TEMPORAL TRENDS (1995 – 2007) OF PCDD, PCDF AND DIOXIN-LIKE PCB CONCENTRATIONS IN COMMERCIAL MEAT AND MEAT PRODUCTS FROM SPAIN

Herrero Laura[1], Bordajandi Luisa R.[1], Gómara Belén[1], Fernández Mario A.[1], Ábalos Manuela[2], Abad Esteban[2], Rivera Josep[2] and González Mª José[1]

[1] Department of Instrumental Analysis and Environmental Chemistry, General Organic Chemistry Institute (CSIC). Juan de la Cierva 3, 28006 Madrid, Spain. e-mail: bgomara@iqog.csic.es
[2] Mass Spectrometry Lab., Department. of Ecothecnologies, IIQAB-CSIC Jordi Girona 18, 08034 Barcelona, Spain.

Introduction

Polychlorinated dibenzo-*p*-dioxins and furans are persistent organochlorinated contaminants widely dispersed in the environment, which accumulate in fatty foods. Polychlorinated biphenyls (PCBs) are structurally related to dioxins, having similar chemical and physical properties. There is a constant public concern about the health hazards of dioxins and related compounds. These compounds are persistent in the environment and tend to accumulate in biological systems. One of the most extensively studied PCDD congeners, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), exhibits a broad range of toxic effects in laboratory animals, some at very low doses. Exposure of the general population to dioxins and dioxin-like PCBs is primarily from food (higher than 90%)[1], especially fat food and products of animal origin. At present date, public concern over the adverse health effects of exposure to these toxicants has been enhanced by a number of dioxin contamination incidents involving food and feedstuffs [2-3]. In 1998, the WHO European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS) conducted a revaluation of the Tolerable Daily Intake (TDI) for dioxins and dioxin-like PCBs. The WHO consultation recommended a decrease of the TDI from 10 to 1-4 pg WHO-TEQ/kg body weight (b.w.)/day [4]. In later revisions, a tolerable weekly intake of 14 pg WHO-TEQs/kg b.w./week [5] and a tolerable monthly intake of 70 pg WHO-TEQs/kg b.w./month[6] have been established. Meanwhile, wide-ranging efforts and more exigent regulations have been in forced in order to reduce the dioxin release[7]. As a result, a new regulation with maximum allowed levels of dioxins in foods and feedstuffs for European Union (EU) countries is now available[5]. Meat products have received a special attention due to both their widespread consumption by the population at large and their contribution of about 35% of the daily intake of TEQs by human population. Although there are substantial databases on PCDD/F levels from Europe, less data are available from Spain, which made difficult to establish background exposure through diet for the general Spanish population.
In this study, we present the results of the first surveillance programme on temporal trends of PCDD, PCDF and dioxin-like PCB concentrations found in Spanish commercially available meat and meat products collected from 1995 to 2007. With this purpose, levels of dioxins and dioxin-like PCBs have been determined in commercial chicken, pork meat and transformed pork meat randomly acquired from supermarkets all over Spain.

Methods and Materials

Sampling

A total of 119 meat and meat products (28 chicken meat, 46 pork meat and 45 transformed pork meat) were analysed in the period 1995-2007. All samples are representative of the foodstuffs available on the Spanish markets. Once at the laboratory, the non-edible part of the food products was removed and the edible part, skin excluded, was stored in stable conditions, either freeze-dried or frozen at -20 °C, until analysis. Each sample consisted of pooled individual samples acquired from the same location. The aggregate samples were prepared depending on the type of food.

