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# Survey of Persistent Organochlorine Contaminants (PCBs, PCDD/Fs, and PAHs), Heavy Metals (Cu, Cd, Zn, Pb, and Hg), and Arsenic in Food Samples From Huelva (Spain): Levels and Health Implications

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Concentrations of PCBs, PCDDs, and PCDFs, heavy metals (Cu, Cd, Zn, Pb, and Hg), and arsenic have been determined in a great variety of food samples purchased in different markets across the city of Huelva, located in southwestern Spain and under strong industrial activity. All samples analyzed presented concentrations below the maximum allowed by the European Community regarding PCDD/Fs, with the exception of samples within the meat group. An estimation of the daily intake resulted in 1.15 pg of $WHO_{PCDD/Fs}$-TEQ/kg of body weight/day for a 70 kg person and 2.63 pg of WHO-TEQ/kg of body weight/day when PCBs were included, therefore accounting for a similar or even higher percentage than PCDD/Fs and showing the importance of their inclusion in monitoring studies. Meat and meat products, together with vegetable oils and dairy products, were the major food groups contributing to the estimated daily intake. For heavy metals and arsenic, the concentrations found were under the value proposed by European regulations, and estimated daily intakes were well below those proposed by the WHO for all metals investigated. PAHs have been analyzed in food samples from marine origin, values ranging from 8.22 to 71.4 ng/g of fresh weight. Pyrene was the most abundant compound, accounting for >80% in the samples investigated. The most carcinogenic PAHs, such as benzo[*a*]pyrene and dibenz[*a*,*h*]anthracene, were in all cases below the limits of detection. Therefore, the samples analyzed in this survey can be considered as safe with regard to the levels obtained and the in-force legislation.

**KEYWORDS: PCBs; PCDD/Fs; heavy metals; PAHs; daily intake**

## 1. INTRODUCTION

It is well-known that the intake of food contaminated with chemicals can lead to intoxication episodes that can be described as acute or, when the disease appears after a latent period of time, long-term intoxications. The chemicals producing the latter tend to accumulate in the body during long periods of time, producing illness when the levels reach critical values in certain tissues (*1*). This is the case of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), highly toxic environmental pollutants distributed worldwide.

Ingestion of contaminated food is the principal way of human exposure to these compounds, accounting for >90% if compared to other ways such as inhalation and dermal contact (*2*). Due to their persistence and lipophilic character, they tend to concentrate in the food chain, particularly associated with fat, foodstuffs of animal origin being one of the main sources (*3*). In the past years attention has been focused on the analysis of food due to a number of contamination episodes concerning dioxins and furans, such as the Belgian PCB/Dioxin Crisis (*4*) and the use of citrus pulp contaminated with PCDD/Fs for compound feed (*5*). To prevent the health risk from PCDD/Fs exposure, the European Commission established in the past years maximum levels permitted in food and feed and recently proposed target and action levels in order to reduce their presence in the different food items (*6*). At the moment these levels refer only to PCDD/Fs, but with the view of including dioxin-like PCBs in the next years. This concern about their

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human health impact and continuous encouragment from scientific committees to monitor food samples across Europe have led to numerous international and local studies on the concentration of dioxins in particular food items (*7*, *8*) or on the estimation of the daily intake from food being part of the average diet of the population (*9*−*11*). Recently, different food surveys have been carried out in Spain, particularly in Catalonia and the Basque country (*12*, *13*). They report new data on the background levels of these types of pollutants and show the most relevant food groups regarding their contribution to the total daily intake, although results should be extrapolated carefully due to the different methodologies and ways of presenting results used by each author.

Polycyclic aromatic hydrocarbons (PAHs) are also ubiquitous environmental pollutants relevant due to their high toxicity and potential carcinogenity. They are formed by the incomplete combustion of organic matter (*14*), the main ways of emission being natural formation and anthropogenic activities. A set of 16 PAHs have been considered by the U.S. Environmental Protection Agency (EPA) as priority pollutants and are commonly used to characterize the PAH load in different samples. Those have been analyzed in sea fish and seafood because they accumulate these kinds of contaminants after deposition in the aquatic environment (*15*).

Another consequence of the increasing pollution levels is the concern about heavy metals exposure. They reach the human body through different ways, mainly inhalation and ingestion. The latter is the main route for the general population, although in very contaminated sites, inhalation could play an important role (*16*). Contamination can happen during food handling and processing and through the feedstuff of animals for human consumption (*17*). In this study the most toxic heavy metals such as lead, mercury, cadmium, and the metalloid arsenic have been determined in a large number of food samples. The nutrients copper and zinc are also analyzed due to their potential toxicity if ingested in excess. The former are subjected to European regulations (*18*), and the World Health Organization (WHO) has recommended values of provisional tolerable weekly intake (PTWI) for lead, cadmium, mercury, inorganic arsenic, and copper based on the results of toxicological studies (*19*).

The main objective of this survey was to obtain representative data on levels of toxic organic pollutants, heavy metals, and arsenic in foodstuff consumed by the general population in a southern Spanish city under the influence of strong industrial activity. The analytical program included the determination of PCDDs, PCDFs, an extensive list of PCBs including dioxin-like congeners (mono-*ortho* and non-*ortho* PCBs), and the most relevant PAHs. Also, heavy metals (cadmium, lead, mercury, zinc, and copper) and the metalloid arsenic were analyzed in the food samples in order to assess the degree of contamination to which the population in the area is exposed. A preliminary estimation of the daily intake of those contaminants is reported.

## 2. EXPERIMENTAL PROCEDURES

**2.1. Sample Collection.** Food products were acquired in different markets across the city of Huelva during the months of February−July 2001. They were selected depending on their acceptance and consumption by the population living in the area. For some food products typical for the city market the scientific name is given as no appropriate English translation could be found. The study involved 42 types of food samples combined into 9 groups of similar foods items as follows: fruits (strawberries and oranges), vegetables (lettuce, potatoes, tomatoes, spring onions, and French beans), milk and dairy products (commercial pasteurized cow's whole milk, butter, cream, and yogurt), chicken eggs, sea fish (tuna, mackerel, sardine, common two-banded sea bream, little sole, tope, canned sardine, and canned tuna), seafood including crustaceans (prawns) and molluscs (cuttlefish and three different types of shellfish), meat and meat products (pork, chicken, loin of pork, processed cold pork meat, cured ham, and chicken liver), vegetable oils (olive oil and sunflower oil), breads and pastries (different types of white bread and madeleines). Once at the laboratory, the nonedible part of the food products was removed, and pooled samples of each food item, consisting of a variable number of individual samples, were stored in stable conditions, either lyophilized or frozen at −20 °C, until analysis. Organic compounds were determined mainly in foodstuff of animal origin, and for this purpose some of the pooled samples of each food item were further composited. This was the case of fish (fatty sea fish subsample consisted of tuna, mackerel, and sardine and lean sea fish subsample consisted of common two-banded sea bream, little sole. and tope), seafood [bivalves subsample consisted of two types of clams (*Tapes* sp. and *Chamelea* sp.)], and meat products (salami-type sausage subsample consisted of three different types of pork sausages).

