# Transfer of lead from shot pellets to game meat during cooking

R. Mateo *, M. Rodríguez-de la Cruz, D. Vidal, M. Reglero, P. Camarero

*Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain*

## Abstract

We evaluated the transfer of Pb from shot to meat during the preparation of breasts of quails with 0, 1, 2 or 4 embedded Pb shot. A traditional Spanish recipe was used which utilizes vinegar during cooking to enhance the long-term preservation of the meat. The effect of the acidic conditions generated by the vinegar on Pb transfer was compared with the same recipe when the vinegar was substituted with water. The effect of up to 4 weeks of storage on Pb transfer has been also evaluated. The transfer of Pb from the embedded shot to the meat was much higher when cooking with vinegar than with water. However, the Pb transfer under acidic conditions did not increase significantly during long-term storage at room temperature. The consumption of only half a pickled quail per week with embedded shot may cause the provisional tolerable weekly intake (PTWI) of Pb by the Spanish consumer to be exceeded.
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## 1. Introduction

Wild birds, especially waterfowl and raptors, are affected by lead poisoning due to the ingestion of Pb shot used for hunting. Waterfowl ingest shot when they confuse it with seeds or more probably with grit, while birds of prey ingest Pb shot embedded in the flesh of their prey (Mateo et al., 1997, 2003). Lead shot pellets retained in the gizzard may become completely eroded in about 21 days, leading to the accumulation of elevated Pb concentrations in the tissues of birds (Sanderson and Bellrose, 1986). Apart from poisoning the birds, Pb shot used for hunting contaminates the edible parts of game animals. In 40% of the waterfowl hunted in Spain between 1990 and 1994 the liver Pb concentration was above 0.5 µg g$^{-1}$ (wet weight, w.w.), which is the maximum

residue level (MRL) established by European regulations for the offal of domestic fowl (Guitart et al., 2002). About 9% of the waterfowl harvested by hunters in Spain had 50–378 µg g$^{-1}$ (dry weight, d.w.) in liver, which implies that ingestion of the liver alone (approximately 6 g d.w.) would represent an intake of 0.3–2.3 mg of Pb (Mateo, 1998; Guitart et al., 2002). The provisional tolerable weekly intake (PTWI) of Pb recommended by FAO/WHO (2000) is 25 µg kg$^{-1}$ of body weight (b.w.) for all age groups within the human population.

In addition to the Pb actually ingested and absorbed by birds, the pathway along which Pb shot travels through the flesh of hunted game birds also tends to be contaminated with small Pb fragments (Frank, 1986). These embedded fragments of metallic Pb are a second potential source of dietary Pb exposure for human consumers of wild game, especially in communities that rely on subsistence hunting and for whom hunter-killed wild game represents a major food source. About 7% of

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\* Corresponding author. Tel.: +34 926295450; fax: +34 926295451.
*E-mail address:* rafael.mateo@uclm.es (R. Mateo).

Inuit newborns from northern Quebec (Canada) between 1993 and 1996 had a cord blood lead concentration equal to or greater than 0.48 µmol $l^{-1}$, which was the intervention level adopted by the Canadian authorities for young children (Lévesque et al., 2003). In comparison, only 0.16% of Caucasian newborns from southern Quebec had cord blood Pb levels equal to or greater than this intervention level. Moreover, the isotopic ratios were different between both populations in Quebec, the ratios for the northern population being closer to that of a Pb shot signature. In another study with communities from northern Ontario (Canada), 24.6% of the naturally exfoliated teeth of children with regular consumption of game meat had elevated dentine-Pb levels ($\geq 10$ µg $g^{-1}$). These levels were comparable to those found in children living near smelters or in urban areas (Tsuji et al., 2001). Also in Canada, 11% of pooled pectoral muscles of hunter-killed game birds had $>0.5$ µg $g^{-1}$ w.w. between 1988 and 1995 (Scheuhammer et al., 1998). This Pb contamination was present in the meat after removing all visible Pb shot, although radiography permitted the detection of numerous smaller ($<1$ mm diameter) metallic fragments in pectoral muscle samples from these birds which helped to explain the Pb contamination reported.

