

LEAD METAL. BIOACCESSIBLE LEAD. ABSORBED FRACTION

- To be absorbed by the human body lead must be *in ionic form* as a water- soluble lead compound. (EFSA scientific opinion 2010).
- Even very small fragments of solid lead metal that may be present in game meat are definitely not in ionic form and is not water soluble!
- Only a small percentage of any lead metal, is converted to a bioaccessible lead compound in the acid gastric environment.
- The in-vitro study "Lead in Game Meat" shows that on average only 1-2 % by weight, depending on weight/surface ratio, of lead metal fragment present in gastrointestinal tract, at normal pH levels in fed state, is converted to a bioaccessible lead compound.

The bioavailable fraction or "oral absorption fraction" is defined as, and equal to, the fraction of an ingested bioaccessible dose that actually crosses the gastrointestinal epithelium and becomes available for distribution to internal target tissues and organs. This in turn varies with the water solubility of the present lead salts released.

(**EPA 2007**: Guidance for Evaluting the oral Bioavailability of Metals in Soil for Human health risk Assessment. OSWER 9285.7-80)

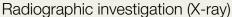
BRIEF ABOUT THE PERFORMANCE OF THE STUDY "LEAD IN GAME MEAT"

Wild boar (sus scrofa) were shot with two different types of lead-based hunting bullets of the same calibre under normal hunting conditions. The un-eviscerated animals are placed prior to rigor mortis in a cradle of jute fabric and fixed in a posture that is natural to the species when they are on the ground. When rigor mortis set in the animals were placed in a freezer, first at -35° Celsius. Then stored in - 25° Celsius for a period of 6 months before CT imaging and collection of meat samples.

Preparing for sampling









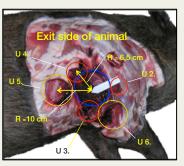
Computed tomography

In what way, and how far, lead fragments are dispersed around the wound channel has been discussed extensively based on common radiographic images.

With the aid of computed tomography (CT), actual dispersal was studied accurately in both dorsal and transversal directions millimetre by millimetre, and also in 3D.

Something that allowed that the most contaminated areas to be easily identified for accurate sampling before the in-vitro investigation.

LEAD IN GAME MEAT Overview of methods and result



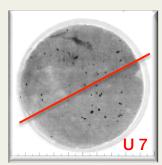
Sampling points exit side



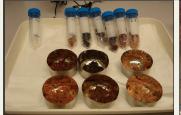
Chest wall under shoulder



All meat and blood from 1st to 7th rib



The homogenized meat X-rayed in mould



Samples prepared



"Ashing" for total lead content



In-vitro simulation in gastric juice (pH 3)

The X- ray samples were visually divided into two parts. One for investigation of total lead. The second part for "in –vitro" gastrointestinal test of present lead metal conversion to a bioaccessible lead compound.

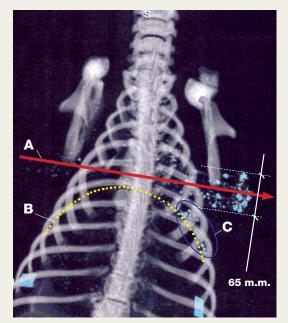
Table 2	Object 1	Gastrointestinal	dissolution
Table 2.	Oblect 1	Gastrointestinai	aissolution

NAME	TOTAL LEAD mg/kg ww	IN VITRO Gastrointestinal mg/kg ww	intestinal mg/kg ww	DIFFERENCES mg/kg ww
I 1	131.00	0.54	0.58	+ 0.04
I 2	72.39	1.12	1.02	- 0.10
I 3	341.00	1.09	1.01	- 0.08
I 4	221.00	2.03	1.21	- 0.82
U 1	1955.00	1.25	0.89	- 0.36
U 3	84.80	0.88	0.62	- 0.26
U 4	22.30	0.34	0.23	- 0.11
U 6	13.8	0.06	0.04	- 0.04
U 7	589.00	1.25	1.17	- 0.08

Samples with a total lead content less than 1 mg/kg w.w were excluded.

