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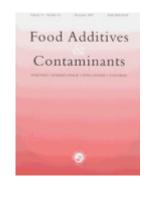
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#### **Food Additives and Contaminants**



# Lead and Cadmium in meat and meat products consumed by a Spanish population (Tenerife Island, Spain)

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 Lead and cadmium in meat and meat products consumed by the population in Tenerife Island, Spain

# Keywords

Lead, cadmium, meat, meat products, graphite furnace atomic absorption spectrophotometry, dietary intake.

#### Abstract

The aim of this study was to determine the levels of lead and cadmium in chicken, pork, beef, lamb and turkey samples (both meat and meat products), collected in the island of Tenerife (Spain). Lead and cadmium were measured by graphite furnace atomic absorption spectrometry (GFAAS). Mean concentrations of lead and cadmium were 6.94 and 1.68  $\mu$ g kg<sup>-1</sup> in the chicken meat samples respectively; 5.00 and 5.49  $\mu$ g kg<sup>-1</sup> in the pork meat samples; 1.91 and 1.90  $\mu$ g kg<sup>-1</sup> in the beef meat samples and 1.35 and 1.22  $\mu$ g kg<sup>-1</sup> in the lamb meat samples. Lead was below the detection limit in the turkey samples and the mean cadmium in the chicken meat products samples were 3.16 and 4.15  $\mu$ g kg<sup>-1</sup> respectively; 4.89 and 6.50  $\mu$ g kg<sup>-1</sup> in the pork meat products samples; 6.72 and 4.76  $\mu$ g kg<sup>-1</sup> in the beef meat products samples. The contribution, in %, of the two considered metals to the PTWIs (Provisional Tolerable Weekly Intake), was calculated for meat and meat products. Statistically significant differences were found for the lead content in meats

between the chicken and pork groups and the turkey and beef groups, whereas for the cadmium concentrations in meats, significant differences were observed between the turkey and the chicken, beef and lamb groups. In meat products, no clear differences were observed for lead and cadmium between the various considered groups.

## Introduction

The main human exposure to heavy metals usually comes from food. After continuously evaluating studies on food additives and their toxicity, the WHO has come to the conclusion that even low levels of some metals, such as lead and cadmium, can give rise to diseases in humans (WHO 2000, WHO 2001). This is produced by the capacity of these metals to accumulate in living organisms. Lead, for example, bio-accumulates in plants and animals. Its concentration is generally magnified in the food chain (Halliwell et al., 2000). Cadmium has a long residence time in human tissues (10-40 years), especially in the kidneys (Rubio et al., 2006). For these reasons, it is of the outmost importance to monitor the lead and cadmium content in dietary intakes.

The main toxic effect of lead is nervous system dysfunction of the foetus and infants. In adults, it causes: adverse blood effects, reproductive dysfunctions; damage to the gastrointestinal track; nephropathies; damage to the central as well as the peripheral nervous system and interferences in the enzymatic systems that synthesise the HEME group (Sierra and Hardisson, 1991; Rubio et al., 2005).

The toxic effects of cadmium are noticeable in various ways. It can interfere with some of the organism's enzymatic reactions, substituting zinc and other metals, manifesting its action in several pathological processes such as renal dysfunctions, hypertension,

arteriosclerosis, inhibition of growth, damages in the nervous system, bone demineralisation and endocrine disruption. In rats, this metal increases the plasma levels of testosterone, induces a phase advance of the nocturnal peak of norepinephrine and abolishes the daily pattern of plasma luteinizing hormone levels (Lafuente et al., 2004 a). Cadmium can, also, stimulate or inhibit the immune activity according to the dose. Doses of 5 and 10 mg/kg of cadmium inhibit the humeral and cellular immune response in rats, whereas doses of 25, 50 and 100 mg/kg produce opposite effects (Montoro et al., 1989; Kosanovic et al., 2002; Lafuente et al., 2004 b).

The health risks produced by lead and cadmium are well known and the levels in food as well as the migration of these metals from food containers are regulated. However, cases of accidental exposure to higher levels of lead and cadmium are often reported within the EU. For example, in Sweden in 2004, a man was poisoned by lead after using ceramics bought in another EU country, which had not been properly fabricated (National Food Administration in Sweden, 2004).

