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Lead ammunition residues in the meat of hunted woodcock: a potential health risk to consumers

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ABSTRACT

Wild meat often retains metallic particles originating from the ammunition fired by hunters. Since ammunition are traditionally lead (Pb)-based, the consumption of game meat may entail the ingestion of Pb embedded in tissues. To assess the related risks to human health, information is needed on the number, dimension and spatial distribution of Pb particles embedded in popular quarry species. In this study, we focused on the Eurasian woodcock (*Scolopax rusticola*), a medium-sized bird intensively hunted across its range. We X-rayed 59 carcasses of woodcock shot by Italian hunters in Ukraine. To check the ammunition types and evaluate the mean weight of the embedded gunshot, we excised a sample of 62 whole pellets from 20 birds. Ammunition residues were found in 57 of the 59 woodcock (96.6%). Radiographs revealed 215 whole pellets and 125 fragmentation centres in 51 (mean = 3.64) and in 48 birds (mean = 2.14), respectively. Most fragmentation centres (75.7%) contained tiny particles (<1 mm). The overall estimated Pb load ranged from 45 to 52 mg/100 g wet weight, most of which (84.6%) in edible parts. The number of embedded pellets per unit of body mass (1.21/100 g of body weight) was higher in comparison with other bird species and also with woodcock shot in the UK, presumably owing to the hunting methods adopted by Italian hunters. The quantity and characteristics of ammunition residues we found suggest that game meat consumers are exposed to a relevant Pb assumption.

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Introduction

Lead (Pb) is a toxic element whose effects on human health are well known (Landrigan & Todd 1994). In the last decades, we have become aware that adverse consequences to organisms may arise even at very low doses when clinical symptoms are not evident. Many epidemiological studies revealed that low Pb exposures (<10 μg/dl in the bloodstream) are especially detrimental to foetuses and children, hampering the development of the nervous system and causing permanent negative effects on cognitive function and behaviour (Canfield et al. 2003; CDC 2005; Lanphear et al. 2005; Chandramouli et al. 2009). In adults, Pb affects information processing and short-term verbal memory, causing psychiatric symptoms and impairment of manual dexterity (Weisskopf et al. 2007). Furthermore, relatively low exposures among adults have been associated with elevated systolic blood pressure, increased risk of myocardial and stroke mortality, cancer and nephropathies (Menke et al. 2006; Schober et al. 2006; EFSA CONTAM 2010; Huang et al. 2013).

These findings prompted international health authorities to reject a safe threshold for Pb exposure as inappropriate and to advocate strongly for reduced intake of Pb as far as possible especially for the most sensitive categories (infants, children and pregnant women) (EFSA CONTAM 2010; JECFA 2010).

Since ingestion is considered the major source of exposure to Pb in developed countries, the intake of Pb for the population of Europe was estimated by analysing Pb contamination in all food categories (EFSA 2012). According to EFSA (2012), Pb levels in food have decreased recently, but the consumption of some food categories may remain a cause of concern. Particularly, high concentrations were recorded in wild boar and pheasant meat, well above the maximum levels admitted by the Commission Regulation No. 1881/2006 for the categories 'meat (excluding offal) of

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bovine animals, sheep, pig and poultry' (max Pb level: 100 μg/kg wet weight) and 'offal of bovine animals, sheep, pig and poultry' (max Pb level: 500 μg/kg wet weight) (Commission of the European Communities 2006). The EFSA food category 'boar (wild pig)' meat gave a mean of 1143 μg/kg, 100-fold higher than 'pork/piglet' meat (mean value 11 μg/kg). Furthermore, some boar samples peaked at 232000 μg/kg. Similarly, 'pheasant' meat reached Pb concentrations 28-fold higher than 'chickens' meat (344 μg/kg versus 12 μg/kg). Similar differences between the game and domestic animals were found in the UK (Pain et al. 2010).

These results are due to the use of Pb ammunition for hunting game animals. High Pb values have been frequently found both in venison and game birds shot by hunters and intended for human consumption (Tsuji et al. 1999; Johansen et al. 2004; Cornatzer et al. 2009; Pain et al. 2010). Soft tissues of large mammals often retain a high number of fragments up to more than 45 cm away from the wound channel, as a result of the frangibility of the various kinds of a rifle bullet. In most cases, fragments are too small and are hardly ever detected or removed during food preparation (Hunt et al. 2006, 2009; Tsuji et al. 2009; Grund et al. 2010; Knott et al. 2010; Lindboe et al. 2012). Contamination is even higher in birds because they are generally killed by several small gunshot pellets instead of a single bullet. Normally, pellets are so small that it is difficult to remove them from the flesh even if they remain whole. Furthermore, they do tend to fragment, creating a large amount of microscopic splinters and particles (Scheuhammer et al. 1998; Mateo et al. 2011; Andreotti & Borghesi 2013).