Extraction and clean-up

The extraction and clean-up methodology has already been described elsewhere[8]. Briefly, meat and meat product samples were homogenised with 1:4 (w/w) silica gel:anhydrous sodium sulphate powder. The mixture was ground to become a fine powder, loaded into a column and spiked with a mixture containing the fifteen $^{13}C_{12}$-labelled 2,3,7,8-substituted PCDDs and PCDFs and the twelve $^{13}C_{12}$-labelled dioxin-like PCBs. A mixture of 1:1 (v/v) acetone:hexane (400 ml) was used as extraction solvent. Clean-up was carried out using a multilayer column filled with neutral silica, silica modified with sulphuric acid (44%, w/w) and silica modified with KOH. The final fractionation step was carried out on a Supelclean™ ENVI™-Carb SPE cartridge (Supelco, Bellefonte, PA, USA) to obtain three different fractions, the first containing the bulk of PCBs, the second non-*ortho* PCBs and the last one corresponding to the PCDD/Fs.

Analysis of dioxin-like PCBs

Dioxin-like PCB congeners were determined by GC coupled to an ion trap detector (GC-ITD) in its tandem operation mode (MS/MS) using a Varian CP-3800 gas chromatograph coupled to a Saturno 2000 ion trap detector (Palo Alto, CA, USA) as previously published [9]. The extract containing the dioxin-like PCBs fraction was evaporated until dry and diluted a solution containing $^{13}C_{12}$-labelled PCBs 70, 111, 138, and 170 as recovery standards. A 4 µl aliquot was injected in the PTV mode (100 ºC, hold for 0.2 min, and then to 300 ºC at 200 ºC/min; splitless time 2.0 min) using two equivalent capillary columns (BPX-5, 60 m × 0.25 mm i.d., 0.25 µm film thickness, SGE, Australia and VF-5MS 55 m × 0.25 mm i.d., 0.25 µm film thickness, Varian). Helium was used as the carrier gas at a constant flow rate of 1 ml/min.

Analysis of PCDD/Fs

Purified extracts were analysed by GC-HRMS/EI(+)SIM on a GC-8000 series (Carlo Erba Instrument, Milan, Italy) coupled to an Autospec Ultima mass spectrometer (Micromass, Manchester, UK) at 10000 resolving power (10% valley definition). Chromatography was run on a DB-5 column (60 m × 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, USA) using helium as carrier gas. A 2 µl sample was injected in the splitless mode and the two major ions of the molecular ion cluster were monitored for each compound as is described elsewhere[10].

Quality control

Quality control criteria such as blanks, recoveries, parallel analysis and participation in interlaboratory studies were carried out during the years. All data were complied with the analytical standards (accuracy, precision, recoveries, etc.) recommended by the recently approved European Union Commission directive for measuring dioxins in food[11]. The laboratory has participated in several international quality control studies for the analysis of PCDD/Fs and PCBs in food matrices, obtaining results always consistent with the consensus means given by the interlaboratory organisations.

In order to make all data presented in this work comparable for the determination of temporal trends, for the analyses conducted before 1998, only data with recoveries higher than 50% and with differences lower than 40% between the upper and lower bound determination levels were considered.

Results and Discussion

To express the temporal trends of PCDD/Fs and dioxin-like PCBs in commercially available foodstuffs from Spain, the results were calculated in pg WHO-TEQ/g on a lipid weight (l.w.) basis, the TEQ values being determined in the lower bound determination levels, *i.e.* the non-detectable congeners were considered zero. These results are shown in Figure 1, in which a different behaviour between PCDD/Fs and dioxin-like PCBs can be observed.

Regarding dioxin-like PCB concentrations, it is important to highlight that, for pork meat samples, a substantial decrease was found between 1995 and 1999. However, from 1999 to 2007, an irregular trend can be observed in the three samples studied. Pork meat samples showed a significant increase from 1999 to 2003, concentrations falling considerably in the next year and fluctuating in those following. On the contrary, for transformed pork meat, a general increase was registered from 1999 to 2006 but this rise has not been continuous over time. After

that, an important decrease was observed in the concentrations corresponding to the 2007 sampling campaign. On the other hand, the concentrations found in chicken meat samples have continued to vary year in, year out; showing the highest values in 2005 and 2006 and the lowest ones in 2004 and 2007.
PCDD/F concentrations in the three types of samples analysed clearly declined from 1995 to 2007. Only chicken meat in 2005 and transformed pork meat in 2001 and 2004 showed a slight increase in their concentration with respect to the general decreasing trend. The greatest decrease was obtained for pork meat samples, falling from 27.2 pg WHO-TEQs/g l.w. in 1995 to 0.06 pg WHO-TEQs/g l.w. in 2007, followed by chicken meat samples and transformed pork meat samples that present concentrations 84 and 24 times lower in 2007 than those found in 1999, respectively.