These are but a few cases which show that there exists a continuous need to monitor and control both possible sources of lead and cadmium in food and items used for food processing. Total diet studies have been performed in various countries during the last decades. For example, lead and cadmium intake has been calculated for total diets in the UK (Ysart et al., 2000), Denmark (Larsen et al., 2002), the USA (Pennington et al., 1986) and, also, in Spain (Cuadrado et al., 1995). However, in Spain, the food pattern varies significantly from region to region (Cuadrado et al., 1995) and, therefore, the intake of heavy metals and its consequent risk, also, differs considerably between regions. Cuadrado et al. (1995), showed that meat and meat products were one of the

most important food groups for the total intake of lead, contributing from 11 to 21%, depending on the part of Spain where the study was performed. However, most heavy metal intake studies carried out in Spain were done in the Iberian Peninsula. Peripheral parts, such as Tenerife (Canary Islands) have never been considered before.

Considering the fact the Tenerife has a very high population density, approximately 365 inhabitants per square kilometre, and that it is annually visited by millions of tourists, it is of great interest to determine accurately the content of different heavy metals on the food products which are consumed in this island. In addition, the Canary Islands depend heavily on food imports due to its limited resources. It is, therefore, essential to assure that local as well as imported primary products reach the maximum standards of quality. As a consequence of what has been mentioned above, the objectives of this study were selected and divided in the following: (1) to analyse the lead and cadmium content of meats and meat products sampled in Tenerife; (2) to check that the measured concentrations were below the maximum values allowed by the current legislation; (3) to compare the measured concentrations with those reported by other authors; (4) to estimate the contribution, in %, of lead and cadmium to the PTWIs for the meat and meat products analysed, and (5) to study if there were any statistical differences between the different groups of meat samples in meat and meat products regarding their lead and cadmium concentrations.

#### Materials and method

### Samples

Meat and meat product samples were divided in the following groups: chicken, pork, beef, lamb and turkey. The criteria of selection were made by the consumption

percentage of these products in Tenerife. This information is published in the Nutritional Survey of Canary Islands (2000), which is called ENCA 1997-1998. In this mentioned survey, 2600 people were considered. The type and amount of consumed food were evaluated using both, a 24-hours intake record and a questionnaire of the consumption frequency of 77 different food types.

The meat samples studied in this work were extracted from: chicken legs (12); chicken breasts (12), pork loins (10), pork steaks (10), bacon from pork (7), beef steaks (10), beef stews (10), minced beef meat (8), lamb legs (8), lamb steaks (7), lamb ribs (7) and turkey breasts (10). The meat product samples analysed were chicken sausages (10), chicken hamburgers (8), chicken pâté (7), pork (boiled ham, dry ham, hard pork, sausage, mortadella, salami) sausages (12), pork sausages (8), pork liver pâté (7), beef hamburgers (10), corned beef (7), chopped beef (7), turkey sausages (8), turkey hamburgers (7) and chopped turkey (7). Lamb meat products were not analysed in his study because in the Canary Islands, this kind of products are hardly consumed. The consumption percentages of liver and kidney are, also, not very high and that is why we decided not to include these meats in our study.

All the samples were collected from January till June 2004 in different commercial centres spread across the island. Once the samples were homogenised, they were stored at -18°C in clean polyethylene recipients. The plastic materials used for storing and treating the samples were cleaned to avoid contamination of the samples with traces of any metal. This procedure was carried out using 5% nitric acid during 24 hours followed with two washes with Milli-Q water.

### Determination of lead and cadmium

Each homogenised sample (2 g) was placed in a porcelain crucible. To avoid crosscontamination between the samples, single used plastic tools were utilised to transfer the material. Each sample was then dried in an oven at a temperature ranging between 60 to 80°C for at least 12 hours. The crucibles with the samples were then introduced in muffle ovens and burned to ash at 450°C. The temperature in the muffle ovens were increased at a rate of approximately 50°C per hour and maintained at 450°C during 18 to 24 hours. The white ashes obtained with this procedure were then dissolved in 5% nitric acid to a volume of 50 mL.