The risk of Pb exposure through the consumption of hunter-killed birds has also been assessed in Greenland. Lead concentrations in the meat of hunted seabirds boiled in salted water following a traditional recipe ('suaasat') was 0.22 µg $g^{-1}$ w.w., which is 10 times higher than in birds not killed with Pb shot (Johansen et al., 2001). In a similar study, Pb concentration in the breast of shot waterfowl was 44 times higher (6.1 µg $g^{-1}$ w.w.) than in drowned waterfowl (0.14 µg $g^{-1}$ w.w.; Johansen et al., 2004).

Here we have evaluated the transfer of Pb from Pb shot to meat after the preparation of game with a Spanish recipe ('escabeche') that traditionally uses vinegar. Moreover, this is a recipe that permits the preservation of the meat for several days or even weeks at room temperature. The presence of a low pH and the time of storage may therefore increase the transfer of Pb to this pickled meat.

## 2. Materials and methods

### 2.1. Cooking procedure

We used quails with manually embedded Pb shot to reduce the variability due to Pb shot fragmentation along the shot path through the meat, and to focus then on the effects of pH and time of storage of meat containing Pb shot. Sixty breasts of non-shot farm reared quails (*Coturnix coturnix japonica*) were obtained ready to cook from a supermarket. Johansen et al. (2004) found 0–5 shot in the breast of thick-billed murres (*Uria lomvia*) and 0–3 shot in common eiders (*Somateria mollissima*). Consequently, we embedded 0, 1, 2, or 4 Pb shot in each breast. Pb shot (size #6) was fired against blocks of paper to produce representative changes in the shot surface due to collisions in the barrel-gun or against the target. Three breasts with each number of Pb shot were processed with or without vinegar to compare the transfer of Pb during cooking under these differing conditions. These samples were prepared for Pb analysis 1 day after cooking. The effect of the storage with vinegar after cooking was studied with 3 breasts for each number of embedded Pb shot and for each storage time (i.e., 1, 7, 14 and 28 days). Cooked samples were stored in glass flasks at room temperature (23 °C) as is traditional. The recipe consisted of boiling 4 groups of 12 breasts (one for each amount of embedded Pb shot) with 300 ml of water, 300 ml of vinegar (acetic acid 6%), 100 ml of sunflower oil (0.2°), garlic, black pepper, laurel and salt. The mean pH ($\pm$SD) of the liquid after cooking was $4.6 \pm 0.1$. Four breasts with the same number of shot were stored in one flask and one breast was removed at each established time. The samples cooked without vinegar were processed in groups of 3 with the same ingredients whilst simply replacing the vinegar with an equivalent volume of water.

In an additional experiment the release of Pb from shot pellets depending on the liquid used and the temperature reached was studied. One #6 Pb shot pellet was introduced into a glass test tube with 5 ml of water or an aqueous solution of acetic acid (3%, pH 2.5). These tubes were maintained for 1 h at 25 °C or 90 °C. The release of Pb from the Pb shot was then measured by the direct analysis of the Pb concentrations in the liquid.

### 2.2. Lead analysis

Lead shot were removed before processing breasts for Pb analysis. The skin was removed and the muscle minced with scissors. The mean ($\pm$SD) weight of the breast samples was $18.02 \pm 3.24$ g. The sample was freeze dried (Christ Alpha 1–2, Braun Biotech), homogenized with a cutting mill and stored at $-20$ °C until analysis. Dry matter content of the cooked meat samples was $35.7 \pm 3.4$%. The cooking liquid (25 ml) left at the end of the entire storage period (4 weeks) was also processed for Pb analysis. Samples (0.5 g) were digested with 3 ml of $HNO_3$ (69% Analytical Grade, Panreac), 1 ml of $H_2O_2$ hydrogen peroxide (30% v/v Suprapur,