The typical practices employed to prepare game meat for consumption (dressing, marinating and cooking in wine, vinegar or in other acidic conditions) facilitate the conversion of metallic Pb into organic compounds, which are more easily absorbed by the digestive system (Mateo et al. 2007, 2011; Hunt et al. 2009). Several studies have revealed a significant association between Pb blood levels and wild game consumption in human populations, not only in arctic regions where wild game is a significant part of the diet (Hanning et al. 2003; Bjerregaard et al. 2004; Johansen et al. 2006) but also at mid-latitude regions (Iqbal et al. 2009). Such evidence has encouraged researchers and several national authorities for health and food safety to assess the risk of Pb poisoning through the consumption of game meat. The results of these assessments concluded that the risks posed by Pb ammunition on human health are not negligible and appropriate measures are needed to minimise those risks (AESAN 2012; Green & Pain 2012, 2015; VKM 2013).

In Europe, a rough estimation of the human population exposed to Pb through the consumption of game meat can be obtained through analysis of the number of hunters and their relatives. According to the European Federation of Associations for Hunting and Conservation, in Europe, there are seven million hunters, without including Russia and Turkey. Therefore, it can be assumed that several tens of millions of Europeans, corresponding to approximately 2–4% of the whole population, more or less regularly eat game meat. The amount of birds shot annually in Europe is estimated to be 101 million (Hirschfeld & Heyd 2005).

To assess the risk to the health of wild meat consumers, it is important to know the quantity and characteristics of the Pb embedded in the flesh of the most popular quarry species. In this study, we focused on Pb contamination in the Eurasian woodcock (*Scolopax rusticola*), a medium-sized bird intensively hunted across its range. We examined adults and juveniles separately since adults may retain Pb fragments in old wounds originating in previous hunting seasons (Falk et al. 2006; Newth et al. 2011; Holm & Madsen 2013). According to Annex II/A of the EU Directive 2009/147/CE, the woodcock can be hunted in all the states within the Union borders (European Parliament and Council of the European Union 2010). Furthermore, the species is intensively shot in the Balkans and in the European countries of the former USSR, both by resident and foreign hunters. Given the popularity of woodcock hunting in Europe, the consumption of its meat is widespread and considerable, but unfortunately, only rough estimates are available on the number of birds annually killed on the continent. Ferrand and Gossmann (2009a) proposed an approximate figure of 3–4 million individuals while Hirschfeld and Heyd (2005) suggested a minimum of 2730125 woodcock shot per year in the EC, Switzerland and Norway.

Specific objectives of our study were: i) to estimate the frequency of both whole pellets and fragments in shot woodcock; ii) to assess if any difference exists in pellet and fragment frequency between adults and juveniles; iii) to estimate the Pb burden embedded in the edible parts and iv) to evaluate implications for the human health.

Materials and methods

We examined a sample of 59 individuals (28 juveniles and 31 adults) selected from a stock of 485 Eurasian woodcock shot by Italian hunters during a hunting trip in Ukraine in October 2011 and seized by the Italian

Custom Agency because they were imported without complying to sanitary regulations. No bird was expressly killed for this study. The birds were frozen whole and stored at −20 °C. On analysis, the woodcock were thawed, aged by plumage and moult status (Ferrand & Gossmann 2009b) and weighed to the nearest gram with a Pesola spring balance. Our sample of juveniles and adults was selected with their body weights within a range of ±15% the average weight of the whole seized stock. We considered both age and weight because: 1) old birds may have embedded pellets remaining from healed wounds in previous hunting seasons (Falk et al. 2006; Newth et al. 2011) and 2) weight is a reliable measure of body mass, that in turn affects the number of embedded pellets (Pain et al. 2010).

We X-rayed the birds with digital radiography equipment (Kodak DirectView CR 800 System, and Kodak DirectView CR cassette 35 × 43 cm with a matrix size of 2048 × 2500) (Eastman Kodak Company, Rochester, NY). Metal particles are easy to distinguish from bone and grit because they are clearly more radio-opaque (Knott et al. 2010). Radiographs were examined at full size to detect whole pellets and large fragments. Micro fragments were counted by zooming into 150%. We considered as 'large' those fragments exceeding 0.5 mm in diameter (Hunt et al. 2006).