The spectrophotometer used to carry out the measurements was a Perkin-Elmer, model 4100 ZL Zeeman, which is equipped with a graphite furnace tube with an automatic sampler.

Table I shows the optimal working conditions in the graphite furnace for the determination of lead and cadmium, indicating the temperature and the times employed in each of the phases. Remark 3 of referee 2: We explain why matrix modifier was not used In the initial lead and cadmium analyses, a mixture of  $NH_4H_2PO_4$  and  $Mg(NO_3)_2$  were used as matrix modifiers. However, no improvements were appreciated in the results and therefore this practice was disregarded later on. The detection limits, calculated as three times the standard deviation of 15 blanks for the acid digest, were 0.28 and 0.065 µg L<sup>-1</sup> for lead and cadmium respectively.

"[Insert Table I about here]"

Quality control of the analytical measurements was performed using blank samples and the following reference materials: the NBS SRH 1577a Bovine Liver from the National

#### **Food Additives and Contaminants**

Institute of Standards and Technology (NIST) and the BCR-278 R Bovine Muscle for the determination of lead and the BCR CRM 184 Bovine Muscle as well as the BCR-278 R Muscle Tissue for the determination of cadmium. The recovery obtained with the reference materials were all above 92% (see table II).

"[Insert Table II about here]"

During all the analytical procedures, each batch of 20 samples were analysed together with a least a blank and a reference sample. The calibration was performed using the calibration curve method.

All the results were tested for normality with the Kolmogorov-Smirnov model (Xu et al., 2002) and for homogeneity in the variance with the Levene test (Pan, 2002). Since our data did not show a normal distribution, the following statistical tests were used (Choy et al., 2001). The non-parametric Kruskal-Wallis test, which allows discriminating individual samples with significantly different results, and the Mann Whitney U test, was used to establish whether there are significant differences between sample groups.

### **Results and Discussion**

The number of samples, number of measurements below detection limits and number of undetectable samples, median value, standard deviation, minimum and maximum for all the meat and meat products analysed for each animal species are shown in table III. "[Insert Table III about here]"

The results show a large variability of lead and cadmium concentrations in some of the meat and meat products groups. However, this variability in biological samples is considered to be normal since the sources of this metal are numerous. Furthermore the

lead and cadmium concentrations, also, depend on the environmental conditions and the food production methods. Even within a certain type of food, concentration variations can be large produced by heterogeneities in the distribution of the metal (Reilly, 1991). In our case, the samples were taken in different times and seasons. Thus the exposure to different metals was not constant contributing to a larger variability.

It can be observed in the data presented in this study that the concentrations of the considered metals are slightly higher in meat product samples than in meat samples. This difference may be explained by the fact that offal (mainly liver and kidney) is often used as an ingredient in meat products and is, also, an important source of lead and cadmium.

The maximum lead and cadmium concentrations which are allowed in meat from different animals are regulated by the EU Directive 466/2001. The authorised levels for lead and cadmium are 100 and 50  $\mu$ g kg<sup>-1</sup> respectively. The concentrations of these metals measured in this work are all within the accepted limits.

Regarding the meat samples, table IV shows a comparison of our results with previously published results (1986-2003). In our study, the lead concentrations in pork are similar to those reported for Sweden (Jorhem et al., 1991), but lower than those measured in Holland, Poland, Finland 1987-1988, Finland 1991, Slovakia and Denmark (Vos et al., 1986; Falandysz et al., 1989; Niemi et al., 1991; Tahvonen and Kumpulainen, 1994; Doganoc, 1996; Larsen et al., 2002). Regarding cadmium concentrations in pork, our values are higher than in Holland, Sweden, Finland and Denmark (Vos et al., 1986; Jorhem et al., 1991; Niemi et al., 1991; Tahvonen and Kumpulainen, 1994; Larsen et al., 2002) but lower than Poland (Falandysz et al., 1989).

