Nickel and copper residues in meat from wild artiodactyls hunted with nickel-plated non-lead rifle bullets

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Abstract A nickel (Ni)-plated copper-solid bullet type released up to 93 µg Ni /10 g bullet mass when immersed into meat juice for 7 days (to simulate fragments remaining in venison). A non-nickel-plated counterpart of identical construction released no Ni, but up to 250 µg copper. During thermal processing of pork cubes with embedded bullets, an average of 2.8 and up to 4.3 (maximum) µg Ni were released from the Ni-plated bullet to the surrounding meat. Average nickel and copper content in meat samples (taken in 2–3 cm distance from the shot wounds) from 30 roe deer (*Capreolus capreolus*) and 3 sika deer (*Cervus nippon*) killed with nickel-plated copper bullets did not differ significantly from those in controls (roe deer haunch). Contamination scenarios would Cu and Ni contents per portion increase moderately by 20 and 3.3 µg, respectively. In order to limit alimentary Ni uptake, the technological need for Ni-plating of bullets should be carefully evaluated.

Keywords Nickel · Copper, rifle bullets · Venison · Consumers' exposure

Introduction

Based on environmental, animal conservation as well as public health concerns on lead, it is widely accepted that bullet-

borne deposition of lead in the environment or in venison should be minimized (BfR 2010). “Non-lead” projectiles are available for this purpose (Thomas 2013), and it seems that precision and killing power issues can be or are already solved (Trinogga et al. 2013; Kanstrup et al. 2016). Concurrently, the deformation and/or fragmentation pattern of bullets/shot has been studied in more detail (Gremse et al. 2014). It has been shown that the energy transfer is less dependent of the bullets' materials used, but more on the constructional features and the velocity of the projectile. Also, non-fragmenting bullets can ensure adequate energy transfer to the animal's tissues. From a conservation point of view, non-fragmenting bullets or bullets fragmenting in few large pieces are preferred since raptors are able to avoid the ingestion of larger metallic particles (Nadjafzadeh et al. 2015). Likewise, such bullet types would ensure that contamination of venison by bullet fragments is kept to a minimum. However, it must be borne in mind that even a non-fragmenting projectile will deposit small metal flakes along the wound channel (Felsman et al. 2016), and that deforming bullets might lose larger single fragments during expansion. The review of Thomas et al. (2016) provides a comprehensive overview on all facets of non-lead hunting ammunition.

Currently available non-lead bullets can be conveniently divided in monolithic copper- or copper alloy types, occasionally with nickel coating or aluminium or plastic tip and such with jacket-core construction, e.g. a tin core replacing lead (Irschik et al. 2014; Paulsen et al. 2015a). Deposition of copper along the wound channel and migration of Cu from embedded bullet fragments during meat storage and preparation have been studied recently (Irschik et al. 2013, 2014; Schuhmann-Irschik et al. 2015; Paulsen et al. 2015a, b), and it was concluded that the amounts released would be of no significance for human health and also not be able to trigger meat spoilage via fat oxidation (Schuhmann-Irschik et al.

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2015; Paulsen et al. 2017). However, it could be shown that lead-free bullets with Ni-plating would release Ni during simulated digestion (Paulsen et al. 2015a) and the question was posed what amounts of Ni would be released from nickel-plated bullets when passing the animal's body or from fragments deposited in edible tissues. To this end, we examined the release of Ni from plated bullets in two model systems in comparison with a non-nickelled counterpart. In addition, we tested tissue from shot wounds of animals killed by two types of nickel-plated copper-solids. Finally, an estimate of foodborne exposure to Ni was made.

Materials and methods

Carcass samples

Samples were obtained from 33 wild ungulates (30 roe deer (*Capreolus capreolus*) and 3 sika deer (*Cervus nippon*)). Animals had been shot by nickel-plated copper-solids with either an open hollow point tip (Kalahari®, norma AB, Amotfors, Sweden; "K"-type) or a hollow tip covered with plastic (RWS HIT®, RUAG Ammotech, Fürth, Germany; "H"-type). Both entry and exit wounds were in the cranial-thoracic region. As samples were obtained from regular hunting events, there was a variation as regards calibres, shooting distance and animal weight. In more detail, 20 carcasses with HIT 8x57IS shot wounds could be sampled (17 roe deer and 3 sika; carcass weights 7–20 kg; shooting distance 30–150 m with a median of 85 m), and 13 roe deer with Kalahari shot wounds (.270 Winchester; .308 Winchester and 7 × 64; carcass weights 8.5–20 kg; shooting distance 40–140 m with a median of 60 m).

The shot wound had not been cleaned or trimmed during evisceration. Within 24 h post-mortem, muscle tissue surrounding entry and exit wounds was taken (radius of 3 cm from the shot wound). From 17 roe deer carcasses, an additional 25 g muscle sample (hind leg) was provided (control).

