ESTIMATED INTAKE OF PCDDs, PCDFs AND CO-PLANAR PCBs IN INDIVIDUALS FROM MADRID (SPAIN) ASSOCIATED WITH AN AVERAGE DIET. STUDIES ON SPANISH REPRESENTATIVE DIETS.

Begoña Jiménez*, Luis Manuel Hernàndez and Marìa Josè Gonzàlez
Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, C.S.I.C., Juan de la Cierva 3, 28006 Madrid, SPAIN
* Present address: *University of Siena, Dept. Environmental Biology, Via delle Cerchia 3, 53100 Siena, ITALY. Fax: 577-298806, e-mail: bjimenez@sidst1.dst.unisi.it*
Etel Eljarrat and Josep Rivera
Laboratory of Mass Spectrometry, Research and Development Center, C.S.I.C., Jordi Girona Salgado 18-26, 08034 Barcelona, SPAIN.

1. INTRODUCTION AND OBJECTIVES

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenils (PCBs) have been detected in human tissues of the general population from several countries with similar patterns and levels[1-5]. Therefore, it is necessary to know the sources of human exposure and the quantities of PCDD/Fs and PCBs intake for appropiate control measures and risk assessment. Food is known to be a major source of PCDDs and PCDFs in the general population. The presence of PCDDs and PCDFs in food has been reviewed recently[6]. It was concluded that most animal-derived food are likely to be contaminated with low levels of PCDD/Fs.

It is most effective to base the samples studied around a typical diet, in order to reflect the trends in food consumption of the region of interest[7]. Various surveys in different countries have found dioxins and furans in samples of fish, milk products, meat and human milk[8,9]. However, little data on Spain food PCDDs and PCDFs are available and any estimates of Spain dioxin intake from food have been performed to date. Since the current daily intake of PCDD/Fs and co-planar PCBs in Spain it is not known, it was considered of great interest to carry out a Dioxin survey in foods. This study intended to investigate the estimated intake of PCDD/Fs and co-planar PCBs associated with an average spanish diet.

Most studies, use analytical data from different food samples and then an "average" concentration of PCDD/Fs in food can be estimated. By combining these "averages" concentrations with food consumption data, a tentative estimate for the average dietary intake of dioxins and furans by the population may be obtained. This study was conducted in a way not reported to date. The idea was to analyze, as a single samples, all the foods, in the way they are eaten, consumed by adult individuals during a whole day instead of analyze all the different food categories separately. In this case it is considered that data from analysis would reflect in a more real way the total dietary intake of PCDD/Fs and PCBs per day for an adult individual since food samples are taken in the amounts and in the way they are normally eaten.

In this Total Diet Study (TDS), composite food groups representing the average diet consumed dailly in Spain by the general population, but not including food consumed outside the home or alcoholic beverages, were collected and analysed for PCDD/Fs and co-planar PCBs.

2. MATERIAL AND METHODS.

Sampling

Food samples, in the way they are consumed, and according to an representative average daily diet consumed in Spain, were collected in Madrid (SPAIN) during April 1995. Regional or seasonal variations which could appear throughout the country were not covered in this study.

During three different days, food items corresponding to all meals consumed by adult individuals during the whole day were collected, according to the standard food composition in Spain[10]. It should be noted that an average spanish diet comprises three meals: breakfast, lunch and evening meal, being the luch the most important and most abundant meal.

All the food items collected were grouped, as reflected in Table 1, in three different groups (FC1, FC2, FC3), one per day, according to all the food which is normally ingested by an adult individual during a whole day.

Food composites were prepared by mixing all the meals constituting each group (FC1, FC2 or FC3) and blending all components with a Turrax apparatous until a fine homogenate was obtained. All the mixtures prepared were of about 2000 grams. Mixtures obtained were freeze dried, being the amount of dry material obtained of about 300 grams for each mixture. Amounts of 5 grams of dry material, mixed with sodium sulfate were used for analysis. All samples prepared were analysed in duplicate.

Extraction and clean up.

Extraction and clean up followed a method previously described in detail in a previous work carried out in the U.K[11] . Basically this comprises low pressure chromatography on neutral and base-modified silica gel, activated carbon dispersed on glass fibers, silica gel impregnated with sulfuric acid, and Florisil. Three fractions were eluted from the carbon column for each sample. These contained ortho-substituted PCBs, non-ortho-substituted PCBs and PCDD/Fs respectively.

