LEVELS OF PCDD/PCDFs AND dl-PCBs IN FOOD COMMERCIAL SAMPLES QUANTIFIED BY HRGC-HRMS

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyles (dl-PCBs) are 3 of the 12 UNEP internationally recognized POPs. They are the most toxic members of POPs. In general, these compounds are the result of the chlorine-containing manufacturing processes or they are produced during incineration (for example, of domestic wastes).[1] PCBs are anthropogenic chemical compounds, with low electrical resistance, which, in combination with their heat stability, makes them very suitable as cooling liquids in electrical equipments. They were produced between 1930 and 1970 and commercialized in relative large quantities for use as dielectrics, hydraulic fluids, plastics and paints.1 PCDD/Fs and dl-PCBs are compounds with at least seven years half-life within human bodies. They are bio-accumulate in humans primarily via the diet, specifically by ingestion of food from animal origin.[2] In humans, a wide variety of health effects have been related to high exposure to dioxins, furans and dioxin-like compounds. Dioxins is a promoting agent of growing and transformation of cancer cells.[3]

Although PCDD/Fs and dl-PCBs are widely distributed throughout the environmental, ingestion via food consumption is the major source of human exposure.[4,5] Dioxin, furan and dl-PCB are ubiquitous environmental contaminants, which are present in all marine plant and animals, birds, mammals and humans and they bioaccumulate through the food chain.[6] In this study, the levels of dioxins, furans and dl-PCBs in three matrixes, were analyzed. The results were reported in total concentrations (pg/g) and in total WHO-TEQ concentrations (pg WHO-TEQ/g) of PCDD/F and dl-PCBs for each of the samples analyzed. Food samples analyzed are commercialized in Colombia, and their marketing and consumption are regulated by both the Ministry of Colombia and European Union.

Materials and methods

Sample treatment

All samples were purchased from a local supermarket in Medellin (Colombia). The soybean oil contained a faction of 5% of palm and olive oil, the fish oil was taken from capsules with 1000 mg of cod liver oil; a butter containing plant seed oils and peeled and deveined shrimp ready for consumption was also used. The samples were spiked with known amounts of standard mixtures of ^{13}C- PCDD/Fs EPA-1613 LCS and dioxin-like ^{13}C-PCBs WP-LCS (Wellington Laboratories Inc., Canada). The samples were treated according to the matrix (oil/fat or solid). In the case of fat and oil, 16 g of sample were treated with two modified silica gel columns, one modified with sulfuric acid and the other, modified with sodium hydroxide, for removing organic components, fat and other interfering compounds. Each column was eluted with n-hexane and was rotary concentrated for the next purification step. The solid food (raw shrimp) samples were filleted and lyophilized in a laboratory of Universidad de Antioquia and then stored at -18 ºC. Then, the samples were sent to the Laboratory of Dioxins in Barcelona for analysis. The sample (14 g of dry weight shrimp) was spiked and after 2 h, it was extracted in a soxhlet for 24 h with a toluene : cyclohexane 1:1 (v/v), solvent mixture. For gravimetric determination, the solvent was removed by leaving them overnight in an oven at 105 °C. Then, fat residues were dissolved in 5 mL of n-hexane and removed using a silica gel column modified with sulfuric acid and sodium hydroxide. The clean-up was performed using manual columns with multilayer silica, alumina and carbon as adsorbents. Two

fractions of PCDD/Fs and dl-PCBs were obtained after passing the extracts trough tandem column, of modified silica and alumina. Then the extracts were purified using a carbon column. Finally, all fractions were rotary concentrated and transferred into vials and spiked with ^{13}C- PCDD/Fs EPA-1613 ISS and ^{13}C-PCBs WP-ISS standards for the analysis.

GC–MS Instrumentation

Samples were analyzed by High Resolution Gas Chromatography coupled to High Resolution Mass Spectrometry (HRGC-HRMS) on a Gas Chromatograph (Thermo Fisher Scientific, Milan, IT) using a 60 m x 0.25 mm i.d. x 0.25 μm film thickness DB-5ms fused silica column (J&W Scientific, CA, USA) coupled to a High Resolution Mass Spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany). Quantification of PCDD/Fs and dioxin-like PCBs were performed using the isotopic dilution method according to EPA[7,8]. Relative response factors (RRFs) for each individual 2,3,7,8-chlorosubstituted PCDD/F and dioxin-like PCB congeners were obtained by calibration with standard solutions. Recovery percent of ^{13}C-labelled compounds was calculated from area comparison between LCS and ISS compounds. Toxic equivalents (WHO-TEQ) were determined using WHO-TEF-2005 factors.

