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► **To cite this version:**

Jesus Sevillano Morales, Rafael Moreno-Rojas, Fernando Perez-Rodriguez, Antonio Arenas Casas, Manuel Angel Amaro Lopez. Risk Assessment of the Lead intake by consumption of red deer and wild boar meat in Southern Spain. Food Additives and Contaminants, 2011, 99999 (1), pp.1. 10.1080/19440049.2011.583282 . hal-00714933

HAL Id: hal-00714933

<https://hal.science/hal-00714933>

Submitted on 6 Jul 2012

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Risk Assessment of the Lead intake by consumption of red deer and wild boar meat in Southern Spain

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2011-031.R2
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	06-Apr-2011
Complete List of Authors:	Sevillano Morales, Jesus; University of Cordoba, Department of Food Science and Technology Moreno-Rojas, Rafael; Universidad de Córdoba, Dto. de Bromatología y Tec. de los Alimentos, Perez-Rodriguez, Fernando; University of Córdoba, Department of Food Science and Technology; Optimum Quality Arenas Casas, Antonio Amaro Lopez, Manuel Angel; University of Cordoba, Department of Food Science and Technology
Methods/Techniques:	Risk assessment, Risk assessment - modelling, Exposure - prob modelling, Exposure assessment
Additives/Contaminants:	Metals
Food Types:	Animal products – meat
Abstract:	The presence of heavy metals in big game meat may pose a risk to human health. The main objective of this work was to carry out a risk assessment study (using a probabilistic and point-estimate approach) of lead intake by consumption of red deer and wild boar meat in Southern Spain based on Spanish data collected in the period 2002-2006. In general, the concentration levels found for wild boar meat (mean= 1291 µg/Kg) were much higher than those observed in red deer meat (mean=326 µg/Kg). The results from a point-estimate risk assessment showed that the estimated average intake of lead among different exposure scenarios varied from 0.1 to 6.5 and from 0.3 to 38 µg/Kg/week for red deer and wild boar meat, respectively; and from 0.3 to 35 µg/Kg/week for individuals

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	<p>consuming both red deer and wild boar meat, and that estimated intake of lead by consumption of big game meat differed significantly between hunters and non-hunters, it being higher for hunters. Besides this, results from the probabilistic risk assessment study corroborated that risk is greater in hunter populations, reaching a maximum in individuals consuming only wild boar and both types of meat, with 0.4 and 0.2 % population above the PTWI (Provisional Tolerable Weekly Intake), respectively. Likewise, the hunter populations consuming wild boar and both types of big game meat (red deer and wild boar meat) were exposed to the maximum Pb level (56 µg/Kg/week) which corresponded to 224 % PTWI, approximately. Further data and studies will be needed to give a complete risk estimation in which it will be crucial to consider the contribution to the Pb intake level of other foods in the diet of both population groups.</p>
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1 Risk Assessment of the lead intake by consumption of red deer 2 and wild boar meat in Southern Spain

3 Sevillano Morales J.S.¹, Moreno Rojas R.¹, Pérez-Rodríguez F.^{1,2} Arenas Casas A.³ and
4 Amaro López M.A.^{1*}

5
6 ¹Departamento de Bromatología y Tecnología de los Alimentos, Universidad de Córdoba,
7 Campus Rabanales, Edif. Darwin-Anexo, 1014 Córdoba, SPAIN.

8 ²Optimum Quality S.L. Pol. Tecnocordoba, C/Estonia, 1 Nave 79, 14014 Córdoba, SPAIN

9 ³Departamento de Sanidad Animal, Universidad de Córdoba, Campus Rabanales, Edificio
10 de Sanidad Animal, 1014 Córdoba, SPAIN

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14 **Keywords:** risk assessment, wild game meat, heavy metals, probabilistic, lead.

15
16
17 *Corresponding author:

18 Dr. Amaro López M.A:

19 Phone: +34- 957 21 20 04

20 Fax: +34- 957 21 20 00

21 E-mail address: bt1amlom@uco.es
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3 **23 Abstract**
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6 24 The presence of heavy metals in big game meat may pose a risk to human health. The main
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9 25 objective of this work was to carry out a risk assessment study (using a probabilistic and
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11 26 point-estimate approach) of lead intake by consumption of red deer and wild boar meat in
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13 27 Southern Spain based on Spanish data collected in the period 2002-2006. In general, the
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15 28 concentration levels found for wild boar meat (mean= 1291 $\mu\text{g}/\text{kg}$) were much higher than
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17 29 those observed in red deer meat (mean=326 $\mu\text{g}/\text{kg}$). The results from a point-estimate risk
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19
20 30 assessment showed that the estimated average intake of lead among different exposure
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22 31 scenarios varied from 0.1 to 6.5 and from 0.3 to 38 $\mu\text{g}/\text{kg}/\text{week}$ for red deer and wild boar
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24 32 meat, respectively; and from 0.3 to 35 $\mu\text{g}/\text{kg}/\text{week}$ for individuals consuming both red deer
25
26 33 and wild boar meat, and that estimated intake of lead by consumption of big game meat
27
28 34 differed significantly between hunters and non-hunters, it being higher for hunters. Besides
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30 35 this, results from the probabilistic risk assessment study corroborated that risk is greater in
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32 36 hunter populations, reaching a maximum in individuals consuming only wild boar and both
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34 37 types of meat, with 0.4 and 0.2 % population above the PTWI (Provisional Tolerable Weekly
35
36 38 Intake), respectively. Likewise, the hunter populations consuming wild boar and both types
37
38 39 of big game meat (red deer and wild boar meat) were exposed to the maximum Pb level (56
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40 40 $\mu\text{g}/\text{kg}/\text{week}$) which corresponded to 224 % PTWI, approximately. Further data and studies
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42 41 will be needed to give a complete risk estimation in which it will be crucial to consider the
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44 42 contribution to the Pb intake level of other foods in the diet of both population groups.
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43 INTRODUCTION

44 Nowadays, hunting has important repercussions in social, economic, environmental and
45 health aspects. Spain is one of the most important countries in production, manufacturing and
46 trade of big game meat and other similar food products obtained from hunting. During the
47 period 2002-2008, a mean production of 21,000 tons of big game meat per year was reported,
48 of which 10,000 tons corresponded to red deer and wild boar (Ministry of Agriculture,
49 Fisheries and Food, 2002, 2003, 2006, and 2008). In addition, according to Bartolomé et al.
50 (2006), 90% of red deer meat produced in Spain is exported to central-European countries.

51 Big game meat is a natural meat of great quality, showing an elevated culinary and nutritional
52 value, highlighting the red deer meat which presents a high protein content and low fat and
53 cholesterol level, together with a reduced caloric contribution. (Bartolomé et al. 2006). In
54 southern Spain, big game meat is free from hormones residues, antibiotics, and other
55 pharmacological compounds, since big game species are wild animals grown in a natural
56 environment (Soler et al. 2007). Nevertheless, big game meat does not imply zero risk, since
57 the presence of contaminants in the environment may increase risk levels through
58 contamination of the meat.