**2.2. Analytical Procedure.** *2.2.1. PCBs and PCDD/Fs Determination.* A total of 23 individual PCB congeners were analyzed including the set of 7 indicators proposed by several countries and international bodies to be monitored (PCBs 28, 52, 101, 118, 138, 153, and 180) (*20*) and also other PCBs relevant due to their presence in commercial mixtures (PCBs 95, 132, 149, 183, 170, and 194) or due their toxicity, such as non- and mono-*ortho* substituted PCBs (PCBs 77, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189), which have been assigned a toxic equivalency factor by the WHO because they are considered to have similar toxicological effects to PCDD/Fs. Congener 81 has not been analyzed in this study. With regard to PCDD/Fs, individual concentrations of the 17 2,3,7,8-substituted congeners and also total PCDD/Fs are reported.

*2.2.1.1. Sample Preparation.* The extraction of PCBs and PCDDs/Fs involved a matrix solid-phase dispersion (MSPD). Samples were ground in a mortar with anhydrous sodium sulfate and silica gel. After addition of known amounts of the 3 $^{13}C_{12}$-labeled non-*ortho* PCBs and 15 $^{13}C_{12}$-labeled 2,3,7,8-PCDD/Fs standards, the samples were extracted with a mixture acetone/hexane (1:1, v/v). Further cleanup and lipid removal were achieved by using acid and basic modified silica gel multilayer columns. Fractionation among the studied compounds was achieved by using Supelclean Supelco ENVI-Carb tubes as described elsewhere (*21*). Three fractions were eluted containing *ortho*-substituted PCBs (including mono-*ortho* PCBs), non-*ortho* PCBs, and PCDDs/Fs congeners, respectively.

*2.2.1.2. Instrumental Analysis. Ortho-Substituted PCBs Instrumental Analysis.* The methodology has been described elsewhere (*22*). Briefly, analyses were performed by GC-$\mu$ECD (Agilent 6890 series II) in the splitless injection mode and using nitrogen as carrier gas. 1,2,3,4-Tetrachloronaphthalene and PCB 209 were used as injection standards. Method limits of detection ranged from 0.04 to 0.40 pg/$\mu$L for all PCBs analyzed.

*Non-ortho PCBs and PCDDs/Fs Instrumental Analysis.* The analysis of non-*ortho* PCBs and PCDDs/Fs was performed by GC-HRMS/EI-(+)-SIM on a GC 8000 series gas chromatograph (Carlo Erba Instruments) coupled to an Autospec Ultima mass spectrometer (Micromass) equipped with a CTC A 200S autosampler, at 10000 resolution (10% valley definition) using the isotopic dilution method for quantification (EPA 1613) (*23*). Details on instrumental conditions are described in Abad et al. (*24*). Method limit of detection ranged from 0.013 to 0.042 pg/$\mu$L for 2,3,7,8-substituted PCDD/Fs and non-*ortho* PCBs.

*Quality Control Criteria.* To ensure the quality of PCBs and PCDD/Fs analysis, blank samples covering the whole analytical procedure were performed and we participated in different interlaboratory studies of PCBs and PCDD/Fs in different food matrices, such as the one recently organized by the National Institute of Public Health (Norway, 2000−2001). Recoveries for the spiked compounds (between 50 and 120% in the case of the three non-*ortho* PCBs and between 60 and 110% for 2,3,7,8-PCDD/Fs) were in the range established by the EPA as acceptable. With regard to mono-*ortho* PCBs, recoveries ranged from 60 to 105%. Further details on quality control in PCDD/Fs analysis are described in Abad et al. (*24*).

**Table 1.** Levels of Total and Mono-*ortho* PCBs and Non-*ortho* PCBs, PCDD/Fs, and WHO-TEQs in the Different Food Samples Analyzed

| | ng/g of lw | | pg/g of lw | | | WHO-TEQs (pg/g of lw) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | total PCBs | mono-*ortho* PCBs | non-*ortho* PCBs | PCDD/Fs | 2,3,7,8-PCDD/Fs | TEQ mono-*ortho* PCBs | TEQ non-*ortho* PCBs | TEQ PCDD/Fs | total TEQs |
| milk and dairy products | | | | | | | | | |
| whole cow's milk[a] | 6.85–51.1 | 1.17–14.6 | 9.32–61.1 | 9.82–55.6 | 3.90–17.0 | 0.118–1.68 | 0.146–0.511 | 0.526–1.50 | 0.881–3.18 |
| cream | 11.2 | 1.68 | 32.7 | 64.8 | 5.35 | 0.176 | 0.585 | 0.775 | 1.54 |
| butter | 9.61 | 1.95 | 24.3 | 26.2 | 4.95 | 0.235 | 0.865 | 1.02 | 2.12 |
| yogurt | 49.0 | 12.0 | na[c] | 9.94 | 6.89 | 1.35 | – | 1.29 | 2.64 |
| sea fish | | | | | | | | | |
| fatty sea fish | 60.6 | 5.07 | 482 | 20.9 | 14.9 | 0.676 (0.0340)[d] | 15.8 (0.796) | 3.38 (0.170) | 19.9 (1.00) |
| lean sea fish | 127 | 21.2 | 245 | 20.3 | 16.6 | 3.16 (0.199) | 7.82 (0.492) | 4.11 (0.259) | 15.1 (0.950) |
| canned tuna | 3.87 | 0.67 | 27.0 | 16.1 | 14.5 | 0.0738 (0.0245) | 0.360 (0.120) | 2.71 (0.899) | 3.14 (1.04) |
| canned sardine | 34.4 | 6.95 | 85.1 | 286 | 199 | 1.01 (0.393) | 3.79 (1.48) | 13.1 (5.11) | 17.9 (6.97) |
| seafood | | | | | | | | | |
| prawn | 58.9 | 15.6 | 259 | 167 | 29.6 | 2.07 (0.171) | 3.47 (0.287) | 4.18 (0.345) | 9.72 (0.802) |
| cuttlefish | 31.3 | 3.34 | 89.5 | 17.9 | 10.4 | 0.410 (0.0199) | 2.34 (0.113) | 2.14 (0.104) | 4.89 (0.237) |
| bivalves | 113 | 14.7 | 132 | 53.2 | 12.5 | 1.83 (0.100) | 1.77 (0.097) | 1.14 (0.063) | 4.74 (0.260) |
| *Donax* sp. | 131 | 35.7 | na | 21.4 | 13.4 | 4.16 (0.267) | – | 11.21 (0.670) | 15.4 (0.937) |
| meat and meat products | | | | | | | | | |
| pork meat | 31.4 | 2.18 | 292 | 18.3 | 11.6 | 0.258 | 1.49 | 1.28 | 3.03 |
| chicken[b] | 40.8–52.1 | 7.61–10.6 | 117–460 | 328–242 | 38.3–117 | 0.805–1.30 | 0.556–3.74 | 1.36–8.93 | 2.72–14.0 |
| salami-type sausage | 5.58 | 1.03 | 30.8 | 50.3 | 23.4 | 0.133 | 0.327 | 0.58 | 1.04 |
| pork sausage | 46.5 | 5.06 | 120 | 76.1 | 72.0 | 0.518 | 0.744 | 1.74 | 3.00 |
| loin of pork | 10.4 | 2.16 | 84.1 | 76.1 | 53.3 | 0.249 | 0.749 | 1.04 | 2.04 |
| chicken liver | 68.4 | 17.1 | 166 | 107 | 29.7 | 2.16 | 1.53 | 3.25 | 6.93 |
| cured ham | 5.32 | 1.14 | 24.7 | 29.1 | 26.8 | 0.137 | 0.300 | 2.90 | 3.34 |
| eggs | | | | | | | | | |
| eggs | 14.6 | 3.44 | 62.7 | 49.3 | 9.53 | 0.422 | 0.725 | 1.00 | 2.15 |
| oils | | | | | | | | | |
| sunflower | 6.26 | 2.2 | 59.6 | 11.4 | 1.90 | 0.261 | 1.44 | 0.339 | 2.04 |
| olive | 2.65 | 0.53 | 20.3 | 15.8 | 3.90 | 0.076 | 0.343 | 0.288 | 0.707 |
| breads and pastries | | | | | | | | | |
| madeleines | 11.00 | 3.09 | 1.79 | 27.7 | 25.6 | 0.364 | 0.0707 | 0.418 | 0.853 |
| white bread | 116 | 25.9 | na | na | na | 3.23 | na | na | na |