NAME	TOTAL LEAD mg/kg ww	IN VITRO Gastrointestinal and intestinal mg/kg ww	BIOACCESSIBLE PART [14]. % of total lead	SAMPLES Nr. Figure 1.
I 1	131.00	0.54	0.41 Centre wound channel. Entr.	1
I 2	72.39	1.12	1.55 " r= 3 cm I	2
I 3	341.00	1.09	0.32 " r= 3 cm II	3
I 4	221.00	2.03	0.92 Chest wall. Entr.	4
U 1	1955.00	1.25	0.06 Centre wound channel. Exit	5
U 3	84.80	0.88	1.04 " r= 6.5 cm I	6
U 4	22.30	0.34	1.52 " r= 6.5 cm II	7
U 6	13.8	0.06	0.43 " r= 10 cm III	8
U 7	589.00	1.25	0.21 Chest wall. Exit	9

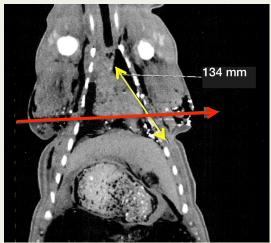
On average only 0.25 % of lead metal present in this object was converted to a bioaccessible lead compound. Taking into account the variables in visually divided samples, gastric pH variations etc. a value of 1-2%. is used



A: Bullet path

B: Diaphragm (midriff)

C: Metal lead fragment in clot



Top: 3D CT image. Bottom: Dorsal CT image, center wound channel

Compilation of measured lead levels in game meat

INVESTIGATOR	TYPE OF ANIMAL	AMMUNITION	SAMPLES		MEDIAN r	ng/kg		MEAN mg/	/kg	MAX mg/kg
а	Game meat		2521	1	0.02		/	3.15		867
b	Wild boar	Bullet	/		0.02			4.7		684(1998)
С	Moose meat*	Bullet	52		0.3			5.6		110
d	Bird	Lead shot	128					2.55		
е	Moose meat*	Bullet	54		0.027		1	0.9		31

^{*}Minced meat

REFERENCES:

- a) EFSA, 2010. EFSA panel on contaminants in the food chain (CONTAM); Scientific opinion on lead in food. EFSA J. 8(4), 1570.
- **b)** Bundesinstitut fur Risikobewertung, "Bleibelastung von Wildbret durch Verwendu", Stellungnahme Nr. 040/2011
- c) Lindboe. M., et al. Lead. Concentration in meat from lead-killed elk and predicted human exposure using Monte Carlo simulation. Food Additives and Contaminants (2012) 1-6, I First.
- d) Mateo.R, et al. 2010. Bioaccessibility of Pb from ammunition in game meat is affected by cooking treatment. PLoS One 6, e15892.
- e) Swedish National Food Agency.

In elastic muscle tissue coarser lead metal fragments occur very close to the wound channel.

In the chest cavity fragments with low weight were halted in the organs soft tissue and by the heavy blood flow and clots.

Claimed intake of Pb in µg/kg bodyweight/day from 18.2 kg of wild boar meat annually

Szenario	Parameter Gehalt	Parameter	Häufigkeit un größen pro J	
	(Wert in mg/kg)	Verzehr	Männer	Frauen ¹
1a Durchschnittlicher Verzehr und gleiche Wahrscheinlichkeit für hohe und niedrige Bleigehalte	Mittelwert (4,7)			
1b Durchschnittlicher Verzehr und sehr geringe Wahrscheinlichkeit für hohe Bleige nalte	- Median (0,02)	Mittelwert	2 x 200 g	1 x 200 g
2a Hoher Verzehr und gleiche Wahrschein ichkeit für hohe und niedrige Bleigehalte	Mittelwert (4,7)			
2b Hoher Verzehr und sehr geringe Wahr- scheinlichkeit für hohe Bleigehalte	Median (0,02)	95. Perzentil	10 x 200 g	5 x 200 g
Ba Bagerhaushalte mit gleicher Wahrscheir ichkeit für hohe und niedrige Bleigehalte	Mittelwert (4,7)			
Bb Jägerhaushalte mit sehr geringer Wahr- scheinlichkeit für hohe Bleigehalte	Median (0,02)	50 g/Tag ²	91 x 200 g	91 x 200 g

How the BfR calculation was performed:

91 servings á 200 grams is: 18.2 kg /365 = 0.05 kg/day

Lead content in meat claimed: $4.7 \text{ mg/kg} = 4700 \mu\text{g} / \text{kg}$

Daily intake: $4700 \mu g \times 0.05 kg = 235 \mu g$

Intake women: 235 μ g/ 60 kg = 3.91 μ g/ kg b.w/day

Intake men: 235 μ g/ 70 kg = 3.35 μ g/ kg b.w/day

	Männer	Frauen
Szenario 1a	0,0736	0,0429
Szenario 1b	0,0003	0,0002
Szenario 2a	0,3679	0,2146
Szenario 2b	0,0016	0,0009
Szenario 3a	3,3479	3,9059
Szenario 3b	0,0142	0,0166
Szenario 4a	0,0399	0,0399

Estimated daily intake of lead for a 70 kg men and 60 kg woman in µg/ kg b.w/day from consumption of 50 grams of wild boar meat/day with a lead content 4.7mg/ kg originating from lead based bullets.

THE MOST IMPORTANT QUESTION IS:

Do this results published by BfR, refere to LEAD METAL OR BIOACCESSIBLE LEAD?