59 The big game species, used frequently as biological indicator of environmental contaminants
60 (Kottferová and Koréneková 1998; Pokorny 2000) are part of the human food chain which
61 can pose a certain health risk derived from consumption with respect to the content of heavy
62 metals. This can be especially relevant to high-risk populations such as the group of hunters
63 (Vahteristo et al 2003; Guitart and Thomas 2005; Mateo et al. 2007; Lazarus et al. 2008).
64 Moreover, there is an additional toxicological risk related to the ingestion of big game meat

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3 65 contaminated by lead (Pb) from ammunition fragments used for shot hunting (Guitart and
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5 66 Thomas 2005).
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9 67 Food Safety is a relevant issue for international organisations, governments and companies.
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11 68 Since the agreement GATT (General Agreement on Tariffs and Trade), Risk Assessment
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13 69 has been set as the framework providing the scientific basis in order to ensure fair
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15 70 commerce avoiding barriers to trade and guaranteeing consumer health. Risk assessment is
16
17 71 a scientific process which provides an estimate of the probability and severity of illnesses
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19 72 attributable to a particular hazard related to food (FAO/WHO 1995). This process is carried
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21 73 out in 4 steps: Hazard Identification, Hazard Characterization, Exposure Assessment and
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23 74 Risk Characterization
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28 75 Risk assessment studies can be carried out from different approaches, i.e. deterministic and
29
30 76 stochastic or probabilistic approach (van Leeuwen and Hermens 1995). The deterministic
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32 77 approach concerns the use of point-estimate values to describe a variable in the model. The
33
34 78 stochastic approach uses distributions of probability to describe uncertainty and/or
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36 79 variability of the model variables. Increasing number of stochastic risk assessments has
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38 80 been carried out in recent years, since it is considered that the inclusion of variability and/or
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40 81 uncertainty in risk assessment better support the decision making process (i.e. risk
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42 82 management).
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47 83 Most of the risk assessment studies on big game meat consumption have been carried out
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49 84 using a deterministic approach, by comparing mean heavy metal content levels in different
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51 85 organs and viscera with levels established by European regulations (Commission Regulation
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53 86 (CE) 1881/2006) in beef, lamb, pork, and poultry (Falandysz 1994; 2005; Santiago et
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55 87 al.1998; Pokorny et al. 2000; Lazarus et al. 2005; Soler et al. 2007) and, in some cases,
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3 88 assessing exposure to heavy metal levels with respect to tolerable ingestion levels set by
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5 89 international organizations (Medvedev 1999; Vahteristo et al. 2003; Mateo et al. 2007;
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8 90 Lazarus et al. 2008).

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10
11 91 Therefore, the aim of this work was to carry out a risk assessment study of lead intake by
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13 92 consumption of red deer and wild boar meat in Southern Spain based on both a probabilistic
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15
16 93 and point-estimate approach.

17 18 19 20 94 **MATERIAL AND METHODS**

21 22 23 95 **Contamination data**

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26 96 Muscle samples were collected from red deer (n=43) and wild boar (n=64), hunted in the
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28 97 period 2003-2006 and from different areas in Cordoba province, Andalusia, (southern Spain).
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30 98 Samples were frozen in individually labeled plastic bags until their preparation for analysis.
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33 99 Then, these samples were lyophilized and homogenized.

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36 100 Pretreatment by wet digestion in a closed system was performed in a *CEM Corporation MDS*
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38 101 *2000* microwave with hermetic and pressure regulated Teflon reactors/vessels. Then, 0.5 g of
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40 102 a lyophilized sample was deposited in the vessels and 6.250 ml of nitric acid (*Hiperpur*
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42 103 *Panreac 69%*) and 0.750 ml of hydrogen peroxide (*Panreac 33%*) were added. Each sample
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44
45 104 was digested by duplicate. Samples were diluted to a final volume of 15 ml with deionized-
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48 105 bidistilled water (>18 MΩ.), obtained from an *Optimum-Maxima Elga Option 3 Water*
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50 106 *Purifier* deionization system.

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54 107 Analytical determinations of Pb were measured by Graphite Furnace Atomic Absorption
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56 108 Spectrophotometry (GF-AAS) using pyrolitic THGF –tubes (transverse heated graphite
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3 109 furnace) in a *Perkin Elmer AA600* equipped with a Zeeman furnace module and an
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5 110 autosampler *AS-800*, controlled by the *WinLab 32* software.
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9 111 The performance characteristics of the analytical method are shown in Table 1 (Thomson et
10
11 112 al., 2002). In order to estimate the trueness, *Certified Reference Material (CRM) N° 184*
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13 113 *Bovine Muscle* was used. The recovery percentage was 97.83 %.
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16 17 114 **Food consumption data**

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20 115 Family consumption data were collected from a survey with a total of 301 interviews
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22 116 realized on those people who initially stated to consume big game meat. The survey was
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24 117 carried out over a period of 12 months in the region of Andalusia (Southern Spain). To our
25
26 118 knowledge, this study is currently the only Spanish survey which provides individual
27
28 119 consumptions data (at home and outside). The collected information included consumption
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30 120 data for different big game animal species, including red deer and wild boar, family size and
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32 121 distinction was made between hunter and non-hunter families.
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39 122 The consumption level was calculated in kg/person/year, dividing the total amount
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41 123 consumed annually for each species (i.e. red deer and wild boar) by the number of members
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43 124 per surveyed family.
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46 125 **Risk assessment**

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49 126 The risk assessment study was performed following two different approaches. The first
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51 127 approach corresponded to a probabilistic one in which variables were defined using
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53 128 probability distributions, and the risk estimation was obtained by simulation of the model
54
55 129 applying the Monte-Carlo analysis (Metropolis and Ulam 1949). The second approach
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3 130 consisted of using point-estimate values to describe variables in risk assessment study (Pocas
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5 131 and Hogg 2009).
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9 132 *Probabilistic Approach*
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12 133 The methodology used in the probabilistic approach followed the guidelines for developing
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14 134 Risk Assessment studies provided FAO/WHO (1995).
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18 135 *Exposure Assessment*
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22 136 FAO/WHO (1995) defines Exposures Assessment (EA) as “The qualitative and/or
23
24 137 quantitative evaluation of the likely intake of a chemical agent via food, as well as exposure
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26 138 from other sources if relevant”. Therefore, in this study, contamination and food consumption
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28 139 data were combined to obtain estimation of the exposure level to Pb by consumption of red
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30 140 deer and wild boar meat in Southern Spain. As concentration levels were always above
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32 141 Detection limit (DL), no left-censored data had to be treated in the statistical analysis.
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34 142 Initially, log-transformed concentration data was utilized to fit probability distributions
35
36 143 describing Uncertainty and Variability on the level of Pb in both types of meat. Consumption
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38 144 data were mathematically treated to represent the average amount (kg) of meat consumed per
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40 145 day based on annual consumption reported by the survey. The data were utilized to construct
41
42 146 an empirical discrete distribution (i.e. no assuming any parental distribution for data) which
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44 147 was simulated as a bootstrap from experimental data.
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52 148 *Hazard Characterization*
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55 149 In this stage, information is compiled about the nature of the adverse health effect associated
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57 150 with the ingestion of a hazard. The chief purpose of this step is to develop a dose-response
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3 151 model (FAO/WHO 1995). This type of model determines the relationship between the
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5 152 ingested dose of a chemical agent and the severity of the adverse health effects (response)
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8 153 However, owing to the lack of appropriate dose-response models for metal elements, usually
9
10 154 chemical food risks to human health are assessed by comparing the dietary exposure with an
11
12 155 adequate safe exposure level such as the Provisional Tolerable Weekly Intake (PTWI)
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14 156 (Renwick et al. 2003). Recently, both the Joint FAO/WHO Expert Committee on Food
15
16 157 Additives (FAO/WHO, 2010) and the European Food Safety Agency (EFSA, 2010) have
17
18 158 concluded that the current value of PTWI (25 µg/Kg/week) should be modified in the light of
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20 159 new scientific data which suggest that such threshold could be no health protective.
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22 160 However, to date, the PTWI has not been updated. Therefore, in this work, the PTWI
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24 161 proposed by the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2000)
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26 162 was utilized as the safe exposure level which corresponds to a intake level of 25 µg/Kg/week.
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33 163 *Risk Characterization: The plug-in (PI) estimator*