Following Andreotti and Borghesi (2013), we subdivided the body of each bird into seven sectors so that the anatomical parts normally consumed by humans could be easily considered separately (Figure 1). We regarded sectors 3, 4, 5 and 6 as edible, i.e. those most commonly eaten by humans.

We counted the number of whole shot pellets, single macro fragments and clusters of radiodense particles (fragmentation centres – FC) embedded in each body sector. The fragments were scored as follows: 0 = none visible; 1 = 1–2 macro fragments; 2 = 2–4 micro-fragments and 3 = > 4 fragments, regardless of their size. We tested whether the number of pellets and fragments observed in the body varied among age classes and body sectors with a chi-square test of independence performed using the R software (R Foundation for Statistical Computing, Vienna, Austria) (R Core Team 2013).

To check the ammunition types used by hunters, we performed a necropsy on 20 woodcock and excised 62 whole pellets (2–4 from each bird). These pellets were accurately washed, dried and weighed by means of a Sartorius analytical balance (accuracy d = 0.1 mg) (A&D Laboratory Balances, Bradford, MA). Furthermore, each pellet was examined for colour, form and size, and tested to see whether they were attracted by a bar magnet.

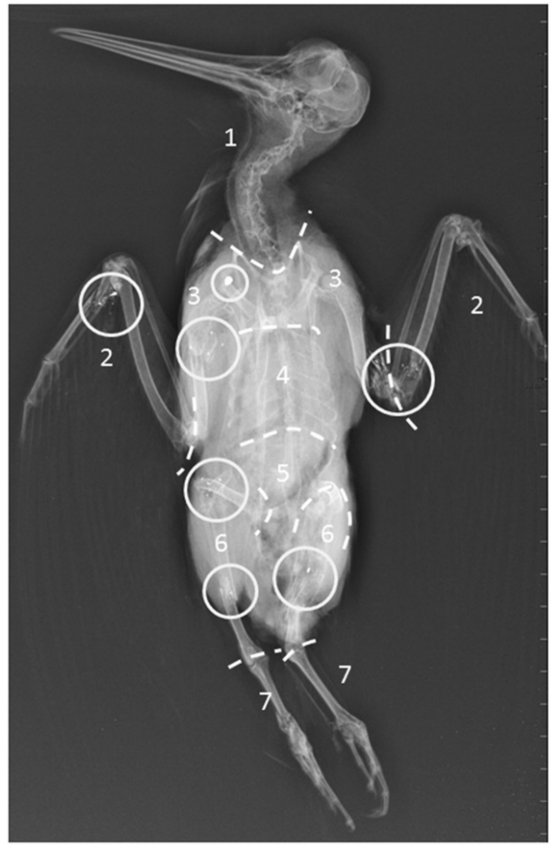

Figure 1. X-ray photograph of a woodcock, showing the body sectors where the position of each shot pellet and fragment was noted. 1, head and neck; 2, wings; 3, humerus and a pectoral girdle; 4, thorax; 5, abdomen; 6, femur and tibiotarsus and 7, tarsus and metatarsus. A whole pellet and six fragmentation centres can be recognised inside the grey circles.

To evaluate the Pb quantity embedded in our sample, we multiplied the number of whole pellets by the mean weight of the excised pellets. Since most pellets lose fragments under the impact, this estimation was repeated considering the mean weight of the heaviest pellets from each woodcock, except in the case of three woodcock with pellets of different sizes, for which the mean weight of the excised pellets was used. Finally, we related the estimated amounts of Pb to the overall weight of the whole woodcock sample to obtain the Pb burden in 100 g wet weight.

Results and discussion

Ammunition residues were found in 57 of the 59 carcasses (96.6%). We observed only whole pellets or fragments in 9 (15.8%) and 6 (10.5%) carcasses, respectively, while in 42 carcasses (73.7%) we found both. Pellets and fragments were embedded in all body sectors (Table 1A and B), but with significant differences in frequency (pellets: chi-square value = 77.17, df = 6, $p<0.001$; fragments: chi-square value = 79.9, df = 6,

Table 1 (A). Distribution of pellets in different body sectors of juveniles and adults of Eurasian woodcock shot during hunting activity.