Figure 2 shows the contributions of the four families of compounds investigated to the total WHO-TEQs for the three types of samples analysed, from 1999 to 2007. For pork meat samples, the contribution of PCDD/Fs to the total TEQ content was always lower than 30%. Regarding PCBs, the contribution of non-*ortho* PCBs showed a continuous increment from 1999 to 2003, the percentages of PCBs being 7% in 1999 and 88% in 2003. The following years, until 2005, the trend changed, and the contribution of mono-*ortho* PCBs increased considerably. In 2005, a percentage higher than 75% was found for mono-*ortho* PCB congeners. After this year and until the present, the trend came back to show an increase of the contribution of non-*ortho* PCBs.
In the case of chicken and transformed pork meat samples, a different profile could be observed between samples from the earliest years and samples from the latest ones. In 1999 and 2001, PCDD/Fs and PCBs presented similar contributions (50% of each approximately), but from 2003 to 2007 the percentage of contribution of PCDD/Fs fell drastically, not exceeding 24% for chicken meat and 27% for transformed pork meat. In recent years, from 2003 to 2007, the major contributor to the TEQs was non-*ortho* PCBs with percentages higher than 82% for chicken meat and 89% for transformed pork meat, except in the case of samples analysed in 2005, in which mono-*ortho* PCBs showed the highest contribution (94% for chicken meat and 81% for transformed pork meat).

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Figure 1. Temporal trends of PCDD/Fs and dioxin-like PCBs (expressed in pg/g WHO-TEQs in a lipid weight) in meat and meat products commercially available in Spain.