[a] Maximum and minimum values corresponding to five samples. [b] Maximum and minimum values corresponding to two samples. [c] Not analyzed. [d] In parentheses are given levels expressed on a fresh weight basis.

*2.2.2. PAHs Determination.* A total of 16 PAHs, namely, naphthalene, acenaphthylene, fluorene, acenaphthen, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, indeno[1,2,3-*c*,*d*]pyrene, and benzo[*g*,*h*,*i*]perylene, were analyzed in sea fish and seafood samples.

*2.2.2.1. Sample Preparation.* Samples were Soxhlet extracted during 8 h with dichloromethane. Further cleanup was performed by using an activated silica gel column (130 °C, 17 h) (*25*), using hexane as elution solvent. A first fraction, eluted with 25 mL of hexane, was discarded, and a second fraction, containing the selected PAHs, was eluted with 25 mL of hexane/dichloromethane (3:2, v/v). The extract was concentrated to a final volume of 1 mL in acetonitrile and filtered using 0.45 $\mu$m syringe filter units.

*2.2.2.2. Instrumental Analysis.* Quantification was carried out by HPLC (Jasco PU-1580) equipped with a Spherisorb ODS2-$C_{18}$ column (25 cm × 4.6 mm and 5 $\mu$m particle size, Waters). A 20 $\mu$L loop was used to inject the sample. The initial mobile phase was acetonitrile/water (Milli-Q) (60:40, v/v), and separation was obtained with a gradient in which acetonitrile increased from 60 to 100% in 20 min at a flow rate of 1 mL/min. Detection was done using a fluorometric detector (Jasco FP-920) with programmable excitation and emission wavelengths, representing a powerful technique for what concerns sensitivity and selectivity. The repeatability, determined at a concentration level of 0.02 ng/$\mu$L, presented relative standard deviations (RSDs) below 7% for all PAHs except pyrene (13%). The reproducibility, determined by analyzing a standard solution at 0.02 ng/$\mu$L within a week, was also satisfactory, with RSD values of <6%. The instrumental limits of detection (LOD), calculated as 3 times the ratio of signal to noise, ranged between 0.0007 and 0.0084 ng/$\mu$L except for dibenz[*a*,*h*]-anthracene (0.013 ng/$\mu$L). Recovery was determined by using spiked samples at two different concentrations, and values ranged between 70 and 110% in all PAHs determined, showing the lowest recoveries of low molecular weight PAHs, such as naphthalene, fluorene, and acenaphthen due to losses during the evaporating step.

*2.2.3. Heavy Metals and Arsenic Determination. 2.2.3.1. Sample Preparation.* For element determination, samples were digested in Teflon containers in an acid medium with $HNO_3$ and $H_2O_2$, at 100 °C for 4 h (*26*).

*2.2.3.2. Instrumental Analysis.* Analyses of zinc and copper were performed using a flame atomic absorption spectrometer (AAS) (SpectrAA-100). Lead, cadmium, and arsenic were measured using a longitudinal AC Zeeman (AAnalyst 600) AAS equipped with a transversely heated graphite atomizer and a built-in fully computer-controlled AS-800 autosampler. Total mercury analysis was carried out with a Perkin-Elmer FI-MHS, using $NaBH_4$ as reductant and coupled to a graphite furnance AAS (Perkin-Elmer 4100 ZL) (*27*).

*Quality Control Criteria.* All samples were analyzed in batches, with method blanks and known standards. Reference material was used to test the confidence of the method: DORM-2 (dogfish muscle, *Squalus acanthias*) from NRC and mussel (NCS, ZC 78003) were used for this purpose, and recoveries ranged from 88 to 110%. RSDs in replicates and reference material were always below 10%.

*2.2.4. Lipid Determination.* The lipid content was determined by MSPD using a mixture of chloroform/methanol (1:1, v/v) as elution solvent. The extract was evaporated until dryness, and the lipid content was determined gravimetrically.

## 3. RESULTS AND DISCUSSION

**3.1. PCBs, PCDDs, and PCDFs Results.** Concentrations of total PCBs, mono- and non-*ortho* PCBs, total PCDD/Fs, and the 17 toxic 2,3,7,8-substituted PCDD/Fs in the food samples

studied are shown in **Table 1**, expressed in nanograms or picograms per gram of lipid weight (lw) and picograms of WHO-TEQ per gram of lipid weight in the upper bound limit of determination (assuming that nondetected values are equal to their corresponding limit of detection). In parentheses and for fish, mollusc, and crustaceans, concentrations are also expressed on a fresh weight (fw) basis.

