THE CONTRIBUTION OF METROLOGY IN TOTAL DIET STUDIES: PORTUGUESE CASE

Marta Ventura, Inês Coelho, Sandra Gueifão, Andreia Rego, Isabel Castanheira

Department of Food and Nutrition, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal, isabel.castanheira@insa.min-saude.pt

Abstract – TDS provide analytical data to estimate dietary exposure of chemical contaminants. In this work was studied Pb, As, Cr and Cd profile in foods from Portuguese TDS Pilot Study. Nine hundred and twenty four foods were pooled in two hundred and thirty one laboratory samples and analysed by ICP-MS under rigorous metrological control. The highest amount (μg/kg) obtained for each chemical substance was: As 9138 ± 237 (Octopus); Pb 282 ± 5.5 (snails); Cr 605 ± 28 (Cured meat); Cd 248 ± 5 (snails). These levels of potential harmful substances do not pose a risk to population.

Keywords: Total Diet Studies, harmful substances, food data, ICP-MS.

1. INTRODUCTION

Total diet studies (TDS) provide analytical data to estimate dietary exposure and risk assessment of contaminants [1]. TDS were adopted by several European countries as the sound scientific basis to strength the linkage between food consumption and food analysis with purpose to assess the effect of harmful substances on health [2].

TDS studies are designed in three steps: planning analysis and communication. The first phase addressing selection of foods from food consumption data, sampling plan, choice of metrological tools (analytical methods, acceptance criteria, CRMs and PT schemes matrix matching) chemical analysis is the second step. Exposure assessment and publication of data are the last phase of TDS [1].
Selection foods are prepared as consumed and analysed in pooled samples. Foods are grouped by similarities taking into account the groups as defined by EFSA, each pool is composed by 12 samples grouped by carbohydrates or fat or protein content [3]. The list of chemical substances was created recently under the frame of Total diet study exposure project.[3] Heavy metals are prioritized substances among TDS exposure chemical substances [4].
ICP- MS is the most appropriate analytical method assisted by microwave. Quality assurance and acceptance criteria were recently discussed under the TDS project [5].
LOD and LOQ are the most relevant method performance criteria. Therefore analytical values below LOQ the so designated left-censored data are used to estimate dietary exposure. In spite of the handling of left-censored data is specified in TDS studies the analytical value used can lead to overestimation of the exposure since LOQ value is used to estimate dietary exposure as the default standard [5]. Analytical uncertainty is another critical parameter. Two approaches are reported to estimate measurement uncertainty. Modelling and experimental approach. However maximum standard measurement uncertainty is established and is one of method and laboratory acceptance criteria in TDS analysis[6]. Analytical data provided by laboratories are then used in combination with food consumption to estimate dietary intake of chemical substance and impact in health of population [7].

The aim of this work was to study the profile of chemical contaminants (Pb, Cr, Cd and As) in foods collect in Portuguese TDS pilot study.

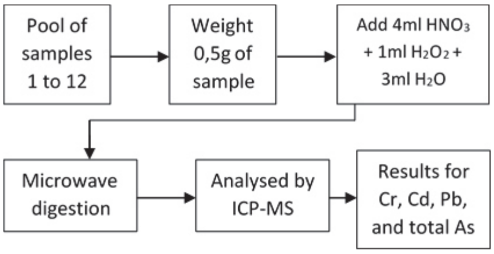
2. MATERIALS AND METHODS

2.1. Food collection

Samples (n=480) were collected according to Portuguese food consumption survey. The food items were coded in FoodEx2 (classification system) and were aggregated into composite samples in the twenty FoodEx2 food groups, and analysed in pooled of twelve samples.

2.2. Sample preparation

Figure 1 – Sample preparation



2.3. Analysis of laboratory sample

Analysis of total elements were carried as described by Coelho et al with slight modifications in brief [8].The digestion was made according the following program; in a first step the power of the microwave was increased to 1150 W and temperature to 180ºC and kept constant for 10 min, in a second step the power decrease to 0 W and the temperature kept at 180ºC during 5 min, in a third step the power was increased to 1150 W and temperature to 210ºC during 12 min, in a fourth step the power decrease to 0 W and the temperature kept at 210ºC during 5 min and in the last step the power was increased to 650 W and temperature to 90ºC during 6 min. Then trace elements were analyzed by ICP-MS (ICP-MS ThermoX Series II). As internal standards were used standard solutions of germanium containing 1000 $mg.L^{-1}$ (inorganics ventures), yttrium and indium containing 1000 $mg.L^{-1}$(Merck).

