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Dioxin and PCB residues in meats from Italy: Consumer dietary exposure

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ABSTRACT

In order to investigate PCB and PCDD/F concentrations and potential human health risk a study has been conducted in meat samples (beef, pork, chicken and turkey) purchased from Italian supermarkets. PCBs were dominant (41.8–77.7 ng g^{-1} l.w.) with respect to PCDD/Fs (20.1–91.1 pg g^{-1} l.w.). The levels were variable and largely dependent upon the type of meat. Accumulation pattern showed a distribution typically reported for meat. PCBs tended to decrease from lowest to highest congeners, while for PCDD/Fs the concentration profile was dominated by highly chlorinated dioxins and furans. Concentrations of PCDD/Fs plus dl-PCBs and indicator PCBs exceeding the EU maximum permissible levels were found in 23.3% and 53.3% of the samples, respectively. Exposure estimates to PCDD/Fs plus dl-PCBs (0.08–4.16 pg WHO-TEQs kg^{-1} b.w. w.$^{-1}$) were within the new limit proposed by the EFSA's expert panel, except for pork sausage, showing a twofold higher value. Concerning the exposure derived from indicator PCBs, the maximum level set by different European countries (10 ng kg^{-1} b.w. d.$^{-1}$) was surpassed solely via consumption of pork sausage (17.22 ng kg^{-1} b.w. d.$^{-1}$), thought also the estimated intake from hamburger consumption (6.88 ng kg^{-1} b.w. d.$^{-1}$) merits attention being close to guideline limit.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs), collectively referred to as "dioxins", and polychlorinated biphenyls (PCBs) are structurally similar halogenated aromatic hydrocarbons. PCBs, in particular, can be divided into two main groups according to their chemical structure and toxicological properties: dioxin-like PCBs (dl-PCBs) consisting of 12 congeners exhibiting either a high toxic potential or a dioxin-like mode of action and non dioxin-like PCBs (ndl-PCBs). It is known that they represent a risk factor for the tumor promotion (Fiore et al., 2019) and alter a number of physiological processes resulting in adverse effects on liver, kidney and neurological and endocrine system (Faroon and Ruiz, 2016). The ndl-PCB group includes six congeners known as indicator PCBs (iPCBs), recommended by the European Food Safety Authority (EFSA) Scientific Panel as the most appropriate markers for PCB contamination level in food, which represents the most important source for human exposure to these pollutants (EFSA, 2005). In consequence the understanding of their levels in foodstuff is a key issue for evaluating the human exposure and to prevent a number of possible diseases. In the past decades, dioxin contamination incidents of feed and foodstuff not only have caused tremendous economic losses, but also brought great hazards to environment and human health. By consequence, many countries have imposed maximum permissible levels (MPLs) of these chemicals in food prior to marketing. Furthermore, internationally recognized organizations have set up toxicological reference values, such as tolerable daily intake (TDI) or tolerable weekly intake (TWI) to which human can be exposed without harm. In this picture, it is important to underline that, EFSA's expert Panel on Contaminants in the Food Chain (CONTAM) has completed, on demand of European Commission, the first comprehensive review of the risks to human and animal health from these substances in food and feed. Owing to this, the data from experimental animal and epidemiological studies were reviewed and it was decided to base the risk assessment on effects observed in humans and to use animal data as supportive evidence. As a result, the Panel reduced of seven-fold the PCDD/F plus dl-PCB tolerable weekly intake, fixing it at 2 pg per kilogram of body weight (EFSA, 2018). This notable reduction might be in line with a general decline in dietary exposure to PCDD/Fs and dl-PCBs observed not only in many European Countries (Baars et al., 2004; Béchaux et al., 2014; Llobet et al., 2008) but also in extra-European countries, such as United States (Charnley and Kimbrough, 2006), Canada (Mayer, 2001) and Taiwan (Lee et al., 2016). The meat,

