

## **Wildlife and Human Health Risks from Lead-Based Ammunition in Europe**

### **Scientific Advances 2014 and 2015**

In 2014 a group of internationally acknowledged scientists signed and published a scientific consensus statement on **Wildlife and Human Health Risks from Lead-Based Ammunition in Europe**<sup>1</sup>, in which the overwhelming scientific evidence on the toxic effects of lead on human and wildlife health has been summarised.

Since the publication of that paper further independent research has been carried out in countries across the European Union on topics, which specifically address the use of lead (Pb) as a component material in hunting ammunition. Links to these studies and summaries are provided in the annex of this paper.

The results of all of these independent studies, both on rifle bullets and for shotgun ammunition, further strengthen the arguments based on the overwhelming evidence for the toxic effects of lead in humans and wildlife, even at very low exposure levels, by adding to the convincing data that the discharge of lead based ammunition into the environment poses significant risks of lead exposure to humans and wildlife, and showing, that the availability and suitability of non-lead alternative products for hunting can be ensured.

On October 20th, 2015, the „Association of European Manufacturers of Sporting Ammunition<sup>2</sup> and the World Forum on Shooting Activities<sup>3</sup> are organising a Symposium<sup>4</sup> under the title

“The Sustainable Use of Lead Ammunition in Hunting and Sports Shooting: Facts and Emotions”

, focusing on the sustainable use of lead in ammunition and its impact on both the environment and human health.

While aiming to “enable delegates to meet a wide cross-section of key leaders in hunting and sports shooting activities, in the fields of research, science, and legislation“, none of the research detailed herein has been included in the programme of this event.

We hereby wish to underline our commitment to make our research publically available, raise awareness for these results and invite all participants into a dialogue based on the scientific evidence.

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<sup>1</sup> <http://www.zoo.cam.ac.uk/departments/conservation-science/European-Statement>

<sup>2</sup> [www.afems.org](http://www.afems.org)

<sup>3</sup> [www.wfsa.net](http://www.wfsa.net)

<sup>4</sup> [www.leadSYMPOSIUM.eu/default.aspx](http://www.leadSYMPOSIUM.eu/default.aspx)

Signed,

**Carl Gremse, M. Sc.**

**Prof. Dr. Siegfried Rieger**

Eberswalde University for sustainable Development (FH)

Departement for Wildlife Biology, - Management & Hunting Practices (FWWJ)

Alfred-Möller-Strasse 1, 16228 Eberswalde

[www.hnee.de](http://www.hnee.de)

**Univ.Prof. Dr. Klaus Hackländer**

University of Natural Resources and Life Sciences, Vienna (BOKU)

Institute of Wildlife Biology and Game Management

Gregor-Mendel-Str. 33, 1180 Vienna, Austria

[www.iwj.at](http://www.iwj.at)

**Ass.-Prof. Dr.med.vet. Peter Paulsen**

Institute for Meat Hygiene, Meat Technology and Food Science

Department of Farm Animal and Public Health in Veterinary Medicine

University of Veterinary Medicine Vienna

[www.vetmeduni.ac.at](http://www.vetmeduni.ac.at)

**Dr. med. vet. Oliver Krone,**

Veterinary specialist for zoo & captive and wild animals, Leibniz

Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Strasse, 17, D-10315 Berlin, Germany

[www.izw-berlin.de](http://www.izw-berlin.de)

**Rafael Mateo Soria, PhD**

Group of Wildlife Toxicology

Instituto de Investigación en Recursos Cinegéticos (IREC) -Spanish Institute of Game and Wildlife Research CSIC-UCLM-JCCM, Ronda de Toledo s/n, 13071 Ciudad Real, Spain

**Prof. Vernon G. Thomas,**

Professor Emeritus, Department of

Integrative Biology, College of Biological Science, University of Guelph, Guelph,

Ontario N1G 2W1, Canada

**Dott. Alessandro Andreotti**

Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA)

Via Ca' Fornacetta 9, 40064 Ozzano Emilia (BO)

[www.isprambiente.gov.it](http://www.isprambiente.gov.it)

**Biol. Niels Kanstrup**

Dansk Jagtakademi

Skrejrupvej 31, 8410 Rønne

[www.danskjagtakademi.dk](http://www.danskjagtakademi.dk)