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36 164 According to FAO/WHO 1995, "Risk is a function of the probability of an adverse health
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38 165 effect and the severity of that effect, consequential to a hazard(s) in food".
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42 166 If X_i is defined as the exposure value to a given contaminant for an individual i ($i=1, \dots, n$) and
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44 167 assuming that exposure values are available for all individuals and expressed in the same unit
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46 168 as the PTWI, a simple way to estimate the previous term risk next term is to use the plug-in
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48 169 (PI) or empirical estimator of the probability to exceed the PTWI, defined as (Tressou et al.
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50 170 2004):
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$$PI = \frac{\#(X_i > PTWI)}{n} \quad \text{Eq. 1}$$

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3 172 where $\#(X_i > PTWI)$ denotes the number of exposure values that exceed the PTWI. In other
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5 173 words, PI is the probability that the exposure of an individual from a given population
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8 174 exceeds the PTWI. For example, if this quantity is equal to 0.05 for a given population, it
9
10 175 means that an unknown individual belonging to that population may exceed the PTWI with a
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12 176 probability of 5%. In a large population, a precise estimation of this quantity is of great
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15 177 importance since even a difference of 1‰ involves a large number of individuals. In our
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17 178 study we applied a $PTWI = 25 \mu\text{g}/\text{kg}/\text{week}$, considering a mean body weight for our study of
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20 179 65 kg (Enríquez, 2007).

21 22 23 180 *Point-estimate approach*

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27 181 The same steps and experimental data as those applied to probabilistic approach were used
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29 182 for the point-estimate approach. However, unlike probabilistic analysis, point-estimate
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31 183 values were used to describe each variable instead of probability distributions. The Pb
32
33 184 concentrations and consumption patterns for both types of meat were described by minimum,
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36 185 geometric mean and 95th values. These values were combined in four different scenarios to
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39 186 estimate the intake level of Pb (i.e. exposure levels) derived from the separate consumption of
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41 187 red deer and wild boar meat in hunter and non-hunter populations. The four calculated
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43 188 exposure scenarios were as follows: Scenario 1 consisting of combining mean values from
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46 189 both consumption data and Pb concentration; scenario 2 combining mean values from
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48 190 consumption data with 95th values from Pb concentration data; scenario 3 using 95th values
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50 191 from consumption data and mean values from metal concentration data; finally, scenario 4
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52 192 combining 95th values from both, consumption and metal concentration data. Then the
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55 193 exposure levels obtained for each scenario were used to estimate the percentage of PTWI
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58 194 reached by each exposure scenario.

195 **Statistical analysis and simulation**

196 Descriptive statistics for consumption and concentration data were applied with Microsoft
197 Excel (Microsoft, Redmond, Wash.). Statistical analysis was performed by means of SPSS
198 8.0 software (SPSS Inc. Chicago, Illinois, USA). Correlation coefficient (r), t-student test,
199 and variance analysis (significance at $P < 0.05$) were applied on consumption data.

200 The probability distributions describing the Pb concentration data were fitted by using @Risk
201 Professional© software (Palisade, Newfield, NY). The fitting to data was assessed by using
202 different statistical tests such as kolmogorov-Smirnov test, Chi-square test and RMSE (Root
203 Mean Square Error). In addition, the visual analysis was equally considered to assess the
204 fitting of the probability distributions to concentration data.

205 The exposure assessment model did not include a separation between the variability and
206 uncertainty of input variables (so-called first order model). The model was simulated using
207 the Latin-Hypercube Technique, implemented in @Risk Professional© software. The
208 simulation was run using 100,000 iterations for each scenario.

209 **RESULTS AND DISCUSSION**

210 **Consumption of red deer meat and wild boar meat**

211 Consumption data indicated that 199 out of a total of interviews ($n=301$), corresponded to
212 individuals who stated that they consume red deer and wild boar meat. No data were
213 collected about other types of meat. Consumers of red deer and wild boar meat were 62 %
214 hunters and 38% non hunters. Consumption data showed that 15% people surveyed
215 consumed only red deer meat (and not wild boar meat) and 14% consumed only wild boar

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3 216 meat (and not wild red deer meat); while 71% out of the people surveyed consumed both
4
5 217 red deer and wild boar meat. Consumption data and main statistics for the different
6
7 218 consumers groups are presented in Table 2. The consumption levels for individuals
8
9 219 consuming only red deer meat were 5.44 ± 8.51 and 1.83 ± 1.73 Kg/person/year; while for
10
11 220 only consumption of wild boar, was 4.97 ± 7.41 and 2.02 ± 3.71 Kg/person/year for hunter
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13 221 and non hunter population, respectively. Overall, big game consumption was significantly
14
15 222 higher for the group of hunters. Red deer meat was most consumed in individuals
16
17 223 consuming only one type of meat, while for individuals consuming both, the consumption
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19 224 level for red deer and wild boar meat was similar. It is noteworthy that the levels of
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21 225 consumption of both types of meat were statically higher in individuals consuming only
22
23 226 one type of meat than individuals consuming both types of meat. This result might be
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25 227 caused by the low number of data obtained for the group of individuals consuming only one
26
27 228 type of meat which could lead to higher mean values. Individuals consuming a great
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29 229 amount of one type of meat also presented a high consumption for the other type of meat.
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31 230 This correlation was quantified by estimating the correlation coefficient (r) between the
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33 231 amount consumed of both types of meat which was equal to 0.31 and 0.39 for hunters and
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35 232 non-hunters, respectively.
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234 Empirical distributions were built based on raw data for the different consumption data set
235 (i.e. hunters, non hunters, and consumption of both or one type of meat). These
236 distributions were used by the Monte-Carlo analysis to account for the different
237 consumption patterns.

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3 238 Consumption data, in our study, for red deer and wild boar meat were higher than those
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5 239 reported by Soler et al. (2007) who carried out an estimation per type of meat through
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7 240 indirect method (0.245 kg/person/year). In turn, our consumption estimation was in
8
9 241 concordance with the studies by Guitart and Thomas (2005) and Lazarus et al. (2008),
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11 242 highlighting that big game meat is most consumed by hunters and their families.
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14 15 16 243 **Concentration of lead in red deer and wild boar** 17