Prior to the initial extraction of the samples, a mixture of $^{13}C_{12}$ PCDD/Fs and non-ortho substituted PCBs internal standards, was added containing one isomer from each homologue group with the exception of OCDF.

Table 1. Food composites prepared in this study.

MEAL	*FC1*	*FC2*	*FC3*
BREAKFAST	Coffee with milk	Coffee with milk	Coffee with milk
		Toast / butter and jam	Orange juice
LUNCH	BREAD/WATER	BREAD/WATER	BREAD/WATER
1	Lentils	Macaroni	Potatoes with meat
2	Chicken/green salad	White fish/green salad	Fried Eggs/Black pudding
3	Orange	Banana	Orange
EVENING MEAL	BREAD/WATER	BREAD/WATER	BREAD/WATER
1	Noodle soup		Jam and cheese sandwich
2	Omelette	Steak/green salad	Pork sausage
3	Yogourt	Pear	Yogourt

Analytical determination.

Resolution and quantification of PCDDs, PCDFs and co-planar PCBs were performed by HRGC-HRMS usig a VG AutoSpec Ultima (VG Analytical, Manchester, U.K.) coupled to a Fisons Series 8000 (8060) Gas Chromatograph. A fused silica capillary DB-5 column (60m, 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, U.S.A.) was used using helium as carrier gas at a column head pressure of 175 Kpa. A minimum resolution of 10,000 was used when operating with the HRMS instrument.

3. RESULTS AND DISCUSSION

Table 2 presents average congener-specific results of PCDD/Fs, co-planar PCBs (values for PCB #126 are not quoted due to analytical interferences) and calculated I-TEQs[12,13] for all the samples analysed. Values reported in Table 2 correspond to total pg found in an average diet, and are calculated on a whole food basis to be more representative of the way food is consumed. In order to compare with other values from similar studies reported in the literature, all values quoted have been calculated three times: firstly assuming that all values less than the limit of detection (LOD) are equal to the LOD, secondly assuming that are equal to the half of the LOD and thirdly assuming that all "not detected" values are equal to zero. In this section discussion is presented on the basis of the first assumption. This is likely to have resulted in a considerable overestimate of the total intake of PCDDs, PCDFs and co-planar PCBs.

Total PCDD mean levels were 19,089.27 pg. Among the PCDDs, it was the OCDD the congener which exhibited the highest levels, as usual in biological samples[14], and the 1,2,3,4,7,8-H_6CDD was the congener which exhibited the lowest average value. The 2,3,7,8-T_4CDD, was found "not detected" in all samples analysed, but assuming the value of the LOD exhibited an average value of 53.68 pg. **Total PCDF** mean levels were found to be 432.37 pg which represent only a 2% of the total PCDD/F levels. This finding differs from the tendency observed for human samples in other studies[14,15] where it was reported that total dioxins contribute 88.5% and dibenzofurans 11.5% to total dioxin levels, but they each contribute almost 50% of the dioxin toxic equivalents. Among the PCDFs, the OCDF was found to be the most abundant with an average value of 168,44 pg. The congener 1,2,3,4,7,8,9-H_7CDF was not detected at any of the samples analysed. **Total co-planar PCB** (#77 and #169) average levels were 12,868.61 pg which represent about the 40% of the total PDDD/F and co-planar PCB mean levels. PCB #77 made the most important contribution (99.8%) to total co-planar PCBs.

The **estimated average total intake** figure for dioxins and furans from an average spanish diet was found to be 142 pg I-TEQ/day (2.4 pg I-TEQ/Kg b.w./day for a 60 Kg person). PCDDs provided almost 80% of the total dioxin equivalents. The 2,3,7,8-TCDD made the most important contribution to dioxin toxicity here, providing almost 38%. This is consistent with results reported by Ono[16]. Also 1,2,3,4,6,7,8-HpCDD, OCDD and 1,2,3,7,8-PnCDD made a substancial contribution, providing 19%, 11% and 9% of the total dioxin toxic equivalents. In total all this four congeners mentioned above contributed 77% of PCDD/Fs I-TEQs, being the remaining 23% distributed between the other PCDDs and the PCDFs. Among PCDF I-TEQs it is only noticeable the contribution made by the toxic 2,3,7,8-PnCDF which represents a 7%, similar to those of the 1,2,3,7,8-PnCDD. All I-TEQ data calculated for each congener could be grouped in three categories depending on the order of magnitude:

1. Range from 0.01 to 1 pg I-TEQ. This group includes five congeners which account 1% of the total I-TEQ value: 1,2,3,7,8-PnCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF and OCDF.
2. Range from 1 to 10 pg I-TEQ. This group includes eight congeners which represent a 16% of the total I-TEQ value, and comprises all the hexa chlorinated dioxins and furans and the 2,3,7,8-TCDF.
3. Range from 10 to 100 pg I-TEQ. Here are grouped five congeners which contribute the 83% of the total I-TEQ: 2,3,4,7,8-PnCDF, 1,2,3,7,8-PnCDD, OCDD, 1,2,3,4,6,7,8-HpCDD and 2,3,7,8-TCDD, which made the most noticeable contribution.