	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	TOT	Mean	SD
Adults (n=31)	9	9	27	29	22	5	1	102	3.29	3.54
Juveniles (n=28)	9	3	33	42	13	11	2	113	4.04	4.43
TOT	18	12	60	71	35	16	3	215	3.64	3.97

Significant differences were found in frequency in different body sectors (chi-square value = 77.17, df = 6, p<0.001). No significant difference was detected between age classes (chi-square value = 10.34, df = 6, p=0.11).
[a]Head neck;
[b]wings;
[c]humerus pectoral girdle;
[d]thorax;
[e]abdomen;
[f]femur tibiotarsus;
[g]tarsus metatarsus.

Table 1 (B). Distribution of fragments in different body sectors of juveniles and adults of Eurasian woodcock shot during hunting activity.

	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	TOT	Mean	SD
Adults (n=31)	15	22	9	1	2	5	5	59	1.90	1.99
Juveniles (n=28)	6	26	15	3	1	8	7	66	2.36	2.54
TOT	21	48	24	4	3	13	12	125	2.14	2.27

Significant differences were found in frequency in different body sectors (chi-square value = 79.9, df = 6, p<0.001). No significant difference was detected between age classes (chi-square value = 7.68, df = 6, p=0.26).
[a]Head neck;
[b]wings;
[c]humerus pectoral girdle;
[d]thorax;
[e]abdomen;
[f]femur tibiotarsus;
[g]tarsus metatarsus.

Table 2 (A). Distribution of different fragment classes in adults of Eurasian woodcock shot during hunting activity (score 0 = none visible; score 1 = 1–2 macro fragments; score 2 = 2–4 micro fragments; score 3 = > 4 fragments, regardless of their size).

	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	TOT
Score 1	1	2	1	0	1	0	3	8
Score 2	13	13	2	1	1	4	2	36
Score 3	1	7	6	0	0	1	0	15
TOT	15	22	9	1	2	5	5	59

[a]Head neck;
[b]wings;
[c]humerus pectoral girdle;
[d]thorax;
[e]abdomen;
[f]femur tibiotarsus;
[g]tarsus metatarsus.

Table 2 (B). Distribution of different fragment classes in juveniles of Eurasian woodcock shot during hunting activity (score 0 = none visible; score 1 = 1–2 macro fragments; score 2 = 2–4 micro fragments; score 3 = > 4 fragments, regardless of their size).

	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	7[g]	TOT
Score 1	0	4	2	1	1	1	1	10
Score 2	3	9	6	2	0	3	3	26
Score 3	3	13	7	0	0	4	3	30
TOT	6	26	15	3	1	8	7	66

[a]Head neck;
[b]wings;
[c]humerus pectoral girdle;
[d]thorax;
[e]abdomen;
[f]femur tibiotarsus;
[g]tarsus metatarsus.

p<0.001). In the anatomical regions most commonly eaten by humans, we found Pb residues in 47 carcasses (79.7%). No significant difference was detected between age classes in frequencies of whole shot and fragments (pellets: chi-square value = 10.34, df = 6, p=0.11; fragments: chi-square value = 7.68, df = 6, p=0.26).

Shotgun pellets

Radiographs revealed 215 pellets in 51 woodcock (mean = 3.64, SD = 3.97, range = 0–17, n = 59). Most of the pellets (n=131, 60.9%) were located in sectors 3 and 4 while only a small fraction (n=15.7%) was found in the distal parts of the wings and legs (sector 2 and 7; Table 1A and B). In the edible sectors, we counted 182 pieces of shot, corresponding to 84.6% of the total amount (mean = 3.08, SD = 3.64, range = 0–17, n=59). In three adults, we detected pellets of different size by examining the radiographs. We ascertained that they were whole pellets of different dimensions by examining them after excision. During the necropsy, we did not find any evidence of connective tissue encapsulation.

Fragments

A minimum of 125 FC were detected in 48 woodcock (mean = 2.14, SD = 2.28, range = 0–10, n = 59). FC were concentrated (n=93, 74.4%) in the head, neck, wings and the pectoral girdle (Table 1B). In the edible sectors, we estimated 44 FC, representing 35.2% of the detected FC (mean = 0.75, SD = 1.32, range = 0–6, n = 59). In most cases, the fragments were assigned to score 2 (n=62, 49.6%) or score 3 (n=36%), revealing a prevalence of small tiny particles (Table 2A and B).