18
19 244 The main statistics based on arithmetic and log-transformed values of Pb concentrations in
20
21 245 red deer meat and wild boar meat are shown in Table 3. Arithmetic values were included in
22
23 246 Table 3 since many scientific papers usually report mean levels based on arithmetic
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25 247 calculation; hence, in our study, comparisons between studies were undertaken by using the
26
27 248 arithmetic mean instead of the geometric mean (based on logarithmic scale). However,
28
29 249 exposure models here developed (mentioned below) were based on concentration probability
30
31 250 distributions built on logarithmic scale.
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37 251 The average level in red deer meat (calculated on arithmetic values) corresponded to 0.326
38
39 252 mg/Kg which was quite similar to data reported by other studies. For instance, Soler et al.
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41 253 (2007) and Taggart et al. (2011) found an average concentration of lead in red deer meat of
42
43 254 0.550 mg /Kg and 0.320 mg /Kg, respectively. In our study, wild boar meat presented a much
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45 255 higher Pb concentration than red deer meat, with an average level of 1.291 mg/Kg. This
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47 256 average value was consistent with the data presented by Taggart et al. (2011) who reported an
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49 257 average level of 1.36 mg/Kg. In turn, Soler et al. (2007) found lower levels showing an
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51 258 average value of 0.68 mg/Kg.
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3 259 In general, lead levels present in big game meats are noticeably higher as compared to levels
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5 260 found in meats from livestock (e.g. pork, beef, calves, etc.). Concentration levels found in
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7 261 lamb meat, cow and calf muscles were markedly lower than those found in red deer and wild
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9 262 boar meat, with average levels of 0.001, 0.012 and 0.006 mg/Kg, respectively (López-Alonso
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11 263 et al. 2000 and 2007; Gonzalez-Weller et al. 2006). Similarly, pork meat presented small
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13 264 levels, with average levels of 0.005 mg/Kg (Gonzalez-Weller et al. 2006). In conclusion,
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15 265 these data indicate that big game meats may contribute to diet with much higher levels of lead
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17 266 than meats from livestock.
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23 267 The Pb mean concentration levels found in this study exceeded the maximum limits (> 0.1
24
25 268 mg/kg) established in the European Regulation (Commission Regulation (CE) 1881/2006).
26
27 269 Thus, 84.4 and 28 % samples belonging to wild boar and red deer meat samples, respectively,
28
29 270 exceeded the established maximum limit. However, values obtained for wild boar meat
30
31 271 samples in this study were different from other studies. Soler et al. (2007) found 50% out of
32
33 272 the wild boar meat samples were above the established maximum limit for Pb. On the other
34
35 273 hand, Falandysz et al. (2005) reported high percentages for red deer meat samples, with
36
37 274 approximately half of analyzed samples above the established maximum level. Note that the
38
39 275 higher concentration found for wild boar could be consequence of both the different feeding
40
41 276 habit between wild boar and red deer and/or environmental contamination (Santiago et al.
42
43 277 1998; Reglero et al. 2008).
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50 278 Statistical tests applied to data (i.e., Kolmogorov-Smirnov test and Chi-square test) indicated
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52 279 that the most suitable probability distribution to represent Pb concentration was the Normal
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54 280 distribution which was defined by mean and standard deviation of log-transformed data
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56 281 (Figure 1 and Table 3).
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3 282 **Risk Assessment**
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7 283 *Point-estimate approach*
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10 284 The Exposure Assessment was carried out using point-estimate values based on four
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12 285 different scenarios as described in material and methods section. Results are presented in
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14 286 Table 4.
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18 287 The mean Pb intake estimated for the different exposure scenarios based on the
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20 288 aforementioned consumption patterns varied from 0.1 to 6.5 and from 0.3 to 37.9 $\mu\text{g}/\text{kg}/\text{week}$
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22 289 for red deer and wild boar meat, respectively; and from 0.3 to 35.4 $\mu\text{g}/\text{kg}/\text{week}$ for
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24 290 individuals consuming both, red deer and wild boar meat.
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29 291 Scenario 4, based on 95th percentile, was the only scenario yielding intake levels of Pb in
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31 292 excess of the established PTWI. Hunters consuming both types of big game meat (red deer
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33 293 and wild boar meat) were exposed to a Pb intake level of 35.4 $\mu\text{g}/\text{kg}/\text{week}$ corresponding
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35 294 with 142 % PTWI, while in hunters consuming only wild boar meat, Pb intake surpassed the
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37 295 PTWI with a percentage of 152%, indicating that the highest exposure level was given in
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39 296 hunters consuming only wild boar meat. In contrast, the non-hunter population obtained a
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41 297 maximum level in scenario 4 of 14.6 $\mu\text{g}/\text{kg}/\text{week}$, approximately (i.e. 58 % PTWI) for
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43 298 individuals consuming wild boar meat. Therefore, similar to the hunter population, wild boar
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45 299 meat was the biggest contributor to the Pb intake levels. Regarding red deer meat
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47 300 consumption in scenario 4, the maximum Pb level was obtained for hunter population with a
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49 301 maximum value of 6.5 $\mu\text{g}/\text{kg}/\text{week}$, which means 26 % PTWI.
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3 302 Results also showed that scenario 2 based on the mean consumption and 95th percentile of Pb
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5 303 concentration presented higher % PTWI than scenario 3 in which 95th percentile of
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7 304 consumption levels was combined with mean Pb concentration. For instance, Pb intake in
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9 305 scenario 2 for group of hunters consuming wild boar meat reached the 37% PTWI, while
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11 306 scenario 3 for the same type of meat only resulted in 12% PTWI. This fact suggests that there
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13 307 was a greater effect of the extreme concentrations of Pb on the intake levels than the extreme
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15 308 consumption levels for the hunter population. However, results from a point-estimate model
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17 309 analysis should not be considered conclusive since there is a great dependence on the
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19 310 magnitude of values (mean, 95th, 99th percentile, etc.) used for designing the scenarios. On
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21 311 the other hand, as expected, lower Pb intake values were derived from scenario 1, in which
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23 312 mean values were used for calculations. In scenario 1, wild boar meat was the big game meat
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25 313 which contributed less to PTWI with values of 3 and 1 % PTWI for groups of hunters and
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27 314 non-hunters, respectively. These values are slightly higher than that reported by Soler et al
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29 315 (2007) which was equal to 0.5%. In turn, data for red deer in scenario 1 (i.e. 0.7 and 0.2 %
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31 316 PTWI for hunter and non-hunter population, respectively) are quite similar to those reported
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33 317 by Soler et al. (2007) and Lazarus et al. (2008) which were 0.5 and 0.9% PTWI, respectively.
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35 318 This difference between studies can be a consequence of different factors, such as the higher
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37 319 levels of Pb found in our study in wild boar meat, and the difference in the consumption
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39 320 patterns between groups of hunters and groups of non-hunters. In our study, for scenario 1,
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41 321 consumption of both types of big game meat resulted in 3 and 1% PTWI in groups of hunters
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43 322 and non-hunters, respectively. None studies were found reporting estimations of Pb intake
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45 323 including consumption of both types of meat hence these data were not comparable with
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47 324 other studies such as the study by Soler et al (2007) or Lazarus et al. (2008) which carried out
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49 325 estimations for both types of meat separately.
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3 326 Overall, results demonstrated that estimated Pb intake by consumption of big game meat was
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5 327 significantly higher in the hunter population due to the major consumption level shown by
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8 328 this group. This fact was also indicated in other studies (Guitart and Thomas 2005; Lazarus et
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10 329 al., 2008). Lazarus et al. (2008) suggested that the specific group, hunters and their families,
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12 330 presents a greater intake of toxic metal species than the general population.

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16 331 Recent studies in Catalonia (Spain) by Llovet et al. (2010) and Martí-Cid et al. (2008), based
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18 332 on deterministic values (mean), reported that the intake level for Pb derived from the overall
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20 333 consumption of meat in general population reached 1.5 and 2.9 % PTWI, respectively. These
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22 334 results are quite similar to the values obtained in scenario 1 for the non-hunter population
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24 335 consuming both types of meat and was lower than that obtained for the hunter population in
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26 336 the same scenario. However, other works, based on the overall consumption of meat reported
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28 337 higher intake levels for Pb, which corresponded with 5.1 and 5.5 % PTWI in Andalusian and
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30 338 Galician population, respectively (Cuadrado et al. 1995; Rubio et al. 2005).

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36 339 *Probabilistic approach*

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40 340 The relationship found between the consumption levels of both types of meat for individual
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42 341 consuming both (i.e. $r = 0.31$ and 0.39 for hunters and non-hunters, respectively) was
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44 342 considered in simulation by applying the Spearman's rank order correlation to the
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46 343 consumption probability distributions.

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50 344 The simulation was carried out by considering the whole range of the probability
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52 345 distributions. Additionally, a simulation was performed by using distributions limited to 95th
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54 346 percentile, in order to enable comparison between both approaches (i.e. probabilistic and
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56 347 point-estimate approaches).

