Science of the Total Environment 916 (2024) 170356

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Difference in concentration of lead (Pb) in meat from pheasants killed using lead and iron (Fe) shotgun ammunition

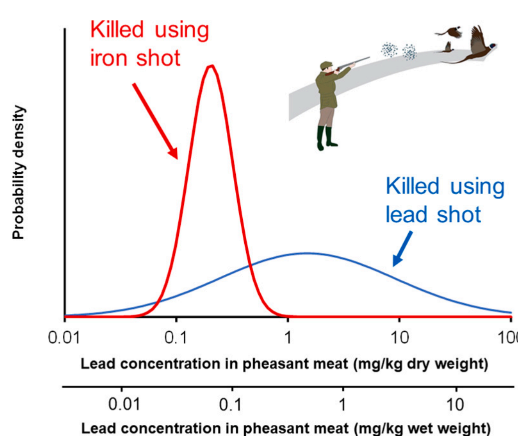
Rhys E. Green [a,b,*], Mark A. Taggart [c], Maider Guiu [c], Hayley Waller [c], Sabolc Pap [c], Rob Sheldon [d], Deborah J. Pain [a,e]

[a] Conservation Science Group, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK
[b] RSPB Centre for Conservation Science, The Lodge, Sandy, Bedfordshire SG19 2DL, UK
[c] Environmental Research Institute, University of the Highlands and Islands, Castle Street, Thurso KW14 7JD, UK
[d] 78 Riverdene Road, Ilford, Essex IG1 2EA, UK
[e] School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK

HIGHLIGHTS

- Concentrations of lead in meat from wild-shot pheasants are compared.
- Lead concentration of birds killed using lead shot was 30 times that for iron shot.
- Using iron rather than lead ammunition would reduce lead levels in game meat.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Rafael Mateo Soria

Keywords:
Bismuth
Zinc
Hunting
Dietary exposure
Human health
Shotgun pellets

ABSTRACT

The use of lead shotgun ammunition for hunting has been banned in a few jurisdictions and habitats, principally to protect wild birds from poisoning by ingestion of spent lead shot. The EU and UK REACH processes have recently considered bans on lead ammunition throughout the European Union and United Kingdom, including assessments of possible health benefits from reduced human dietary exposure to lead from game meat. Comparisons of the mean lead concentrations in meat from gamebirds killed using lead and non-lead shotgun ammunition have not been published. We compared lead concentrations in meat from wild-shot pheasants from which lead shotgun pellets were recovered ($n = 27$) with those from which iron pellets were recovered ($n = 20$), having removed all pellets from the meat before analysis. The mean concentration of lead in meat from pheasants killed using lead shot was 2.10 mg/kg w.w., which is >20 times the European Union's maximum permitted level

* Corresponding author at: Conservation Science Group, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.
E-mail address: reg29@cam.ac.uk (R.E. Green).

https://doi.org/10.1016/j.scitotenv.2024.170356
Received 13 October 2023; Received in revised form 30 December 2023; Accepted 20 January 2024
Available online 23 January 2024

for the lead concentration in meat from domesticated animals. For pheasants killed using iron shot the mean was 0.07 mg/kg w.w., which is below the maximum permitted level.

1. Introduction

Meat from free-ranging wild-shot small game animals (< ca. 6 kg body weight) killed by hunters using shotguns is often eaten by humans in Europe and elsewhere (Green and Pain, 2015; ECHA, 2021). In the United Kingdom (UK), the common pheasant (*Phasianus colchicus*) is the species of small game animal mostly frequently killed by hunters (Aebischer, 2019) and almost all are killed using shotgun ammunition composed principally of lead (Pb) (Pain et al., 2010; Green et al., 2021, 2022a, 2023). Lead shotgun pellets often fragment upon impact with the bodies of gamebirds, leaving widely-dispersed small lead particles embedded in the meat (Pain et al., 2010; Green et al., 2022b). These fragments represent a small proportion of the mass of lead in the pellets which strike the bird (~0.3 %; Pain et al., 2010), but are sufficient to account for the concentrations of lead measured in the meat (Green et al., 2022b). The fragments are difficult for consumers to detect and remove and a proportion of the lead in them is likely to be absorbed from them because of their large surface-to-volume ratio (Pain et al., 2010; Green and Pain, 2012; Green et al., 2022b). Absorbed lead from any source can impair nervous, cardiac, renal, immune, endocrine and other functions in humans (EFSA, 2010; Advisory Committee on Childhood Lead Poisoning Prevention, 2012). The EFSA CONTAM Panel concluded that there was no evidence for minimum lead concentrations in blood plasma below which effects on IQ, systolic blood pressure and chronic kidney disease do not occur (EFSA, 2010). Since the introduction of regulations to reduce lead in, for example, petrol and paint, the primary route of exposure of humans to lead in the European Union (EU) and the UK has become the diet (EFSA, 2010). Dietary lead derived from lead ammunition can contribute substantially to this exposure in people who consume game meat frequently (EFSA, 2010; Green and Pain, 2012). The European Commission has set 0.1 ppm w.w. (wet weight) as the maximum level (EUML) permitted for lead in marketed meat (muscle) from domesticated animals, (Regulation EC1881/2006 and Regulation 2023/915). No EUML has been set for lead in wild game meat for reasons which are not clear (Thomas et al., 2020).

There is evidence that meat from wild-shot small game animals contains concentrations of lead much higher than those set by the EU as Maximum Levels for meat from domesticated animals (Pain et al., 2022). This applies even when whole shotgun pellets have been removed from the meat sample prior to chemical analysis (Pain et al., 2010, 2022). Replacement of lead shotgun ammunition by non-lead ammunition has been suggested as a potentially effective way to reduce dietary exposure of high-level consumers of game meat to ammunition-derived lead. The European Chemicals Agency (ECHA) was asked by the European Commission in July 2019 to prepare a proposal to restrict the placing on the market and use of lead in ammunition under the EU Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (EU REACH). This would make hunting with lead shotgun ammunition and bullets unlawful in the European Union. This request was complementary to the restriction on the use of lead gunshot in wetlands which was considered earlier. The proposal may be implemented in 2024, if it is accepted and there are no delays to the regulatory process (https://www.echa.europa.eu/web/guest/hot-topics/lead-in-shot-bullets-and-fishing-weights). Similar legislation is being considered by the UK Government under the UK REACH process, which has similar proposed timing. Although it is known that meat from wild-shot game animals often has a high concentration of lead (EFSA, 2010; Pain et al., 2022), we know of no published reports comparing mean concentrations for meat from gamebirds known to have been killed using lead shotgun ammunition with those killed with non-lead shot.