Cutting and mincing of samples was done with ceramic knives (Graefe, Germany) on polypropylene cutting boards, in order to avoid contamination with nickel from stainless steel surfaces. Blood clots and bruised tissues were removed, and only meat in a distance of 2–3 cm from the wound was taken. Such meat was chopped, vacuum packed and stored frozen at –25 °C until analysis. Samples were then portioned in 0.5 g subsamples for analysis. Within one sample, the number of 0.5 g subsamples varied from 2 to 7. These subsamples were analysed separately, but the results were averaged in order to represent the sample.

From five roe deer, samples of ruminal content were provided.

Experimental procedure to determine solubility of nickel and copper

For these experiments, HIT® and its non-nickel-plated counterpart (Barnes TTSX®, Barnes Bullets, Mona, USA, "T"-type), both 0.308 in. diameter and 10.7 g weight were used. Bullets were obtained by pulling from live rounds with an inertia hammer. For HIT bullets, the Ni-plating was scraped off from one third of the cylindrical part of the bullet, in order to simulate the abrasion taking place when the bullet passes the barrel rifling.

To estimate the solubility of nickel and copper from bullets in acidic liquids, bullets were placed tip-down in a reagent tube and 5 ml freshly pressed beef juice (pH 5.7) were added so that the bullet was completely covered with liquid. Tubes were then stored at 0–2 °C in the dark for 7 days under static conditions. Then, bullets were removed and copper and nickel concentration in the liquids were determined. For each bullet type, $n = 6$ specimens were tested.

In addition, the release of nickel and copper into meat was studied. Pork loin was obtained 24 h after slaughter and cut into 50 g cubes. In each cube, a bullet was inserted so that it was fully covered by meat. Meat cubes were vacuum packed and then placed in a water bath set at +72 °C and were boiled for 45 min. to +70 °C internal temperature. The bag was opened and the meat was allowed to reach room temperature. Subsequently, the meat cube was cut up with a ceramic knife and a 2-mm-thick meat slice around the bullets was removed; meat sticking on the bullet surface was removed with toothpicks. Meat was then chopped and stored at –20 °C until analysis. Per bullet type, five meat cubes were spiked with a bullet and tested. Number of control meat cubes was also five.

Determination of copper and nickel content

From meat samples, aliquots of 0.5 g were combined with 5.5 ml nitric acid (65%) and 1.5 ml hydrogen peroxide (30%) and subjected to microwave-assisted digestion (details see Irschik et al. 2013). Meat juice samples (4 ml) were fumed with 10 ml nitric acid (65%) and 0.6 ml hydrogen peroxide (30%), the residue was dissolved in 0.6 ml HNO₃ and made up to 20 ml with distilled water (Paulsen et al. 2015a).

Nickel contents were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Perkin-Elmer Optima 3000 XL; Perkin-Elmer, USA) using a program validated for meat and offal (Sager 2005). Each run contained two blanks in appropriate dilutions for adequate corrections. Copper was determined by flame AAS (Perkin-Elmer AAnalyst 300) in dilutions of the nitric acid-hydrochloric acid digest (Irschik et al. 2013). Limits of determination were 0.06 and 0.08 mg/kg (or µg/ml) for Ni and Cu, respectively.

Estimation of consumer's exposure to copper and nickel via meat from game shot by solid nickel-plated copper bullets

For the estimation of the nickel uptake, we used two approaches. For the first, the amount of Cu or Ni released from one bullet embedded into meat (juice) during storage or boiling was added to the metal content of a portion of 90 or 250 g (with average Cu or Ni content of the venison control samples; “background level”), and for the second, the median as well as the highest Cu or Ni content of a 0.5 g meat subsample in 2–3 cm distance from the shot wound was added to the metal content of a meat portion.

Statistical processing of data

Descriptive statistics were done with MS Excel. Data below the limit of determination (LOD) were set to $\frac{1}{2}$ LOD (“middle bound”). *T* test was used to determine if the release of Cu and Ni from bullets into meat juice or meat differed significantly from controls, with $P < 0.05$ as level of significance. Significance of differences between copper and nickel contents in tissues near to the shot wound to those of control samples was assessed by Kruskal-Wallis test. In case that the test yielded $P < 0.05$, differences between groups were examined by comparing the medians $\pm 1.96 \times$ standard errors of the medians (Lozan and Kausch 1998).