Merk) and 4 ml of $H_2O$ (Milli-Q grade) with a microwave oven (Ethos E, Milestone). The digestion program used began at 750 W and ramped for 15 min until 180 °C was reached, this temperature was then held for 10 min at 800 W. Samples were diluted to a final volume of 50 ml with Milli-Q $H_2O$. Lead analysis was done by graphite furnace-atomic absorption spectroscopy in an AAnalyst 800 (Perkin Elmer) using 50 µg $NH_4H_2PO_4$ and 3 µg $Mg(NO_3)_2$ as matrix modifiers in each atomization. Calibrations were prepared from a commercial solution with 1 g/l of Pb (Panreac). The limit of Pb quantification was 0.01 µg $g^{-1}$ d.w. Blanks and samples with spiked amounts of Pb (0.5, 1 or 2 µg) were processed in each batch of digestions. Recovery (mean± SE) with these spiked samples was 95.4±4.9%. A reference sample of pig kidney (CRM N° 186) with a certified Pb level of 0.306 µg $g^{-1}$ d.w. was also analyzed and a concentration of 0.311±0.006 µg $g^{-1}$ d.w. was determined.

### 2.3. Statistical analysis

The effect of the presence of vinegar during cooking was studied with a 2-way analysis of variance (ANOVA) with the samples processed 1 day after cooking. The factors included in the analysis were the number of embedded Pb shot (0, 1, 2, and 4) and the presence/ absence of vinegar. The effect of the storage time was studied with the samples cooked with vinegar with a 2-way ANOVA. The factors in this analysis were the number of embedded Pb shot and the time of storage (1,
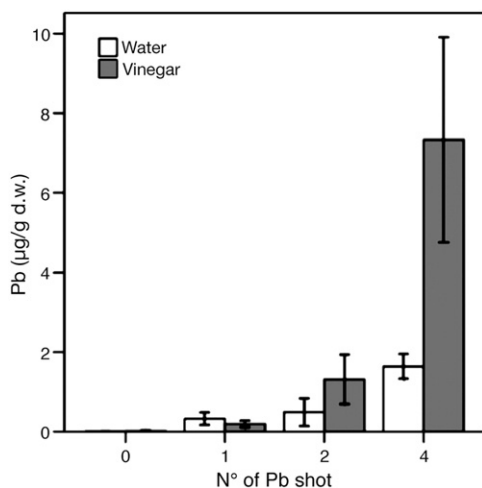


Fig. 1. Lead concentrations (mean±SE) in the breasts of quails with different numbers of embedded Pb shot after cooking with a traditional Spanish recipe containing vinegar (pickled) or substituting this with water (non-pickled).
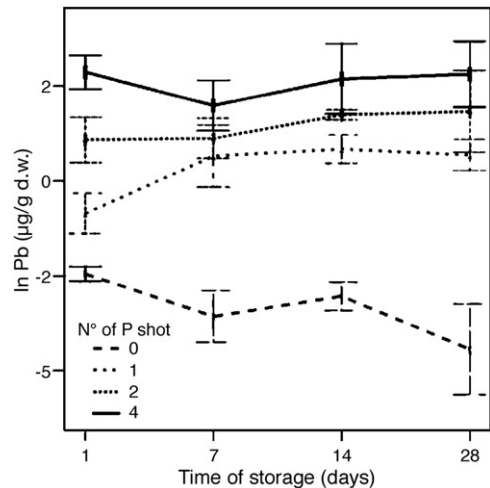


Fig. 2. Logarithm transformed Pb concentrations (mean±SE) in breasts of pickled quails stored at room temperature for differing time periods and with different numbers of embedded Pb shot.

7, 14, 28 days). Non detected values were considered as half of the limit of quantification (0.005 µg $g^{-1}$ d.w.). The Pb concentrations were log-transformed to approach a normal distribution of the data. The relationship between the number of embedded Pb shot and the concentration of Pb in the cooking liquid was studied using Spearman correlation coefficients. The results of the assay to investigate the release of Pb using different liquids and temperatures in test tubes were analyzed with a 2-way ANOVA (with liquid and temperature as factors). The significance of the results was set at $p<$ 0.05. The analyses were performed with the SPSS v 13.0 statistical package.

## 3. Results

The presence of vinegar during cooking quail breasts with Pb shot increased significantly the transfer of Pb to the meat ($F_{1, 16}=12.5$, $p=0.003$; Fig. 1). The number of Pb shot was also positively related with the Pb concentration in meat after cooking ($F_{3, 16}=35.9$, $p<0.001$). The interaction between the number of embedded shot and the presence of vinegar was also significant ($F_{3, 16}=3.75$, $p=0.033$).