Results and discussion

Table 1 shows the total sum of concentrations and WHO-TEQs for PCDD/F and dl-PCBs in terms of pg/g for all samples analyzed. The highest levels of PCDD/F, expressed as the sum of the 17 congeners, were found in shrimp with a value of 44.8 pg/g dry weight followed by soybean oil and butter which have a close concentration, while the lowest levels were found in fish oil. In terms of total WHO-TEQ concentrations for dioxins and furans, shrimp also showed the highest value (1.7 pg/g WHO-TEQ dry weight) and the lowest levels were obtained in fish oil (1.0 pg/g WHO-TEQ fat).

Table 1. Total concentrations and WHO-TEQ values (upperbound) for PCDD/Fs and dl-PCBs in different foods commercialized in Medellín-Colombia, expressed in pg/g of fat for the oils and butter and in pg/g of dry weight for shrimp

Compound	*Soybean oil*	*Fish oil*	*Butter*	*Shrimp*
∑ PCDD/Fs	13.2	8.0	13.0	44.8
WHO-TEQ (PCDD/Fs)	**1.3**	**1.0**	**1.0**	**1.7**
∑ non-ortho-PCBs	38.0	149.9	23.3	23.4
WHO-TEQ (non-orthoPCBs)	0.05	2.8	0.05	0.2
∑ Mono-ortho-PCBs	89.0	6381. 2	69.0	170.3
WHO-TEQ (Mono-orthoPCBs)	0.003	0.1	0.002	0.005
∑ dl-PCBs	127.0	6531.0	92.3	193.5
WHO-TEQ (dl-PCBs)	**0.06**	**2.9**	**0.05**	**0.2**
WHO-TEQ (PCDD/Fs + dl-PCBs)	**1.4**	**4.0**	**1.1**	**1.9**

Figure 1 shows concentrations of individual PCDD/Fs congeners expressed in pg/g oil. In all the cases, the most representative value corresponds to the concentration of OCDD. Similarly, the other congeners which contribute significantly to the total are 1234678-HpCDD and OCDF.

For the case of the non-ortho and mono-ortho PCBs, a similar trend was observed. Again, fish oil showed the highest concentration, expressed as 12 total congeners PCBs (4 non-ortho and 8 mono-ortho PCBs), table 1. The content of dl-PCBs in fish oil is much higher compared with the content found in vegetable oil. For the fish oil, the PCB-118, PCB-156, PCB-167 and PCB-105 congeners present the largest contribution. In the case of the sample of shrimp, it was observed that mono-ortho PCBs correspond to 88% of the total content of dl-PCBs, whereas in the butter sample, the mono-ortho-PCB corresponds to 75% of the total.

Figure 2 shows the concentrations of individual dl-PCBs congeners in different food commercialized in Medellín-Colombia. For most of the cases, a similar trend is observed in the content of individual dl-PCBS congeners, in this case the most representative value corresponds to the concentration of PCB-118. Similarly, it is observed that most contribution for food samples correspond to PCB-77, PCB-105 and PCB-118 congeners. It is important to mention that the sample of fish oil has the highest content of dl-PCBs which is 6531.0 pg/g of fat. In this case, the largest contributions to the total content are PCB-118, PCB-105, PCB-167 and PCB-156 congeners.

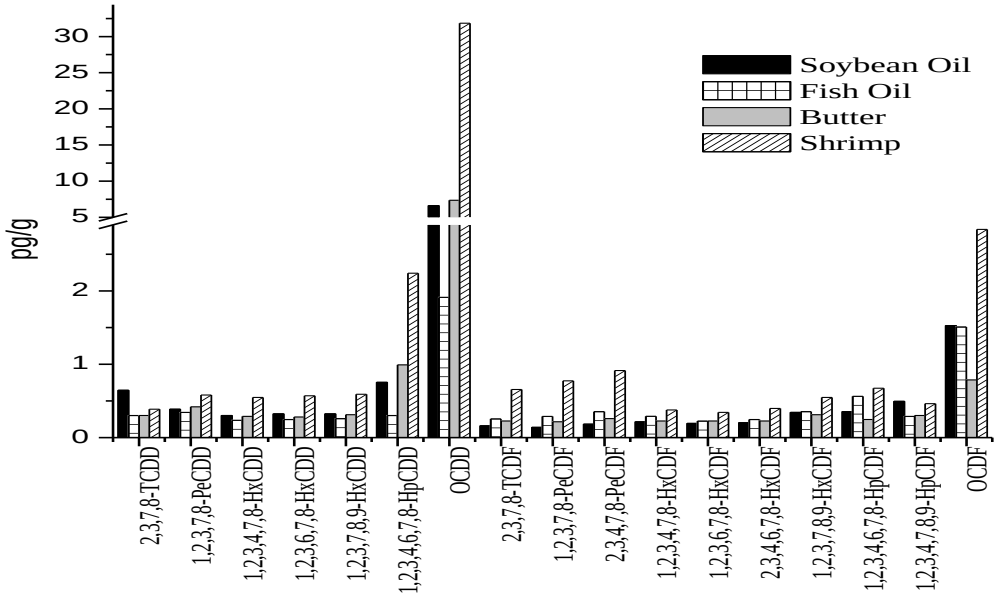

Figure 1. Concentrations of individual PCDD/Fs congeners in different food commercialized in Medellín-Colombia expressed in pg/g of fat for the oils and butter and in pg/g of dry weight for shrimp