Results and discussion

Release of Ni and cu from bullets embedded in meat juice or meat

Nickel content in meat juice with nickel-free “T” bullets ($0.2 \pm 0.2 \mu\text{g Ni}/10 \text{ g bullet mass}$) was not significantly different from that of the control ($0.3 \pm 0.0 \mu\text{g Ni}$; $P > 0.05$). Conversely, the Ni-plated “H”-type released significantly higher amounts of Ni ($76.5 \pm 10.4 \mu\text{g}/10 \text{ g bullet}$; highest single result 92.6). The amount of Ni released from the “H”-type corresponds well to results presented for the Ni-plated Kalahari (“K”) bullet (Paulsen et al. 2015a). Significantly, more Cu was released from the non-plated “T”-type ($215.6 \pm 37.8 \mu\text{g}/10 \text{ g bullet mass}$) compared to control ($0.6 \pm 0.0 \mu\text{g}$), also in similar amounts as reported for other Cu-solids (i.e., Barnes TSX; Paulsen et al. 2015a). The Cu release from “H”-type ($31.1 \pm 10.1 \mu\text{g}/10 \text{ g bullet mass}$) was significantly higher than the Cu content of the control and can be explained by the exposed Cu surfaces on the cylindrical part of the bullet (where the Ni-cover had been scraped off). Since these were smaller in area than in the “T”-type, comparably lower amounts of Cu were released.

Metal contents in meat parts adjacent to the embedded bullet followed the same patterns as meat juice with embedded bullets. Cu contents in meat cubes with embedded “T”-type bullets were $66.1 \pm 27.9 \text{ mg Cu/kg}$ (maximum 102.7 mg/kg), and thus, significantly higher than in controls ($0.49 \pm 0.14 \text{ mg/kg}$; maximum 0.65 mg/kg), whereas the Cu content in meat with embedded “H”-type bullets was $4.3 \pm 3.1 \text{ mg Cu/kg}$ (maximum 8.49 mg/kg), and not significantly different from controls. Nickel contents in controls and in meat embedded with “T” bullets were $<0.06 \text{ mg/kg}$, and $1.4 \pm 0.8 \text{ mg Ni/kg}$ (maximum 2.14 mg/kg) in meat with embedded “H” bullets, corresponding to an average of $2.8 \mu\text{g Ni}$ per 2 g meat adjacent to the bullet.

Contents of cu and Ni in meat tissue near to the shot wounds

The median Cu values from meat near the shot wound (1.6 and 1.9 mg/kg for “H” and “K”) were somewhat higher than in the control (1.3 mg/kg), see Fig. 1. This was, however, of no statistical significance. Single 0.5 g subsamples contained up to 8.9 mg Cu/g, which was similar to findings for pure-copper bullets of comparable construction (Irschik et al. 2013). Contents of nickel were below the limit of detection (0.06 mg/kg) in most controls, with a maximum of 0.29 mg/kg, whereas the medians for meat near “H” and “K” shot wounds were 0.1 and 0.3 mg/kg, with corresponding maximum values of 0.2 and 1.8 mg/kg, respectively. Cu contents in control samples are in the range as reported in a recent study on Austrian game meat, with average contents of 1.3 and 1.6 mg/kg for red and roe deer, respectively (Ertl et al. 2016). These authors also determined Ni contents, and, similar to our study, contents were often below the limit of detection, in this case, $<1 \mu\text{g/kg}$ (whereas in our study, LOD was $60 \mu\text{g/kg}$). Because non-detectable concentrations have been

Fig. 1 Box-and-whiskers plot of Cu and Ni contents in meat in 2–3 cm distance from the shot wounds, in milligram per kilogram fresh matter. Thick horizontal bar indicates the median; the box, the first and third quartile. Ranges indicate minimum and maximum metal content per sample. Blank denotes control from distant meat parts (haunch, $n = 17$); H, the “H” bullet type ($n = 20$); and K, the “K” bullet type ($n = 13$)

assumed to be half of the detection limits, Ertl et al. (2016) report lower average Ni contents for venison than we do.

Within a sample, the variation between the 0.5 g subsamples was sometimes considerable. For “K”-type, the ratio for highest to lowest copper content within a sample was >3:1 for 3/13 samples, with a maximum ratio of 18.1:1 and a maximum single result of 34.9 mg Cu/kg meat. Ratio of Ni contents within a sample was >3:1 for 5/13 samples, with a maximum single result of 6.7 mg Ni/kg meat. For “H”-type, the ratio for highest to lowest copper content within a sample was ≤3:1 for all 20 samples, with a maximum ratio of 2.1:1 and a maximum single result of 6.9 mg Cu/kg meat. Likewise, ratio of Ni contents within a sample was >3:1 for 8/20 samples, with a maximum single result of 1.4 mg Ni/kg meat. In sum, “K”-type subsamples had higher maximum Ni and Cu contents, which was considered when meat-borne exposure to Cu and Ni was estimated.