#### **Food Additives and Contaminants**

It can be observed that lead concentrations in beef are lower than the values that have been measured in other areas. The cadmium concentrations, except for the value found by López-Alonso for Galicia (Northern Spain), our values are similar than those found in the literature.

The lead and cadmium concentrations measured in chicken are within the range given for Poland, Finland and Denmark by Falandysz, 1991; Tahvonen and Kumpulainen, 1994 and Larsen et al., 2002. In lamb, one can see that lead concentrations are lower than in Denmark, although cadmium concentrations are similar, whereas in turkey our lead concentrations are non detected and cadmium concentrations are higher than Denmark (Larsen et al., 2002).

"[Insert Table IV about here]"

Regarding the analysed meat product samples, both the lead and cadmium concentrations measured in this study were well below the values found in recent literature.

"[Insert Table V about here]"

Considering that the total meat intake by the population in the Canary Islands is 78 g day<sup>-1</sup> and the total meat products intake is 25.9 g day<sup>-1</sup> (ENCA 1997-1998), the daily intake of lead due to the consumption of meats and meat products is 0.296  $\mu$ g day<sup>-1</sup> (2.072  $\mu$ g week<sup>-1</sup>) and 0.155  $\mu$ g day<sup>-1</sup> (1.082  $\mu$ g week<sup>-1</sup>) respectively. This represents, for an average person weighting 65 kg, approximately 0.127 % of the PTWI due the meat consumption and 0.066 % of the PTWI due the meat products consumption, as established by FAO/WHO (25  $\mu$ g kg<sup>-1</sup> week<sup>-1</sup>) (WHO, 1993). The average dietary cadmium intake due to the consumption of meats and meat products calculated in this study is 0.246  $\mu$ g day<sup>-1</sup> and 0.138  $\mu$ g day<sup>-1</sup> respectively. This represents about 0.4 % of

the PTWI due the meat consumption and 0.2 % of the PTWI due the meat products consumption for this metal. Meats contribute to approximately 9% of the total lead and cadmium intake in Tenerife (Canary Islands) and this is the reason why one should consider that there are other sources of these two metals to the diet.

A statistical comparison of the lead and cadmium concentrations in the different meat groups and meat products showed the following: (1) regarding the lead concentrations measured in meats, no significant differences were found between the diverse meat groups when they were considered individually. However, significant differences were measured between the chicken and pork groups and the turkey and beef groups; (2) regarding the cadmium concentrations measured in meats, significant differences were observed between the turkey and the chicken, beef and lamb groups. In the case with the pork, no significant differences were measured due to the high variability found between this and all other groups; (3) in meat products, no clear differences were observed for the lead and cadmium between the various considered groups. This was, most likely, produced by the high variability of the samples that were analysed here.

## Conclusions

The lead and cadmium levels in meat and meat products analysed in this study were well below the legal limits established by the current EU legislation. These levels are, also, comparable to results recently published for other countries. It has been shown here that the consumption of meats in the Canary Islands contribute to a 0.1 % and 0.4 % of the PTWI for lead and cadmium respectively, whereas the consumption of meats products contribute to a 0.1 % and 0.2 % of the PTWI for lead and cadmium. Nevertheless, meats and meat products contribute to approximately 9 % of the total lead

 and cadmium intake in Tenerife (Canary Islands) and there are other sources of exposure of these two metals. In addition, the results of this study show that there exist needs to evaluate and monitor lead and cadmium levels in foodstuffs to establish the intake values which are safe for the population.

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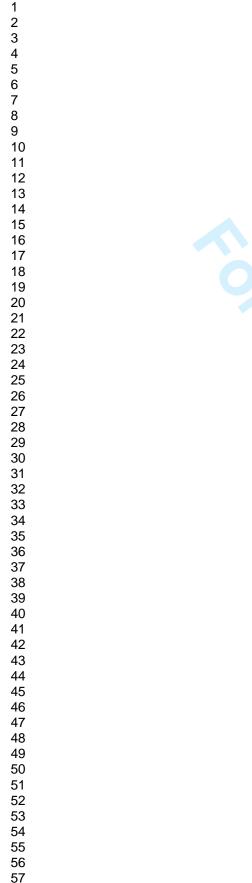


