DETERMINATION OF POLYCHLORINATED DIBENZO-*P*-DIOXINS (PCDDs), DIBENZOFURANS (PCDFs) AND DIOXIN-LIKE POLYCHLORINATED BIPHENYLS (DL-PCBs) IN COMMERCIAL HONEYS FROM BRAZIL AND SPAIN

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Introduction

For the general population, dietary intake is the main route of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) and polychlorinated biphenyls (PCBs) exposure, contributing with more than 90% to the daily exposure[1]. The dioxin-contamination incidents involving food and feedstuffs happened in recent years[2-3] and the re-evaluation of the tolerable daily intake (TDI) of dioxins[4], have prompted wide-ranging efforts and the tightening of regulations to reduce dioxin release into the environment[5]. In 2006, the World Health Organization (WHO) re-evaluated the toxicity equivalent factors (TEFs) assigned to dioxins and dioxin-like PCBs (DL-PCBs) for the calculation of the toxic equivalent quantities (TEQs)[6] and the European Commission has recently established maximum permissible levels of dioxins and DL-PCBs in foods[7].

Honey is a natural product produced by *Apismellifera* bees from the nectar or secretions of plants, and has been consumed by many people around the World as a natural food, in medical therapies, and as an alimentary supplement. Its chemical composition is variable, due to contribution of the plant, climate, environmental conditions and the ability of the beekeeper[8]. Nowadays, bee products are being produced in an environment polluted by different sources of contamination, which can be transported by honeys bees to the hive and incorporated into honey[9]. It is difficult to protect food and animal feed from the sources of toxic chemicals ubiquitous in the environment[10], particularly in the case of honey, since honeybees travel long distances and come close to many plants.

Residues of some persistent organic pollutants (POPs) have been found in honey samples, such as organochlorine pesticides[11-13] and non-dioxin-like polychlorinated biphenyls (NDL-PCBs)[14-15], but there are very limited report regarding PCDDs, PCDFs, and DL-PCBs levels in the related literature[16].

The concentrations of seventeen 2,3,7,8-substituted PCDD/F and twelve DL-PCB congeners found in 26 honey samples commercially available in Brazil and Spain markets in 2010 and 2011 were presented. The differences in dioxin and DL-PCB concentrations and profiles between the two countries were also reported.

Materials and methods

The sample preparation and extraction was based on Blasco *et al.* (2004)[17], with some modifications. Samples were diluted with water, spiked with a mixture containing eleven $^{13}C_{12}$-labeled PCBs and fifteen $^{13}C_{12}$-labeled 2,3,7,8-substituted PCDDs and PCDFs and extracted three times with ethyl acetate and petroleum ether (9:1, v/v). The extract was clean-up in a multilayer column filled with neutral silica gel, silica gel activated and modified with sulfuric acid (44% and 22%, w/w) and anhydrous sodium sulfate, eluted with *n*-hexane. A final fractionation step was carried out on Supelclean^{TM} ENVI^{TM}-Carb SPE cartridges (Supelco, Bellefonte, PA, USA) to obtain the PCDD/Fs and DL-PCBs in separate fractions. A standard solution containing 50 pg μL^{-1} of $^{13}C_{12}$-labeled PCBs 70, 111, 138, and 170 was used as injection standard for recoveries calculation of the labeled PCBs added as surrogates, as well as a standard solution containing 200 pg μL^{-1} of $^{13}C_{12}$-labeled 1,2,3,4-tetrachloro-dibenzo-*p*-dioxin and 1,2,3,7,8,9-hexachloro-dibenzo-*p*-dioxin was used as injection standard for recoveries calculation of the labeled PCDDs and PCDFs added as surrogates. The sample extracts were analyzed by GC-QqQ(MRM) on a TRACE GC Ultra (Thermo Fisher Scientific, Milan, Italy) coupled to a TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Fisher Scientific, Bremen, Germany), operating in EI mode (40 eV, electron energy). Injections were performed in the PTV mode in a capillary HP-5ms column (30 m, 0.25 mm i.d., 0.25 µm film thickness) purchased from Agilent Technologies (Palo Alto, CA, USA). The oven temperature was programmed from 90 ºC (2 min) to 160 ºC at a rate of 15 ºC min^{-1}, then to 225 ºC at 4 ºC min^{-1}

and then to 290 ºC (5 min) at 7 ºC min^{-1}. Helium was used as the carrier gas at a constant flow rate of 1.2 mL min^{-1}. The temperature of the transfer line and the MS source were set at 300 ºC and 240 ºC, respectively. Collision gas (Ar) pressure was set to 1.5 mTorr for all the experiments. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode, with two transitions monitored using argon as the collision gas following the method of García-Bermejo *et al.* (2011)[18]. The isotope dilution technique was used as quantification method.