The time of storage did not affect the transfer of Pb from shot to the meat ($p=0.744$). As happened with samples 1 day after cooking, meat Pb concentration increased with the number of embedded shot ($F_{3, 32}=$ 58.0, $p<0.001$; Fig. 2). The concentration of Pb in the cooking liquid with vinegar was correlated with the number of embedded shot ($rs=0.604$, $p=0.049$; Fig. 3). Mean Pb concentrations in the liquid with vinegar were
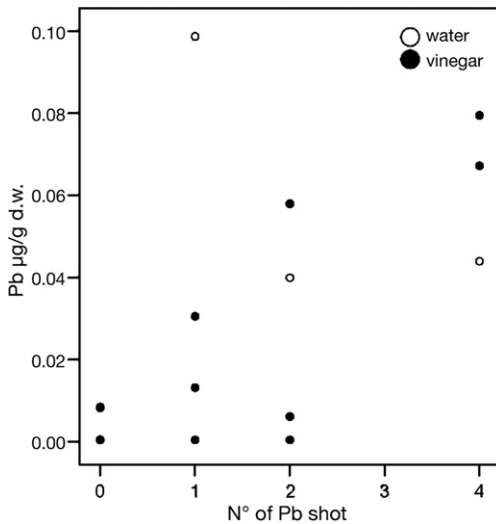
Fig. 3. Relationship between the number of embedded Pb shot and the Pb concentration in the liquid resulting from cooking quail breasts with vinegar or water. Two samples with vinegar were lost during the process. One of the samples with water shows an unexpected high value that was confirmed by repeated analyses.

2, 9, 15 and 50 ng ml$^{-1}$ for breasts with 0, 1, 2 and 4 Pb shot, respectively. The liquid without vinegar had Pb concentrations ranging from 6 to 66 ng ml$^{-1}$. This result shows that most of the lead is retained in the meat independently of the presence of vinegar.

In order to permit comparisons with other studies or regulatory levels, Pb concentrations were expressed in wet weight (Table 1). Values ranged from below the limit of Pb quantification in quails without embedded Pb shot to values of 2.12 $\mu$g g$^{-1}$ and 31.5 $\mu$g g$^{-1}$ in non-pickled and pickled quails with 4 embedded Pb shot, respectively.

The acidic conditions and the high temperature used during cooking were confirmed to be important in the experiment done with Pb shot directly immersed in aqueous solutions (Table 2). The amount of Pb released

Table 1
Lead concentrations ($\mu$g g$^{-1}$ w.w.) in breasts of quails with different numbers of embedded Pb shot

| Recipe | No. of shot | $n$ | Mean | SE | Minimum | Maximum |
|---|---|---|---|---|---|---|
| Pickled | 0 | 3 | <0.01 | – | <0.01 | 0.01 |
| quails | 1 | 3 | 0.32 | 0.16 | 0.10 | 0.63 |
| | 2 | 3 | 0.49 | 0.35 | 0.10 | 1.19 |
| | 4 | 3 | 1.64 | 0.31 | 1.07 | 2.12 |
| Non-pickled | 0 | 12 | 0.02 | 0.00 | <0.01 | 0.04 |
| quails | 1 | 12 | 0.81 | 0.25 | 0.06 | 3.13 |
| | 2 | 12 | 2.90 | 1.51 | 0.38 | 19.37 |
| | 4 | 12 | 8.63 | 3.12 | 0.69 | 31.50 |

Table 2
Amount of lead ($\mu$g, mean$\pm$SE, $n=3$) extracted from one #6 Pb shot pellet (0.110 g) with two different solutions (5 ml) at two different temperatures during 1 h

| | 25°C | 90°C |
|---|---|---|
| Water | 0.62$\pm$0.29 | 6.52$\pm$1.03 |
| Water with acetic acid (3%) | 93.03$\pm$6.88 | 306.7$\pm$27.88 |

from shot pellets was significantly higher with the presence of acetic acid ($F_{1,\ 8}=186.6$, $p<0.001$) and with higher temperatures ($F_{1,\ 8}=58.4$, $p<0.001$).

