Human Exposure to Polychlorinated Diphenyl Ethers through the Diet in Catalonia, Spain

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Although polychlorinated diphenyl ethers (PCDEs) are recognized environmental pollutants, information concerning human exposure to these organic substances is very scarce. For the present study the concentrations of PCDEs in a number of foodstuffs acquired in Catalonia, Spain, were determined. The dietary intake of PCDEs was estimated for various age groups of the general population living in this Spanish region. With the exception of fish and shellfish, PCDE concentrations were under the limit of detection in the 10 remaining food groups analyzed. For an adult (20−65 years old) male of 70 kg average body weight, the estimated total dietary intake of PCDEs was 41 ng/day. It was assumed that if a PCDE congener was below the detection limit, the concentration was equal to half of the limit of detection. The highest exposure to PCDEs through the diet corresponded to the group aged 51−65 years, whereas the lowest intake corresponded to the youngest group (4−9 years). With the exception of the group aged >65 years, PCDE intake was always higher in males than in females. The results of this study should be of interest for future assessments of time trends in human exposure to PCDEs through the diet.

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/PCDFs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), polybrominated diphenyl ethers (PBDEs), and polychlorinated diphenyl ethers (PCDEs) are polyhalogenated persistent organic pollutants (POPs), which are lipophilic substances with long half-lives (*1*, *2*).

In recent years, information concerning levels of PCDD/PCDFs or PCBs in foodstuffs has been relatively abundant. However, studies about other POPs such as PCNs, PBDEs, and PCDEs have not been frequent and have been generally limited to a small number of foods, mainly fish and shellfish (*2*). PCDEs have been utilized as industrial compounds and biocides (*3*). However, because PCDEs are persistent pollutants that can bioaccumulate in the food chain, nowadays, the essential interest in PCDEs is directly related to their role as environmental contaminants (*2*, *4*−*7*). In fact, PCDEs have been detected in a number of environmental samples including sediments, fish and mussels, and birds (*6*−*10*), as well as in human adipose tissue (*7*). PCDEs are structurally and by physical properties similar to PCBs (*2*). Like these, PCDEs consist of 209 congeners in which the number of chlorines varies from 1 to 10. The occurrence in the environment is mainly the result of their presence as impurities in chlorophenol preparations, substances

that have been widely used as wood preservatives, fungicides, herbicides, etc. (*3*, *10*, *11*).

In mammals, individual PCDE congeners have been shown to induce aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD) activities (*11*). With respect to the toxicity of PCDEs, oral treatment of adult rats with three PCDE congeners (one pentaCDE and two hexaCDEs) for 28 days showed that the three congeners might only be considered moderately toxic at the doses administered (0.04, 0.4, 4.0, and 40 mg/kg/day) (*12*). In a subsequent study of the same investigators, one pentaCDE, one hexaCDE, and one heptaCDE congener were given in the diet of rats at 0,5, 5.0, 50, and 500 ppm for 4 weeks. The no-observable-effect levels (NOELs) were between 5 and 50 ppm for the three PCDEs (*13*).

Despite the potential concern about PCDEs as toxic environmental contaminants, studies concerning PCDEs in general, and their toxic effects in particular, are very scarce. Moreover, information concerning PCDE levels in foodstuffs, as well as dietary intake of PCDEs, has not been reported. As for other POPs, it is expected that for the general population food is probably the main source of exposure to PCDEs. During the period 2000−2002, we carried out in Catalonia (Spain) a wide study on human exposure from dietary sources to a series of metals and POPs (*14*−*19*). In this paper, we present the concentrations of PCDEs in food samples collected in various cities of Catalonia, as well as an estimate of dietary intake of PCDEs by the general population of this region. To the best of

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our knowledge, this is the first study to quantitatively examine levels of PCDEs in foods and evaluate the dietary intake of these organic pollutants.