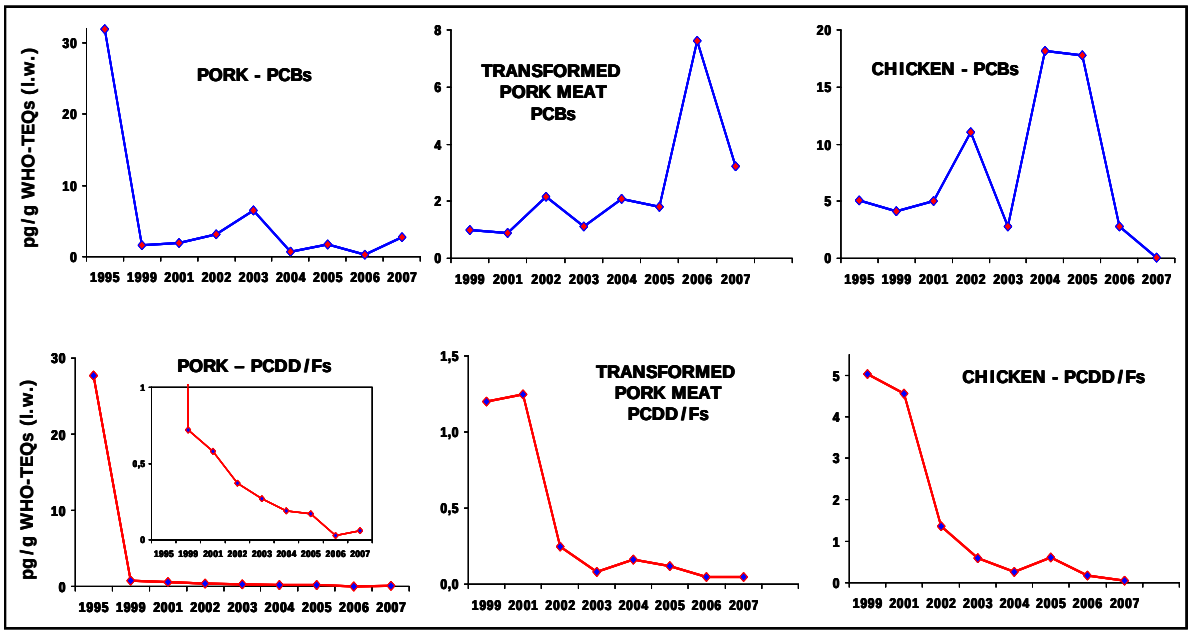
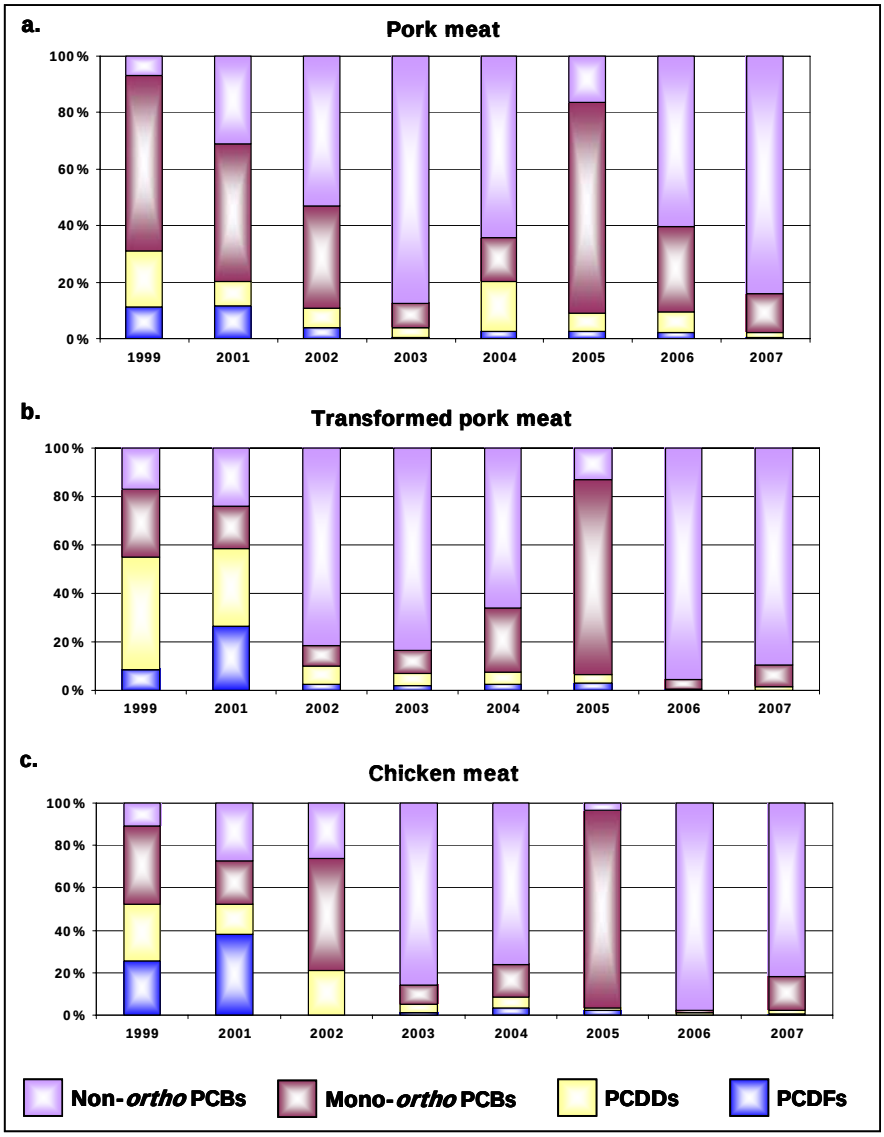


Figure 2. Percentage of contribution of PCDD/Fs, mono-*ortho* PCBs and non-*ortho* PCBs to the total WHO-TEQ content of (a) pork, (b) transformed pork and (c) chicken meat samples.



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