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3 348 The results derived from the simulation of the probabilistic models indicated that the
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5 349 exposure levels of Pb were above the established PTWI. Main statistics of the simulated
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8 350 distributions of Pb intake ($\mu\text{g}/\text{kg}/\text{week}$) for the different groups of consumers are shown in
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10 351 Table 5. Figure 2 represents simulated distributions of the percentage of Pb intake with
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12 352 respect to the PTWI for both models using the whole distributions and distributions truncated
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14 353 at 95th percentile. All models for non-hunters showed similar results (see Figure 2A-C)
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16 354 yielding *PI* values equal to 0.00 % which means that the PTWI level was not exceeded by
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18 355 the non-hunter population for all simulated cases.
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23 356 For hunters, the calculated *PI* was 0.00 and 0.40 % for red deer meat and wild boar meat
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25 357 consumption, respectively when the whole distributions were simulated (see Figure 2D-E). In
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27 358 this same model, when both types of big game meat were considered together in the diet, the
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29 359 *PI* was situated in 0.20 %. On the contrary, when distributions were truncated at 95th
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31 360 percentile, that intake of Pb did not exceed the established PTWI (i.e. *PI*=0.00 %) for all
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33 361 simulated cases. In summary, based on models with whole distributions, for hunters the risk
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35 362 were derived from wild boar meat since this type of meat presented higher Pb levels in
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37 363 addition to a major consumption level in this population group, while the non-hunter
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39 364 population was not exposed to risk levels when consumed red deer and/or wild boar meat
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41 365 (*PI*=0.00%).
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48 366 The mean Pb intake from the different probabilistic models was quite similar (i.e. 0.2-0.9
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50 367 $\mu\text{g}/\text{Kg}/\text{week}$) except for hunters consuming wild boar or both types of meat in which the
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52 368 intake level was around 1 $\mu\text{g}/\text{Kg}/\text{week}$ (i.e. ~4 % PTWI). With respect to 95th percentile
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54 369 values, these showed a similar pattern to mean values, obtaining higher values again for
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3 370 hunters consuming wild boar or both types of meat with approximately 6 and 4 $\mu\text{g}/\text{Kg}/\text{week}$,
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5 371 respectively which means 24 and 16 % PTWI (Table 5). In general, truncated distributions
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7 372 produced lower Pb intake levels than models with whole distributions. This fact was
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9 373 especially noticeable for maximum values obtained in the simulation. For instance, in models
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11 374 with truncated distributions, the highest intake value was obtained for hunter population
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13 375 consuming wild boar meat with approximately 18 $\mu\text{g}/\text{Kg}/\text{week}$ (72 % PTWI). A similar value
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15 376 may also be seen for hunter population consuming both types of meat (16 $\mu\text{g}/\text{Kg}/\text{week}$).
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17 377 Likewise, for models with whole distributions, the largest maximum value was found in
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19 378 hunter population consuming only wild boar and both types of meat, with values as high as
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21 379 56 $\mu\text{g}/\text{kg}/\text{week}$ which corresponds with 224 % PTWI. These results are not unexpected since
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23 380 wild boar meat presented the highest maximum level of Pb together with a very high
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25 381 maximum consumption level (i.e. 30 kg/person/year). However, it is noteworthy that those
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27 382 maximum values took place on rare occasions, with very few iterations during simulation
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29 383 (<10). Concerning the intake level associated with red deer meat consumption, the maximum
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31 384 values were obtained for non-hunter population with 24.8 and 11.8 $\mu\text{g}/\text{kg}/\text{week}$ using whole
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33 385 distributions and distributions truncated at 95th percentile, respectively. This result was
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35 386 caused by the maximum consumption value used in the simulation (i.e. maximum limit
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37 387 applied to the consumption distribution) which was equal to 50 Kg/year. This value
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39 388 corresponded with a single person in the survey who stated to consume only red deer meat,
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41 389 thereby representing a very extreme exposure scenario. In contrast, 95th percentile values
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43 390 were higher for hunters, being 1.3 and 0.6 $\mu\text{g}/\text{Kg}/\text{week}$ for whole distributions and
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45 391 distributions truncated at 95th, respectively. While for non-hunter population, the 95th
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47 392 percentile values were 0.6 and 0.5, respectively.
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3 393 The comparison of the point-estimate and probabilistic approaches evidenced similarities
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5 394 between deterministic scenarios using mean and the mean values obtained from the
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8 395 simulation (see Table 4 and 5). The convergence between results was especially marked for
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10 396 models simulated with truncated distributions and results in scenario 1 (deterministic
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12 397 approach). For instance, values for hunters obtained from the point-estimate approach for
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14 398 scenario 1 and from the simulation (i.e. truncated distributions) were in the same variation
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17 399 range, 0.2-0.7 $\mu\text{g}/\text{kg}/\text{week}$ (Table 4 and 5). On the contrary, estimations in the scenario 4
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19 400 (using 95th values) from the point-estimate approach were much higher than 95th percentile
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21 401 values from the probabilistic approach, demonstrating that the deterministic approach using
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23 402 extreme percentiles could lead to overestimating the risk. Furthermore, most of scenarios
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25 403 combining 95th percentiles and mean values resulted in higher values than those obtained in
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27 404 the simulation, and were more evident in models with truncated distributions. For example,
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29 405 values obtained from the hunter population in scenario 2 oscillated between 2 and 9
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31 406 $\mu\text{g}/\text{Kg}/\text{week}$, while simulation with truncated distributions for hunters obtained 95th percentile
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33 407 values between 0.6 and 3 $\mu\text{g}/\text{Kg}/\text{week}$.

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39 408 Overall, results from the probabilistic model gave a rise to a more accurate risk estimate than
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41 409 those obtained in the point-estimate approach. Nevertheless, both approaches agreed to
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43 410 indicate that hunters consuming only wild boar meat and hunters consuming both types of
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45 411 meats posed a serious risk (i.e. > PTWI). Moreover, both approaches similarly showed that
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47 412 the major consumption level associated with the hunter population was responsible for high
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49 413 Pb intake levels. Conclusions from truncating distributions at 95th percentile (none scenarios
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51 414 led to levels above the PTWI) did not coincide with either the point-estimate approach or the
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53 415 probabilistic approach with whole distributions. In our opinion, this result highlights the
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3 416 importance of considering the right-tail of distributions to accurately estimate the risk.
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5 417 Furthermore, we stress the idea that unlike the deterministic calculation; the probabilistic
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8 418 model is able to quantify which percentage of the population was exposed at risk levels
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10 419 (above PTWI) by using the *PI* concept and at which levels they were exposed. Nevertheless,
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12 420 scenario analysis using a deterministic approach may be useful to gain insight into those
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14 421 ranges or values of the variable and variables most contribute to the final risk (or intake
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16 422 level).
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21 423 This information is crucial to accurately assess the risk by Pb intake associated with
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23 424 consumption of red deer meat and wild boar meat by different consumers group providing a
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25 425 more adequate quantitative measure of risk which may be used by risk manager to make
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27 426 decisions on recommendations and risk mitigation strategies to be implemented.
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31 427 **Conclusions**