Contents of cu and Ni in rumen content

Median Cu level in rumen content was 1.7 mg/kg fresh matter, with a range from 1.4 to 2.3 mg/kg, whereas the median Ni content was 0.1 mg/kg, with a maximum of 0.3 mg/kg fresh matter. Since inexperienced shots or failures during evisceration can cause contamination of venison with rumen content or faeces, sampling of such contaminated venison could result in biased Cu and Ni results. We tried to avoid such bias by sampling only carcasses with cranial-thoracic shot wounds and without visible “green” contamination of the meat.

Estimates for copper and nickel intake via consumption of meat from game

Estimates for the copper and nickel uptake are shown in Table 1. In essence, the additional quantity of Cu would be

in the range of 10–20 µg per portion, which is a negligible amount compared to a tolerable daily intake of 35 mg (based on a TDI of 0.5 mg/kg; JECFA 1982). As the surfaces were Ni-plated, it is conceivable that the amounts of copper are lower than those reported for Cu-solids without Ni-plating (Irschik et al. 2013; Paulsen et al. 2015a).

Nickel is known to elicit a variety of adverse health effects (EFSA 2015; Thomas 2016). For oral uptake of Ni, EFSA (2015) derived benchmark dose levels of 1.1 and 2.8 µg/kg body mass for acute (systemic chronic dermatitis) and chronic (foetal loss) adverse health effects. As expected, nickel amounts in median contamination scenarios did not differ from that of control. Based on 70 kg body mass, the consumption of a 250 g portion with maximum contamination would contribute to 28% or 11% of the benchmark dose levels for acute or chronic adverse health effects of Ni. This calculation includes both natural nickel content as well as bullet-borne nickel contamination. As regards the naturally occurring Ni content, actual contents might be lower than we calculated (“middle bound” approach), since studies with a lower limit of detection reported lower Ni contents in venison (Ertl et al. 2016).

Maximum concentrations of Ni in venison attributable to Ni-plated bullets reached 4.3% of the EFSA benchmark dose for acute and 1.7% for chronic adverse health effects. To avoid any kind of additional Ni contamination, it should be evaluated if ballistic benefits (e.g. reduced barrel fouling) outweigh any possible negative health effects. Permanent contact to nickel can cause allergic reactions such as skin irritations.

Conclusions

Nickel-plated copper-solids will release measurable quantities of nickel when stored in meat juice and—in lower amounts—

Table 1 Estimates for the copper and nickel uptake via game meat

Contamination scenario	Bullet ^d	Cu, mg per portion		Ni, µg per portion	
		90 g	250 g	90 g	250 g
Control, background level ^a	None	0.13	0.37	6.75	18.75
Background level plus average Cu (4.3 µg/g)/Ni (1.4 µg/g) in 2 g meat adjacent to embedded bullet ^b	“H”	0.14	0.38	9.40	21.40
Background level plus median Cu (1.9 µg/g)/Ni (0.3 µg/g) content in 0.5 g meat ^c	“H”	0.13	0.37	6.76	18.76
	“K”	0.13	0.37	6.86	18.86
Background level plus highest Cu (34.9 µg/g)/Ni (6.66 µg/g) content in 0.5 g meat ^c	“H”	0.13	0.37	7.00	19.00
	“K”	0.15	0.38	10.04	22.04

^a Example how calculation was made: Median Cu content in controls (1.5 µg/g) × 90 (g) = 135 µg or 0.13 mg Cu per 90 g portion; corresponding concentration for Ni was 0.075 µg/g, which resulted in 6.75 µg Ni per 90 g portion

^b Example, Ni in 90 g portion was calculated as 88 g of meat from control (0.075 µg/g) = 6.6 µg plus content in 2 g meat near bullet (1.4 µg/g) = 2.8 µg; the sum is 9.4 µg

^c Subsample in 2–3 cm distance from the shot wound^d It was assumed that per portion (90 or 250 g), one bullet was embedded.

when heated in a meat matrix. In wild game killed with nickel-plated bullets, meat in 2–3 cm distance from the shot wounds had the same median Cu and Ni contents than meat from distant region (haunch). Only maximum contamination scenarios did increase Cu and Ni contents for ca. 20 and 3.3 µg, respectively. Whereas the increase in Cu contents is of no concern, the known adverse effects of Ni should restrict its use, especially as at least for some bullet types, equivalent non-nickel-plated bullets made of copper are available. Higher Ni levels in venison could be a food safety concern, but more likely in terms of contamination—which could render meat unfit for consumption (EC 2002)—rather than posing a health hazard.

Data were obtained by testing the entire bullets and not fragments; thus, the actual quantities of Ni deposited in tissues could be lower, when smaller fragments with correspondingly smaller outer surfaces come in contact with meat.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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