If the mean I-TEQ value calculated for co-planar PCBs is used in the estimation of the dietary intake, the estimated average total intake figure is 272 pg I-TEQ/day (4.5. pg I-TEQ/Kg b.w./day for a 60 Kg person). In this case PCDD/Fs contributes 52% and co-planar PCBs 48% to total I-TEQ value.

This estimated discussed above assumes that values of all analytes in foods below the LOD are equal to the LOD and represents the ***upper bound*** estimate.

Table 2. Estimated dietary intake of congener specific PCDDs, PCDFs and co-planar PCBs and calculated I-TEQs in total pg/person/day.

ISOMER	+	*AVER. (a)*	*STAND. DEVIAT.*	*I-TEQ (a)*	*AVER. (b)*	*STAND. DEVIAT.*	*I-TEQ (b)*	*AVER. (c)*	*STAND. DEVIAT.*	*TEQ (c)*
2,3,7,8-TCDF	5	47.52	21.45	4.75	47.21	21.96	4.72	46.91	22.46	4.69
2,3,7,8-TCDD	0	53.68	51.34	53.68	26.84	25.67	26.84	0	0	0
1,2,3,7,8-P_5CDF	5	16.02	8.04	0.80	15.84	8.46	0.77	14.95	8.96	0.75
2,3,4,7,8-P_5CDF	6	20.46	10.54	10.23	20.46	10.54	10.23	20.46	10.54	10.23
1,2,3,7,8-P_5CDD	3	25.59	11.66	12.79	21.88	15.49	10.94	18.18	19.43	9.09
1,2,3,4,7,8-H_6CDF	6	19.71	10.81	1.97	19.17	10.81	1.97	19.17	10.81	1.97
1,2,3,6,7,8-H_6CDF	6	19.05	6.85	1.90	19.05	6.85	1.90	19.05	6.85	1.90
2,3,4,6,7,8-H_6CDF	4	22.06	12.63	2.21	21.10	13.46	2.11	20.15	14.30	2.02
1,2,3,7,8,9-H_6CDF	6	45.58	15.35	4.56	45.58	15.35	4.56	45.58	15.35	4.56
1,2,3,4,7,8-H_6CDD	2	25.00	15.43	2.50	19.61	20.00	1.96	14.23	24.64	1.42
1,2,3,6,7,8-H_6CDD	3	31.56	26.78	3.16	27.31	30.44	2.73	23.08	34.26	2.31
1,2,3,7,8,9-H_6CDD	2	30.28	21.78	3.03	24.35	26.81	2.43	18.43	31.92	1.84
1,2,3,4,6,7,8-H_7CDF	6	54.91	36.30	0.55	54.91	36.30	0.55	54.91	36.30	0.55
1,2,3,4,7,8,9-H_7CDF	0	18.62	11.79	0.19	9.58	6.36	0.09	0	0	0
1,2,3,4,6,7,8-H_7CDD	6	2345.83	2776.80	23.46	2345.83	2776.80	23.46	2345.83	2776.80	23.46
OCDF	6	168.44	145.50	0.17	168.44	145.50	0.17	168.44	145.50	0.17
OCDD	6	16144.96	18802.17	16.14	16144.96	18802.17	16.14	16144.96	18802.17	16.14
SUM PCDD/Fs		19089.27		**142.08**	19032.34		**119.59**	18974.87		**81.10**
SUM PCDDs		18656.90		114.76	18610.81		84.51	18564.71		54.27
SUM PCDFs		432.37		27.32	421.53		27.08	410.16		26.83
PCB # 77	6	12840.88	773.41	128.41	12840.88	773.41	128.41	12840.88	773.41	128.41
PCB # 169	3	27.73	21.20	1.39	25.94	23.06	1.3	24.16	24.94	1.21
SUM PCBs (#77,#169)		12868.61		**129.80**	12866.82		**129.71**	12865.04		**129.62**
SUM PCDD/Fs & PCBs		31957.88		**271.88**	31899.16		**241.30**	31839.91		**210.72**

+ Number of positives; **(a)** Average calculated assuming that not detected are equal to the Limit of Detection; **(b)** Average calculated assuming that not detected are equal to LOD/2; **(c)** Average calculated assuming that not detected. are equal to zero.