**Dr. Ruth Cromie,**

Head of Ecosystem Health, Wildfowl & Wetlands Trust, Slimbridge,

Gloucestershire GL2 7BT, UK

<http://www.wwt.org.uk>

**DI Dr. Friedrich Völk**

Österreichische Bundesforste AG

Pummgasse 10-12,

A-3002 Purkersdorf

[www.bundesforste.at/jagd](http://www.bundesforste.at/jagd) **Research from Germany:**

## Further investigations into the killing effect of non-lead rifle ammunition

[http://www.bmel.de/SharedDocs/Downloads/Landwirtschaft/Wald-Jagd/BLE-Forschungsbericht-Jagdmunition.pdf?\\_\\_blob=publicationFile](http://www.bmel.de/SharedDocs/Downloads/Landwirtschaft/Wald-Jagd/BLE-Forschungsbericht-Jagdmunition.pdf?__blob=publicationFile)

In this study for the Federal German Ministry for Food and Agriculture, 11,371 standardised reports on the harvest of game (roe deer, red deer, fallow deer and wild boar) in hunting practice in Germany were analysed. 68 bullet types were used. Both lead and non-lead bullets were used. For 15 bullet types (8 non-lead, 6 lead) laboratory terminal ballistic testing was conducted representing the bullet velocities present in hunting practice. For 2,881 field reports, situation specific terminal ballistic data were obtained, allowing observational data to be linked with bullet performance data. Strong effects of the variables “shot placement”, “animal mass” and “terminal ballistic performance” influencing the distance, an animal runs after being shot (running distance) were detected. **Bullet material was shown to have no impact on the running distance, when terminal ballistic performance is above a threshold of 1,500 Joules in centimetres 0-15 in simulant material, that has been established as a result in this study.**

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0102015>

This study compares terminal ballistic testing methods in ballistic soap. 36 blocks of ballistic soap were shot over a range of impact velocities and analysed using two methods. Method 1 used a novel approach to analyse computed tomography data of the 36 blocks of ballistic soap. This non-invasive method was used to measure the terminal ballistic performance of the tested bullet at each impact velocity and was used to measure the bullet material deposited at each impact velocity. Method 2 was the traditional approach of measuring the cavities in segmented blocks of material using scaled photography. A very close correlation was found, validating the use of Method 1 for terminal ballistic testing. This method also greatly enhances the value of such testing by very accurately detecting size and shapes of bullet particles deposited in the target material. **The direct comparison of three non-lead-bullets and one lead bullet, frequently used in the above field trials showed, that a) non-lead-bullets can be equally terminally effective as the tested lead bullet and b) deposit significantly less material into the target.**

[http://www.wageningenacademic.com/doi/pdf/10.3920/978-90-8686-238-2\\_30](http://www.wageningenacademic.com/doi/pdf/10.3920/978-90-8686-238-2_30)

A summary chapter on the projects carried out in Germany; also detailing the aspects of food safety of game meat produced through hunting (in English).

## Aspects of Food Safety of game meat produced through hunting

<http://www.bfr.bund.de/cm/350/alles-wild-bfr-symposium-zu-forschungsvorhaben-zum-thema-wildbret-tagungsband.pdf>

In 2012 a research program into the deposition of bullet material into marketable meat was launched by German federal and state governments in cooperation with the private sector (meat processors, vendors and ammunition manufacturers, and federal and state non-governmental organisations). The scope of this study was to monitor the content of lead (Pb), copper (Cu) and zinc (Zn), in marketable game meat and to determine which fractions are attributable to bullets and which to other environmental sources. The results were presented in 2014 at a BfR-Symposium<sup>5</sup>. Using non-lead bullets significantly reduces lead content in marketable game meat. Use of leaded bullets of “bonded” construction – a process countering the separation of the bullets jacket and its lead core on impact – does not reduce the lead content of marketable game meat shot with such bullets, when compared to lead bullets of conventional construction.