*3.1.1. Levels in the Different Food Group Samples.* Total PCB concentrations (as the sum of the different individual congeners analyzed) in the different food groups presented great variations showing the highest concentrations food samples in the sea fish and seafood groups. Bivalves (*Donax* sp.) presented the highest concentrations (131 ng/g of lw) followed by lean sea fish (127 ng/g of lw). Meat and meat products showed in general values below those in marine samples (ranging from 5.32 to 68.4 ng/g of lw). For the rest of the food samples analyzed, values remained below 49.0 ng/g of lw with the exception of white bread. With regard to just the three non-*ortho* congeners (PCBs 77, 126, and 169), again marine samples presented the highest values (ranging from 85.1 to 482 pg/g of lw), although canned tuna presented lower concentrations (27.0 pg/g of lw). These levels were followed by those in meat and meat products. Total PCDD/Fs levels presented a great variation between the different groups, the meat and meat products group showing the highest values (ranging from 18.3 to 328 pg/g of lw) and the concentrations being noticeable in canned sardine (286 pg/g of lw) and in prawns (167 pg/g of lw). Milk and dairy products, eggs, and oils presented similar values, ranging from 9.82 to 64.8 pg/g of lw. For the most toxic PCDD/Fs, those with a 2,3,7,8-substitution pattern, differences in their contribution to the total PCDD/Fs values were found between the groups: whereas in general for milk, dairy products, eggs, and oils they accounted for $<25\%$, for sea fish they represented $>70\%$. On the other hand, for the meat and meat products group, they accounted for $<20\%$ in the case of chicken samples but for $>50\%$ in pork products.

*3.1.2. Congener Profiles.* PCB profiles showed congeners 138, 153, and 180 together with less chlorinated congeners such as PCBs 52, 95, and 101 to be the ones with a higher relative weight. With regard to non-*ortho* PCBs, PCB 77 was the predominant congener, especially in the meat and meat products group, accounting for $>80\%$ of the non-*ortho* PCB content. It is remarkable the presence in sea fish of the most toxic PCB congener, PCB 126, with a contribution to the total of $\sim30\%$. In all cases PCB 169 represents a low percentage to the total ($<8\%$). In 2,3,7,8-PCDD/Fs profiles, OCDD appeared to be the most abundant congener in all samples analyzed, as also observed by other studies regarding "non contaminated samples" (*28*, *29*). Also, 2,3,4,7,8-PeCDF and 1,2,3,4,6,7,8-HpCDD accounted for a relatively high percentage of the total, being remarkable that in fish and seafood a higher prevalence of TCDF and 2,3,4,7,8-PeCDF was found when compared to other types of food samples studied.

*3.1.3. TEQs Calculation and Health Implications.* To assess the toxicity associated with the presence of PCDD/Fs and dioxin-like PCBs (mono-*ortho* and non-*ortho* PCBs) in foodstuffs, the WHO has proposed toxic equivalency factors (WHO-TEFs) for each of the toxic congeners, which relate the toxicity of the different congeners to that of 2,3,7,8-TCDD, the most toxic one (*30*). Combining the concentration found for each congener with its corresponding WHO-TEF, a single WHO-TEQ value is obtained, suitable to compare levels between different studies and with the maximum content in food allowed by different international bodies. **Table 1** shows the concentrations obtained expressed in picograms of WHO-TEQ/g of lw in the upper bound limit of determination. The group presenting the highest total TEQ values (sum of WHO-TEQs due to dioxin-like PCBs and PCDD/Fs) was sea fish and seafood products, fatty sea fish exhibiting the highest values (19.9 pg of WHO-TEQ/g of lw) and canned tuna the lowest (3.14 pg of WHO-TEQ/g of lw). Meat and meat products presented lower values ranging from 1.04 to 14 pg of WHO-TEQ/g of lw, chicken meat and entrails presenting higher values than pork and pork products. The rest of the samples analyzed presented values well below those found for fish and meat samples, ranging from 0.707 to 3.18 pg of WHO-TEQ/g of lw. When the TEQ PCDD/Fs concentrations obtained are compared with those found in previous studies, the decrease of the concentrations observed in the last years should be taken into account (*9*). Regarding milk, the concentrations were lower than or in the same range as those reported in studies carried out in Spain or in other countries, and dairy products presented similar or in some cases higher concentrations (*7*, *11*, *12*, *31*, *32*). Sea fish and seafood levels were in general lower than those reported in Spain by Llobet et al. (*12*) and by Focant et al. (*11*) in Belgium. Regarding eggs, the value was higher than those reported in Spanish studies (*12*, *31*) but in the lower range of those described by Liem and Theelen (*32*). In general, the concentrations in meat and meat products were higher than those reported in the literature, especially in the case of chicken meat, presenting values above those described in Spain (*24*, *31*) and other European countries (*11*, *33*) but, nevertheless, well below those reported after a contamination episode (*34*). Pork showed concentrations in the same range as those reported in a recent study in Catalonia (*31*) and Germany (*35*) but higher than those reported in Belgium (*11*) and by Liem and Theelen (*32*).

It is of high interest to evaluate the relative abundance of PCDD/Fs and dioxin-like PCBs with regard to the total TEQ value. Many studies in the past did not include the determination of dioxin-like PCBs, but recently, it has been stated that they dominate the TEQ content in many foodstuffs (*36*). In this study, depending on the food matrix, variations in their contribution varied as shown in **Figure 1**. For milk and dairy samples similar contributions were found (52 vs 48%, for PCDD/Fs and dioxin-like PCBs, respectively). This was also observed for meat and meat products, although cured ham presented a clearly higher contribution of PCDD/Fs (87%). For sea fish, the contribution of dioxin-like PCBs was particularly noticeable, representing $>70\%$ to the total content, whereas for canned samples the opposite was observed (PCDD/Fs TEQ represented $>70\%$). In most cases the largest contribution to the TEQ value due to dioxin-like PCBs corresponded to non-*ortho* PCBs, demonstrating the importance of the determination of these congeners and the need of further research to assess their distribution and accumulation within the food chain.

Concerning the contribution of individual congeners to the total TEQ value, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD seemed to be the most important ones together with PCB 126 in all food groups. In the sea fish and seafood groups, this pattern is maintained, although for canned fish samples 2,3,7,8-TCDD had a similar or even a higher weight than the two penta congeners before mentioned. Also in the meat and meat products group was remarkable the percentage of tetradioxin to the total ranging from 9 to 16%. It is worth noticing that although the congeners exhibiting the highest concentrations in most of the samples were the highest chlorinated ones (OCDD and HpCDD), when