If concentrations less than the LOD are taken as equal to zero, then the estimated average intake would be 81 pg I-TEQ/day (1.35 pg I-TEQ/Kg b.w./day for a 60 Kg person) which is the ***lower bound*** estimate and is a 43% lower than the upper bound estimate. In this case the toxicity percentage is reduced due to the absence of figure for 2,3,7,8-TCDD which made the most important contribution in the first estimate, while the I-TEQ values contributed by the other congeners remain with similar values in both estimates. In the case of the *lower bound* estimate, when the mean I-TEQ value calculated for co-planar PCBs is used in the estimation, then the estimated average total intake figure is 210 pg I-TEQ/day, which is a 23% lower than the *upper bound* estimate.

An expert group convened by the WHO/EURO has recommanded a TDI of 10 pg/Kg b.w./day for 2,3,7,8-TCDD which is equivalent to an intake of 600 pg TEQ/day for a 60 Kg person. Thus the estimated average dietary intake of dioxins and furans in Spain is considerably below the TDI recommended by the WHO/EURO Expert Group[17], even considering calculated I-TEQ value for co-planar PCBs in the upper bound estimate.

There is evidence that high levels of PCDDs, PCDFs and co-planar PCBs may be derived from the consumption of dietary supplements based on fish oils[11]. This study conducted on dietary supplement fish oils obtained in Spain reported that the daily intake of PCDD/Fs and co-planar PCBs via fish oil capsules would be 4.84 pg I-TEQ/day for an individual weighing 60 Kg. Although not consumed by a large portion of the population, the potential contribution via these products is worthy of attention, so the contribution from this source to the total average I-TEQ daily intake should be taken into account.

Calculations reported in this study may be compared with those from similar studies carried out in other countries. A 1989 Canadian report[18] used analysis of composite food smaples from ten food classes. The estimated total dietary intake of dioxins and furans was calculated as 92 pg TEQ (1.5 pg TEQ/Kg b.w/day) assuming all undetected congeners were absent. This is consistent with the figure 81.10 pg I-TEQ reported in this study. Two German studies[8,19] carried out in 1989 and 1990 respectively estimated daily intake of dioxins and furans in Germany analysing numerous samples of meat, dairy products, eggs, fish, vegetables and fruits. In the first study[8], the estimated total dietary intake of PCDD/Fs was calculated to be 94 pg TEQ/person/day, for a 60 Kg person by assuming undetected congeners present at half the LOD. In the second study[19]the estimated total dietary intake was 72 pg TEQ/person/day for a 60 Kg person by assuming undetected congeners present at half the LOD. The value 119 pg I-TEQ/person/day quoted in Table 2 is slightly higher than both values reported for Germany. Other studies in the United Kingdom[20] and United States[21] have indicated similar intakes of PCDD/Fs using the now commonly accepted I-TEF. In the U.K. estimated dietary intake was calculated to be 125 pg I-TEQ/person/day for a 60 Kg person, assuming not detected values equal to the LOD. In USA intakes were indicated in the range of 18 to 192 pg I-TEQ/person/day for an adult weighing 65 Kg. In Japan[16] similar studies were conducted using food items based on the standard energy and food compositions of Japanese. 2,3,7,8-TCDD equivalent (USEPA) of the daily intake through food was calculated to be 63 pg/man/day for a person weighing 50 Kg, assuming "not detected" values as equal to zero.
Estimates from Canada, Germany, U.K., U.S.A. and Japan are in good agreement with the estimated dietary intake of dioxins and furans in Spain. For co-planar PCBs, data could not be compared because they were not reported in the mentioned studies since the analysis and interest of co-planar PCBs is of relative recent origin.

The pattern of PCDD/PCDF isomers in human tissues from Spain[5,9,14] and foods reported here are different from each other. This difference may result from the fact that this xenobiotics in food are metabolised in the human body. Also, other sources of human exposure to this chemicals are not negligibles.