## Lead exposure and food processing in white-tailed eagles and other scavengers

<http://link.springer.com/article/10.1007%2Fs10344-015-0953-1>

Lead poisoning caused by feeding on shot game animals containing fragments of lead-based bullets is a serious hazard for avian scavengers. Both obligate and facultative scavenging species are affected, such as the **white-tailed sea eagle (*Haliaeetus albicilla*)** for which **lead intoxication is the most important mortality factor**. However, the exploitation of hunter-killed animals by facultative avian scavenger communities is little studied. Here, we used an experimental approach to identify lead-exposed species and explore the food processing of white-tailed sea eagles and other scavengers. We conducted feeding experiments with free-ranging scavengers in six eagle home ranges in north-eastern Germany and with six eagles temporarily held in captivity. We provided ungulate carcasses containing non-toxic iron particles of different diameters, simulating bullet fragments, as a food source. Primarily, avian scavengers such as ravens, eagles and buzzards exploited the carcasses, indicating them to be exposed to lead fragments and poisoning. With increasing diameter of experimental particles, free-ranging scavengers and captive eagles increased the percentage of

<sup>5</sup> [http://www.bfr.bund.de/de/veranstaltung/bmelv\\_bfr\\_symposium\\_alle\\_s\\_wild\\_stand\\_der\\_wissenschaft\\_zum\\_verhalten\\_bleifreier\\_munition](http://www.bfr.bund.de/de/veranstaltung/bmelv_bfr_symposium_alle_s_wild_stand_der_wissenschaft_zum_verhalten_bleifreier_munition)

particles avoided during feeding. Scavengers almost completely avoided fragments of 8.8 mm diameter. Behavioural observations of captive eagles showed that they detected iron particles predominantly by touching the experimental carcass with their bill tip, suggesting that they use mechanoreceptors to judge food quality. Our findings indicate that the use of bullets that deform or fragment into particles greater than 9 mm in size, such as numerous lead-free bullets, may prevent metal ingestion and poisoning in avian scavengers.

## Research from Austria:

### Suitability of non-lead rifle ammunition for hunting practice

[http://www.dib.boku.ac.at/fileadmin/data/H03000/H83000/H83200/NEWS/Bleifreie\\_Buechsenmunition\\_Bericht\\_IWJ.pdf](http://www.dib.boku.ac.at/fileadmin/data/H03000/H83000/H83200/NEWS/Bleifreie_Buechsenmunition_Bericht_IWJ.pdf)

In this study, 1226 protocols on hunting events by professional hunters covering 55 variables on hunter, rifle, ammunition, shot conditions, hit point, behaviour of game (roe deer, red deer, sika deer, fallow deer, chamois, mouflon, wild boar and marmot) and game meat evaluation were analysed. The protocols compile the use of 15 expandable bullet types in 14 calibres. Apart from three established lead bullet types, 12 lead-free bullet types were used. The statistical analysis with the help of regression trees revealed that the bullet material (lead vs. non-lead) did not affect killing efficacy, blood trails, or evaluation of game meat quality. Instead, other factors such as hit point, exit wound size, calibre etc. were important. These results are in line with various studies and **underline the general option to switch from lead to lead-free rifle ammunition.**

### Release of metals from bullet fragments during storage and food ingestion

<http://link.springer.com/article/10.1007%2Fs10344-015-0926-4>

<http://www.sciencedirect.com/science/article/pii/S0309174015300061>

A major part of this work focuses on model studies to assess the amount of copper (Cu) in meat from game hunted with Cu-bullets, and the release of Cu from embedded fragments into the surrounding meat during meat storage, culinary processing, ingestion and digestion. In essence, no indication was found that a single embedded Cu fragment per meat portion would be of toxicological relevance. The release of metals from selected „lead-free“ bullets after embedding-ingestion-digestion depended on the nature of the metal and on its accessibility (i.e. exposed surfaces). For food safety reasons, the release of Al, Ni, Pb from bullets should be critically evaluated. When venison with embedded Cu bullets was subjected to different culinary processing procedures, the amount of Cu released from the embedded bullet was affected primarily by the retention period of the bullet in the meat during cool storage, but much less by the different heating protocols. The presence of Cu fragments had no significant effect on secondary products of fat oxidation as well as on primary\* fat oxidation products. Cu doping of 7-28 mg/kg as dust or sulphate\* did not increase fat oxidation. In an attempt to provide a first estimate of exposure assessment of consumers who would ingest meat with embedded rifle bullet fragments, we simulated the release of metals to the meat environment as well as into gastric or duodenal juice. Studies comprised: a) elemental composition of eight commercially available lead-free bullets (0.308-in. calibre); b) the release of metal when bullets were kept for 7 days in meat juice (0–2 °C), to simulate embedded bullet fragments; and c) the quantities of metals released when bullets were subjected to a combined artificial gastric and duodenal digestion, to simulate accidental ingestion of meat containing bullet fragments. In six of the eight bullet types, Pb was <0.02% of bullet mass. Release of Cu and Zn into meat juice did not exceed 0.0025 and 0.0015% of the bullet mass, respectively. During simulated digestion, bullets released ≤0.01 % Cu and ≤0.0025% Zn. From these data, a worst-case was calculated to amount to an additional quantity of 2.03 mg Fe, 1.18mg Cu and 0.39 mg Zn (originating from 10 g embedded bullet material) per meat portion, which still would not exceed the recommended daily intake values. Aluminium tips of bullets released a max. of 0.61 mg Al per bullet (normalized to 10 g). In contrast, release of nickel from coatings and of lead from alloys may be of concern. Although experiments were conducted in simulated environments, results indicate that Al, Ni and Pb contents in rifle bullets should be kept as low as possible.