In this paper, we test the hypothesis that the concentration of lead as a contaminant of game meat available to human consumers differs according to the type of shotgun ammunition used to kill small game animals. In particular, we compare the mean concentration of lead in meat from wild-shot, free-ranging common pheasants known to have been killed by hunters using lead shot with that for birds killed using iron shot and report on smaller samples for two other types of shotgun ammunition (bismuth Bi and zinc Zn).

2. Material and methods

2.1. Compliance with ethics requirements

Carcasses of the dead common pheasants used in the study were all from birds killed legally by hunters in the UK and obtained from food retailers and game meat wholesalers. The study did not involve human subjects or any animal experiments.

2.2. Provenance of pheasant carcasses

We obtained 101 carcasses of free-ranging common pheasants, all of which had been killed by hunters on shooting estates in England in 2022 and processed and packaged for sale to consumers as 'oven-ready' prior to us acquiring them. We were unable to determine from the suppliers exactly where the birds had been shot. Carcasses had been plucked and the head, neck, tarsi, feet and viscera had been removed. The carcasses were obtained as six batches, which differed as to source business, date of acquisition and the co-author who processed the carcasses. Details of the batches are given in Supplementary Table S1. Batches 1 and 2 were provided to us for research purposes by a game supply business which intended to supply its customers with pheasant carcasses killed only using non-lead shotgun ammunition but was unsure about the types of ammunition actually being used by hunters on its source estates. We purchased the other four batches from retailers of wild-shot pheasant carcasses without indicating to them that they would be used for research purposes.

2.3. Recovery of shotgun pellets

We unpacked and examined each carcass and attempted to find shotgun pellets by removing the skin and dissecting the muscles. We did not X-ray the carcasses and therefore were unable to perform a complete count of the number of imaged embedded shot present. We did not always attempt to recover all the pellets which might have been present in each carcass and sometimes stopped searching when we had recovered one or two. It would have been preferable to standardize the amount of dissection effort so that it was the same for all carcasses, but this was not done. Methods used to find shotgun pellets are described elsewhere (ERI, 2023). We recovered 105 shotgun pellets from 54 carcasses (range 1–6 per carcass with recovered shot). Pellets from each carcass were stored in a screw-topped polyethylene tube marked with a unique code.

2.4. Identification of the principal chemical element in shotgun pellets

When more than one pellet was recovered from a carcass, we performed qualitative tests to determine whether they were all likely to be principally composed of the same metallic element or included pellets of more than one type. We determined surface colour, deformability/brittleness, attraction to a magnet and whether or not the pellet melted when touched with a hot soldering iron. These tests do not identify unequivocally the principal metallic element from which the pellet is composed, but they allow pellets with differing characteristics and composition recovered from the same carcass to be distinguished. The methods and the metals they distinguish between are described elsewhere (Green et al., 2021, 2022a). Numbers of pellets recovered which were and were not attracted to a bar magnet are shown in Supplementary Table S2. If the tests indicated that all pellets from the same carcass were of the same type, we selected one of the pellets at random for chemical analysis. If our tests found pellets of different types from the same carcass, we analysed one pellet of each type. We attempted to dissolve each of the pellets selected for chemical analysis in nitric acid. If the pellet did not dissolve in nitric acid alone, we used a 1:1 mixture of nitric and hydrochloric acids. We used an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Agilent 5900 with SPS4 autosampler) to measure the concentrations of metals in the solution and to estimate from them the proportion of the mass of each pellet comprised of each metallic element. This method is described in more detail elsewhere (Green et al., 2021, 2022a, 2023). We assigned each pellet to a principal metal type according to which metallic element comprised >50 % of its mass.

2.5. Sampling of meat from pheasant carcasses

For Batches 1 and 3 one of us (REG) dissected off as much as possible of the edible meat from both breasts (pectoral muscles) and legs, diced it into approximately 1 cm cubes, mixed these thoroughly and took a sample of 30-50 g of the mixture. A similar procedure was followed for Batches 2, 4 and 5, which were processed by DJP, but with only breast meat being sampled. For Batch 6, which was processed by RS, four samples, each of approximately 10 g of meat were taken from each breast and leg, to give an overall sample of 30-40 g. The samples were placed into individually-coded polythene bags, sealed and frozen before being sent for analysis.

2.6. Measurement of the concentration of lead in pheasant meat

We thawed the frozen samples of meat and examined them macroscopically to find and remove any whole shot not already detected and removed during dissection of the carcasses. This was aided by flattening the sample within the sample bag, then uplighting it with a large flatbed 44 W LED work light. Any shot present were then silhouetted within a thin layer of translucent tissue. One lead shot was recovered in this way and is included in the totals presented in Table S2. Pellets of the same type had been recovered during dissection. Samples were weighed, dried to constant mass, weighed again and milled to a fine powder. From each milled sample, 0.4 g was digested in nitric acid and hydrogen peroxide and the samples, certified reference material and blanks were analysed using an inductively coupled plasma optical emission spectrometer (ICP-OES; Agilent 5900). We expressed the concentration of lead in meat on a dry weight (d.w.) and wet weight (w.w.) basis. These concentrations and the limits of detection (LOD) for each analysis are shown in Supplementary Table S2.

2.7. Statistical analysis

We first compared lead concentrations in meat between samples from pheasant carcasses from which only lead shot were recovered and those from which only iron shot were recovered using data for carcasses within the same batch. Carcasses with these two shot types were present in two of the batches. We used Wilcoxon-Mann-Whitney non-parametric tests for this preliminary analysis to compare median concentrations between the two shot types because the test makes no assumptions about the form of the probability distribution function of the data (Seigel and Castellan, 1988). We also compared lead concentrations in meat among samples from pheasant carcasses from which only lead shot were recovered for all five batches for which data from carcasses of this kind were available. We tested for differences among median concentrations among batches using the non-parametric Kruskall-Wallis One-Way Analysis of Variance by Ranks (Seigel and Castellan, 1988).