Table I. Thermal programme used for the determination of lead and cadmium in the graphite furnace.						
Phase	Temperature (°C)	Rise Time	Hold Time	Argon Flux (mL/min)		
Evaporation of the solvent	100	10	20	250		
drying	130	25	20	250		
Pirolysis	450	15	20	250		
Pre-treatment	$\frac{800^{(*1)}}{700^{(*2)}}$	5	$10^{(*3)}$ $5^{(*4)}$	250		
Atomisation	1250	0	3	0		
Cleaning	2500	2	3	250		

(\*1) Pre-treatment temperature for the Pb

(\*2) Pre-treatment temperature for the Cd

reatment of the second se (\*3) Time at which the temperature for the pre-treatment of the Pb is maintained

<sup>(\*4)</sup> Time at which the temperature for the pre-treatment of the Cd is maintained

*Injection temperature: 20° C* 

Wavelength of the Pb: 283,3 nm Wavelength of the Cd: 228,8 nm

*Volume of the injection: 20 \mu L* 

Table II. Lead and cadmium recovery study.						
<b>Element</b>	Material	$\mathbf{N}^{a}$	Concentration			Procedure
			Measured <sup>b</sup>	Certified <sup>c</sup>	Recovery %	
			$(\mu g K g^{-1})$	$(\mu g K g^{-1})$		
Pb	NBS SRH	11	130±20	140±20	92,85	AAS
	1577a					graphite
	Bovine					furnace
	Liver					
Pb	BCR-278	11	2.000±40	1.970±70	101,52	AAS
	R Mussel					graphite
	Tissue					furnace
Cd	BCR CRM	11			92,30	AAS
	184 Bovine		12±1	13±2		graphite
	Muscle					furnace
Cd	BCR-278	11	348±7	347±8	100,28	AAS
	R Mussel					graphite
	Tissue					furnace

<sup>a</sup> N<sup>o</sup> of samples

<sup>b</sup> Mean  $\pm$  standard deviation

<sup>c</sup> Confidence interval: 95%

Table III. Concentrations of lea	d and cadmi	ium in meat an	d meat produc	cts (ug kg <sup>-1</sup> ).
	Μ	leat	Meat p	roducts
	Chicken		Chi	cken
	Lead	Cadmium	Lead	Cadmium
Number of samples	24	24	25	25
Number of not detectable samples	0	0	2	0
Median value (µg kg <sup>-1</sup> )	6,94	1,68	3,16	4,15
Standard deviation	4,63	1,76	0,48	1,34
Minimum ( $\mu g k g^{-1}$ )	0,93	0,52	2,76	2,09
Maximum (µg kg <sup>-1</sup> )	13,43	5,21	3,71	5,22
	Р	ork	Po	ork
	Lead	Cadmium	Lead	Cadmium
Number of samples	27	27	27	27
Number of indetectable samples	1	0	5	0
Median value ( $\mu g k g^{-1}$ )	5,00	5,49	4,89	6,50
Standard deviation	4,79	5,11	3,84	3,93
Minimum ( $\mu g k g^{-1}$ )	1,02	0,42	0,35	0,52
Maximum (µg kg <sup>-1</sup> )	11,55	13,24	13,23	13,54
	В	eef	Be	eef
	Lead	Cadmium	Lead	Cadmium
Number of samples	28	28	24	24
Number of indetectable samples	3	0	3	0