In another study\*\*, we assessed what number of metal fragments could be expected in meat from animals shot with certain lead-free rifle bullets. Some 108 meat packs obtained from hunted animals and pork shoulder experimentally contaminated by shooting it with bullets were examined for radiodense fragments and after storage and boiling, subjected to simulated gastric and duodenal digestion. In both the digested and undigested fraction, contents of Al, Cr, Cu, Fe, Ni, Pb, Zn and other elements were determined. Whereas the metal contents in the digested fractions did not differ markedly from those in controls, higher metal contents were detected in undigested residues of three samples (indicating metal fragments), whereas in the other samples, dense particles were obviously bone material (assessed by P and Ca levels). But even in the meat portions with high metal contents, the digested and thus, bioavailable fraction of metals was in the same range as in meat portions with low metal levels. Although the data originate from a small sample set and should be considered a preliminary finding, the approach of studying meat portions instead of small

aliquots - and the assessment, which fraction of metal particles will actually be available for absorption - has its merits, when contamination with metal particles is studied.

\*Book chapter in press

\*\*in: Paulsen et al. (2015) J.Food Safety and Food Quality 66:128-131

## Research from Denmark:

### Suitability of Non-Lead Ammunition for the hunting of Mammals

[http://www.danskjagtakademi.dk/fileadmin/user\\_upload/NK/Bly/150615\\_Blyfri\\_riffelammunition\\_RAPPORT.pdf](http://www.danskjagtakademi.dk/fileadmin/user_upload/NK/Bly/150615_Blyfri_riffelammunition_RAPPORT.pdf)

Lead rifle ammunition is allowed for hunting in Denmark. Despite the focus on the subject in neighbour countries such as Germany and Sweden, there is no governmental and public interest in the matter in Denmark. However, due to the risk of lead contamination of human game meat consumers, predators, scavengers and ecosystems an increasing number of individual private hunters wish to improve their hunting practice and ask for more knowledge to switch to non-lead alternatives. Particularly, hunters ask for guidance concerning efficacy and lethality of non-lead ammunition. With this background The Danish Academy of Hunting, which is an individual consultant, took the initiative to begin this project in 2012. "15. Juni Fonden" supported it financially. Aarhus University has assisted with statistical analyses of the material. During three hunting seasons 15 Danish hunters shot 657 animals. Most animals were shot in Danish districts, some in Sweden and Ireland, a few in other countries. Normal Danish hunting types were used: High seat, stalking, driven. All hunters used standard rifle calibres with lead and non-lead (copper) bullets. Almost 50% of the sample was red deer, 25% roe deer and the remaining 25% distributed on other species such as wild boar, sika and fallow deer. The most common calibres used were 30-06, 308, 6,5x55 and 270 (totally 75% of the sample) and the remaining distributed on small calibres like .222 and .223 and big calibres like 9,3-62. Some 30% of the sample was taken with lead bullets (a majority RWS, Lapua and Nosler), 70% with copper bullets (a majority Barnes, Lapua and Hornady). Shooting distances ranged from 7 to 380 meters, with 80% < 100 m. Flight distances ranged from 0 to 1,500 meter, with 90% < 100 m. Precision of the shot was measured roughly by monitoring frequency of hits in the following anatomic sections: head, neck, anterior body (low and high), abdomen (low and high), haunch and legs (front and hind). Movement of the animal (in the moment of the shot) was registered in the following categories: "stands", "walks", "runs" and "gallops". All hunters involved had extensive experience and during the last data collection season (2014/2015) participants were asked to give their overall evaluation of the single shot episode. This was monitored in a simple "smiley" system: "happy", "neutral", "un-happy". Flight distance (=escape distance) was used as the overall response parameter to test the efficacy of ammunition. The effect of lead versus copper cannot be separated from effects of shooting distance, bullet weight and precision of the shot. Further, flight distances differ between species. Hence, our statistical model incorporated these conditions, and testing was made only on three groups of species: red deer, roe deer and fallow/sika deer. Overall, the study shows that there is no consistent and significant difference in flight distance for lead and copper ammunition. We conclude that the tested non-lead bullets have an efficacy similar to traditional lead ammunition and meet all lethality requirements for ammunition used in traditional hunting in Denmark. This corresponds with findings concluded in other, similar studies, e.g. Knott et al. (2010) and Gremse and Rieger (2012). From an animal welfare point of view, non-lead ammunition within the tested range of calibres can be recommended as an alternative to lead ammunition.