All analyses such as blanks, recoveries, and parallel analyses were complied with analytical standards as recommended by the EU Commission in the directive for measuring dioxins in food[19]. A method blank in each set of analysis (three analyses and a blank) was carried out. Satisfactory repeatability and intermediate precision were achieved when analyzing standard solutions, with relative standard deviation (RSDs) generally below 10 and 19%, respectively. Recoveries for the spiked compounds were in the range established by the EPA as acceptable for PCBs (40 to 87%) and 2,3,7,8-PCDD/Fs (42 to 90%). Method detection limits (LODs) for the honey samples were in the range 0.008 to 0.17 pg g^{-1} for PCBs, and 0.018 to 0.141 pg g^{-1} for PCDD/Fs. The laboratory has participated in different international inter-laboratory studies and several international quality control studies for the analysis of PCBs and PCDD/Fs in different food matrices. The results were consistent at all times with the consensus means given by the inter-laboratory organization.

Results and discussion

The average levels of PCDDs, PCDFs and DL-PCBs expressed in pg g^{-1} on a fresh weight basis, found in Spanish and Brazilian commercial honey products are shown in Figure 1 and 2. Detectable levels of the individual DL-PCBs were found in almost all of the investigated samples, except PCBs 123 and 169 that were not detected in any of the Spanish honey samples. Regarding PCDD/Fs, detectable and very low levels of tetra- hepta- and octa- CDD/Fs were found in very few Brazilian honey samples, while OCDD and OCDF congeners were found in all Spanish honeys.

The average and standard deviation of total DL-PCBs found in Spanish honey samples (122 ± 63 pg g^{-1} f.w.) was slightly higher than that found in Brazilian ones (103 ± 61 pg g^{-1} f.w.), being the contribution of mono-*ortho* PCBs to total DL-PCB concentrations higher than 96% in both cases. The DL-PCB profiles found in honeys from both countries are very similar. PCBs 118 and 105, both mono-*ortho* congeners, exhibited the highest DL-PCBs mean values in Spanish (83 ± 43 pg g^{-1} f.w. and 26 ± 14 pg g^{-1} f.w., respectively) and Brazilian (72 ± 40 pg g^{-1} f.w. and 18 ± 13 pg g^{-1} f.w., respectively) samples, while the non-*ortho* PCBs 81, 126 and 169, had the lowest ones in both countries (Figure 1).

Concerning PCDD/Fs, the average and standard deviation concentration levels found in Spanish honey samples (0.46 ± 0.17 pg g^{-1} f.w.) was similar to the levels found in Brazilian ones (0.43 ± 1.3 pg g^{-1} f.w.), being the contribution of PCDDs to total PCDD/Fs higher than 84% in both cases. The PCDD/Fs profiles found in honeys from both countries are very similar and dominated by OCDD and OCDF congeners. In the Spanish samples only these two PCDD/F congeners were detected, while in the case of Brazilian honeys the two tetras- and two heptas- CDD/Fs were also detected in some of the samples (Figure 2).

The mean WHO-TEQs $_{PCDD/Fs+DL-PCBs}$ levels, expressed in pg g^{-1} f.w., found in the honeys from both countries were quite similar and in the low pg g^{-1} f.w. levels, although it was slightly higher in Brazilian (0.050 pg WHO-TEQs g^{-1} f.w.) than in Spanish (0.034 pg WHO-TEQs g^{-1} f.w.) honey samples. The most remarkable findings in this study were the large contribution of the high chlorinated PCDD/Fs, and PCBs 105 and 118 to the total PCDD/Fs and DL-PCBs in the honey samples from both countries. The detection of PCDD/Fs and DL-PCBs in honeys from different countries highlights the risk that their presence poses to the health of humans and wildlife, since honey is a non-fatty natural product which is highly consumed all over the World.

It is difficult to compare the present results with those obtained in other monitoring programs.To our knowledge, only one previous study has been performed to investigate levels of PCDD/Fs and DL-PCBs in honey samples from China and Taiwan[16], in which none detected levels of those compounds were found. In some other studies honeybees or honeybee products (honey, wax, pollen) have been used as marker for assessing environmental pollution in agricultural and industrial areas, but most of them have being performed with pesticides[11-13]and their results are not comparable with dioxin compounds. In any case, honey is a natural product that must be free of any chemical contaminants and safe for human consumption.

Contamination in honey can be due to its constituents (nectar and pollen) and/or it can be transferred by bees when transforming nectar [20]. Nectar, the basic constituent of honey, has low lipid content and so its potential to bioaccumulate POPs is very low. That may help explain the low concentrations of dioxins detected in the honey

samples. The PCDD/Fs and DL-PCBs found in honeys in the present study are very far from the maximum allowed levels established by the UE legislation for high lipid content food[7].