MATERIALS AND METHODS

Sampling. From June to August 2000, food samples were randomly acquired in local markets, big supermarkets, and grocery stores from seven cities (Barcelona, Tarragona, Lleida, Girona, L'Hospitalet de Llobregat, Badalona, and Terrassa) of Catalonia, which have populations between 150,000 and 1,800,000 inhabitants. Detailed information on the collection of food samples was recently reported (*17*). A total of 54 samples were analyzed for PCDE concentrations. Two samples composed by 10 individual subsamples were analyzed for the following foodstuffs: meat of beef (steak, hamburger), pork (loin, sausage), chicken (breast), and lamb (steak); fish (hake, sardine) and shellfish (mussel); vegetables and tubers (lettuce, tomato, potato, green beans, cauliflower); fresh fruits (apple, orange, pear); and eggs. For the following foodstuffs, cow's milk (whole, semiskimmed) and dairy products (yogurt, cheese), cereals [bread (loaf and packet sliced), pasta, rice], pulses (lentils, beans), fats (margarine) and oils (olive, sunflower), tinned fish (tuna, sardine), and meat products (ham, hot dogs, salami), one sample of each item, composed by eight individual subsamples, was analyzed.

Chemical Analysis. Food samples were homogenized and blended using a domestic mixer. Composite samples were lyophilized prior to analyses of PCDEs, which were performed in accordance with the U.S. EPA 1625 method (semivolatile organic compounds by isotope dilution GC-MS). Prior to extraction, dried samples were homogenized. The freeze-dried solid samples (5−10 g) were mixed with a small amount of Na_2SO_4 and spiked with a mixture of $^{13}C_{12}$-labeled standards. Samples were extracted for 24 h with the following organic solvents (Soxhlet extraction): toluene for vegetables, fruits, cereals, eggs, milk, and dairy products; hexane/dichloromethane (1:1) for meat, fresh fish, and mussels; and petroleum ether for fish in oil. For oil and margarine, 2 g of the sample was dissolved in hexane and immediately used for the cleanup procedure.

The cleanup procedure and fractionation of the sample aliquot was carried out as a multiple cleanup using adsorption chromatography, a multilayer silica column [from top to bottom: Na_2SO_4/silica (1.2 g)/ silica (sulfuric acid, 44%) (9 g)/silica (1.2 g)/silica (sodium hydroxide, 33%) (3 g)/silica (1.2 g); pretreatment, hexane; elution, hexane 400 mL], an alumina column (Na_2SO_4/35 g alumina basic super I; pretreatment and rising, toluene, 70 mL; elution, 50% dichloromethane/ hexane v/v 200 mL), and a gel chromatography column (70 g of Bio-Beads SX3; pretreatment and rising, cyclohexane/ethyl acetate, 150 mL; elution, cyclohexane/ethyl acetate 150 mL). Finally, the PCDE-containing fractions were reduced to the volume necessary for the analysis. Prior to PCDE analysis, $^{13}C_{12}$-labeled standards (^{13}C-CDE-68, ^{13}C-CDE-86, ^{13}C-CDE-180) were added for calculation of recovery ratios. The cleaned extract was analyzed using a Fisons 8060 high-resolution gas chromatograph with a 60 m DB5ms capillary column and splitless injection coupled with a Micromass Autospec Ultima system high-resolution mass spectrometer with electronic impact and a multiple ion detection mode (resolution ≥ 10000). Internal standards were used for PCDE quantification. Mean recovery ratios varied from 80% for the sum of tetraCDEs (range = 51−123%) to 88% for the sum of heptaCDEs (range = 59−118%). Double determinations were performed to check the quality of the procedure. For fat and oil samples, the limit of detection was between 2 and 10 ng/kg of dry weight, whereas for the remaining foodstuffs the detection limits varied from 5 to 40 ng/kg of dry weight, depending on the specific food item and the respective homologues. The condition of the laboratory was checked before and during the analyses by analyzing blank samples in regular intervals (together with every sample batch analyzed). No problems could be identified with regard to the blanks.