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whose nutritional content is unequivocal, being a source of high biological value proteins, iron, vitamin B12 as well as essential nutrients, is an important component of a balanced diet. This food also provides a good contribution of saturated and unsaturated fatty acids, which can serve as a sink for lipophilic pollutants as PCDD/Fs and PCBs. Despite this, scientific literature on the levels of these harmful chemicals in meat products is not certainly abundant (Ankarberg et al., 2007; Bramwell et al., 2017; Cederberg et al., 2010; De Mul et al., 2008; Domingo, 2017; Domingo and Nadal, 2017, 2016; Gonzáles et al., 2018; Marin et al., 2011; Perelló et al., 2012; Quijano et al., 2017; Rauscher-Gabernig et al., 2013; Schwarz et al., 2014; Shen et al., 2017; Tlustos et al., 2014; Volatier et al., 2006), especially for Italy (Diletti et al., 2018, 2007; Fattore et al., 2008, 2006; Turci et al., 2006). Considering this gap in knowledge, the present study shows the results of congener-specific analysis of PCBs and PCDD/Fs performed on different meat kinds including meat of beef, pork, chicken and turkey purchased from Italian supermarkets. The objectives are to verify whether the contaminant concentrations exceed the maximum permissible levels set by the European Council directives (Official Journal of the European Union, 2011) and whether the estimated intake level satisfies the requirements of the new criterion of the EFSA's expert panel (EFSA, 2018).

2. Materials and methods

2.1. Sample collection

In March–April 2018, a total of 445 meat samples including meat of beef (50 steak and 83 hamburger samples), pork (50 loin and 122 sausage samples), chicken (70 breast samples) and turkey (70 breast samples) were randomly acquired in supermarkets representing the five most popular retailer brands in Italy (Table 1). The various supermarkets were located in 7 towns (Barletta, Bari, Brindisi, Gallipoli, Foggia, Lecce, Taranto) in Apulia, Southern Italy. The samples of each meat type were mixed to prepare composite samples according to purchase site (pool = 5), homogenized and stored below −20 °C pending analysis.

2.2. Chemicals and standards

All solvents were of ultra-trace analysis grade and were obtained from Carlo Erba (Levanchimica, Bari, Italy) and Sigma-Aldrich (Milan, Italy). Silica gel (0.063–0.100) was purchased from Merck (Darmstadt, Germany), basic alumina (150 mesh) and florisil (60–100 mesh) were obtained from Sigma-Aldrich (Milan, Italy). For dl-PCB determination, all standards were purchased from **AccuStandard** Inc. (New Haven, USA) while standard reference material (ERM – BB445) was obtained from European Joint Research Center (JRC, Geel, Belgium). For PCDD/Fs analysis, native and labelled standards were acquired from Wellington laboratories Inc. (Guelph, Ontario, Canada) whereas standard reference material (CARP-2) was bought from National Research Council of Canada (NRCC, Ottawa, Canada).

Table 1
Sample collection.

Samples	Supermarkets					Total
	1	2	3	4	5	
Beef meat						
Steak	12	8	10	13	7	50
Hamburger	15	15	20	13	20	83
Pork meat						
Loin	9	11	10	10	10	50
Sausage	24	22	26	28	22	122
White meat						
Chicken breast	10	15	15	20	10	70
Turkey breast	15	15	15	10	15	70
N° of samples purchased in each supermarket	85	86	96	94	84	**445**

