µg/L) was calculated as 4.03 times the standard deviation of six replicate blank determinations. Taking into account the digest volume, dilution of the digest and the sample weight, the mean tissue LoD was 0.05 µg/g dry weight (dry wt.). Three replicate samples of two certified reference materials, Tort 2 and Dolt 4 (both from National Research Council Canada, Ottawa, Canada) were run concurrently with the sparrowhawk and red kite tissues. The mean recoveries for total lead from samples of Tort 2 and Dolt 4 were 94.5% and 105.2%, respectively.

The determined total Pb concentrations informed dilution of the digests in 2% nitric acid to create solutions with matched Pb concentration (i.e. 1 µg l⁻¹ Pb in the acid digest solution). This enabled analysis of all stable Pb isotopes (204, 206, 207,208) by ICP-MS using the pulse counting mode; the intensity of each Pb isotope was quantified using the mean of five 0.4 second integrations. Instrumental mass bias on the ICP-MS was determined by measurement of the NIST SRM981 Common Lead Isotopic Standard ran after every fifth 'unknown' sample. Lead isotopic ratio values were corrected for mass bias using the ICPMS operating software and by applying an identical correction to that required to achieve the certified values for NIST SRM98. The NIST SRM981 consisted of 99.99% pure Pb with a certified isotopic composition of Pb 206: 24.1442±0.0057%, Pb 207: 22.0833±0.0027%, Pb 208: 52.3470±0.0086%. Furthermore, the mass bias correction detailed above was checked by spiking all isotopic standards and 'unknown' samples with 10 µg l⁻¹ Tl and comparing the found isotope ratio value for Tl 203 and Tl 205 with the expected value for naturally occurring Thallium.

The data for birds that died in 2011 were combined with those for birds that died in 2010 and were reported by Walker et al., (2012). Overall, there were data for 60 sparrowhawks from locations across Britain and for 38 red kites from three clustered regions that roughly reflected the areas where birds were reintroduced. (Figure 1).

Lead concentrations in the liver were not normally distributed. As a result, data were logarithm-transformed (base 10) prior to statistical analysis. Unless otherwise stated, concentration are presented here as µg/g dry wt. of sample and have been statistically analysed using either GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA) or Minitab version 16.1 (Minitab Inc., Coventry, U.K.).