Dietary Exposure Estimates. Average daily consumption data were obtained from recent studies carried out in Catalonia (*20*, *21*). The population was divided into 12 groups according to different ages and sexes (6 × 2). Food daily consumption for a standard adult male was calculated as the mean value for males in the three age groups between

Table 1. PCDE Concentrations (Nanograms per Kilogram of Fresh Weight) in Different Fish and Shellfish Samples Acquired in Catalonia, Spain

	hake, n^a = 2	mussel, n = 2	sardine, n = 2	tuna in vegetable oil, n = 1	sardine in vegetable oil, n = 1
tetraCDEs	0.3–1.5	0.7–4.6	4.8–5.9	0.2	5.0
pentaCDEs	2.3–12.8	6.5–12.1	9.8–115	0.2	5.2
hexaCDEs	18.4–270	39.8–76.8	209–1531	1.5	27.2
heptaCDEs	10.6–150	4.4–5.7	72.0–14	0.4	16.7
octaCDEs	14.4–272.	8.4–8.1	104–914	1.0	17.9
sum PCDEs	**45.9–707**	**59.8–107**	**400–2707**	**3.3**	**71.9**

^{a} n = number of analyzed samples.

20 and 65 years old. PCDE intake was estimated for each food group assuming that when a congener was below the detection limit, the concentration was equal to half of the respective limit of detection (LOD) (ND = $^1/_2$ LOD).

RESULTS AND DISCUSSION

With the exception of the samples of fresh fish (hake and sardine), shellfish (mussels), and tinned fish (tuna and sardine), the concentrations of PCDEs in the remaining food groups were, for all homologues (tetra- to octaCDE), under the respective detection limits. Moreover, in tinned tuna, only hexaCDE was detected. The results (fresh weight) corresponding to the fish and shellfish group are summarized in **Table 1**, where the sum of tetra-, penta-, hexa-, hepta- and octaCDEs and the total PCDE concentrations are shown. PCDE levels were higher in fresh than in tinned fish. Especially remarkable was the total PCDE concentration found in fresh sardine (400 and 2707 ng/kg of fresh weight for the two samples analyzed). The differences between PCDE levels in fresh and tinned sardine are probably due to the different sources of origin, with very probably different rates of PCDE pollution. Moreover, the number of analyzed samples of each item was rather small, and consequently the current differences are more relevant. In all species analyzed, a predominance of the homologues hexaCDEs was observed in the sum of total PCDEs, whereas the lowest contribution corresponded to tetraCDEs.

Information on PCDE levels in fish is not especially abundant. In marine species it is very scarce; most available data correspond to freshwater species (*2*, *10*). Salmon, pike, and bream from two Finnish rivers contained ∼25−800 ng/g (lipid weight) total PCDEs (*5*), whereas trout from the Great Lakes in Canada contained between 54 and 303 ng/g (wet weight) total PCDEs (mean = 824 ng/g lipid weight) (*22*). With respect to marine species, no PCDE congeners were detected in Arctic cod muscle, whereas in liver the total PCDE concentration was 14 ng/g (lipid weight) (*7*). In turn, levels of tetraCDEs from 24 to 416 ng/g (dry weight) were reported in mussels from a bay in Rhode Island (*2*). In the present study, all food items were randomly acquired independent of the source of origin. No data on PCDE levels in hake, sardine, and tuna were previously reported. Therefore, a comparison of the current data with previous results could not be carried out.

Table 2 shows data on food intake and dietary intake of PCDEs by a standard male adult of 70 kg body weight and aged between 20 and 65 years. Total dietary intake of PCDEs was estimated to be 41 ng/day, calculated assuming that if a congener was below the detection limit, the concentration was equal to half of the LOD. Although in the present survey the total number of food samples analyzed (composites) for PCDE

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Table 2. Estimated Dietary Intake of PCDEs by a Standard Male Adult (20–65 Years of Age) of Catalonia, Spain[a]

food group	daily consumption (g)	PCDE intake (ng/day) (ND = $^1/_2$ LOD)
vegetables	226 (15.7)[b]	0.1
pulses	24 (1.7)	0.03
cereals	206 (14.3)	1.0
tubercles	74 (5.1)	0.08
fruits	239 (16.6)	0.2
fish and shellfish	92 (6.4)	38
meat and meat products	185 (12.8)	0.3
eggs	34 (2.4)	0.04
dairy products	106 (7.3)	0.2
milk	217 (15.0)	0.1
fats and oils	41 (2.8)	0.5
total intake	1444 (100)	**41**
		0.59[c]