We assigned a value of half of the LOD to the three carcasses with lead concentrations in meat which were below the LOD. We considered this approximation to be acceptable because of the small proportion of <LOD samples (6 %). We then calculated arithmetic means of d.w. and w.w. lead concentrations in meat from carcasses from which we had recovered each type of shotgun pellet, after excluding results for two carcasses in which we found two different types of shot. We chose to calculate arithmetic means because they can be used to calculate directly measures of mean levels of cumulative dietary exposure to lead from successive meals, such as weekly and annual intake rates, which are often used in assessments of public health outcomes (EFSA, 2010; Green and Pain, 2012). We obtained 95 % confidence intervals for the arithmetic means using a bootstrap method (Manly, 2007). We took n concentration values at random, with replacement, from the n observed values for a given shot type and calculated the arithmetic mean from this bootstrap sample. We repeated this procedure 10,000 times, ranked the bootstrap values and took the bounds of the central 9500 values to be the 95 % confidence interval for the mean. We obtained confidence intervals for the ratio of the mean concentration for birds killed using lead shot to that for birds killed using iron shot and the percentage of biologically incorporated environmental lead by aligning the 10,000 bootstrap values for each shot type in random order, calculating the derived parameters for each bootstrap samples and finding their confidence intervals as described above.

All of our parametric analyses required the assumption that the concentration values were log-normally distributed. We therefore wished to check that the forms of the empirical distributions of lead concentrations in meat samples separately for carcasses of pheasants from which only lead shot and only iron shot were recovered conformed approximately to log-normal models. To do this, we plotted cumulative distributions of the dry weight lead concentrations in meat for both shot types for carcasses from all batches combined and compared them by eye with the expected log-normal distributions based upon the mean and standard deviations of the $\log_e$-transformed values for each type. We then used Kolmogorov-Smirnov one-sample tests to assess the statistical significance of the maximum deviation of the empirical cumulative distributions from those expected from the fitted log-normal models (Seigel and Castellan, 1988).

We used Welch's unequal variance t-test (Ruxton, 2006) to test the significance of differences between the means of the $\log_e$-transformed lead concentrations in meat from carcasses of pheasants killed using shotgun pellets principally composed of lead and of iron. We fitted ordinary least squares regression models to dry weight lead concentration data from carcasses of pheasants killed using shotgun pellets principally composed of lead and of iron. The dependent variable was the $\log_e$-transformed dry weight concentration of lead in the meat. Independent variables were shot metal type (binary variable: lead = 1, iron = 0) and batch code (a five-level factor). Although there were six batches (see Supplementary Table S1), the sixth batch only included pheasants killed using bismuth shot and therefore was not included. We considered that it was necessary to include the potential effect of batch in the model because the shooting locations and protocols used to collect meat samples differed among batches (see 2.5). The differences in protocol were slight and we therefore expected that resulting differences, if any, in mean lead concentration would be small. However, we modelled the possible effect of batch as a precaution. We fitted four models: (1) the null model with no main effects, (2) the main effect of metal type only, (3) the main effect of batch only and (4) the main effects of both metal type and batch. We calculated the small-sample version of the Akaike Information Criterion (AIC_c) and AIC_c weights for each of the four models and the relative importance of the two variables across the whole model set (Burnham and Anderson, 2002). We selected the model with the highest AIC_c weight. We calculated the relative importance of the two variables from the AIC_c weights of the models (Burnham and Anderson, 2002).

3. Results

3.1. Types of shotgun pellets recovered

We recovered at least one shotgun pellet from 54 of the 101 carcasses studied. Qualitative tests indicated that the shotgun pellets recovered from 52 of the carcasses were of one type and that two carcasses had pellets of two types. We therefore conducted chemical analyses to identify the principal metallic element of 56 pellets. Of these, 28 (50 %) were composed principally of lead, 22 (39 %) were composed principally of iron and three each (5 %) of bismuth and of zinc. The mean percentages by weight of each principal element in pellets assigned to each type were: lead – 94.9 % (range 84.8–100.0 %), iron – 96.7 % (81.0–100.0 %), bismuth – 98.5 % (96.3–100.0 %) and zinc 99.3 % (97.7–100.0 %). One carcass had at least one zinc and at least one iron pellet and another had both lead and iron pellets.

3.2. Concentration of lead in meat in relation to the metal type of shot used to kill the bird

We excluded results from the two carcasses with two different types of recovered shot from our analyses of the concentration of lead in meat from carcasses of birds in relation to the types of shot used to kill them (Supplementary Table S2: #22 and #33). We first compared the distributions of individual values for the concentration of lead in meat from carcasses of pheasants from which only lead shot and only iron shot were recovered by plotting a graph of dry weight lead concentration in meat in relation to batch code and shot metal type (Fig. 1). Inspection of this graph suggested that the lead concentration in meat tended to be higher in carcasses with lead shot than those with iron shot in both of the two batches in which carcasses with both shot types were present, though this impression was much more convincing for Batch 1, for which sample sizes were larger (lead $n = 8$; iron $n = 14$), than for Batch 2 (lead $n = 2$; iron $n = 6$). The median concentration of lead was significantly higher in the carcasses with only lead shot recovered than for those with only iron shot recovered in Batch 1 (Wilcoxon-Mann-Whitney test: $W_{8,14} = 131$; two-tailed $P = 0.008$). The difference was in the same direction but did not approach statistical significance for Batch 2 (Wilcoxon-Mann-Whitney test: $W_{2,6} = 10$; two-tailed $P = 0.742$). After combining the data for carcasses from Batches 1 and 2, the median concentration of lead was significantly higher in the carcasses with only lead shot recovered than for those with only iron shot (Wilcoxon-Mann-Whitney test: $W_{10,20} = 219$; two-tailed $P = 0.005$). The results of these tests were the same for dry weight and wet weight concentrations.

Considering only the 27 samples from carcasses of pheasants from which only lead shot was recovered, inspection of Fig. 1 did not indicate any obvious pattern of consistent differences in dry weight lead concentration in meat among the five batches. Our tests supported this impression in finding no indication of statistically significant variation among batches for dry weight concentrations (Kruskall-Wallis test; $KW_4 = 4.60$; $P = 0.331$) or wet weight concentrations (Kruskall-Wallis test; $KW_4 = 4.53$; $P = 0.338$).