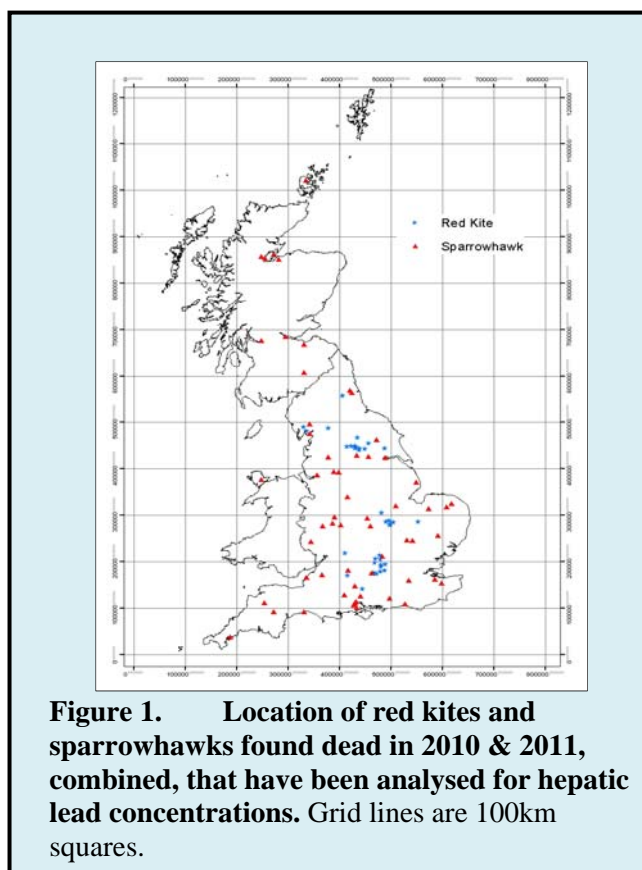


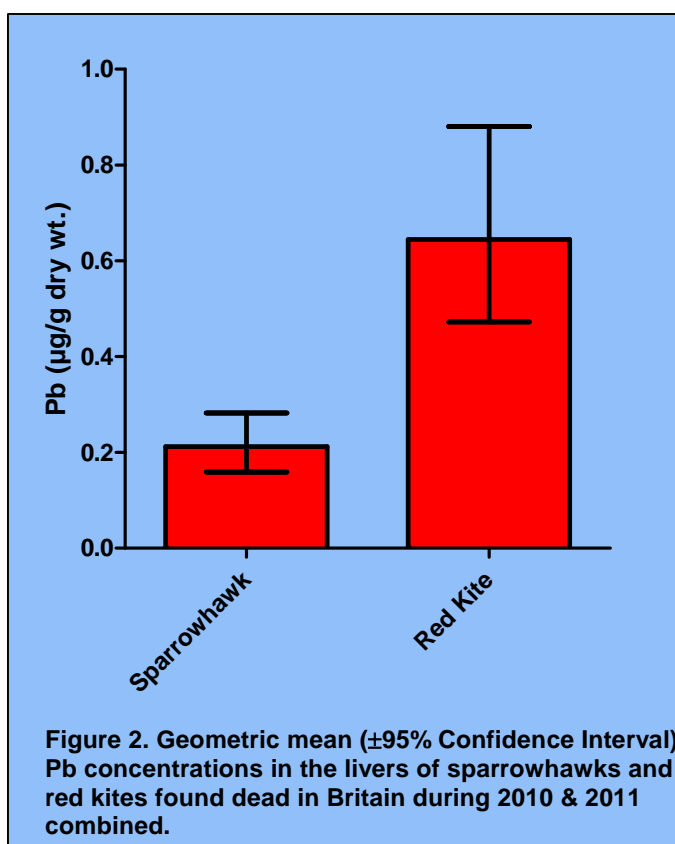
Figure 1. Location of red kites and sparrowhawks found dead in 2010 & 2011, combined, that have been analysed for hepatic lead concentrations. Grid lines are 100km squares.

3. Results and Discussion

3.1 Total lead concentrations in livers of sparrowhawks and red kites

Birds with no history of lead poisoning usually have liver Pb concentrations of $<2 \mu\text{g/g}$ wet weight and frequently of $<1 \mu\text{g/g}$ wet weight (wet wt.) (Franson and Pain, 2011). These “background” values are equivalent to dry wt. Pb concentrations of <7.1 and $<3.6 \mu\text{g/g}$ dry wt. respectively in the red kite and sparrowhawk livers that were analyzed.

The geometric mean liver Pb concentrations in sparrowhawks and red kites were 0.2 and $0.6 \mu\text{g/g}$ wet wt respectively (Figure 2) and only one bird from either year, an adult female sparrowhawk, had a liver residue ($12.6 \mu\text{g/g}$ dry wt., equivalent to $3.3 \mu\text{g/g}$ wet wt.) greater than “background” concentrations. The bird was found dead in an urban garden with injuries consistent with a collision. The bird had good fat reserves and appeared to be in good condition prior to death. For Falconiforms, sub-clinical poisoning has been reported in birds with liver concentrations of between 2 and $6 \mu\text{g/g}$ wet wt., although the associated physiological effects are insufficient to severely impair normal biological functioning and do not result in external signs of poisoning (Custer et al., 1984, Henny et al., 1991, Kramer and Redig, 1997).



Interspecies and inter-year differences in \log_{10} -transformed total Pb liver concentration were tested using a General Linear Model. Although in general the concentrations of Pb in both species were low, hepatic Pb concentrations were significantly higher in red kites than in sparrowhawks ($F_{1,94}=26.15$, $P<0.001$; Figure 2). There was no significant difference in Pb concentrations between years ($F_{1,94}=0.01$, $P=0.934$), nor for the species-year interaction term ($F_{1,94}=1.27$, $P=0.263$). It may have been expected that if the higher Pb concentrations observed in red kites had resulted from sporadic exposure to lead, for example from ingested of lead ammunition, then the variability of lead concentration would be higher in this species than in sparrowhawks. However, there was no significant difference between the two species in the variability of liver Pb concentrations (Levene’s test for equal variance: $W=0.89$, $P=0.35$).

Liver Pb concentrations have been measured in red kites from the United Kingdom previously. The median (inter-quartile range) liver Pb concentration in 20 red kites that had died between