2.4. ICP-MS determination procedure

ICP-MS measurements were performed using software Xseries PlasmaLab 2.5. Further details of the instrumental settings are given in table 1.

Table 1 ICP-MS Operating conditions

ICP-MS Thermo X series II	
Extraction	-113,7
Focus	10
Pole Bias	-0,1
Hexapole Bias	-3
Nebulizer flow rate (L min^{-1})	0,87
Forward Power (W)	1404
Cool gas flow rate (L min^{-1})	13
Auxiliary gas flow rate (L min^{-1})	0,9
Sampling Depth	120
Standard Resolution	135
High Resolution	150
Analogue Detector	1902
PC Detector	3353

2.5. Quality assurance and traceability

A rigorous quality assurance program followed the ISO 17025 requirement was implemented for analysis of Total elements as laboratory holds an accreditation for these assays. Certified Reference Material GBW 10014 Cabbage and NIST 1548a Typical Diet was used to test the accuracy and precision of the method of analysis of total elements. Recoveries between 80% and 120% were obtained. Repeatability was under 10% for all analysis. The detection limits were estimated by repetitive analysis of procedural blank samples (n=10) as described in EFSA document [1]. Laboratory performance was demonstrated through a successfully participation in PT schemes runs by an accredited provider. All samples were analysed in triplicate. A Quality Control (QC) sample was included in each group of twelve test samples to validate the test run. Instrumental drift was monitored by difference between result of first and last QC samples (< 5%).

3. RESULTS

Results of accurancy of the method are presented in table 2. Values found are within the laboratory acceptance criteria. In table 3 results of PT schemes are presented expressed by z scores. All the values are < 2 z score reveling that laboratory sucessufly participate in this schemes and demonstrated appropriate performance. Highest desviation was obtained for Chromium in canned fish may due to the large variablilty that occurs for this element when concentrations are near LOQ as in this case.

Table 2- Method accuracy using matrix matched certified reference materials ($\overline{x}$ ± s mg/kg).

Element	Certified value	Determined value
NIST 1548a Typical Diet		
As	0.20 ± 0.01	0.181 ± 0.006
Cd	0.035 ± 0.0015	0.024 ± 0.003
Pb	0.044 ± 0.009	0.037 ± 0.002
GBW 10014 cabbage		
Cr	1.8 ± 0.3	1.6 ± 0.1

Table 3 – Results obtained during the PT scheme for the analysis of heavy metals in foodstuffs.

Matrix	Cr	As	Cd	Pb
Canned crab meat	-0.5	0.4	0.4	-0.2
Milk powder	0.64	1.9	0.8	0.6
Powdered rice	0.24	-1.3	-1,0	-0.6
Canned fish	1.49	-0.1	-0.9	-0.3
Edible oil	0.68	0.9	1.1	-0.1

Occurrence data is presented on table 4 and table 5, for inorganic contaminants determined by ICP-MS. Cr was found in all samples excepted for kiwi, on the others foods Cr contents is presented in the range of 47 µg /kg (pineapple) to 605 µg /kg (cured meat). Chromium can be a Cr(III) or Cr(VI) when Chromium is presented as Cr(III) it is important in glucose metabolism among other biological functions [9]. However if it is presented as hexavalent Cr it is a harmful substance. When speciation is absent as in the case of TDS Cr is consider as the worst case assuming that all is present as Cr(VI). Human exposure occurred in other variety of ways such as enviromental and occupational however food is one of the main sources of exposure to Cr(VI). Cr concentrations in foods consumed by Portuguese population is similar to those obtained in another European TDS studies [11].

As is present in 35% of the analysed food samples, in the range of 21 µg /kg (dried fruits) to 9138 µg /kg (octopus). In the case of Arsenic EFSA guidelines are available reporting the occurrence of chemical arsenic species. High arsenic values were found in fish which is probably, arsenobetaine, the arsenic no toxic form. However inorganic arsenic(iAs) is the predominant form in dried foods. Arsenic (iAs) is a know carcinogen in humans causing lung, liver, skin and bladder cancer[10]. Nevertheless results are comparable to those published in 1st and 2nd French TDS and are not in levels of concerned [11].