The arithmetic mean concentration of lead in meat was much higher for carcasses of pheasants from which only lead shot were recovered than for those with iron, bismuth and zinc shot (Table 1). The arithmetic mean concentration of lead in meat from pheasants killed using lead shot was about 30 times higher than that for those killed using iron shot (Table 1). We assumed that all of the lead in meat from birds killed using iron shot was biologically-incorporated, rather than being derived from the shotgun pellets which struck the bird, whereas meat from birds killed using lead shot was derived from both sources. Hence, the ratio of arithmetic mean concentration for birds killed using iron shot to that for birds killed using lead shot is an approximate estimate of the mean proportion of lead in the meat of birds killed using lead shot that is biologically-incorporated. Our results suggest that about 3 % of the lead in meat in pheasants killed using lead shot is from environmental sources (Table 1). The difference between the means of $\log_e$-transformed concentrations for birds killed using lead and iron was highly

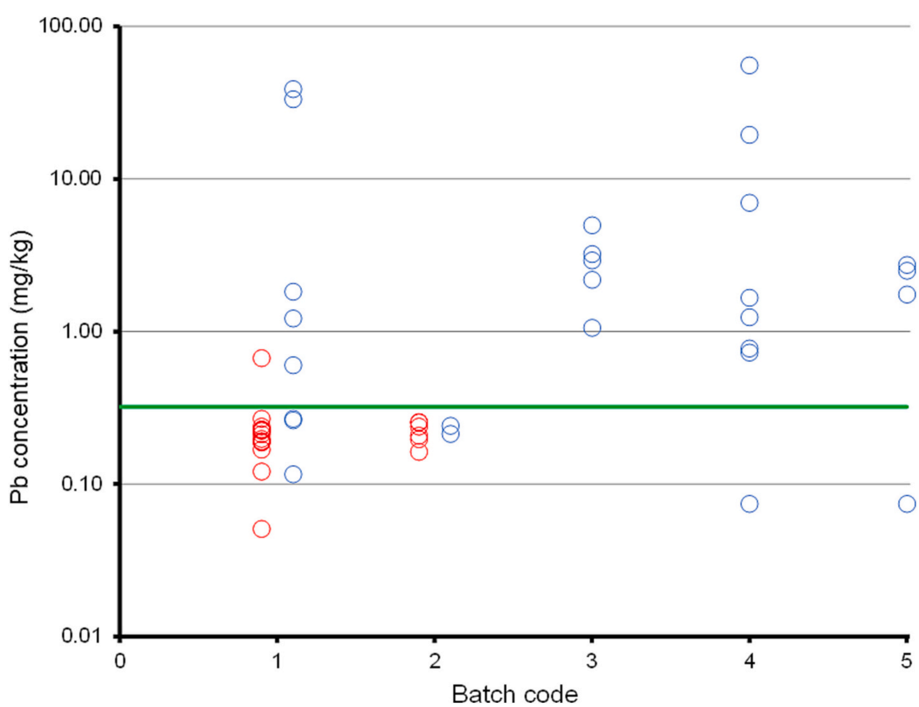


Fig. 1. Dry weight concentrations of lead in individual samples of meat from carcasses of common pheasants from which only lead shot (blue symbols) and only iron shot (red symbols) were recovered. Results are also shown in relation to the five batches of carcasses which were collected and processed separately and labelled with arbitrary codes 1–5. The lowest three values are those for samples with concentrations <LOD, for which we assumed a concentration of LOD/2. The horizontal green line shows the EU Maximum Level for the wet weight concentration of lead in meat from domesticated animals (0.1 mg/kg w.w.) converted to its dry weight equivalent (0.32 mg/kg d.w.). The vertical axis is $\log_{10}$-transformed, but the axis label values are not transformed.

Table 1
Arithmetic mean concentrations of lead in samples of meat from wild-shot common pheasants in relation to the principal element of which a shotgun pellet recovered from each carcass was composed. Bootstrap 95 % confidence intervals (C.I.) are shown for each parameter.

Principal shot metal	*N*	Mean d.w. concentration (mg/kg)		Mean w.w. concentration (mg/kg)	
		Mean	95 % C.I.	Mean	95 % C.I.
Lead	27	6.85	2.43–12.55	2.10	0.75–3.89
Iron	20	0.22	0.18–0.28	0.07	0.06–0.09
Bismuth	3	0.78	–	0.23	–
Zinc	2	0.16	–	0.05	–
Ratio of means Lead:Iron		30.5	10.7–58.6	28.9	10.2–56.4
Percentage environmental		3.3	1.7–9.4	3.5	1.8–9.8

statistically significant, both for dry weight (Welch's $t_{30.7} = 5.48$, two-tailed $P < 0.0001$) and wet weight concentrations (Welch's $t_{31.3} = 5.30$, two-tailed $P < 0.0001$). Inspection of Fig. 2 suggests that the distributions of individual concentration values were approximately log-normal for both types of shot. The cumulative distributions for both shot types did not deviate significantly from those expected from the fitted log-normal models (Kolmogorov-Smirnov one-sample tests: lead, $D = 0.113$, $P > 0.2$; iron, $D = 0.227$, $P > 0.2$).

Our regression models of $\log_e$-transformed dry weight concentrations in meat were intended to assess the relative importance of differences between birds killed using lead ($n = 27$) and iron ($n = 20$) shot and among the five batches of carcasses. Comparison of AIC_c weights among the four models considered (see Statistical Analysis) showed that the model with only the effect of metal type (lead v. iron) had by far the highest AIC_c weight (0.955). The next highest AIC_c weight value was 0.042 for the model with effects of both metal type and batch. The relative importance of the variable metal type was much higher (0.996) than for batch (0.045). We conclude that the most important variable influencing the concentration of lead in the meat of these pheasants was whether they were killed using lead shot or iron shot.

Our study included too few carcasses of birds killed using only bismuth and zinc shot ($n = 3$ and $n = 2$ respectively) to give reliable estimates of mean lead concentration and their confidence limits for these shot types. Meat from both carcasses of birds killed using zinc shot had low concentrations of lead (0.031 and 0.073 mg/kg w.w.). The mean lead concentration in meat from birds killed using bismuth shot was much lower than for those killed using lead shot (Table 1; individual lead values 0.069, 0.106 and 0.509 mg/kg w.w.).