## Research from Spain:

### Reducing Pb poisoning in birds and Pb exposure in game meat consumers: the dual benefit of effective Pb shot regulation.

Mateo R, Vallverdú-Coll N, López-Antia A, Taggart MA, Martínez-Haro M, Guitart R, Ortiz-Santaliestra ME. Environment International 2014 Feb;63:163-8.

<http://www.sciencedirect.com/science/article/pii/S0160412013002663>

The use of lead (Pb) ammunition in the form of shot pellets has been identified as a Pb exposure risk in wildlife and their human consumers. We explore the hypothesis that Pb shot ban enforcement reduces the risk of avian Pb poisoning as well as Pb exposure in game meat consumers. We assessed compliance with a partial ban on Pb shot commencing in 2003 by examination of 937 waterbirds harvested by hunters between 2007 and 2012 in the Ebro delta (Spain). Prevalence of Pb shot ingestion was determined, as were Pb concentrations in liver and muscle tissue to evaluate the potential for Pb exposure in game meat

consumers. Hunted birds with only embedded Pb shot (no steel) declined from 26.9% in 2007-08 to <2% over the following three hunting seasons after ban reinforcement. Pb shot ingestion in mallards decreased from a pre-ban value of 30.2% to 15.5% in the post-ban period. Liver Pb levels were predominantly defined by the presence of ingested shot, whereas muscle levels were defined by the presence of both ingested and embedded shot. Only 2.5% of mallard muscle tissue had Pb levels above European Union regulations for meat (0.1µg/g wet weight) in the 2008-09 season, when Pb shot ingestion prevalence was also at a minimum (5.1%). Effective restrictions in Pb ammunition use have a dual benefit since this reduces Pb exposure for game meat consumers due to embedded ammunition as well as reducing Pb poisoning in waterbirds.

### **Altered immune response in mallard ducklings exposed to lead through maternal transfer in the wild.**

Vallverdú-Coll N, López-Antia A, Martínez-Haro M, Ortiz-Santaliestra ME, Mateo R.  
Environmental Pollution 2015 Oct;205:350-6.

<http://www.sciencedirect.com/science/article/pii/S0269749115003073>

Lead (Pb) poisoning has caused significant mortality in waterfowl populations worldwide. In spite of having been banned since 2003, prevalence of Pb shot ingestion in mallards (*Anas platyrhynchos*) from the Ebro delta was still 15.5% in 2011-12. We collected mallard eggs from this area to study the effects of maternally transferred Pb on eggshell properties and on immune response and oxidative balance of ducklings. Eggshell Pb levels were positively correlated with Pb levels in the blood of ducklings. Ducklings with blood Pb levels above 180 ng mL<sup>-1</sup> showed reduced body mass and died during the first week post hatching. Blood Pb levels positively correlated with humoral immune response, endogenous antioxidants and oxidative stress biomarkers, and negatively correlated with cellular immune response. Pb shot ingestion in birds can result in maternal transfer to the offspring that can affect their developing immune system and reduce their survival in early life stages.

### **Sublethal Pb exposure produces season-dependent effects on immune response, oxidative balance and investment in carotenoid-based coloration in red-legged partridges.**

Vallverdú-Coll N, Ortiz-Santaliestra ME, Mougeot F, Vidal D, Mateo R.  
Environmental Science & Technology 2015 Mar 17;49(6):3839-50.