2.3. Analytical method

The concentrations of 18 individual PCB congeners (indicator PCBs (iPCBs): 28, 52, 101, 138, 153 and 180 and "dioxin-like" PCBs (dl-PCBs): non-*ortho* PCBs 77, 81, 126, 169 and mono-*ortho* PCBs 105, 114, 118 123, 156, 157, 167, 189) together with the seventeen 2,3,7,8-substituted PCDD/F congeners were determined. A complete description of the experimental procedures relative to PCBs and PCDD/Fs has been described and validated (Barone et al., 2018). Briefly, homogenized samples (0.5–3.0 g) were ground in a porcelain mortar and pestle with Na_2SO_4 and spiked with PCB 143 used as internal standard. The mixture was extracted with hexane and the extracts were concentrated via a rotory evaporator (60 °C) and subsamples were taken in order to determine the tissue fat content by gravimetry. An aliquot (about 100 mg) of the remaining extract was dissolved in hexane and cleaned by passing through 8 g of acid silica (H_2SO_4, 44% w.w.), using 50 mL of a mixture of hexane/dichloromethane (1/1, v/v) for elution of the analytes. The eluate was evaporated to dryness and redissolved in 100 µL of iso-octane. For the determination of PCDD/Fs (US EPA method 1613) the samples extracted, as above reported, were added with a clean-up standard solution (50 µl) at a concentrations of 0.8 ng ml^{-1} and subjected to a multi-step clean up to remove the matrix and the potential interfering components. The first stage was a fat destruction step consisting of a treatment of the sample solution with sulphuric acid and base back-extraction. The concentrated extract was sequentially subjected to multilayer silica gel, basic alumina and florisil chromatography columns for further cleanup. The multilayer silica gel column (glass column with 15 mm inner diameter and 30 cm long) was packed from bottom to top with 1 g activated silica, 4 g basic silica (1.2%, w/w), 1 g activated silica, 8 g acidic silica (30%, w/w), 1 g activated silica, 2 g $AgNO_3$ silica (10%, w/w) and 4 g anhydrous sodium sulphate in according to EPA 1613. The first eluted solvent was discarded, while the second eluate containing PCDD/Fs was collected. The extracts were evaporated to dryness and redissolved in iso-octane. Appropriate C^{13}-labelled extraction standards (Wellington laboratories Inc., Guelph, Ontario, Canada) were added to the samples in order to control the whole sample preparation process. The final obtained PCBs and PCDD/Fs extracts were injected and analysed separately.

2.4. Instrumental analysis

PCDD/Fs and dl-PCBs analysis were performed on a HRGH/HRMS system consisting in a MAT 95 XL mass spectrometer, coupled with a GC Trace series 2000 (Thermo Electron, Darmstadt, Germany). Chromatographic separation was carried out with a Trace Gold™ TG-Dioxin capillary column (60 m × 0.25 mm i.d. X 0.25µn film thickness; Thermo Fisher Scientific, Waltham, MA, USA) for PCDD/Fs and with a Trace TR-PCB8 MS capillary column (50 m × 0.25 mm i.d. X 0.25µn film thickness; Thermo Fisher Scientific, Waltham, MA, USA) for dl-PCBs. Helium (99.9999% purity) at 1 mL/min was used as carrier gas and the temperatures of ion source and transfer line were set at 260 and 290 °C, respectively. Injections were performed in splitless mode (for 1 min with a 1 ml vol. and split rate of 140 ml/min) on an A200S autosampler (Thermo Electron, Darmstadt, Germany). For PCB determination the oven programme was the following starting at 100 °C and held for 1 min, then increased to 200 °C in 5 min and manteined for 1 min, augmented to 270 °C in 14 minand hence maintained for 11 min, finally increased to 310 °C in 5 min, held for 6 min. For PCDD/Fs, instread, the temperature ramp started at 90 °C, was held for 6.5 min and increased to 220°c in 10.5 min, hence held for 10 min, then augmented to 235 °C in 5 min, maintained for 7 min; increased again to 300 °C in 16 min and held for 5 min. Electron ionization mode (E.I.), as well as, voltage selected ion recording mode (VSIR) was chosen as operating

Table 2
Mean concentrations of PCBs (ng g^{-1} lipid weight), PCDD/Fs (pg g^{-1} lipid weight) and percentage of individual congeners for each meat group.