3.3. *Proportions of samples for which the concentration of lead in meat exceeded the EUML for meat from domesticated mammals and poultry*

The EU Maximum Residue Level (0.1 mg/kg w.w.) for lead in meat from domesticated mammals and poultry was exceeded in meat samples from 74 % of carcasses of pheasants known to have been killed using lead shot (Supplementary Table S2). The proportion of samples exceeding the EUMLwas much lower (5 %) for carcasses of pheasants in our study known to have been killed using iron shot (Supplementary Table S2). This difference in proportions exceeding the EUML between lead and iron was statistically significant (Fisher exact test, two-tailed $P < 0.0001$). None of the samples from pheasants killed using zinc shot had lead concentrations in the meat which exceeded the EUML. Concentrations for two of the three carcasses of birds killed using bismuth shot exceeded the EU Maximum Residue Level (67 %), though only by a relatively small amount (largest value, 0.509 mg/kg w.w.). This proportion was significantly greater than that for birds killed using iron shot (Fisher exact test, two-tailed $P = 0.034$).

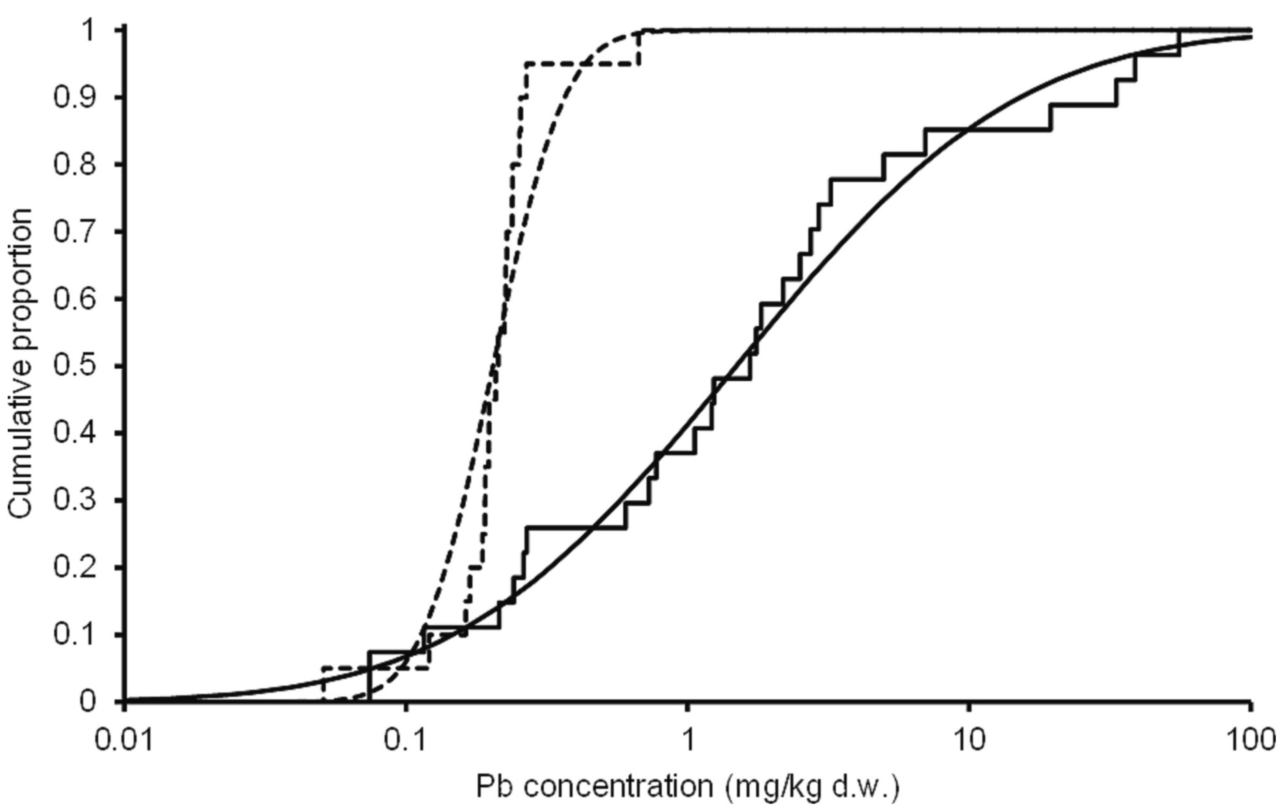


Fig. 2. Cumulative distributions (stepped lines) of the dry weight concentration of lead in the meat from carcasses of wild-shot common pheasants from which a lead shotgun pellet (solid line) or an iron pellet (dashed line) was recovered. Curves show fitted log-normal distributions.

4. Discussion

4.1. Concentration of lead in meat in relation to the metal type of shot used to kill the bird

Our most robust finding was that the arithmetic mean concentration of lead in meat samples from pheasants killed using lead shot, from which shotgun pellets were removed before analysis, was about 30 times greater than that from birds killed using iron shot. Mateo et al. (2014) reported that the geometric mean dry weight concentration of lead in muscle tissue of waterbirds (mostly ducks) was 8–17 times greater in birds killed using lead shot than those killed using iron shot. A comparison of lead concentrations in meat from roe deer (*Capreolus capreolus*) and wild boar killed using lead and non-lead (mostly copper) bullets showed that the concentration of lead was considerably higher in animals killed using lead than non-lead bullets for both species (Gerofke et al., 2018). Our study included too few carcasses of birds killed using only bismuth and zinc shot to give reliable estimates of mean lead concentration and their confidence limits for these shot types, but suggests that meat from both carcasses of birds killed using zinc shot had low concentrations of lead and the concentration of lead in meat from birds killed using bismuth shot was lower than for those killed using lead shot.

We included batch code as a categorical nuisance variable in our analyses because carcass batches almost certainly included pheasants shot at different (but unknown) locations in England and the three co-authors used different methods when collecting meat samples for analysis. However, both the non-parametric analysis of variance and parametric regression analyses indicated no additional effect of batch on lead concentration in meat after shot metal type was taken into account. We conclude that source location and the different processing methods did not have a detectable additional effect on the mean concentration of lead in the meat.