## 4. Discussion

The use of Pb shot for small game hunting contributes significantly to the contamination of this meat, especially if it is cooked with vinegar. However, the Pb transfer from the embedded Pb shot to the meat under acidic conditions did not increase significantly during a long storage period at room temperature. These findings show that most of the transfer of Pb in the presence of vinegar is produced during cooking and is favoured by the high temperature of the process. Similarly, a higher release of Pb was obtained with Pb shot immersed in an acid aqueous solution (acetic acid 3%) that in water, and this was significantly increased by heat. However, it is interesting to notice the significant release of Pb in the acid aqueous solution at 25 °C after only 1 h of immersion of shot pellets, because this contrasts with the non-significant increase of the release of Pb from the embedded shot during the storage of the pickled quails. This may be explained by the lower pH in the test tubes solution than in the liquid after cooking quails.

We found that one #6 Pb shot in a piece of meat of 18 g w.w. can release a quantity of lead sufficient to contaminate the product to levels above the maximum level established at 0.1 $\mu$g g$^{-1}$ w.w. for meat in EU countries (European Commission, 2001). All but one of the samples cooked with embedded Pb shot were above this level and Pb levels were up to 300 times higher in the samples with 4 embedded Pb shot that were cooked with vinegar. All the quails without embedded Pb shot were below the MRL established in the EU.

The transfer of Pb from shot to the meat during cooking is not the only important cause of contamination. The path of the shot is contaminated by the disintegration of the Pb shot as it passes through the flesh of the animal. Scheuhammer et al. (1998) detected extremely high concentrations of Pb (3,910 $\mu$g g$^{-1}$ d.w.) in the breast of waterfowl killed with Pb shot even after removing the primary Pb shot pellets from the flesh.

These authors found that 21% of individual breasts from pools with $>0.5 \mu g$ of Pb $g^{-1}$ w.w. contained $>5 \mu g \ g^{-1}$ d.w. and an average concentration of $211 \pm 634 \mu g \ g^{-1}$ d.w. Johansen et al. (2004) detected means of 6.1 and 0.73 $\mu g \ g^{-1}$ w.w. for Pb in the breasts of thick-billed murres and common eiders, respectively, killed with Pb ammunition. These studies show that the contamination of meat would most probably have been even higher in this study if the quails had been shot instead of artificially embedded with shot.

Although the transfer of Pb along the shot path to the meat can be highly variable, depending on factors such as collision with bones or the length of the path, we can establish an assessment of the minimum risk of exposure to Pb based on the number of Pb shot present at the time of cooking the meat. Johansen et al. (2001) considered 200 g of meat to be a regular meal of small game. If we calculate the intake of Pb with the pickled meat with embedded Pb shot we can show that just taking one such meal would result in the 'lowest observed adverse effect level' (LOAEL) being exceeded for different age groups in the human population, and in certain conditions, the PTWI will also be exceeded (Fig. 4). The LOAELs considered are associated with

impaired neurobehavioral and cognitive development and electrophysiological deficits in children, reduced gestational age and birth weight in infants, and elevated blood pressure in adults (Carrington et al., 1993). These LOAELs are 60 $\mu g \ day^{-1}$ for children $\leq 6$ years old, 150 $\mu g \ day^{-1}$ for children $\geq 7$ years old, 250 $\mu g \ day^{-1}$ for pregnant women and 750 $\mu g \ day^{-1}$ for adults. Although the meat of small game is probably not eaten daily by the general population, it is something which is commonly eaten within the families of hunters in rural areas in Spain. Considering daily consumption, 8% and 50% of the pickled quails with 2 and 4 embedded Pb shot respectively, would generate Pb intake of $>750 \mu g$ per 200 g meal (Fig. 1). In the case of pregnant women, 25%, 66.6% and 91.6% of the quails with 1, 2 and 4 Pb shot respectively, would contribute $>250 \mu g$ of Pb per meal. However, since a weekly consumption of game meat is probably more common in Spain nowadays, these figures may represent an overestimate in intake for the majority of the population. Even so, the PTWI is currently established at 25 $\mu g \ kg^{-1}$ of b.w. (FAO/WHO, 2000), which equates to 1,500 $\mu g$ of Pb for humans weighing 60 kg. This 'threshold level' would be exceeded by 8.3% and 33.3% of 200 g meals with pickled meat containing 2 and 4 Pb shot/breast, respectively (Fig. 4).