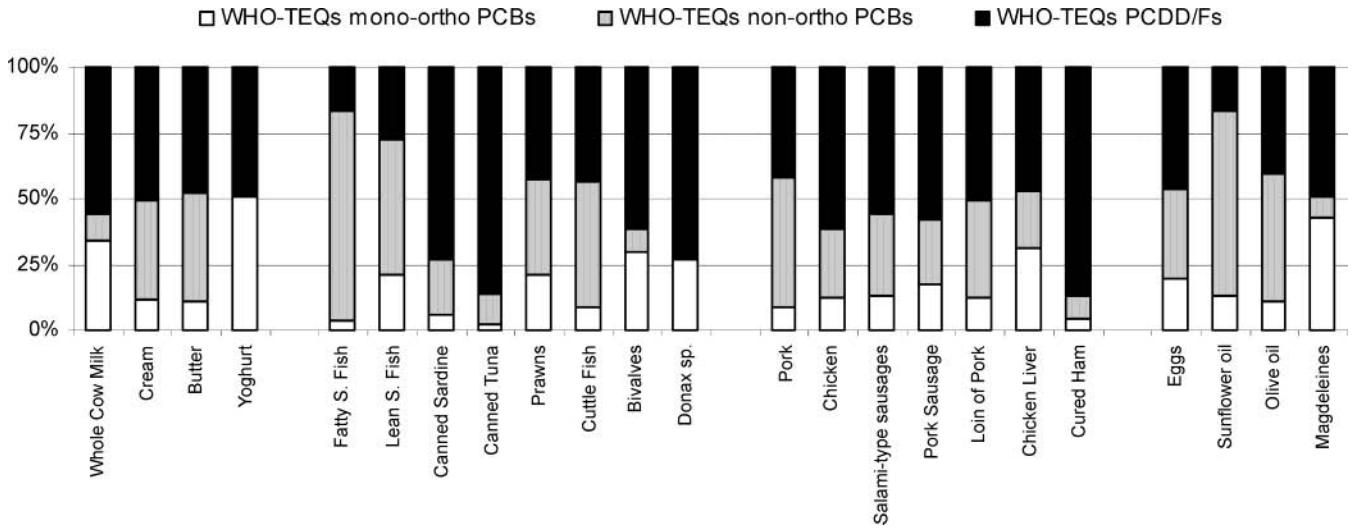


**Figure 1.** Percentage contribution to the total TEQ of mono- and non-*ortho* PCBs and PCDD/Fs in various food items from Huelva (Spain).

data are expressed in WHO-TEQ, they represent a very low relative contribution to the TEQ value due to their low TEF value.

None of the food samples analyzed, except some mentioned below, exceeded the maximum levels, expressed as WHO-TEQs$_{PCDD/Fs}$ in the upper bound level, established by the European Community (*6*). Neither the two sea fish samples nor the mollusc and crustacean samples analyzed presented values above the limit set by the European Union for these kinds of products when expressed on a fresh weight basis (4 pg of WHO-TEQ PCDD/Fs fresh weight), except the canned sardine. In the case of meat and meat products, three samples, including pork meat, presented values above the limit of 1 pg of WHO-TEQ/g of lw established for pork and pork products. For chicken meat, one sample presented a value higher than that established for chicken meat (2 pg of WHO-TEQ/g of lw). Chicken entrails remained under the maximum. From the time being European Union legislation sets the maximum levels permitted only for PCDD/Fs but with the view of including dioxin-like PCBs in the coming years. As discussed above, the important contribution of dioxin-like PCBs to the WHO-TEQ content will lead to a re-evaluation of the maximum values permitted to adjust the levels to the so-called "background levels" in noncontaminated matrices.

Although only a limited number of samples were analyzed, an estimation of the dietary intake was done in order to assess the food groups having a higher contribution to the total daily intake of the studied compounds. Dietary exposure calculations are done by combining data on consumption habits with the different concentrations of PCBs and PCDD/Fs expressed in WHO-TEQ found in food samples. In this study the consumption habits data used were from the Spanish Food and Nutrition Survey (ENNA) carried out by the National Institute of Statistics (INE) in 1991 for the region of Spain under study. The dietary intake was calculated by multiplying the concentration value of each food item by its consumption. The estimated daily intake of PCDD/Fs resulted in 1.15 pg of WHO$_{PCDD/Fs}$-TEQ/kg of body weight/day for a 70 kg person (always referring to upper bound values) and on a lipid basis. When dioxin-like PCBs were also included in the calculation, the value raised twice to 2.63 pg of WHO-TEQ/kg of body weight/day, consistent with other studies describing that dioxin-like PCBs account for a similar or even higher percentage than PCDD/Fs to the dietary intake (*9*). Nevertheless, those values are well within those recommended by the WHO for PCDD/Fs and dioxin-like PCBs (1−4 pg of WHO-TEQ/kg of body weight/day) (*3*). Comparisons with dietary intake values described in the literature should be done carefully due to the different methodologies used for their calculation. In each study there are great differences in the sampling strategy (coverage of products purchased), the way of expressing concentrations (upper, medium, and lower bound), the use of international TEF (I-TEF) or WHO-TEFs values, the inclusion of dioxin-like PCBs in the calculation, and many other factors influencing the final result. Taking into account all of these limitations, the values found in this study are in the same range or below those recently reported for other Spanish areas such as Catalonia (1.36 pg of WHO$_{PCDD/Fs}$-TEQ/kg of body weight/day, considering ND = $^1/_2$LOD) (*12*) and the Basque country (1.9 pg of I-TEQ$_{PCDD/Fs}$/kg of body weight/day, considering ND = LOD) (*13*). Compared to European studies, the values are in the same range as those reported for Germany (0.73 pg of I-TEQ$_{PCDD/Fs}$/kg of body weight/day, considering ND = $^1/_2$LOD) (*10*), Finland (0.66 pg of I-TEQ/kg of body weight/day, considering ND = 0) (*37*), Belgium (1 pg of WHO-TEQ$_{PCDD/Fs}$/kg of body weight/day, considering ND = 0) (*11*), and those reported by Liem et al. (9) from different European countries for the period after 1995, values ranging from 0.4 to 1.5 pg of I-TEQ/kg of body weight/day. With regard to the food groups contribution to the PCDD/Fs daily intake, meat and meat products representing 54%, followed by vegetable oils (21%), milk and dairy products (15%), and finally sea fish and seafood samples (4%), are the ones responsible for the major intake (**Figure 2**). This pattern has also been observed in many studies (*11*, *38*, *39*) but can be subjected to variations depending on the specific consumption habits in the studied area.

**3.2. PAHs.** The concentrations of the 16 priority PAHs analyzed are shown in Table 2 expressed in nanograms per gram of fresh weight (fw). As before mentioned just some of the most frequently consumed marine samples in the area were taken for analysis.