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35 428 This work demonstrates that big game meat from red deer and wild boar is a significant
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37 429 source of lead for consumers, though higher levels were detected in wild boar. In this study,
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39 430 the hunter population is identified as a risk population since results showed that there is
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41 431 certain percentage of the population which is exposed to levels above PTWI. This fact is a
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43 432 consequence of elevated big game meat consumption and also due to the high lead
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45 433 concentration levels found especially in wild boar meat. According to our results, a
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47 434 moderate consumption of big game meat might be recommended as a significant means to
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49 435 reducing the risk associated with the Pb intake. Nevertheless, in order to obtain a more
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51 436 complete estimate of the lead levels at which the different population groups are exposed,
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53 437 we should take into consideration the contribution of other foods to intake. Therefore, risk
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3 438 assessment studies considering new factors and data as well as an updated PTWI level
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5 439 should be carried out to obtain a more definitive conclusion
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9 440 **Acknowledgements**

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12 441 This study was supported by the Concerted CO3-84 project, the Andalusian Institute of
13
14 442 Agrarian Research and Training, Fishing, Food and Organic Production (IFAPA),
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16 443 Department of Innovation, Science and Enterprise, Andalusia, Spain.
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3 Figure 1. Fitted probability distributions to experimental lead concentration obtained in red deer
4 (A) and wild boar (B) meat.
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7 Figure 2. Output probability distributions of the plug-in (*PI*) estimator for non-hunters
8 population (A) and hunters population (B) obtained by Monte-Carlo analysis using whole input
9 probability distributions (————) and truncated input probability distribution at 95th (
10 - - - - -). Vertical lines are situated at 1 and 100 % PTWI (i.e. 0 log (%PTWI) and 2 log
11 (%PTWI), respectively). Numbers on the top horizontal bars (black colour: whole distributions,
12 and grey colour: truncated distributions at 95th) correspond with the percentages of individuals
13 accumulated at the levels of log (%PTWI) marked by the vertical lines. The percentages at the
14 right side of the horizontal bars correspond with the *PI* estimator (i.e. percentage of individuals
15 exceeding the PTWI).
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Figure 1

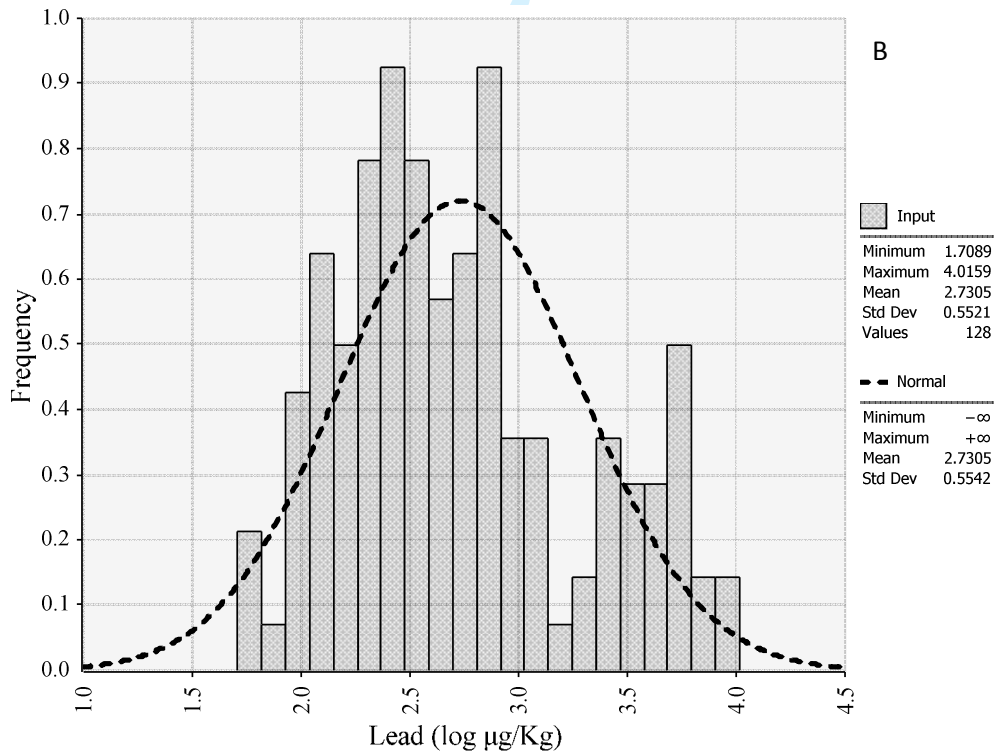
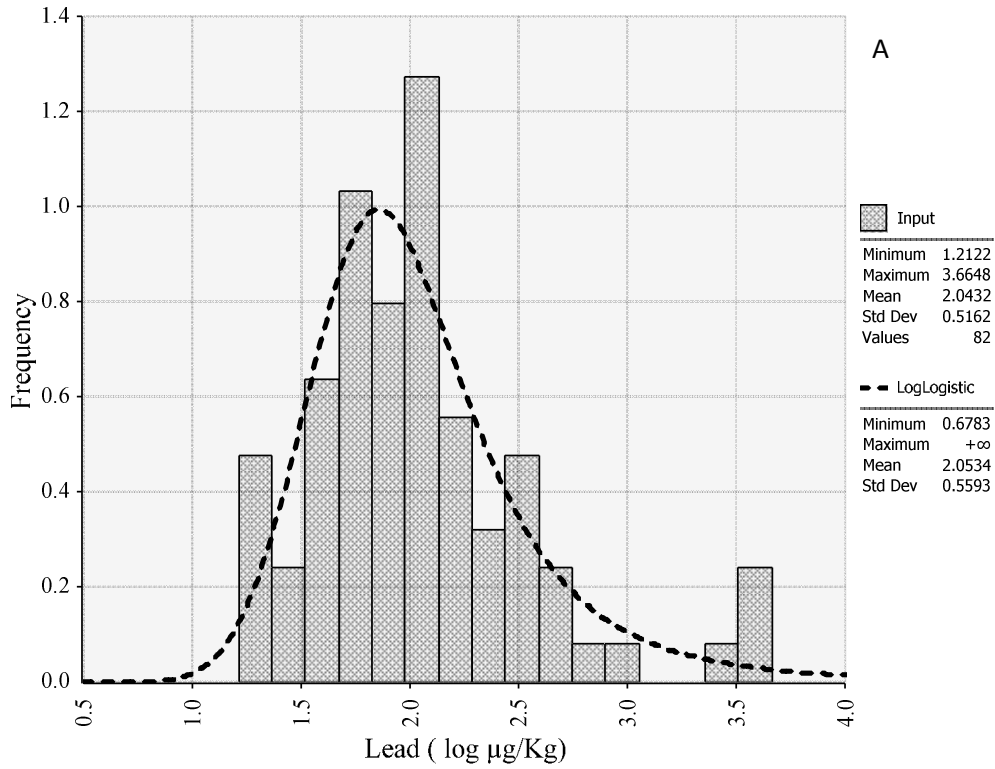
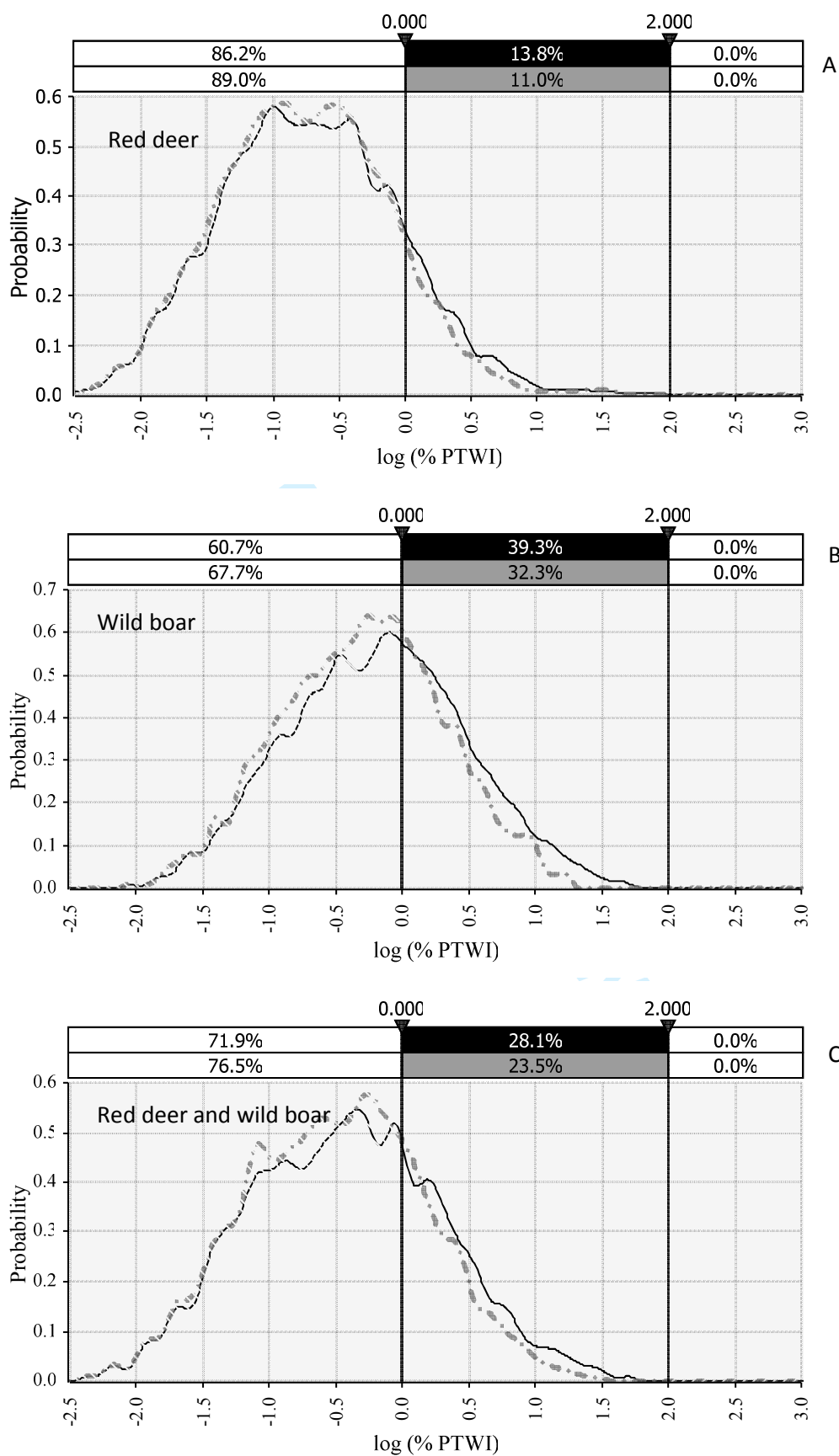