The intake of Pb through the diet in humans has been studied in several regions of Spain. Results range between 28.4 and 574 $\mu g \ day^{-1}$ (Cuadrado et al., 1995; Moreiras et al., 1995; Llobet et al., 2003; Falcó et al., 2005) and show a mean of 48 $\mu g \ day^{-1}$ (Rubio et al., 2004). We can estimate that the ingestion of 200 g of quail breasts with 1, 2 or 4 Pb shot contributes 162, 580 or 1,727 $\mu g$ of Pb, respectively, which is 3.4, 12 or 36 times this estimated mean daily intake. If we consider an estimated weekly intake of Pb in Spain of 336 $\mu g$, the inclusion in the diet of only half a quail with 31.5 $\mu g \ g^{-1}$ w.w. (the highest concentration found in the pickled quails studied) per week would be enough to exceed the PTWI.

This risk assessment must be taken as only a first approach because the experiments have been designed to control some of the factors involved in the process of the transfer of Pb from shot to meat. However, further studies should address the actual number of Pb shot found in harvested small game and the risk of exposure in humans to Pb with real field samples which would include the variability due to the transfer of Pb along the shot path.

Many countries such as the USA, Canada, Denmark, Norway, Finland, Netherlands, Switzerland, Australia, Belgium, Cyprus, Ghana, Israel, Japan, Latvia, Malaysia,
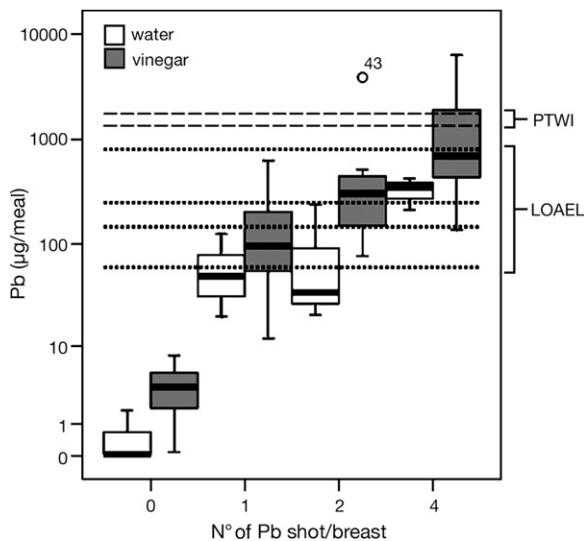


Fig. 4. Plot boxes (median, 25–75%, range) of Pb concentrations in breasts of pickled and non-pickled quails with different numbers of embedded Pb shot and relationships with several threshold levels established for dietary Pb exposure in humans. PTWI: provisional tolerable weekly intake for humans of 50–70 kg body weight (1350–1750 $\mu g$ week$^{-1}$). LOAEL: lowest observed adverse effects level for children $\leq$ 6 years old (60 $\mu g \ day^{-1}$), children $\geq 7$ years old (150 $\mu g \ day^{-1}$), pregnant women (250 $\mu g \ day^{-1}$) and adults (750 $\mu g \ day^{-1}$). These calculations of exposure are very conservative because a significant transfer of Pb can occur through the shot path before cooking the meat (Scheuhammer et al., 1998; Johansen et al., 2004).

Russia, South Africa, Sweden and United Kingdom have implemented total or partial bans on the use of Pb shot for hunting (Morehouse, 1992; Scheuhammer and Norris, 1996; AEWA, 2002). Since 2001, lead shot has been banned in Spain for hunting in protected wetlands only (Ministerio de Medio Ambiente, 2001). However, it is still widely used to kill upland game-birds and rabbits and it is exactly these game species which are more commonly cooked with vinegar. The increased use of non-toxic Pb shot alternatives would not only protect upland birds from Pb shot ingestion (Mateo et al., 2003; Soler-Rodríguez et al., 2004; García-Fernández et al., 2005), but also prevent exposure to Pb in humans who consume meat containing embedded shot residues.

## Acknowledgments

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