[a] Results are given for an individual of 70 kg of body weight. [b] In parentheses are given percentages of total consumption. [c] Total intake expressed in ng/kg of body weight/day.
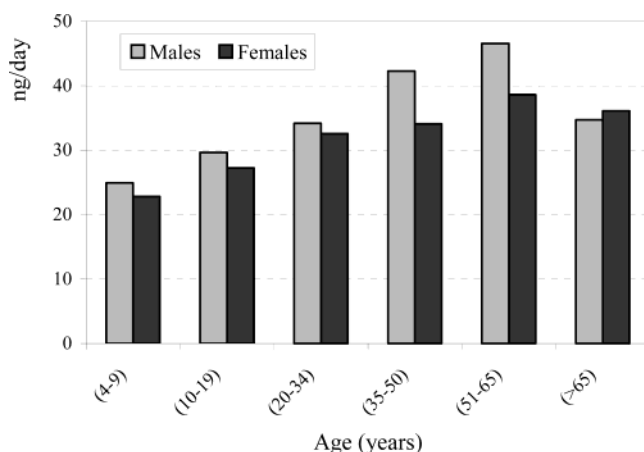
Figure 1. Estimated dietary intake of PCDEs (nanograms per day) by the general population of Catalonia (Spain), in relation to age and sex.

concentrations was relatively notable, the fact that these pollutants were detected in only fish and shellfish, together with the total absence of previous data about dietary intake of PCDEs in other countries, suggests caution should be taken in establishing conclusions on the current dietary intake of these pollutants.

The estimated PCDE intake by the population of Catalonia classified according to age and sex is depicted in **Figure 1**. For each sex, data are shown for the following age groups: children (4−9 years), adolescents (10−19 years), adults (20−34, 35−50, 51−65 years), and seniors (>65 years). The highest intake of PCDE through the diet corresponds to males and females in the group aged 51−65 years, whereas the lowest intake corresponds to both sexes in the youngest group (4−9 years). A continued increase with the age in the dietary PCDE exposure can be observed until 65 years, when a decrease is then noted. With the exception of the group aged >65 years, PCDE intake was always higher in males than in females, which is due to a greater food intake by males. However, the small differences between male and female groups are not statistically significant. On the other hand, when expressed in nanograms per kilogram of body weight per day, children aged 4−9 years showed the highest PCDE intake.

A number of studies have confirmed the common receptor-mediated mechanism of action of polyhalogenated aromatic compounds such as PCDD/PCDFs, PCBs, PCNs, PBDEs, and PCDEs (*1, 12, 13*). The reported information suggests that the structure−activity relationships (SARs) for PCDEs and PCBs are comparable (*23−25*). Recently, we have also determined the dietary intake of PCDD/PCDFs, PCBs, PCNs, and PBDEs by the population of Catalonia (*15−18*). For a standard male adult (70 kg body weight), the daily intake of these pollutants through fish and shellfish, the only food group in which PCDEs could be detected, was the following: PCDD/PCDFs, 28.7 pg, WHO-TEQ; PCBs, 82.9 pg, WHO-TEQ; PCNs, 3.6 ng; and PBDEs, 30.7 ng. Quantitatively, the current intake of PCDEs (41 ng/day) is similar to that of PBDEs and ∼10-fold higher than the intake corresponding to total PCNs from fish and shellfish.

A toxicity similar to that of PCBs has been suggested for PCDEs (*1, 24, 26*). However, currently, TEFs for the calculations of TEQ for PCDEs (as well as for most PCN and PBDE congeners) are not available. Therefore, although in this study we might not calculate the TEQ value corresponding to PCDEs, the PCDE contribution to total TEQ should not be underrated in a global context of foodstuffs polluted by various subfamilies of POPs that are acting under the same mechanism of toxicity. For those individuals daily consuming notable amounts of fish and shellfish it could be of concern.

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