**3.2.1. Levels in the Different Marine Species Analyzed and Health Implications.** The 16 PAHs selected for this preliminary monitoring were those considered by the Environmental Protection Agency (EPA) as relevant contaminants due to their potential toxicity. Some of them, including benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene and benz[*a*]anthracene, have been classified as carcinogenic group 2A by the International Agency for Research on Cancer (IARC). The highest total PAHs concentrations were found for both fish species studied (71.4 and 65.7 ng/g fw, respectively for common two-banded sea

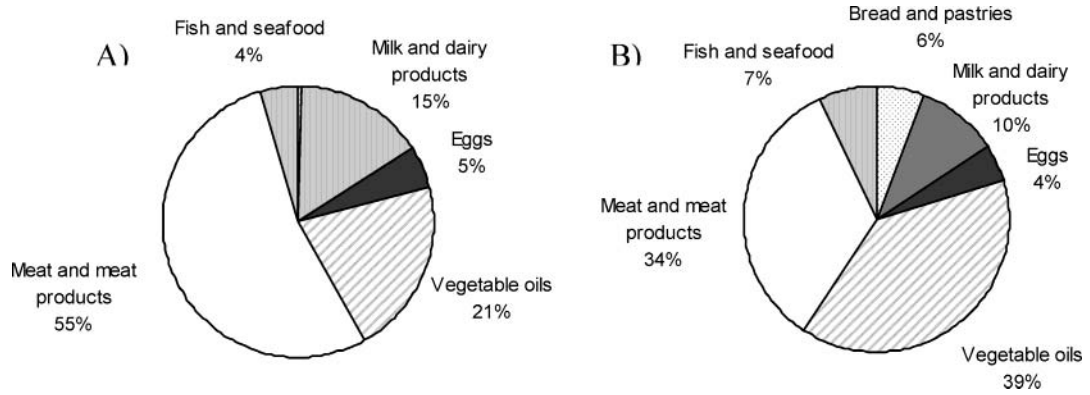


**Figure 2.** Contribution of major groups to the estimated daily intake calculated in this study: (**A**) PCDD/Fs; (**B**) PCBs (mono- and non-*ortho* PCBs).

**Table 2.** Levels of PAHs in the Different Food Samples Analyzed

| | ng/g of fresh wt | | | | | |
|---|---|---|---|---|---|---|
| | little sole | common two-banded sea bream | cuttlefish | prawns | Chamelea *sp.* | Tapes *sp.* |
| naphthalene | 1.53 | 2.00 | 1.57 | 4.02 | 2.27 | 3.16 |
| acenaphthylene | nf[a] | nf | nf | nf | nf | nf |
| fluorene | ND[b] | 0.985 | NQ[c] | ND | 0.358 | ND |
| acenaphthen | 0.353 | 0.568 | ND | 0.700 | ND | ND |
| phenanthrene | 2.82 | 3.86 | NQ | 2.30 | 1.76 | 1.68 |
| anthracene | 0.0777 | NQ | ND | 0.130 | ND | 0.399 |
| fluoranthene | 5.23 | 5.48 | 1.64 | 0.343 | 3.63 | 2.79 |
| pyrene | 61.4 | 52.8 | 18.6 | 0.613 | 32.4 | 44.2 |
| chrysene + benz[*a*]anthracene | NQ | NQ | ND | NQ | NQ | NQ |
| benzo[*b*]fluoranthene | NQ | NQ | ND | 0.115 | NQ | NQ |
| benzo[*k*]fluoranthene | NQ | NQ | ND | NQ | NQ | NQ |
| benzo[*a*]pyrene | NQ | NQ | ND | ND | NQ | NQ |
| dibenz[*a*,*h*]anthracene | NQ | ND | ND | ND | NQ | NQ |
| indeno[1,2,3-*c*,*d*]pyrene | ND | ND | ND | ND | NQ | ND |
| benzo[*g*,*h*,*i*]perylene | ND | ND | ND | ND | NQ | ND |
| **sum PAHs** | **71.4** | **65.7** | **21.8** | **8.22** | **40.4** | **52.3** |

[a] No fluorescence. [b] Not detected. [c] Not quantified.

bream and little sole), followed by the bivalves samples (52.3 and 40.4 ng/g fw), and cuttlefish (21.8 ng/g fw). Prawns presented the lowest concentrations (8.22 ng/g fw). It is remarkable that PAHs such as benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, indeno-[1,2,3-*c*,*d*]pyrene and benzo[*g*,*h*,*i*]perylene, presented levels below the limits of detection. Those high molecular weight PAHs (3−5- benzenoid rings) are also the most potential carcinogenic to living organisms. These results are in good agreement with those reported by other authors that concentrations of the most toxic PAHs in muscle of bottom fish as the ones studied are typically near or below the limits of detection (*40*−*41*), because they are readily metabolized to intermediates which binds to liver DNA or form conjugates that pass into the bile (*42*). This metabolism depends on the specific species, exposition period and also the presence of other xenobiotics that could influence their transformation (*43*). Regarding the profiles found, pyrene appeared to be the most abundant compound (accounting for more than 80% in all samples), except in prawns, where naphthalene accounted for the highest percentage (49%). It's also noticeable the presence of phenanthrene, reported as one of the major PAH component of crude oil (*44*). Profiles with a prevalence of low molecular weight PAHs (with 2 or 3 rings) are typical of PAHs mixtures generated by petrogenic pollution (*45*) and similar profiles have been observed for areas with an intense petroleum activity (*41*).

In general, the concentrations obtained were within the average concentrations of PAHs in aquatic organisms, especially marine organisms, reported from various studies, ranging from 1 to 100 ng/g of fw (*43*). Comparison of our results with those reported in the literature regarding sea fish samples places them in the range of those described for unpolluted areas (values from <0.5 to 148.0 ng/g of fw) (*44*) and in the lower range of values described for fish in areas with an intense petroleum activity (*41*). With regard to molluscs and crustaceans, and especially the former, they have been used in many studies for monitoring purposes because they concentrate pollutants from the marine environment and have a lower rate of metabolism (*44*). The concentrations found are similar to or even lower than those found in oysters collected in Germany and France (41.1 and 65.4 ng/g of fw, respectively) (*46*).

No European or Spanish regulation was found regarding this type of contaminant in fish or seafood.

**3.3. Heavy Metals and Arsenic. Table 3** shows the concentrations of the different heavy metals determined in the food items expressed in micrograms per gram of fresh weight for Cu and Zn and expressed in nanograms per gram of fresh weight for Cd, Pb, total As, and Hg.