<http://pubs.acs.org/doi/abs/10.1021/es505148d>

Ingestion of lead (Pb) shot pellets constitutes the main cause of Pb poisoning in avifauna. We studied the effects of sublethal Pb exposure on immunity, carotenoid-based coloration, oxidative stress and trade-offs among these types of responses during spring and autumn in red-legged partridges (*Alectoris rufa*). We evaluated constitutive immunity testing lysozyme and natural antibody levels, and blood bactericidal and phagocytic activities. We studied induced immunity by testing phytohaemagglutinin (PHA) and humoral responses. We analyzed fecal parasite and bacterial abundance and oxidative stress biomarkers. Pb exposure in spring reduced natural antibody levels, whereas in autumn, it reduced lysozyme levels and increased phagocytic activity. Pb exposure increased PHA response in both seasons, and decreased T-independent humoral response in autumn. Pb exposure also increased noncoliform and decreased coliform Gram-negative gut bacteria. In spring, Pb exposure decreased antioxidant levels and increased coloration in males, whereas in autumn, it increased retinol levels but reduced coloration in both genders. Our results suggest that in spring, Pb-exposed females used antioxidants to cope with oxidative stress at the expense of coloration, whereas Pb-exposed males increased coloration, which may reflect an increased breeding investment. In autumn, both genders prioritized oxidative balance maintenance at the expense of coloration.

### **Effects of lead shot ingestion on bone mineralization in a population of red-legged partridge (*Alectoris rufa*).**

Álvarez-Lloret P, Rodríguez-Navarro AB, Romanek CS, Ferrandis P, Martínez-Haro M, Mateo R.  
Science of the Total Environment 2014 Jan 1;466-467:34-9.

<http://www.sciencedirect.com/science/article/pii/S0048969713007559>

The effect of lead (Pb) toxicity on bone mineralization was investigated in a wild population of red-legged partridge (*Alectoris rufa*) inhabiting a farmland area contaminated with Pb-shot from recreational hunting activities in Albacete, a southeastern province of Spain. Femora from 40 specimens of red-legged partridge

were analyzed for Pb by graphite furnace atomic absorption spectroscopy (GF-AAS), and for bone composition by Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD). The FTIR and DRX data of bone were analyzed in detail to determine possible alterations in bone mineral chemistry and crystallinity due to Pb toxicity. Results showed a marked decrease in the degree of mineralization as Pb concentrations in bone tissue increased while XRD analyses showed that the crystallinity of apatite crystals increased with the Pb load in bone. These load-dependent effects are indicative that Pb contamination altered bone remodeling by reducing new bone mineral formation and demonstrate that bone quality is a sensitive indicator of adverse effects on wild bird populations exposed to Pb pollution.

### ***In vitro* evaluation of cell death induced by cadmium, lead and their binary mixtures on erythrocytes of Common buzzard (*Buteo buteo*).**

Hernández-García A, Romero D, Gómez-Ramírez P, María-Mojica P, Martínez-López E, García-Fernández AJ.

Toxicology In Vitro. 2014 Mar;28(2):300-6.

<http://www.sciencedirect.com/science/article/pii/S0887233313003093>

Cadmium and lead are persistent and ubiquitous metals that can cause several deleterious effects in living beings. Apoptosis and necrosis are two types of cell death that can be found after in vivo and in vitro exposure to these metals. In this study, isolated red blood cells from living captive Common buzzard (*Buteo buteo*) were exposed in vitro to different concentrations of lead, cadmium, and the mixture lead-cadmium in a proportion of 1:10 (similar to that found in previous field studies). Data obtained from dose-response curves were used to evaluate the interactive effects of metal mixtures on cell viability. In general, except for the exposure to NOEC, additivity was the most frequently observed response. As described in human, after in vitro exposure, lead was highly accumulated in buzzard erythrocytes, while cadmium accumulation was scarce. Finally, the type of cell death (apoptosis or necrosis) induced by the exposure to different concentrations of these heavy metals and their mixtures was evaluated in the red blood cells. Apoptosis was found to be the main type of cell death observed after cadmium and/or lead exposure. However, this exposure caused an increase in lysis or necrosis, especially if red blood cells were exposed to high doses.

### **Effects of heavy metals on biomarkers for oxidative stress in Griffon vulture (*Gyps fulvus*).**

Espín S, Martínez-López E, Jiménez P, María-Mojica P, García-Fernández AJ.

Environmental Research 2014 Feb;129:59-68.