Pellet characteristics

All pellets were non-magnetic, dark, dull and deformed, and, therefore, were assumed to be Pb. Overall they weighed 2319.8 mg (mean = 37.4 mg, SD = 16.7, range = 13.1–76.7, n=62). When taking the heaviest pellets from each necropsied woodcock, we obtained a mean weight of 43.8 mg (SD = 16.7, range = 20.0–76.7 n=20).

Woodcock weights

The mean weight of the whole stock of seized woodcock did not differ significantly from the examined sample (whole stock: mean = 313.8 g, SD 30.1, n=403; examined woodcock: 304.0 g, SD = 1.7, n=59; t-test= −1.94, p=0.05). The mean weights of adults and juveniles did not differ (juveniles: mean = 304.0 g, SD = 15.4, n=28; adults: mean = 300.4 g, SD = 19.5, n=31; t-test= −0.18, p = 0.40).

Pb mass

On the basis of both the mean weight of the excised pellets and the heaviest pellet subset, we estimated the Pb burden embedded in woodcock ranging from 45 to 52 mg/100 g wet weight.

Comparison with previous studies

Our study revealed that the majority of the X-rayed carcasses contained visible particles of metallic Pb deriving from spent ammunition. In comparison with data collected in previous studies on different bird species, woodcock had a higher rate of embedded whole pellets per unit of body mass, i.e. 1.21 pellets/100 g of body weight, versus 0.93 in European starlings (Andreotti & Borghesi 2013), 0.73 in red-legged partridges (Mateo et al. 2011), 0.52 in common eiders (Johansen et al. 2004) and 0.41 in thick-billed murres (Johansen et al. 2001). Moreover, the frequency of pellets counted in our study is relatively high when compared to the figures reported by Pain et al. (2010) for the Eurasian woodcock hunted in the UK. The authors X-rayed oven-ready carcasses (without feathers, viscera and heads) and recorded numbers of pellets and large radio-dense fragments. They found a mean of one pellet per woodcock, a low value with respect to the mean of 3.1 detected in our study considering edible sectors only.

These discrepancies in pellet frequency may be due to different hunting methods adopted with regard to the target species and traditional hunting practices. The low shot resistance of woodcock leads many hunters to prefer cartridges with numerous pellets of smaller size than those used to shoot game birds with a similar body mass. In addition, the hunting technique may influence the firing distance, which affects the spread pattern of projectiles hitting the prey. For example, in the UK, woodcock are often flushed towards the guns by a line of 'beaters' walking through the wood, while Italian hunters prefer to walk behind a pointing dog, shooting the woodcock at a shorter distance over the dog's point (Spanò & Fadat 2014). Hence, the quantity of Pb embedded in quarry bird species appears to be related not only to the species but also to the hunting technique. These results should be borne in mind when assessing Pb contamination levels in game meat from different countries.

The concomitant presence of pellets of different size embedded in three adults might be explained by either the use of cartridges loaded with mixed grains or the existence of old wounds originating in previous hunting seasons. Since only slight changes occur in tissues surrounding Pb gunshot embedded in old wounds (Sanderson et al. 1998), the circumstance that we did not observe connective tissue encapsulation does not allow us to exclude the presence of pellets from previous hunting seasons.

We did not find a higher frequency of whole pellets in adults when compared to juveniles, as found in long-lived species such as sea ducks and geese that can accumulate pellets in subsequent hunting seasons (Falk et al. 2006; Newth et al. 2011; Holm & Madsen 2013). This result might be influenced by two factors: (i) woodcock have little or no resilience to the shot and hardly ever survive when injured and (ii) adults are more cautious than juveniles and flush earlier when stalked leading to them being shot at longer distances by a wider pellet spread. This latter factor is relevant in assessing the Pb load in woodcock meals because juveniles are generally far more abundant than adults in hunting bags (more than 65% juveniles in France (Boidot & Aurousseau 2013); 81.1% juveniles in our sample).

If we compare data on fragments recorded in starlings in a study carried out with a similar methodology (Andreotti & Borghesi 2013), we observe a higher density of FC in woodcock (0.72 FC per 100 g of body weight, versus 0.60 in starlings), even if the fragmentation rate of pellets, calculated as the ratio FC/whole pellets, appears to be slightly lower in woodcock than in starlings (0.58 versus 0.64). The difference in the fragmentation rate might be related to a higher frequency of pellets that have passed through the starlings leaving Pb particles in the tissues, but have not remained in the carcass due to the smaller body mass of this species. Fragment distribution in woodcock differs significantly from starlings when considering all body sectors (chi-square value = 27.7, df = 6 p<0.0001), but there is no difference when taking into account the edible parts only (chi-square value = 0.4, df = 3, p=0.9).