*3.3.1. Levels in the Different Food Group Samples.* In general, the concentrations obtained in this study for the different food samples analyzed showed zinc (Zn) as the element presenting the highest levels, followed by copper (Cu), total arsenic (As), lead (Pb), mercury (Hg), and cadmium (Cd). Sea fish and seafood presented the highest heavy metal content, except for Zn, for which meat and meat products showed the highest accumulation. Milk and dairy products, together with vegetables,

**Table 3.** Levels of Heavy Metals and Arsenic in the Different Food Samples Analyzed

| | μg/g of fresh wt | | ng/g of fresh wt | | | |
|---|---|---|---|---|---|---|
| | Cu | Zn | Cd | Pb | As (total) | Hg |
| fruits and vegetables | | | | | | |
| strawberries | 0.576 | 1.22 | 2.25 | 23.7 | 7.17 | 7.42 |
| oranges | 0.866 | 1.21 | 1.61 | 23.1 | 5.00 | 5.39 |
| potatoes | 1.22 | 3.35 | 15.1 | 4.30 | 15.3 | 3.01 |
| tomatoes | 1.38 | 2.65 | 4.96 | 18.8 | 15.9 | 2.97 |
| spring onions | 0.567 | 2.67 | 16.4 | 7.18 | 11.5 | 3.28 |
| French beans | 1.73 | 3.29 | 3.54 | 5.30 | 12.4 | 2.48 |
| lettuce | 0.493 | 7.31 | 7.50 | 7.50 | 4.77 | 1.81 |
| milk and dairy products | | | | | | |
| whole cow's milk[a] | 0.0500–0.0880 | 2.90–4.10 | 0.0730–0.696 | 1.24–24.1 | ND–4.98 | 3.23–3.34 |
| cream | 0.268 | 3.24 | 2.14 | 10.1 | 16.1 | 3.29 |
| butter | ND[d] | 0.559 | ND | 17.1 | 3.67 | na[e] |
| yogurt | 0.014 | 3.18 | ND | 7.98 | 11.7 | na |
| sea fish | | | | | | |
| sardine | 2.41 | 26.6 | 12.5 | 217 | 2645 | 46.73 |
| mackerel | 0.884 | 7.70 | 12.6 | 15.1 | 907 | 100 |
| tuna | 0.316 | 5.14 | 9.04 | 21.1 | 516 | 296 |
| two-banded sea bream | 0.545 | 21.5 | 4.79 | 478 | 4114 | 248 |
| little sole | 0.946 | 8.84 | ND | 130 | 1201 | 69.3 |
| tope | 0.379 | 3.37 | 4.18 | 29.3 | 22008 | 549 |
| canned tuna | 0.431 | 3.48 | 6.78 | 17.1 | 234 | 278 |
| canned sardine | 1.49 | 18.4 | 53.6 | 69.6 | 278 | 234 |
| seafood | | | | | | |
| prawns | 13.2 | 16.6 | 50.4 | 50.4 | 16080 | 148 |
| cuttlefish | 7.80 | 14.0 | 13.3 | 88.9 | 10563 | 83.4 |
| *Tapes* sp. | 7.09 | 13.1 | 159 | 200 | 2008 | 23.4 |
| *Venus* sp. | 2.58 | 17.9 | 52.0 | 484 | 4067 | na |
| meat and meat products | | | | | | |
| pork meat | 1.51 | 41.6 | ND | 6.62 | 62.4 | 18.8 |
| chicken[b] | 0.138–1.27 | 7.21–13.9 | 3.07–22.3 | 24.8–55.7 | ND–17.8 | 19.9–27.6 |
| salami-type sausage | 3.33 | 45.5 | 58.0 | 156 | 3.52 | 34.8 |
| pork sausage | 2.16 | 54.6 | 12.1 | 52.0 | 16.1 | 66.5 |
| loin of pork | 2.16 | 54.6 | 13.3 | 66.7 | 1.92 | 237 |
| chicken liver | 4.74 | 36.3 | 22.5 | 20.0 | 31.5 | 3.50 |
| cured ham | 1.56 | 9.03 | 6.27 | 81.6 | 2.88 | 15.7 |
| eggs | | | | | | |
| eggs[c] | 0.665–0.788 | 10.8–11.9 | 0.591–0.837 | 1.82–24.2 | 3.56–4.01 | 5.68 |
| oils | | | | | | |
| sunflower | ND | ND | 0.243 | 18.3 | 0.745 | 2.00 |
| olive | ND | ND | ND | 4.27 | 2.23 | 1.00 |
| breads and pastries | | | | | | |
| madeleines | 0.652 | 3.44 | 3.51 | 12.8 | ND | na |
| white bread | 2.28 | 13.3 | 16.8 | 67.2 | 22.7 | 13.4 |

[a] Maximum and minimum values corresponding to five samples. [b,c] Maximum and minimum values corresponding to two samples. [d] Not detected. [e] Not analyzed.

exhibited the lowest values. With regard to Cu accumulation, the highest values were found for seafood, both molluscs and crustaceans (values ranging from 2.58 to 13.2 μg/g of fw), followed by meat and meat products and sea fish. The highest Zn values were reported for meat and meat products (values ranging from 7.21 to 54.6 μg/g of fw), chicken liver presenting higher values than chicken meat. These concentrations were followed by those in sea fish and seafood. For Pb, again sea fish presented the highest concentrations (ranging from 15.1 to 478 ng/g of fw), together with molluscs and crustaceans. These levels were followed by meat and meat products. The rest of the samples presented values below 67.2 ng/g of fw. The cadmium profile was very similar to that observed for Cu, presenting concentrations always below 58 ng/g of fw except for seafood, which showed values ranging from 13.3 ng/g of fw found in cuttlefish to 159 ng/g of fw found in *Tapes* sp., consistent with the fact that molluscs and crustaceans usually present higher values due to their ability to concentrate cadmium from contaminated waters (*47*). Milk and dairy products showed the lowest concentrations. With regard to total As, all food samples presented values below 62.4 ng/g of fw except marine samples. Sea fish values ranged between 234 ng/g of fw (canned tuna) and 22008 ng/g of fw (tope), and with regard to molluscs and crustaceans, prawns presented the highest values and *Tapes* sp. the lowest. These concentrations are well above those observed for the rest of the food samples, consistent with the fact that sea fish and seafood tend to accumulate higher levels of this metalloid as previously reported by other authors (*17*). Emphasis should be placed on the fact that the toxicity of arsenic is related to the chemical form present in the matrix, inorganic species such as As(III) and As(V) being the most toxic ones. Mercury also showed higher concentrations in marine samples (values ranging from 23.4 to 549 ng/g of fw), tope showing the highest concentrations. The toxicity of mercury, and similarly to arsenic, depends mainly on its chemical form. In this case, the organic form, usually monomethylmercury (MeHg), presents a higher toxicity than the inorganic and elemental forms. Marine organisms are known to turn inorganic mercury into organic forms, thus making Hg more easily transferable through the aquatic food chain (*17*). Within the rest of the food groups analyzed, values were below 66.5 ng/g of fw. When the values found in this study were compared with those reported in the literature, similar or even lower concentrations for fruits, vegetables, milk, dairy products, meat, and meat products were