<http://www.sciencedirect.com/science/article/pii/S0013935114000024>

Metals are involved in the formation of reactive oxygen species (ROS) which may result in metal-related oxidative stress that can lead to oxidative damage to lipids, DNA and proteins. It is necessary to understand the mechanisms of metal toxicity in wild birds, and the concentrations that cause effects on oxidative stress biomarkers. The aim of this study is to assess the concentrations of lead (Pb), cadmium (Cd), mercury (Hg), copper (Cu) and zinc (Zn) with regards to oxidative stress in blood samples of 66 Griffon vultures (*Gyps fulvus*) from two areas of the Autonomous Community of Valencia (East of Spain). The two study areas (Alcoy n=36 and Cincorres n=30) were selected as random locations of interest that had not yet been studied, and are feeding stations where supplementary food, mainly of pork origin, is provided for vultures. Given that the two study areas are not considered polluted sites, we expected to find low metal concentrations. However, there are no known threshold concentrations at which metals can affect antioxidant systems, and low metal levels may have an effect on antioxidant biomolecules. In this study, since sampling was done at the beginning of the hunting season, the low Pb levels found in most Griffon vultures from Alcoy and Cincorres (median=12.37 and 16.26µg/dl, respectively) are suggestive of background levels usually found in vultures that feed on pork carcasses all year round. The ingestion of game meat with bullet fragments in carcasses or with Pb shots embedded in the flesh could be the cause of the high blood Pb concentrations found in three vultures from Cincorres (83, 290 and 362µg/dl). Griffon vultures feeding in Cincorres had enhanced CAT and GST activities and tGSH concentrations, which may be interpreted as protective response against the higher TBARS levels. This study provides threshold concentrations at which metals affect antioxidant system derived from 66 samples of Griffon vulture. Blood Cd concentrations greater than 0.05µg/dl produced an induction of 33% in GPx and of 44% in CAT activity in erythrocytes of vultures from Alcoy. Hg concentrations in blood higher than 3µg/dl produced an induction of 10% in SOD activity. Concentrations of Pb above 15µg/dl in blood produced an inhibition of 12.5% in GPx and 11.3% in CAT activity, and a TBARS induction of 10.7% in erythrocytes of Griffon vultures.

## **Delta-aminolevulinic acid dehydratase ( $\delta$ ALAD) activity in four free-living bird species exposed to different levels of lead under natural conditions.**

Espín S, Martínez-López E, Jiménez P, María-Mojica P, García-Fernández AJ.  
Environ Res. 2015 Feb;137:185-98.

<http://www.sciencedirect.com/science/article/pii/S001393511400468X>

The purposes of this study were: (1) to determine the  $\delta$ ALAD activity and  $\delta$ ALAD ratio in blood of four free-living bird species (Griffon vulture, Eagle owl, Slender-billed gull and Audouin's gull); (2) and to investigate the correlations between  $\delta$ ALAD activity/ratio and Pb concentrations in blood samples. A decrease was observed in  $\delta$ ALAD activity in Griffon vultures and Eagle owls exposed to Pb. In addition, negative relationships were found between  $\delta$ ALAD ratio or  $\delta$ ALAD activity and Log blood Pb levels in Griffon vultures and Eagle owls, and these relationships were stronger in areas with the highest Pb exposure. We provide equations that may be helpful to estimate  $\delta$ ALAD activity and  $\delta$ ALAD ratio using blood Pb concentrations. Regarding gull species,  $\delta$ ALAD activity found in the present study may be considered the normal activity in Slender-billed gull and Audouin's gull species, since very low blood Pb concentrations and no correlations were found in these species. Although both  $\delta$ ALAD activity and  $\delta$ ALAD ratio are sensitive biomarkers of Pb exposure and effect in birds, the use of  $\delta$ ALAD ratio may improve the results. Besides, this study provides blood threshold concentrations at which Pb bears effects on  $\delta$ ALAD enzyme (5 $\mu$ g/dl in Eagle owl; 8 $\mu$ g/dl in Griffon vulture; and probably >2 $\mu$ g/dl in Slender-billed gull and Audouin's gull). Our findings show that Eagle owl seems to be more sensitive to  $\delta$ ALAD enzymatic inhibition by Pb than Griffon vultures. Eagle owls and Griffon vultures exhibited up to 79% and 94% decrease in  $\delta$ ALAD activity when blood Pb concentrations exceeded 19 and 30 $\mu$ g/dl, respectively. Regarding the effects related with  $\delta$ ALAD inhibition, significant negative correlations were found between  $\delta$ ALAD activity and hematocrit in Eagle owls and Griffon vultures, which may be related to compensatory response associated with a decrease in  $\delta$ ALAD activity. In addition, an effect on creatine kinase activity and total proteins in plasma was found in Griffon vultures. The significant negative correlations found between  $\delta$ ALAD activity and tGSH in Griffon vulture, and between  $\delta$ ALAD activity and Log CAT activity in Eagle owls, may be related to a protective response of antioxidant system against reactive oxygen species (ROS). The negative relationship found between  $\delta$ ALAD activity and TBARS levels in Griffon vulture is probably related to an induction of lipid peroxidation by ROS that may be generated by  $\delta$ ALA accumulation when  $\delta$ ALAD activity is depressed.  $\delta$ ALAD activity and  $\delta$ ALAD ratio in blood are suggested as important nondestructive biomarkers for Pb exposure and effect for future biomonitoring studies in Griffon vulture and Eagle owl. Further studies are recommended to provide new data on Pb concentrations at which  $\delta$ ALAD activity is affected in different wild bird species and to elucidate why different species tolerate Pb in different ways.