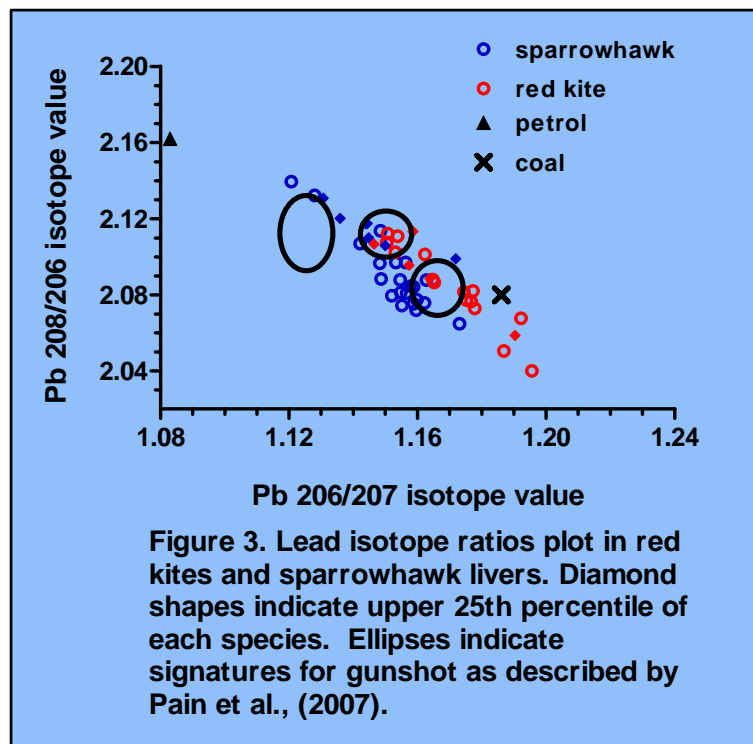
1994 and 1999 was found to be 0.99 (<1.13-2.24) $\mu\text{g/g}$ dry wt. (Shore et al., 2000), similar to the levels found in the current study. However, another study of 44 birds that had been found dead between 1995 and 2003 found that seven of 44 red kites had elevated (>6 $\mu\text{g/g}$ dry wt.) liver Pb concentrations, and six (14% of total sample) had concentrations greater than 15 $\mu\text{g/g}$ dry wt., compatible with fatal Pb poisoning (Pain et al., 2007).

The Pb concentrations detected in the present sample of sparrowhawks, are similar to those reported for birds that died between 1982 and 1992 (median: 0.55 $\mu\text{g/g}$ dry wt., maximum 12.3 $\mu\text{g/g}$ dry wt; Pain et al., 1995), suggesting that there has been little change in Pb contamination in sparrowhawks over the last 20 years. This contrasts with declines of over 70% in bone Pb concentrations in a freshwater vertebrate sentinel, the otter *Lutra lutra* over a similar time period (Chadwick et al., 2011). The differences in temporal trends in sparrowhawks and otters may reflect exposure to different sources of lead and differences between habitats in the extent to which Pb is distributed and accumulated.

3.2 Lead isotope ratios

Analysis of the isotope ratios of liver lead residues may be one means of ascertaining the likely origin of Pb found in the liver; Pb isotope ratios in liver tissues can be compared with Pb ratios for lead shot, industrial sources and petrol. In a previous study, (Pain et al., 2007), lead isotopic signatures (Pb 208/206; Pb 206/207) in liver samples of some of the kites that were analysed were compatible with those found in lead shot extracted from regurgitated pellets (Pb 206/207 ratios for shot of 1.12-1.17, Pb 208/206 ratios of 2.07-2.13; Figure 3). Lead isotope ratios found in the livers of kites with very low Pb concentrations were distinct from UK petrol Pb isotopic signatures, and this was taken to indicate that birds were exposed to little residual petrol Pb.

In the current study, there was considerable overlap in the liver Pb isotope ratios of red kites and sparrowhawks (Figure 3). There was also some overlap with the isotope signature for coal and for Pb shot, but the signatures in the birds were distinct from that of petrol (Figure 3), as found by Pain et al., (2007). There was no clear evidence that birds with the higher residues (those with the top 25 percentile of total liver Pb concentrations) in either species had isotope signatures that particularly resembled that of shot or ammunition. The Pb isotope pattern observed in the red kites and sparrowhawks in the current study may reflect the fact that liver Pb concentrations were low in the



birds we analysed and were likely to have been a result of exposure to multiple diffuse sources. Interestingly however, there appears to be some separation in the isotope signatures between the two species (Figure 3), with sparrowhawks having a higher Pb 208/206 ratio and lower Pb 206/207 ratios compared with red kites. This isotopic segregation will be examined further when amalgamated datasets are of sufficient size for detailed analysis.

4. Conclusions

- The majority of sparrowhawks and all red kites analysed had liver Pb concentrations below those thought to cause clinical and sub-clinical adverse effects in Falconiforme species.
- Red kites had significantly higher Pb concentration than those measured in sparrowhawks.
- Pb isotopic ratios in the two species overlapped but there was evidence of species separation.
- The isotopic ratios for both species overlapped the signature for gunshot and, in the case of red kites, that for coal, but the signatures were distinct from that of petrol.

5. Acknowledgements

We thank all the members of the public who have submitted birds to the Predatory Bird Monitoring Scheme. Their efforts are key to the success of the scheme. Fieke Molenaar, Rebecca Vaughan and Gabriela Peniche, all from the Institute of Zoology, carried out post mortem examinations on the red kites. We also thank Andy Bright (www.digiscoped.com) and Gerry Whitlow (www.hyelms.com) for providing photographs of predatory birds and Jacky Chaplow for providing the maps for this report.

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