Figure 2



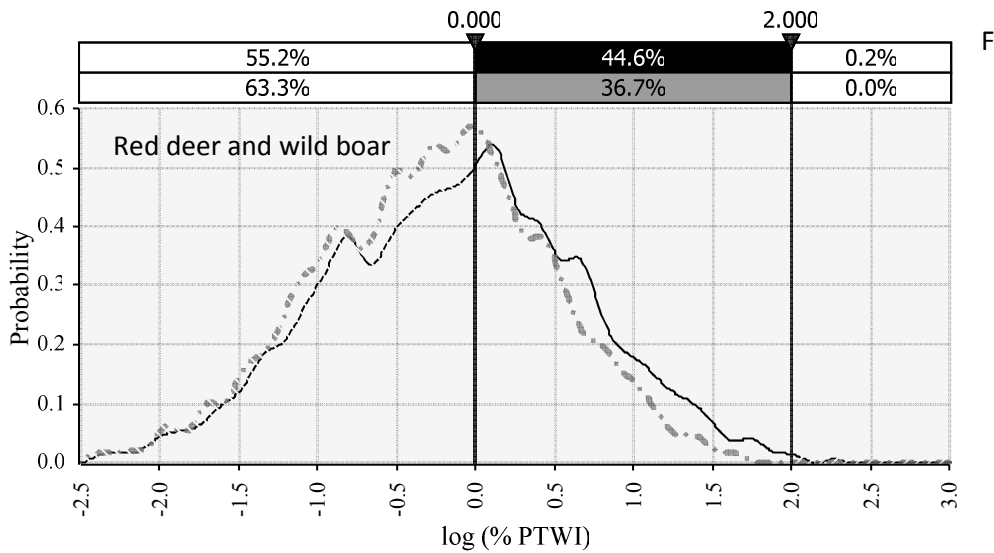
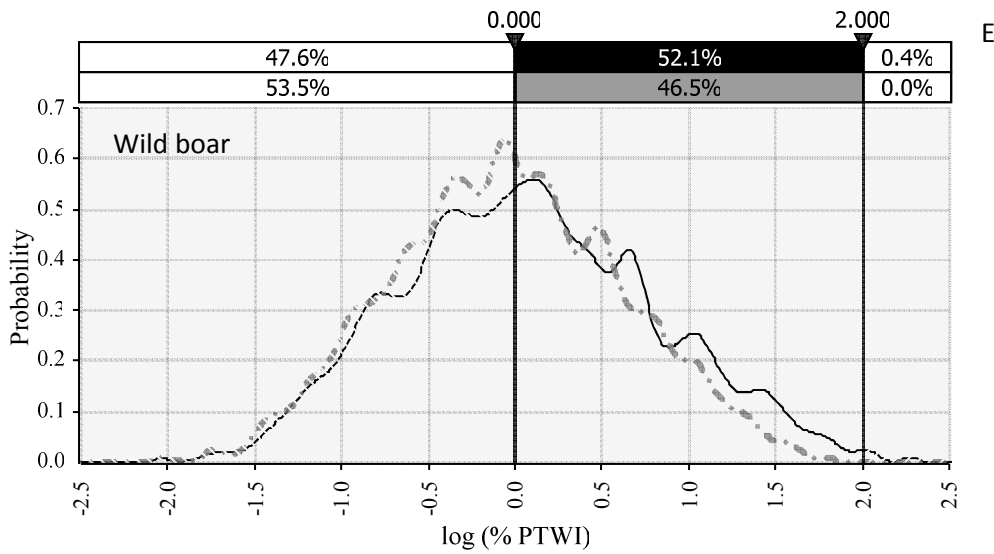
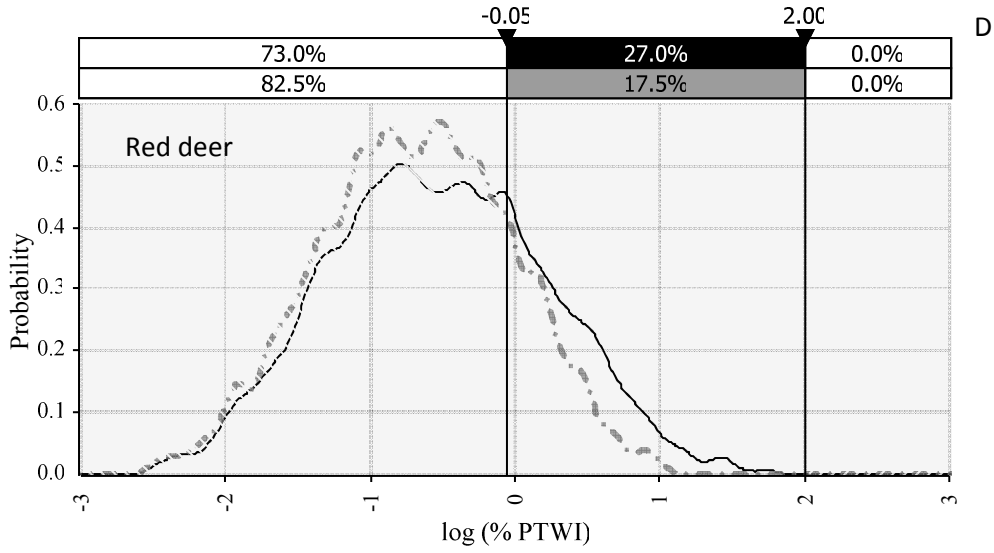


Table 1. Performance characteristics of the analytical method applied to quantify the lead content in samples of red deer and wild boar meat (Thompson, 2002).