Levels of Pb contamination in woodcock meat found in our study are much higher than those reported for venison examined by X-ray. As far as we know, there is only one estimate of the total mass of bullet fragments left in carcasses of deer (Knott et al. 2010). This corresponds to 1.6 mg/100 g wet weight, 30-fold lower than the estimates of Pb embedded in woodcock. Pb concentrations determined through chemical analysis confirm a higher burden of Pb in birds than in ungulates, but these differences are less relevant. For example, in partridges values have been found 2–8-fold higher than in red deer or wild boar (Mateo et al. 2011; Sevillano Morales et al. 2011). This discrepancy can be explained considering that in birds there is a relatively higher amount of large Pb particles (essentially whole pellets) that are generally removed before performing chemical analyses.

Implication for the human health

Our findings reveal that woodcock meat derived from animals shot by traditional Pb ammunition retains a considerable quantity of metallic Pb in the form of both large particles (i.e. macro fragments and whole pellets) and tiny fragments. Even when substantial pieces of Pb are removed from game meat, the presence of Pb is still significant, especially where tiny fragments are spread widely. Chemical analyses have found high levels of Pb contamination in game tissue samples of quarry species where Pb particles were detected by X-ray (Tsuji et al. 1999, 2009; Johansen et al. 2001; Pain et al. 2010; Mateo et al. 2011). Furthermore, embedded Pb can be easily absorbed through the intestine by game meat consumers, given the amount of tiny fragments, easily attacked by the gastric acid, and the leaching processes during cooking treatments (Mateo et al. 2007, 2011; Hunt et al. 2009). Hence, regular woodcock meat consumers are exposed to real health risks.

Hunters are not usually woodcock specialists, killing both ungulates and small game during the same hunting season. This implies that people consuming woodcock often eat other game birds, small mammals and ungulates. It has been found that frequent consumption of big game meat alone is enough to expose the hunter population to the risk of Pb poisoning (Sevillano Morales et al. 2011; Lindboe et al. 2012). Consumption of contaminated woodcock meat will increase the intake of Pb in game consumers already exposed to the risk of Pb poisoning. In Italy, more than 230 000 ungulates are estimated to be shot annually, producing 6828.7 tons of game meat (Ramanzin et al. 2010), in addition to at least 17 million birds (Hirschfeld & Heyd 2005) and an unknown number of hares and rabbits. Since the use of Pb ammunition is banned exclusively in wetlands of special protection areas and in some hunting districts for the hunting of cloven-hoofed games, only a negligible fraction of game birds and mammals is killed with Pb-free shot or bullets. This means that the 750 000 licenced hunters in Italy and their relatives consume approximately tens of millions of meals derived from ungulates and no less than 5 million meals from game birds, killed with Pb ammunitions.

The high frequency of Pb pieces and, in particular, of tiny fragments recorded in this study reveals that precautions during the preparation for consumption of shot game birds are not enough to guarantee Pb-free meat. The removal of whole pellets before cooking is not easily achieved, given their small size and abundance, and does not prevent the ingestion of a significant quantity of the smallest particles. Additionally, Pb residues are almost uniformly distributed in game bird carcasses, making removal more difficult. It is a different case to ungulates, where fragments of ammunition are concentrated in a radial zone around the wound channel, allowing a small concentrated area of the carcass to be removed and disposed of. The distribution of Pb in bird carcasses makes it impossible to define handling procedures which reduce the risk for game bird consumers. The use of Pb-free pellets is the only effective solution to avoid the consumption of Pb when eating shot small game. It could also reduce the Pb pollution in the environment. Positive effects have been observed in wildlife and ecosystems, where Pb ammunition has been banned (Mateo et al. 2014).

Conclusions

The considerable amount of embedded particles of metallic Pb found in our study suggests that a significant risk to human health is associated with the frequent consumption of woodcock killed using traditional Italian hunting techniques. Therefore, a risk assessment is recommended.

To reduce the hazard of Pb poisoning on regular woodcock consumers and more in general on game meat consumers, actions should be promoted to raise awareness of the risks among exposed groups (hunters, gamekeeper, etc.). A mandatory Pb-free certification mark could be used to provide a guarantee of safety for game meat consumers. At the same time, the adoption of a total ban for the use of Pb ammunition in both aquatic and terrestrial ecosystems is strongly recommended (Group of Scientists 2014).

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