4.2. Probable sources of lead in pheasant meat

Lead shotgun pellets that strike pheasants and other gamebirds often fragment (Pain et al., 2010) and leave small metallic fragments embedded and widely-distributed in the edible tissues of the carcass (Green et al., 2022b). The lead shot we recovered were comprised of about 95 % of lead by mass, whereas ten iron shot recovered from carcasses of wild-shot pheasants killed in the UK and analysed in the same laboratory by the same ICP-OES method contained <0.01 % lead (Green et al., 2023). Hence, our finding of higher lead concentrations in meat from pheasants killed using lead shot than for those killed using other shot types is consistent with the hypothesis that most of the lead in the meat of pheasants killed using lead shot is derived from small fragments of lead detached from the shot which struck and killed the bird, rather than to biologically-incorporated lead from environmental sources. Biologically-incorporated environmental lead accumulates in body tissues of vertebrate animals, especially the liver, kidney and bone. A study of stable isotopes of lead in the bones of wild red grouse (*Lagopus lagopus scoticus*) at three UK sites where the species was hunted by shooting indicated that the birds at two sites had been exposed long-term principally to lead from shotgun ammunition by ingesting spent pellets. At the other site, bone isotope ratios were consistent with a combined exposure both to ingested lead gunshot and to lead residues from past mining of a lead ore (galena) in the region (Thomas et al., 2009). Concentrations of biologically-incorporated lead are usually lower in muscle than in bone, though lead concentrations in muscle may be elevated in lead-poisoned birds (Longcore et al., 1974; Fimreite, 1984; Gasparik et al., 2012). Although the mean concentration of lead in carcasses of wild-shot pheasants from which iron shot were recovered was low, it was considerably higher than that for meat from farmed domestic fowl (*Gallus domesticus*) obtained from UK retailers and reported in a previous study (Pain et al., 2010) (0.073 mg/kg w.w. cf. 0.019 mg/kg w.w. respectively). The 95 % confidence intervals of the two means did not overlap (pheasant: 0.058–0.092 mg/kg; fowl 0.014–0.025 mg/kg). This suggests that there may be environmental exposure of free-ranging pheasants to lead in excess of that of farmed poultry. The most likely pathway for such exposure is by ingestion by pheasants of spent lead shotgun pellets on shooting estates, which occurs frequently in Britain (Butler et al., 2005) when the birds mistake spent shotgun pellets for grit or seed. Our results suggest that approximately 3 % of the lead in the meat of pheasants killed using lead shot is of environmental origin, with the remainder being from embedded fragments of lead shot.

4.3. Implications for human dietary exposure to ammunition-derived lead

Our results for pheasants killed using lead shot are likely to be representative of the concentration of lead in meat eaten by consumers more widely because the arithmetic mean concentration of lead in the meat of pheasants known to have been killed using lead shot found in our study was similar to the mean derived from a large number of published studies reporting concentrations in meat from small game animals (including pheasants) killed using unknown types of ammunition and obtained from many locations in Europe, including the UK. The mean Europe-wide wet weight concentration of lead for wild-shot small game animals killed with unknown ammunition types and sampled during the period 1991–2021 was 2.47 mg/kg w.w. (Pain et al., 2022), compared with 2.10 mg/kg w.w. for pheasants known to have been killed in England using lead shot in our study. The 95 % confidence intervals of the two means overlap substantially. Our mean lead concentration for pheasants killed using lead shot is also similar to, and not significantly different from, that from previously reported samples of pheasants killed in the UK using unknown shot types (two reported values: 0.98 and 2.01 mg/kg w.w.; Pain et al., 2022). The similarity of the mean concentration of lead in meat from pheasants known to have been shot using lead shotgun ammunition from our study and that for pheasants and other small game killed using unknown ammunition types is consistent with the principal ammunition types used for hunting small game in most European countries being composed of lead. Although some food wholesalers and retailers in the UK have attempted to supply only meat products from gamebirds killed using non-lead shotgun ammunition, these voluntary efforts have not been successful so far (Green et al., 2023). As an example of this, the carcasses we obtained for research purposes (Batches 1 and 2) came from a supplier of a large UK food retail business which wished to verify that their intention to market only meat products from pheasants killed using non-lead ammunition was being fulfilled. Both of these batches included carcasses with embedded lead shot.

X-ray microtomography has indicated that many fragments of lead are present in carcasses of wild-shot pheasants killed using lead ammunition and that they are mostly small and widely dispersed in the edible meat (Pain et al., 2010; Green et al., 2022b). Hence, they are unlikely to be detected and discarded during food preparation and consumption. Lead concentrations might be higher close to wound channels, but these are difficult to detect and too widely distributed within most gamebird carcasses for it to be practical to discard affected meat without substantial wastage (Green et al., 2022b). This contrasts with the situation for meat from deer and wild boar killed using lead rifle bullets, where the concentration of lead is considerably higher close to the wound channel than distant from it (Dobrowolska and Melosik, 2008) and careful removal of wound channel tissues during food preparation can therefore reduce dietary exposure to ammunition-derived lead. Therefore, our results indicate that dietary exposure to lead of people who eat meat from small game would be likely to be substantially reduced if they consumed meat from animals killed using iron shot rather than lead shot.

The EU Maximum Residue Level (0.1 mg/kg w.w.) for meat from domesticated mammals and poultry was exceeded in 74 % of carcasses of pheasants in our study known to have been shot using lead shot, but only 5 % of those killed using iron shot. The proportion of samples exceeding the EUML for pheasants killed using lead shot in our study was broadly similar to that found in a Europe-wide small game study of game killed using unknown shot types (Pain et al., 2022). We obtained too few data for birds killed using zinc and bismuth shot to draw firm conclusions about dietary exposure to lead, though it is likely that exposure would be lower than from game shot using lead ammunition. However, it is of concern that three pheasants in our sample were shot with zinc shotgun pellets (two only with zinc and one with zinc and iron shot) because zinc is toxic when ingested by waterbirds (Levengood et al., 1999) and probably by other bird species and has not passed the US system for approval as a non-toxic shot type. It is important that all shot types proposed as alternatives to lead are not toxic to wildlife. The chemical composition of non-toxic ammunition used for hunting is regulated only in the USA and Canada (Thomas, 2019).