## **Assessment of the exposure to heavy metals in Griffon vultures (*Gyps fulvus*) from the Iberian Peninsula.**

Carneiro M, Colaço B, Brandão R, Azorín B, Nicolas O, Colaço J, Pires MJ, Agustí S, Casas-Díaz E, Lavin S, Oliveira PA.  
Ecotoxicol Environ Saf. 2015 Mar;113:295-301.

<http://www.sciencedirect.com/science/article/pii/S0147651314005715>

Griffon vultures (*Gyps fulvus*), by virtue of their position at the top of the food chain and as obligate scavengers, are at risk of accumulating and concentrating heavy metals in their tissues and may be more predisposed to their toxic effects. The aim of this study is to investigate heavy metal concentrations in Griffon vultures in Portugal and Catalonia, Spain and to determine if heavy metal concentrations in the blood of weak and/or injured Griffon vultures admitted to wildlife rehabilitation centres (WRC) reflect contamination profiles in the local, free-living and outwardly healthy population. Whole-blood samples taken from 121 Griffon vultures caught in the wild or admitted to WRC in Portugal and Catalonia, Spain were examined for cadmium (Cd), mercury (Hg) and lead (Pb) by inductively coupled plasma mass spectrometry (ICP-MS). Cd and Hg were not detected in most samples (98.3% and 95%, respectively), while Pb was detected in all birds in concentrations ranging between 4.97 and 300.23  $\mu$ g/dl. Birds admitted to WRC had significantly lower Pb concentrations (24.15  $\pm$  15.07 and 25.98  $\pm$  18.04  $\mu$ g/dl in Portugal and Catalonia, Spain, respectively) than animals caught in the wild (29.67  $\pm$  13.19 and 42.22  $\pm$  50.08  $\mu$ g/dl in Portugal and Catalonia, Spain, respectively) ( $p < 0.05$ ). This may be explained by the fact that malnutrition was the main cause of admission of Griffon vultures to WRC, as ingestion has been described as the most significant pathway for Pb exposure in raptors. Therefore Griffon vultures admitted to WRC do not seem to be representative of the local, free-flying popula-



tions, so it remains necessary to continue catching when one intends to monitor Pb exposure in this species. The population of vultures captured in Catalonia, Spain showed the highest mean blood Pb concentration, perhaps due to the municipal rubbish dump located near the feeding station, with rubbish providing a significant fraction of their trophic needs. The ingestion of game meat with bullet fragments in carcasses or with Pb shots embedded in their flesh could also be the cause of the high blood Pb concentrations found in some vultures. The potential risk of Pb exposure in Griffon vulture populations must be given consideration, since most individuals evaluated had Pb concentrations between 20 and 100µg/dl, which is considered to be sub-clinical exposure to Pb, and which is above the threshold level at which Pb can affect antioxidant system in this species (15 µg/dl).

## **Research from UK:**

### **The Proceeding of the Oxford Lead Symposium**

The Proceedings (from the December 2014 symposium hosted by the Edward Grey Institute, the University of Oxford), will be published soon on the following website:

<http://www.oxfordleadsymposium.info> These proceedings provide recent analyses of risks to human and wildlife health from lead ammunition in the UK together with broader European perspectives on the political, legal and practical aspects of use of lead and non-lead ammunition.

## **Suggested citation:**

Group of Scientists, 2015. Wildlife and Human Health Risks from Lead-Based Ammunition in Europe - Scientific Advances 2014 and 2015.

Available from:

[University of Applied Sciences Eberswalde Germany](#)