Cadmium is a contaminant presented in foods originated from zones in contact with industrial workplaces. Apart from environmental and occupational, food are main route of human exposure to cadmium. In this study Cd is quantified in 12.5% of 480 food samples analysed, the others were below the LOQ. For all food samples, no value exceeded the maximum levels fixed by Commission Regulation (EU) No 488/2014 [12].

Lead is the most recognised toxic environmental pollutant of the 480 food samples analysed 10% of Pb values were quantified, the others were below the LOQ. For all food samples, no value exceeded the maximum levels fixed by Commission Regulation (EC) No 1881/2006 [13].

Table 4 - Contents of chemical substances in samples in rich protein foods. Concentration $\bar{x} \pm s$ µg /kg.

Samples	Cr	As	Cd	Pb
Bovine	188 ± 8	n.d.	n.d.	n.d.
Calf	179 ± 6	n.d.	n.d.	n.d.
Swine	186 ± 4	n.d.	n.d.	n.d.
Sheep	135 ± 10	n.d.	n.d.	n.d.
Rabbit	286 ± 15	n.d.	n.d.	n.d.
Chicken	343 ± 6	n.d.	n.d.	n.d.
Turkey	126 ± 9	n.d.	n.d.	n.d.
Cured meat	605 ± 28	31 ± 5	n.d.	n.d.
Sausage	293 ± 17	n.d.	n.d.	n.d.
Salmon	562 ± 13	680 ± 34	n.d.	n.d.
Sea Bream	407 ± 18	850 ± 35	n.d.	n.d.
Fresh Cod	116 ± 9	3555 ± 63	n.d.	n.d.
Sardine	119 ± 3	2695 ± 101	39 ± 1	50 ± 3
Tuna	188 ± 3	809 ± 36	n.d.	n.d.
Shrimps	178 ± 11	4104 ± 211	93 ± 1	n.d.
Molluscs	379 ± 16	3667 ± 233	125 ± 17	260 ± 23
Octopus	103 ± 12	9138 ± 237	115 ± 3	n.d.
Dried Cod	241 ± 12	1060 ± 25	n.d.	76 ± 2
Snails	182 ± 15	29 ± 3	248 ± 5	282 ± 6
Eggs	260 ± 13	n.d.	n.d.	n.d.

Table 5 - Contents of chemical substances in samples in poor protein foods. Concentration $\bar{x}$ ± s µg/kg.

Samples	Cr	As	Cd	Pb
Orange	74 ± 13	n.d.	n.d.	n.d.
Aple	62 ± 3	n.d.	n.d.	n.d.
Pear	57 ± 1	n.d.	n.d.	n.d.
Grapes	114 ± 5	n.d.	n.d.	n.d.
Strawberries	51 ± 2	n.d.	n.d.	n.d.
Peach	69 ± 2	n.d.	n.d.	n.d.
Kiwi	n.d.	n.d.	n.d.	n.d.
Banana	146 ± 4	n.d.	n.d.	n.d.
Pineapple	47 ± 3	n.d.	n.d.	n.d.
Dried fruits	343 ± 50	21 ± 2	n.d.	n.d.
Dried figs	217 ± 15	31 ± 1	n.d.	n.d.
Jam	182 ± 10	n.d.	n.d.	n.d.
Fruit salad	124 ± 19	n.d.	n.d.	n.d.
Canned fruit	108 ± 5	n.d.	n.d.	n.d.
Broad bean	103 ± 9	n.d.	n.d.	n.d.
Peas	103 ± 5	n.d.	n.d.	n.d.
Beans	93 ± 6	n.d.	n.d.	n.d.
Cowpea	119 ± 7	n.d.	n.d.	n.d.
Lupin	121 ± 14	43 ± 4	n.d.	n.d.
Table olives	212 ± 3	n.d.	n.d.	51 ± 3

4. CONCLUSIONS

The results of TDS Portuguese Pilot study showed that contents of inorganic chemical contaminants are similar to those published by studies carried out in other European Countries.

The values for cadmium and lead did not exceed the maximum levels set up in legislation.