4.4. *Limitations of the study*

Only two of the six batches of pheasant carcasses we examined included some from which lead shot was recovered and also others from the same batch from which iron shot was recovered. This limitation restricts the general applicability of our findings to meat from all UK pheasants killed using iron shot. Had we obtained data from more batches of carcasses of birds killed using both lead and iron shot at a wide range of known locations, we might have found some batches in which the lead concentration in meat from birds killed using iron shot was higher than that reported here because of greater exposure to environmental lead. Hence, it would be desirable to have more results for matched samples of birds killed using lead and iron shot at a wide and representative range of shooting locations and with their meat processed in the same way. However, such a study would be difficult to conduct at present, without shooting by experimenters using different ammunition types at several sites, because a recent survey of large gamebird shoots in the UK reported that only 2 % of them require hunters to use non-lead ammunition (Green et al., 2023). Critical further evidence, not available from our study, would be a comparison of lead concentrations in meat from small game animals between carcasses of animals killed using lead shotgun ammunition and much larger samples of those killed using other non-lead shot types including zinc and bismuth.

We did not conduct an analysis of stable isotopes of lead to compare isotopic ratios between pheasants killed with lead shot and those killed using other shot types. Isotopic characteristics have recently been determined for the types of lead shotgun ammunition most widely used in the UK (Taggart et al., 2020). Using those data for this comparison might reveal a difference. However, we suggest that much of the biologically-incorporated lead in the tissues of pheasants killed using iron shot may be derived from spent shot ingested by the birds and therefore have similar isotopic ratios to lead derived from embedded fragments of the lead shot used to kill pheasants. This might make the difference between isotopic characteristics for pheasants killed with lead and non-lead ammunition quite small, but such a study might still be useful in allowing biologically-incorporated lead in pheasant meat to be partitioned between that derived from ingested lead shot and that from other sources, such as soil.

We did not attempt to assess the correlation between the concentration of lead in meat with the number of lead pellets recovered from the carcass because we are unlikely to have recovered all of the shot present in the carcasses. Determination of the number of embedded shot present can only be achieved reliably when the number of shot in the carcass has been established by X-radiography before dissection, which we did not do. A previous study (Pain et al., 2010) found that the concentration of lead in the meat of pheasants and other wild-shot gamebirds was positively correlated with the number of shot detected in the carcass on X-radiographs, but lead concentration was elevated even in carcasses in which no whole pellets were present. The authors attributed this finding to some pellets passing through the bird's body without embedding in tissue but leaving behind detached fragments of lead. Unpublished observations by one of us (REG) suggest that it is especially likely that no embedded shot will be present in the carcass when birds are shot at close range (< ca. 20 m), probably because the high impact velocity at close range results in all or most pellets passing through the bird's body.

5. Conclusions

As far as we are aware, our study is the first to compare lead concentrations in meat from gamebirds killed using lead and non-lead shotgun ammunition and therefore gives a useful first quantification of the difference in lead concentration in meat in relation to shot metal type. The mean lead concentration for samples of meat from carcasses of wild-shot common pheasants from which only lead shotgun pellets were recovered was about 30 times greater than for birds from which only iron pellets were recovered. The mean lead concentration in meat from pheasants known to have been killed using lead shot was similar to mean concentrations of lead reported previously for many samples of meat from wild-shot small game animals obtained from across Europe which had been killed using unknown types of ammunition. Mean lead concentrations in the meat of pheasants killed using bismuth and zinc shot were lower than for those shot using lead, but the sample sizes for these two shot types were too small to reach firm conclusions. Our results indicate that changing the type of shotgun ammunition in use for hunting from lead to iron would reduce the concentration of lead in meat from wild-shot small game and the dietary exposure to lead of consumers.

CRediT authorship contribution statement

Rhys E. Green: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Mark A. Taggart:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **Maider Guiu:** Data curation, Investigation, Methodology, Validation, Writing – review & editing. **Hayley Waller:** Data curation, Investigation, Methodology, Validation, Writing – review & editing. **Sabolc Pap:** Data curation, Investigation, Methodology, Validation, Writing – review & editing. **Rob Sheldon:** Investigation, Writing – review & editing. **Deborah J. Pain:** Conceptualization, Investigation, Methodology, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We are grateful to Lincolnshire Game Ltd. for assisting us with the supply of pheasant carcasses and to John Gregson (Waitrose & Partners) and Mark Avery for advice. There was no specific funding for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.170356.

References

Advisory Committee on Childhood Lead Poisoning Prevention, 2012. Low level lead exposure harms children: A renewed call for primary prevention. Report of the Advisory Committee on Childhood Lead Poisoning Prevention of the Centers for Disease Control and Prevention. Atlanta, Georgia; 2012. http://www.cdc.gov/nceh/lead/acclpp/final_document_010412.pdf.

Aebischer, N.J., 2019. Fifty-year trends in UK hunting bags of birds and mammals, and calibrated estimation of national bag size, using GWCT's National Gamebag Census. Eur. J. Wildl. Res. 65, 64.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference, 2nd ed. Springer.

Butler, D.A., Sage, R.B., Draycott, R.A.H., Carroll, J.P., Potts, D., 2005. Lead exposure in ring-necked pheasants on shooting estates in Great Britain. Wildl. Soc. Bull. 33, 405–971.

Dobrowolska, A., Melosik, M., 2008. Bullet-derived lead in tissues of the wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*). Eur. J. Wildl. Res. 54, 231–235.

ECHA, 2021. ANNEX XV RESTRICTION REPORT–Lead in outdoor shooting and fishing. VERSION NUMBER: 2.0 DATE: 24 March 2021. https://echa.europa.eu/documents/10162/da9bf395-e6c3-b48e-396f-afc8dcef0b21.

EFSA, 2010. Scientific opinion on lead in food. EFSA Panel on Contaminants in the Food Chain (CONTAM). EFSA Journal. 2010; 8:1570. https://www.efsa.europa.eu/en/efsa-journal/pub/1570.

ERI, 2023. Shot-switch. https://eri.ac.uk/research/major-projects/shot-switch/.

Fimreite, N., 1984. Effects of lead shot ingestion in willow grouse. Bull. Environ. Contam. Toxicol. 33, 121–126. https://doi.org/10.1007/BF01625520.

Gasparik, J., Venglarcik, J., Slamecka, J., Kropil, R., Smehyl, P., Kopecky, J., 2012. Distribution of lead in selected organs and its effect on reproduction parameters of pheasants (Phasianus colchicus) after an experimental per oral administration. J. Environ. Sci. Health A 47, 1267–1271. https://doi.org/10.1080/10934529.2012.672127.