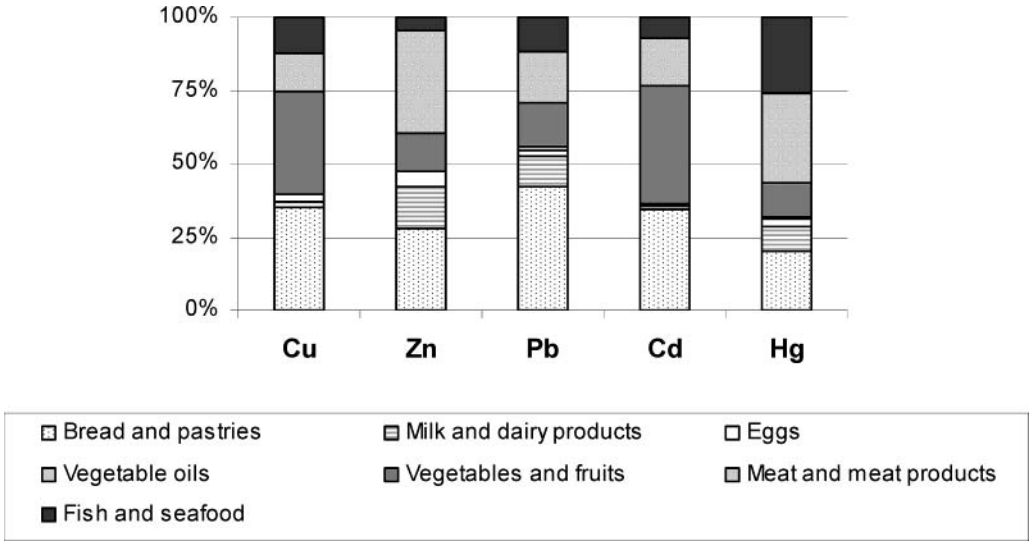


**Figure 3.** Contribution of the different food groups to the estimated heavy metals intake calculated in this study.

found (*16*, *48*). With regard to marine samples, a previous study concerning similar fish species from the same area in 1999 (*49*) reported similar values of Cd, Pb, Cu, and Zn but higher total As concentrations. Compared to the values obtained by Canli et al. (*50*) in sardine from an area under the influence of strong agricultural and industrial activity, the Pb, Cd, and Cu contents were also lower than those reported but Zn contents were higher. Concentrations were also lower than those found in different marine species from the Canary Islands (*51*) and those found in the North and Baltic Seas (*52*). Muñoz et al. (*53*) recently conducted a study reporting levels of total As in marine samples consumed in the Basque country. The concentrations were in the same range as the above-described, ranging from 0.3 to 26.22 $\mu$g/g of fw.

*3.3.2. Health Implications.* The European Community has recently established maximum allowed levels in several food matrices for some of the heavy metals analyzed in this study (*18*, *54*). With regard to Hg, Pb, and Cd none of the food samples analyzed in this study presented levels above those permitted. European Union legislation does not regard maximum values of Zn and Cu. For copper, the Spanish government established in 1991 a maximum level of 20 $\mu$g of Cu/g of fw for fish, crustaceans, cephalopods, and bivalves. For the latter group an exception was made in the case of oysters and *coquina* clam (*Donax* sp.), establishing a value of 60 $\mu$g of Cu/g of fw (*55*). Again, all marine samples were below those limits. With regard to As, none of the European countries regulate its content as total As, although some countries such as Australia and New Zealand establish a maximum content concerning inorganic As in fishery products.

WHO has also established for Pb, Cd, Hg, inorganic As, and Cu provisional tolerable intakes on a weekly basis (PTWI). Dietary exposure calculation has also been done as described for organic pollutants, combining data on consumption habits and concentration in the food samples analyzed. Data are reported on a daily basis and for a 70 kg person. For Pb, a value of 0.38 $\mu$g of Pb/kg of body weight was found, far below the maximum intake proposed by WHO (3.57 $\mu$g of Pb/kg of body weight). The group contributing the highest percentage to the daily intake was bread and pastries (42%), followed by meat and meat products (17%) and marine food samples (12%) (**Figure 3**). For Cd, the intake was found to be 0.11 $\mu$g of Cd/kg of body weight, also below the WHO provisional intake level of 1 $\mu$g of Pb/kg of body weight. In this case, vegetables (38%) and bread and pastries (35%) are the major contributors to the intake. For Hg, the value obtained, 0.14 $\mu$g of Hg/kg of body weight, is also below the level of 0.71 $\mu$g of Hg/kg of body weight proposed by WHO, meat and meat products together with marine samples being the groups contributing the highest percentages to the total intake (30 and 26%, respectively). For As, WHO established a provisional intake value regarding inorganic As of 2.14 $\mu$g of As/kg of body weight/day. No value of As intake is reported in this study because the As determined refers to total arsenic. Further investigation is focused on the determination of the different arsenic forms in marine samples from the area under study to assess the real toxicity associated to the presence of this metalloid in fish and seafood. Regarding Cu, a value of 15.28 $\mu$g of Cu/kg of body weight was found, well below the recommended value of 500 $\mu$g of Cu/kg of body weight. The major contributors to the daily intake were bread and pastries together with vegetables and fruits (35 and 28%, respectively). Although no tolerable intake value is established for Zn, the daily intake was calculated in order to assess which of the food groups studied presented a higher relative weight in the total intake, being in this case meat and meat products (35%), followed by bread and pastries (28%).

Compared to the values described in the literature for cadmium, lead, and mercury daily intake, the levels found in this study were lower than those reported for Spain in a previous study (0.14 $\mu$g of Cd/kg of body weight/day, 0.55 $\mu$g of Pb/kg of body weight/day, and 0.17 $\mu$g of Hg/kg of body weight/day) (*56*). With regard to data reported for different countries, Cd daily intake in this study is also lower than that described for Sweden and France (0.17 and 0.38 $\mu$g of Cd/kg of body weight/day, respectively) (*57*, *58*), Pb daily intake is in the range of those reported for other European countries (0.24, 0.74, and 0.45 $\mu$g/kg of body weight for Sweden, France, and The Netherlands, respectively) (*57*, *59*, *60*), but mercury presented a higher intake value than those described in the literature: 0.01 $\mu$g of Hg/kg of body weight/day for The Netherlands (*60*), 0.025 $\mu$g of Hg/kg of body weight/day for Sweden (*57*), and 0.057 $\mu$g of Hg/kg of body weight/day for the United Kingdom (*61*).

To conclude, the levels obtained in the present survey seem to be below those established by European legislation regarding PCDD/Fs, except in the case of some meat products, showing that no risk is associated with the consumption of the food samples analyzed. When dietary intake is calculated, including also PCBs, the value obtained is within the range proposed by

WHO (1−4 pg of WHO-TEQ/kg of body weight/day). In the case of the PAHs analyzed, concentration levels are in the range of those considered for unpolluted areas. The levels found of heavy metals such as lead, cadmium, and mercury are all under the maximum level proposed by European legislation. Dietary intake calculations presented values below those recommended by WHO for the elements analyzed. In the case of arsenic, further speciation should be carried out in order to assess the toxicity associated with the presence of inorganic arsenic.

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