Deleted: 1

Median value (µg kg <sup>-1</sup> ) Standard deviation	1,91 0,007	1,90 1,86	<u>6,72</u> 1,49	4,76 4,93
Minimum (µg kg <sup>-1</sup> )	1,91	0,50	5,66	1,76
Maximum (µg kg <sup>-1</sup> )	1,91	4,45	7,78	13,50
	1,72	т,т5	7,70	15,50
	L	amb	L	amb
	Lead	Cadmium	Lead	Cadmium
Number of samples	22	22	0	0
Number of indetectable samples	1	0	-	-
Median value (µg kg <sup>-1</sup> )	1,35	1,22	-	-
Standard deviation	0,80	0,59	-	-
Minimum (μg kg <sup>-1</sup> )	0,59	0,72	-	-
Maximum ( $\mu g k g^{-1}$ )	2,20	2,09	-	-
	Τι	ırkey	Τι	ırkey
	Lead	Cadmium	Lead	Cadmium
Number of samples	10	10	22	22
Number of indetectable samples	10	0	1	0
Median value (µg kg <sup>-1</sup> )	N.D.	5,49	9,12	5,98
Standard deviation	-	0,68	8,65	3,10
Minimum (µg kg <sup>-1</sup> )	-	4,70	2,56	1,03
Maximum ( $\mu g k g^{-1}$ )		5,90	21,30	9,46

Reference	Origin	Type of meat	Pb ( $\mu g k g^{-1}$ )	Cd (µg kg <sup>-1</sup>
Vos et al., 1986	Netherlands	Pork meat	10	2
Falandysz et al., 1989	Poland	Pork meat	130	21
Jorhem et al., 1991	Swedish	Pork meat	5	1
Niemi et al., 1991	Finland	Pork meat	15	1,5
Tahvonen and	Finland	Pork meat	9	N.D.
Kumpulainen, 1994				
Doganoc, 1996	Slovenia	Pork meat	< 50	10
Larsen et al., 2002	Denmark	Pork meat	< 15	< 1
This study	Tenerife	Pork meat	5,00	5,49
	(Spain)			
		-		-
Jorhem et al., 1991	Swedish	Beef	< 5	1
Niemi et al., 1991	Finland	Beef	13	1,3
Falandysz, 1993	Poland	Beef	40	6
Tahvonen and	Finland	Beef	10	N.D.
Kumpulainen, 1994				
Doganoc, 1996	Slovenia	Beef	50	4
López Alonso et al., 2000	Galicia (Spain)	Beef	9	1
Larsen et al., 2002	Denmark	Beef	< 16	< 1
López Alonso et al.,	Galicia	Beef	14,7	35,7
2003	(Spain)			
This study	Tenerife	Beef	1,91	1,90
	(Spain)	*		
			-	
Falandysz, 1991	Poland	Chicken	< 10-20	< 5
Tahvonen and Kumpalainem, 1994	Finland	Chicken	6	N.D.
Larsen et al., 2002	Denmark	Chicken	< 14	< 1
This study	Tenerife (Spain)	Chicken	6,94	1,68
Larsen et al., 2002	Denmark	Lamb	< 14	1,8
This study	Tenerife	Lamb	1,35	1,22
	(Spain)			
		1		
Larsen et al., 2002	Denmark	Turkey	< 14	< 1
This study	Tenerife	Turkey	N.D.	5,49
	(Spain)			

Reference	Origin	Type of meat	Pb (µg kg <sup>-1</sup> )	Cd (µg kg <sup>-1</sup> )
	_	product		••••
Catalá et al., 1983	Spain	Ham	320	32
Cattaneo and Balzareti 1984	Italy	Ham	380	67
Becerra et al., 1987	Spain	Ham	2.240	N.D.
Cervera et al., 1988	Spain	Ham	29	6
Brito et al., 1990	Spain	Ham	570	84
Karavoltsos et al., 2002	Greece	Ham	-	0,6
Dabeka and McKenzie,	Canada	Cured Ham	90	3
1991				
Noirfalisse, 1980	France	Pork liver paté	268	37
Nuurtamo et al., 1980	Finland	Pork liver paté	< 20.000	20
Becerra et al., 1987	Spain	Pork liver paté	360	N.D.
Cervera et al.,1988	Spain	Pork liver paté	30	28
Brito et al., 1990	Spain	Pork liver paté	300	73
Ybáñez et al., 1982	Spain	Sausages	380	36
Beltrán et al., 1987	Cuba	Sausages	-	9
Karavoltsos et al., 2002	Greece	Sausages	-	1,6
Meah, 1989	UK	Corned beef	666	-
Onianwa et al., 2000	Nigeria	Corned beef	-	70

\* N.D. = Non Detected

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 Sausages

 Corned beef

 Corned beef