In general, PCDD/F levels in the oil samples analyzed are low, except for the case of vegetable oil. The sum of WHO-TEQ for PCDD/Fs (1.3 pg/g of fat) and PCDD/Fs + dl-PCBs (1.4 pg/g of fat) in the soybean were above of the maximum established by the European Regulation, which is 0.75 pg/g of fat and 1.25 pg/g of fat respectively[9].

Moreover, according to the maximum levels for dioxins established in the resolution 2154 of 2012 of the Ministry of Health and Social Protection of Colombia, the content of WHO-TEQ PCDD/Fs (0.75 pg/g of fat) of soybean oil is above the limit, 1.3 pg/g of fat. However, in the case of the sum of PCDD/Fs + dl-PCBs (1.5 pg/g of fat) the value obtained in the analysis is within the allowable range. This indicates that the commercial product of soybean oil is allowed to be marketed consumed; however, it is not possible to export the product to the European Union for not complying with the requirements of the content of contaminants in foodstuffs.

Other food products (oil fish, butter and shrimp) show that the sum of WHO-TEQ for PCDD/Fs and PCDD/Fs + dl-PCBs is within the parameters established by both the European Regulation and for Colombia, where the fishery products are regulated according to Resolution 122 of 2012 of the Ministry of Health and Social Protection, which established a maximum levels of 4 pg/g of dry weight WHO-TEQ for PCDD/Fs and 8 pg/g of dry weight WHO-TEQ for the sum of PCDD/Fs + dl-PCBs. On the other hand, the European Regulation[9] established that maximum levels for fishery products correspond to 3.5 pg/g of dry weight WHO-TEQ PCDD/Fs

and 6.5 pg/g of dry weight WHO-TEQ PCDD/Fs + dl-PCBs. Similarly, the regulation[9] established for dairy products a maximum levels of 2.5 pg/g of fat WHO-TEQ and for 5.5 pg/g of fat WHO-TEQ for PCDD/Fs and sum of PCDD/Fs + dl-PCBs, respectively.

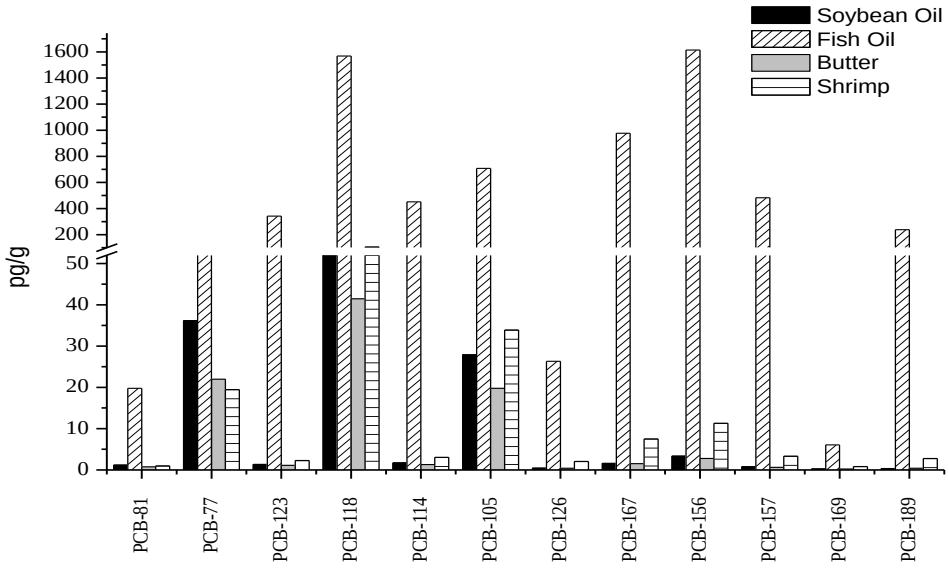

Figure 2. Concentrations of individual dl-PCBs congeners in different food commercialized in Medellín-Colombia expressed in pg/g of fat for the oils and butter and in pg/g of dry weight for shrimp

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