The highest values for Arsenic were found in fish which is mostly arsenobetaine, an arsenic no toxic form. The highest contents of chromium were found in cured meat and salmon, the other results are similar to those observed in other TDS carried out in Europe, which can be due to the similarity of the samples.

Chemical contaminants are presented in foods in small amounts and appears do not pose a risk to portuguese population.

REFERENCES

[1] FAO, EFSA, and WHO, "Towards a harmonised Total Diet Study approach: a guidance document," *EFSA J.*, vol. 9, no. 11, pp. 1–66, 2011.

[2] N. Arnich, V. Sirot, G. Rivière, J. Jean, L. Noël, T. Guérin, and J. C. Leblanc, "Dietary exposure to trace elements and health risk assessment in the 2nd French Total Diet Study," *Food Chem. Toxicol.*, vol. 50, no. 7, pp. 2432–2449, 2012.

[3] A. Papadopoulos, I. Sioen, F. Cubadda, H. Ozer, H. I. Oktay Basegmez, A. Turrini, M. T. Lopez Esteban, P. M. Fernandez San Juan, D. Sokolić-Mihalak, M. Jurkovic, S. De Henauw, F. Aureli, K. Vin, and V. Sirot, "TDS exposure project: Application of the analytic hierarchy process for the prioritization of substances to be analyzed in a total diet study," *Food Chem. Toxicol.*, vol. 76, pp. 46–53, 2015.

[4] K. Vin, A. Papadopoulos, F. Cubadda, F. Aureli, H. I. Oktay Basegmez, M. D'Amato, S. De Coster, L. D'Evoli, M. T. López Esteban, M. Jurkovic, M. Lucarini, H. Ozer, P. M. Fernández San Juan, I. Sioen, D. Sokolic, A. Turrini, V. V. Sirot, H. Imge, O. Basegmez, M. D. Amato, S. De Coster, L. D. Evoli, M. Teresa, L. Esteban, M. Jurkovic, M. Lucarini, H. Ozer, P. Mario, F. San, I. Sioen, D. Sokolic, A. Turrini, and V. V. Sirot, "TDS exposure project: Relevance of the Total Diet Study approach for different groups of substances," *Food Chem. Toxicol.*, vol. 73, pp. 21–34, 2014.

[5] S. Millour, L. Noël, R. Chekri, C. Vastel, A. Kadar, and T. Guérin, "Internal quality controls applied in inductively coupled plasma mass spectrometry multi-elemental analysis in the second French Total Diet Study," *Accredit. Qual. Assur.*, vol. 15, no. 9, pp. 503–513, 2010.

[6] F. Ulberth, "Metrological concepts required for food safety and quality testing," *Accredit. Qual. Assur.*, vol. 16, no. 12, pp. 607–613, 2011.

[7] V. Sirot, J. M. Fremy, and J. C. Leblanc, "Dietary exposure to mycotoxins and health risk assessment in the second French total diet study," *Food Chem. Toxicol.*, vol. 52, pp. 1–11, 2013.

[8] I. Coelho, S. Gueifão, A. S. Matos, M. Roe, and I. Castanheira, "Experimental approaches for the estimation of uncertainty in analysis of trace inorganic contaminants in foodstuffs by ICP-MS," *Food Chem.*, vol. 141, no. 1, pp. 604–611, 2013.

[9] Ş. Tokalıoğlu, "Determination of trace elements in commonly consumed medicinal herbs by ICP-MS and multivariate analysis," *Food Chem.*, vol. 134, no. 4, pp. 2504–2508, 2012.

[10] S. Kapa, H. Peterson, K. Liber, and P. Bhattacharya, "Human Health Effects From Chronic Arsenic Poisoning–A Review," *J. Environ. Sci. Heal. J. J. Environ. Sci. Heal. Part A*, vol. 41, no. 10, pp. 1093–4529, 2006.

[11] S. Millour, L. Noël, A. Kadar, R. Chekri, C. Vastel, V. Sirot, J. C. Leblanc, and T. Guérin, "Pb, Hg, Cd, As, Sb and Al levels in foodstuffs from the 2nd French total diet study," *Food Chem.*, vol. 126, no. 4, pp. 1787–1799, 2011.

[12] U. Europeia, "Jornal Oficial L 136," vol. 25, 2014.

[13] D. A. S. C. Europeias, "20.12.2006," vol. 2006, no. 8, pp. 5–24, 2006.