Gerofke, A., Ulbig, E., Martin, A., Müller-Graf, C., Selhorst, T., Gremse, C., Spolders, M., Schafft, H., Heinemeyer, G., Greiner, M., Lahrssen-Wiederholt, M., Hensel, A., 2018. Lead content in wild game shot with lead or non-lead ammunition – does "state of the art consumer health protection" require non-lead ammunition? PLoS One 13 (7), e0200792. https://doi.org/10.1371/journal.pone.0200792.

Green, R., Taggart, M., Pain, D., Smithson, K., 2022b. Implications for food safety of the size and location of fragments of lead shotgun pellets embedded in hunted carcasses of small game animals intended for human consumption. PLoS One 17, e0268089. https://doi.org/10.1371/journal.pone.0268089.

Green, R.E., Pain, D.J., 2012. Potential health risks to adults and children in the UK from exposure to dietary lead in gamebirds shot with lead ammunition. Food Chem. Toxicol. 50 (11), 4180–4190. https://doi.org/10.1016/j.fct.2012.08.032.

Green, R.E., Pain, D.J., 2015. Risks of health effects to humans in the UK from ammunition-derived lead. Pp. 27-43. In: Proceedings of the Oxford Lead Symposium. Lead ammunition: Understanding and minimising the risks to human and environmental health. Eds. Delahay, R.J. & C.J. Spray, C.J. Oxford, UK: The Edward Grey Institute.

Green, R.E., Taggart, M.A., Pain, D.J., Clark, N.A., Clewley, L., Cromie, R., Elliot, B., Green, R.M.W., Huntley, B., Huntley, J., Leslie, R., Porter, R., Robinson, J.A., Smith, K.W., Smith, L., Spencer, J., Stroud, D., 2021. Effect of a joint policy statement by nine UK shooting and rural organisations on the use of lead shotgun ammunition for hunting common pheasants Phasianus colchicus in Britain. Conserv. Evid. 18, 1–9. https://doi.org/10.52201/CEJ18ROTZ8607.

Green, R.E., Taggart, M.A., Pain, D.J., Clark, N.A., Clewley, L., Cromie, R., Dodd, S.G., Elliot, B., Green, R.M.W., Huntley, B., Huntley, J., Pap, S., Porter, R., Robinson, J.A., Sheldon, R., Smith, K.W., Smith, L., Spencer, J., Stroud, D., 2022a. Effectiveness of actions intended to achieve a voluntary transition from the use of lead to non-lead shotgun ammunition for hunting in Britain. Conserv. Evid. 19, 8–14. https://doi.org/10.52201/CEJ19/SAFD 8835.

Green, R.E., Taggart, M.A., Pain, D.J., Clark, N.A., Clewley, L., Cromie, R., Green, R.M.W., Guiu, M., Huntley, B., Huntley, J., Leslie, R., Porter, R., Roberts, J., Robinson, J.A., Robinson, R.A., Sheldon, R., Smith, K.W., Smith, L., Spencer, J., Stroud, D., 2023. Voluntary transition by hunters and game meat suppliers from lead to non-lead shotgun ammunition: changes in practice after three years. Conserv. Evid. 20, 1–7. https://doi.org/10.52201/CEJ20/OQWU5273.

Levengood, J.M., Sanderson, G.C., Anderson, W.L., Foley, G.L., Skowron, L.M., Brown, P.W., Seets, J.W., 1999. Acute toxicity of ingested zinc shot to game-farm mallards. Illinois Natur. History Surv. Bull. 36, 1–36.

Longcore, J.R., Locke, L.N., Bagley, G.E., Andrews, R., 1974. Significance of lead residues in mallard tissues. US Fish and Wildlife Service Special Scientific Report—Wildlife No. 182.

Manly, B.F.J., 2007. Randomization, Bootstrap and Monte Carlo Methods in Biology, 3rd edition. Chapman & Hall/CRC.

Mateo, R., Vallverdú-Coll, N., López-Antia, A., Taggart, M.A., Martínez-Haro, M., Guitart, R., Ortiz-Santaliestra, M.E., 2014. Reducing Pb poisoning in birds and Pb exposure in game meat consumers: The dual benefit of effective Pb shot regulation. Environ. Int. 63, 163–168.

Pain D.J., Cromie R.L., Newth J., Brown M.J., Crutcher E., Hardman P., Hurst, L., Mateo, R., Meharg, A.A., Moran, A.C., Raab, A., Taggart, M.A., Green R.E., 2010. Potential hazard to human health from exposure to fragments of lead bullets and shot in the tissues of game animals. PLoS One; 5: e10315. doi:10.1371/journal.pone.0010315.

Pain, D.J., Green, R.E., Taggart, M.A., Kanstrup, N., 2022. How contaminated with ammunition-derived lead is meat from European small game animals? Assessing and reducing risks to human health. Ambio 51, 772–1785. https://doi.org/10.1007/s13280-022-01737-9.

Ruxton, G.D., 2006. The unequal variance t-test is an underused alternative to Student's t-test and the Mann–Whitney U test. Behav. Ecol. 17, 688–690. https://doi.org/10.1093/beheco/ark016.

Seigel, S., Castellan, N.J., 1988. Nonparametric Statistics for the Behavioral Sciences, 2nd ed. McGraw-Hill.

Taggart, M.A., Shore, R.F., Pain, D.J., Peniche, G., Martinez-Haro, M., Mateo, R., Homann, J., Raab, A., Feldmann, J., Lawlor, A.J., Potter, E.D., Walker, L.A., Braidwood, D.W., French, A.S., Parry-Jones, J., Swift, J.A., Green, R.E., 2020. Concentration and origin of lead (Pb) in liver and bone of Eurasian buzzards (Buteo buteo) in the United Kingdom. Environ. Pollut. 267, 115629.

Thomas, V.G., 2019. Chemical compositional standards for non-lead hunting ammunition and fishing weights. Ambio 48, 1076–1078.

Thomas, V.G., Scheuhammer, A.M., Bond, D.E., 2009. Bone lead levels and lead isotope ratios in red grouse from Scottish and Yorkshire moors. Sci. Total Environ. 407, 3494–3502.

Thomas, V.G., Pain, D.J., Kanstrup, Green, R.E., 2020. Setting maximum levels for lead in game meat in EC regulations: an adjunct to replacement of lead ammunition. Ambio 49, 2026–2037. https://doi.org/10.1007/s13280-020-01336-6.