EFSA 2010 endpoint for bioaccessible lead exposure is set at $0.63 \,\mu\text{g}/\,\text{kg}$ b.w/day for adults. It must be assumed that $4.7 \,\text{mg}/\,\text{kg}$ is the presence of lead metal fragments determined by random sampling. If the results $3.91 \,\text{and} \, 3.35$ is bioaccessible lead, $100 \,\%$ of the $4.7 \,\text{mg/kg}$ lead metal in the wild boar meat must be converted in gastrointestinal tract. This is not likely at all.

CORRECT METHOD OF CALCULATING EXPOSURE TO BIOACCESSIBLE LEAD FROM A GIVEN LEAD METAL CONTENT IN GAME MEAT

Mean lead metal level in actual meat: 4.7 mg/kg (from BfR)

Daily annual consumption: 0.05 kg/Day (18.2 kg/year)

Body weight of the consumer: 60 kg

Converted lead metal to bioaccessible form in gastric tract: 1.5% (Lead in Game Meat)

4.7 mg lead metal = $4700\mu g/kg$ (micrograms)

Daily consumption = $4700 \times 0.05 \text{ kg} = 235 \mu \text{g/day}$

Daily intake of lead metal = $235\mu g / 60 kg = 3.91 \mu g / kg b.w / day$

Exposure of bioaccessible lead = $1.5 \times 3.91 / 100 = 0.059 \mu g/kg b.w / day$

This exposure is 9.4% of the EFSA recommended endpoint for adults 0.63 µg/kg b.w /day.

Or equal to a daily consumption of 3.52 decilitres of tap water at the EU limit 10 µg /litre.

THIS SIMPLE FACT ABOVE SHOWS THAT GAME MEAT CAN HAVE RELATIVELY HIGH LEVELS OF LEAD METAL WITHOUT LEADING TO AN ALARMINGLY HIGH LEVEL OF EXPOSURE. EVEN IF GASTRIC pH WOULD BE CLINICALLY LOW.

PREDICTING IMPACT ON B-Pb WITH USE OF EPA SLOPE FACTOR MODEL

The most important biomarker for general exposure from all sources of an absorbed fraction of bioaccessible lead is blood lead level (B-Pb) normally expressed in micrograms per litre ($\mu g/L$), $\mu g/dL$ or $\mu mol/L$.

From a known exposure of dietary bioaccessible lead per kilogram body weight and day actual impact on B-Pb can be calculated using the *Carlisle and Wade* empirical equation (California EPA 1992) slope factor model, applied for adults, adopted by CONTAM panel and described in **EFSA** 2010 (page 99).

Exposure: μ g /kg b.w/per day x b.w x 0.4 = B-Pb μ g/L. (The factor 0.4 is the bio kinetic slop factor BKFS, from EPA diagram)

Estimate from previous page set in context:

 $0.059 \mu g / kg b.w/day \times 60 kg \times 0.4 = 1.42 \mu g/L$

Endpoint	Population	BMDL	Corresponding dietary Pb exposure		
egraph, subbanc to roughers or manages, conference readings or	et from 0.35 to	B-Pb (μ g/L)	μg/kg b.w. per day	μg/person per day	
Developmental neurotoxicity	Children	12	0.50	10 ^(a)	
Nephrotoxicity	Adults	15	0.63	37.5 ^(b)	
Cardiovascular effects	Adults	36	1.50	90 ^(b)	

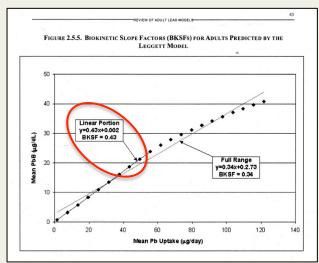


Diagram from EPA, **Review of Adult Lead Models** (Final draft 2001) BKSF, (bio kinetic slope factor)

Annual exposure of lead from meat is estimated to be 8-10% of the total dietary exposure.

From the example with a consumption of 18.2 kg of wild boar meat annually, with 4.7mg lead metal content/kg exposure is ≈ 9.5 % which far from alarming.

Recommended endpoints for dietary exposure of lead. (EFSA 2010)



Game meat from animals shot with lead ammunition when properly slaughtered and butchered is an extremely safe foodstuff in comparison to many others

- It is difficult to measure the actual quantities of metallic lead in game meat. Result obtained from random sampling from small biopsies can easily be overestimated.
- In order to make a meaningful risk assessment it is necessary to take in consideration the accepted science regarding the chemical form that lead must be in to actually be absorbed by the human body.
- In calculating exposure and impact on B-Pb established methods must be used.

THANKS FOR YOUR ATTENTION!