Element	LQ* (µg/Kg)	LD* (µg/Kg)	Precision (%)	Sensitivity (µg/L)	Trueness (%)	CWR* (µg/Kg)
Pb	36.35	8.04	3.13 ^a /1.60 ^b	2.0-3.1	97.8	36-500

^ared deer.

^bwild boar

LQ: Limit of detection; LD: Limit of quantification; CWR: concentration working range.

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Table 2. Main statistics for the consumption data of wild boar and red deer meat (Kg/person/year) obtained in a survey carried out in the region of Andalusia, Spain (n=199). Data were grouped based type of population (i.e. hunter or non-hunter population) and on the type of consumption, that is said, individuals consuming only one or both types of meat.

	Consumption Red deer		Consumption Wild boar		Consumption Red deer & wild boar	
	Hunter (14/199)	Non hunter (17/199)	Hunter (20/199)	Non hunter (8/199)	Hunter (90/199)	Non hunter (50/199)
Standard deviation	8.51	1.73	7.41	3.75	6.25/5.63*	7.05/1.43
Mean	5.44	1.83	4.97	2.02	4.58/3.82	2.85/1.56
Maximum	30.00	6.67	26.66	11.25	26.67/30.00**	50.00**/5.00
95 th percentile	21.87	5.33	20.33	7.83	19.43/15.91	6.37/5.00
Minimum	0.25	0.14	0.25	0.25	0.67/0.67	0.13/0.13

* Red deer/wild boar

**Maximum consumption levels for red deer and wild boar meat corresponded with two persons who stated to only consume big game meat which represents an extreme consumption scenario.

Table 3. Main statistics based on logarithmic and arithmetic values of the lead levels found in red deer meat (n=43) and wild boar meat (n=64) and estimated probability distributions.

Main statistics	Red deer		Wild boar	
	Logarithmic scale log ($\mu\text{g/Kg}$)	Arithmetic scale $\mu\text{g/Kg}$	Logarithmic scale log ($\mu\text{g/Kg}$)	Arithmetic scale $\mu\text{g/Kg}$
Standard deviation	0.5	852	0.6	2017
Mean	2.0	326	2.7	1291
Maximum	3.7	4622	4.0	10372
95 th percentile	3.0	915	3.8	6088
Minimum	1.2	16	1.7	51
Distribution	Normal (2.0; 0.5)	-	Normal (2.7; 0.6)	-

Table 4. Estimated lead intake ($\mu\text{g}/\text{Kg}/\text{week}$) and percentage (between parenthesis) with respect to PTWI ($25 \mu\text{g}/\text{Kg}/\text{week}$) based on a mean body weight of 65 Kg for different exposure scenarios in hunter and non-hunter population, distinguishing individuals consuming one type of meat (i.e. wild boar or red deer meat) and individuals consuming both types of meat.

Specie	Consumer	Scenario 1 ^a	Scenario 2 ^b	Scenario 3 ^c	Scenario 4 ^d
Red deer	Hunter	0.2 (0.7)	1.6 (6.4)	0.7 (2.8)	6.5 (26.0)
	Non-hunter	0.1 (0.2)	0.5 (2.2)	0.2 (0.7)	1.6 (6.3)
Wild boar	Hunter	0.7 (2.9)	9.2 (37.11)	3.0 (12.0)	37.9 (151.8)
	Non-hunter	0.3 (1.2)	3.8 (15.08)	1.2 (4.6)	14.6 (58.5)
Red deer +Wild boar	Hunter	0.7 (2.9)	8.5 (34.0)	3.0 (12.0)	35.4 (142.0)
	Non-hunter	0.3 (1.3)	3.7 (15.0)	0.9 (3.8)	11.2 (44.9)

^a using mean value from both consumption data and Pb concentration

^b using mean value from consumption data and 95th value from Pb concentration

^c using 95th value from consumption data and mean value from Pb concentration

^d using 95th values from both, consumption and Pb concentration

Table 5. Lead intake ($\mu\text{g}/\text{Kg}/\text{week}$) based on a mean body weight of 65 Kg obtained in the probabilistic risk assessment model for hunters and non-hunters population, considering individuals consuming one type of meat (i.e. wild boar or red deer meat) and individuals consuming both types of meat.

	Non-hunter population						Hunter population					
	<u>Red deer meat</u>		<u>Wild boar meat</u>		<u>Red deer and wild boar meat</u>		<u>Red deer meat</u>		<u>Wild boar meat</u>		<u>Red deer and wild boar meat</u>	
	Whole distributions	Truncated distributions	Whole distributions	Truncated distributions	Whole distributions	Truncated distributions	Whole distributions	Truncated distributions	Whole distributions	Truncated distributions	Whole distributions	Truncated distributions
Minimum	0.001	0.001	0.002	0.002	0.001	0.001	0.001	0.001	0.002	0.002	0.001	0.001
Maximum	24.83	11.84	22.27	5.12	15.04	9.69	17.90	3.51	56.01	18.44	56.01	15.93
Mean	0.18	0.14	0.51	0.32	0.36	0.24	0.31	0.15	1.25	0.69	0.89	0.48
SD*	0.74	0.50	1.12	0.54	0.92	0.53	0.86	0.27	3.29	1.37	2.62	1.07
Mode	0.00	0.01	0.02	0.02	0.00	0.01	0.00	0.01	0.04	0.01	0.01	0.00
5th**	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.02	0.02	0.01	0.01
20th	0.01	0.01	0.04	0.04	0.02	0.02	0.02	0.01	0.07	0.06	0.04	0.03
40th	0.03	0.03	0.11	0.09	0.06	0.05	0.04	0.03	0.18	0.15	0.12	0.09
50th	0.04	0.04	0.16	0.13	0.09	0.08	0.07	0.05	0.28	0.22	0.19	0.14
60th	0.07	0.06	0.24	0.19	0.15	0.12	0.11	0.08	0.42	0.32	0.31	0.22
70th	0.11	0.09	0.37	0.27	0.23	0.19	0.19	0.12	0.71	0.51	0.49	0.34
80th	0.17	0.15	0.60	0.42	0.39	0.30	0.34	0.20	1.26	0.84	0.90	0.57
90th	0.34	0.27	1.19	0.79	0.80	0.58	0.74	0.37	3.00	1.74	1.95	1.14
95th	0.61	0.46	2.12	1.31	1.51	0.97	1.29	0.61	5.81	3.02	3.89	2.04
PI***	0.00 %	0.00 %	0.00%	0.00 %	0.00 %	0.00 %	0.00%	0.00 %	0.40 %	0.00%	0.20%	0.00 %

*SD: Standard deviation; **Percentile; *** $PI = \frac{\#(X_i > PTWI)}{n}$ where $\#(X_i > PTWI)$ denotes the number of exposure values that exceed the PTWI ($25\mu\text{g}/\text{Kg}/\text{week}$)