However these estimates are based on very limited data sets and further studies are required for more accurate estimates. In fact, here is reported the estimated intake associated with an average diet, but it is also of interest further studies which are being conducted in order to know which foods make significant contributions to the estimated dietary intake of dioxins, furans and PCBs; and the influence of localised sources of this xenobiotics.

4. ACKNOWLEDGEMENTS

B. Jimènez would like to thank to the C.S.I.C. and C.A.M. for founding.

5. REFERENCES

1. Nygren, M., C. Rappe, G. Lindstrom, M. Hansson, P.-A. Bergqvist, S. Marklund, L. Domellof, L. Hardell and M. Olsson.(1985) In: Chlorinated Dioxins and Dibenzofurans in The Total Environment, L.H. Keith, C. Rappe and G. Choudhary, eds., Butterworth, Stoneham, Mass.

2. Van den Berg, M., F.W.M. Van den Wielen, K. Olie and C.J.V. Boxtel (1986) Chemosphere 15, 693-706.

3. Ryan, J.J., R. Lizotte and B.P.-Y. Lau (1985) Chemosphere 14, 697-706.

4. Schecter, A., J.J. Ryan, R. Lizotte, W.F. Sun, L. Miller, G. Gitlitz and M. Bogdasarian (1985) Chemosphere 14, 933-937.

5. Gonzàlez, M.J., B. Jimènez, L.M. Hernàndez and J. Rivera (1993) Chemosphere 27(1-3), 97-104.

6. Jimènez, B., M.J. Gonzàlez and L.M. Hernàndez (1991) Food Chemistry 39, 257-271.

7. Travis, C. and D. Armes (1990) In: Toxic Chemicals, Health and the Environment, L.B. Lave and A.C. Upton, eds. Johns Hopkins University Press.

8. Beck, H., K. Eckart, W. Mathar and R. Wittkowski (1989) Chemosphere 18, 417-424.

9. Gonzàlez, M.J., B. Jimènez, L.M. Hernàndez and M.F. Gonnord (1996) Bull. Environ. Contam. Toxicol. 56, 197-204.

10. MAPA (1990). La alimentaciòn en España. 1990. Ministerio de Agricultura, Pesca y Alimentaciòn. Madrid, Spain.

11. Jimènez, B., C. Wright, M. Kelly and J.R. Startin (1996) Chemosphere 32(3), 461-467.

12. NATO. Pilot Study on International Information Exchange on Dioxin and Related Compounds. Scientific Basis for the Development of the International Toxicity Equivalency Factor (I-TEF): Method of Risk Assessment for Complex Mixtures of Dioxins and Related Compounds. No. 176. North Atlantic Treaty Organization, Committee on the Challenges of Modern Society, 1988.

13. Safe, S. (1992) Chemosphere 25, 61-64.

14. Jimènez, B., L.M. Hernàndez, E. Eljarrat, J. Rivera and M.J. Gonzàlez (1996) Levels of PCDDs, PCDFs in serum samples from non exposed individuals living in Madrid (Spain). Environmental Research, submitted.

15. Schecter, A., K. Jiang, O. Papke, M. Ball, J.J. Ryan, H.D. Cau, L.C. Dai, H.R. Quynh, H.Q. Cuong, N.T.N. Phuong, P.H. Piet, A. Beim, J. Constable, J. Startin, M. Samedy and Y.K. Seng (1994) Environ. Health Perspect. Supplements 102, 159-171.

16. Ono, M., Y. Kashima, T. Wakimoto and R. Tatsukawa (1987) Chemosphere 16(8/9), 1823-1828.

17. World Health Organization, Regional Office for Europe (1991). Summary report. Consultation on Tolerable Daily Intake from food of PCDDs and PCDFs. Bilthoven, Netherlands, 4-7 December 1990. **EUR/ICP/PCS 030(S) 0369n**, publ. WHO Regional Office for Europe, Copenhagen.

18. Birmingham, B., B. Thorpe, R. Frank, R. Clement, H. Tosine, G. Fleming, J. Ashman, J. Wheeler, B.D.Ripley and J.J. Ryan (1989) Chemosphere 19, 507-512.

19. Fürst, P., C. Fürst and W. Groebel (1990) Chemosphere 20, 787-792.

20. Ministry of Agriculture, Fisheries and Food. *Dioxins in Food.* Food Surveillance Papar No. 31. HMSO. London, 1992.

21. Schecter, A., J. Startin, C. Wright, M. Kelly, O. Papke, A. Lis, M. Ball and J. Olson (1994) Chemosphere 29(9-11), 2261-2265.