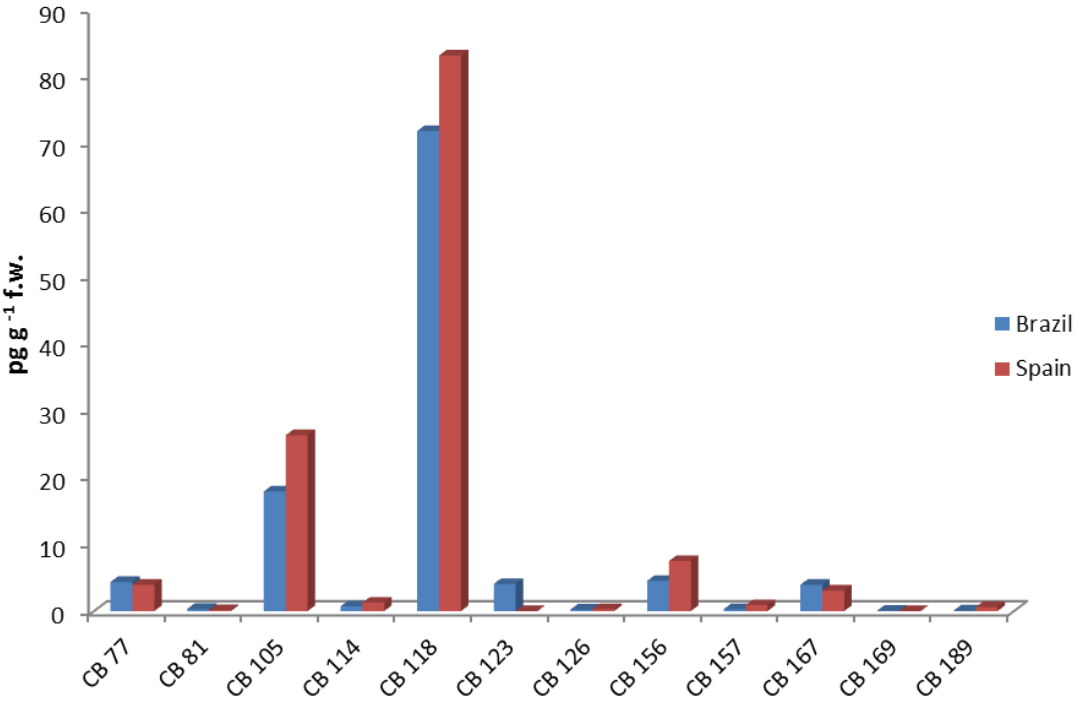

Figure 1: DL-PCB concentrations found in commercial honey samples from Spain and Brazil.

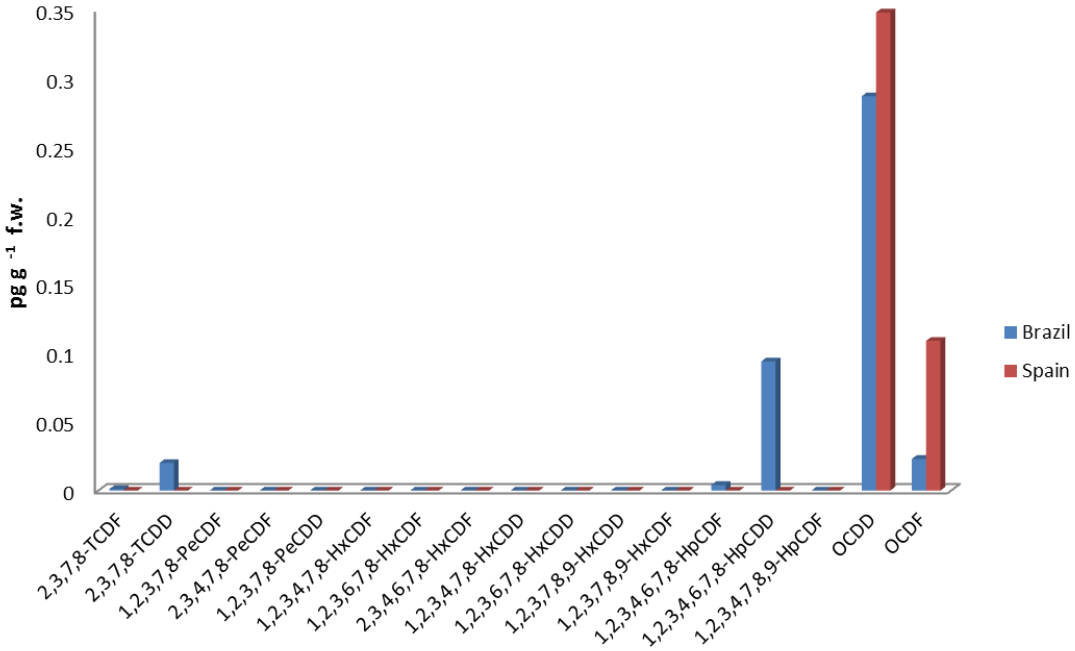

Figure 2: PCDD/F concentrations found in commercial honey samples from Spain and Brazil.

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