Congener	Beef steak	Hamburger	Pork loin	Pork sausage	Chicken breast	Turkey breast	Total meat
Indicator PCBs							
PCB 28	7.4 ± 1.52	8.1 ± 2.36	5.1 ± 1.70	6.2 ± 2.05	5.6 ± 0.94	6.6 ± 1.02	39.0 (14.5%)
PCB 52	7.7 ± 0.55	9.7 ± 4.03	5.2 ± 1.32	6.9 ± 1.74	5.4 ± 1.26	6.6 ± 1.89	41.4 (15.4%)
PCB 101	10.6 ± 0.53	12.1 ± 5.97	7.1 ± 2.00	9.0 ± 0.90	6.1 ± 1.18	7.3 ± 2.69	52.1 (19.3%)
PCB 138	10.7 ± 0.58	13.2 ± 5.2	7.5 ± 1.88	8.1 ± 2.03	8.1 ± 1.04	9.3 ± 2.84	56.9 (21.1%)
PCB 153	12.4 ± 1.27	15.1 ± 6.09	5.6 ± 3.47	6.6 ± 1.65	9.1 ± 1.29	10.3 ± 3.61	59.0 (21.9%)
PCB 180	4.0 ± 0.14	4.9 ± 1.11	3.0 ± 2.02	3.7 ± 1.02	2.2 ± 0.72	3.1 ± 0.99	20.9 (7.8%)
Σ Indicator PCBs	52.7 ± 3.1	63.0 ± 20.42	33.4 ± 9.75	40.6 ± 6.98	36.5 ± 2.95	43.2 ± 12.37	269.3 (100%)
dl-PCBs							
Non-*ortho* PCBs							
PCB 77	ND	0.04 ± 0.05	ND	ND	0.07 ± 0.10	ND	0.11 (0.2%)
PCB 81	ND	ND	ND	ND	ND	ND	–
PCB 126	ND	ND	ND	ND	ND	ND	–
PCB 169	ND	ND	ND	ND	ND	ND	–
Mono-*ortho* PCBs							
PCB 105	1.0 ± 0.22	2.0 ± 1.28	1.5 ± 1.70	3.3 ± 3.00	1.3 ± 0.81	2.7 ± 0.53	11.8 (17.2%)
PCB 114	ND	0.01 ± 0.01	ND	0.01 ± 0.004	ND	ND	0.01 (0.02%)
PCB 118	1.7 ± 0.27	3.0 ± 1.67	1.9 ± 1.16	3.3 ± 0.84	1.0 ± 0.73	2.1 ± 1.22	13.0 (18.9%)
PCB 123	1.3 ± 0.20	2.7 ± 1.43	1.4 ± 1.35	3.7 ± 0.63	0.6 ± 0.54	2.0 ± 1.86	11.7 (17.0%)
PCB 156	1.3 ± 0.14	2.7 ± 1.93	1.2 ± 1.71	3.5 ± 0.74	0.10 ± 0.14	1.3 ± 1.27	10.1 (14.6%)
PCB 157	0.004 ± 0.01	0.01 ± 0.01	0.01 ± 0.004	0.004 ± 0.01	0.004 ± 0.01	0.01 ± 0.01	0.03 (0.05%)
PCB 167	0.01 ± 0.004	0.01 ± 0.01	0.004 ± 0.01	0.01 ± 0.004	0.002 ± 0.004	0.004 ± 0.01	0.03 (0.05%)
PCB 189	3.3 ± 0.50	4.2 ± 1.11	3.3 ± 2.0	5.6 ± 1.11	2.3 ± 1.20	3.3 ± 2.12	22.0 (32.0%)
Σ dl-PCBs	8.6 ± 0.89	14.7 ± 6.09	9.4 ± 3.11	19.3 ± 3.81	5.3 ± 2.20	11.5 ± 3.98	69.0 (100%)
Total PCBs	61.3 ± 3.04	77.7 ± 23.61	42.8 ± 12.70	59.9 ± 8.49	41.8 ± 3.96	54.6 ± 15.43	338.2
Dioxins							
2,3,7,8-TCDD	0.19 ± 0.08	0.20 ± 0.06	0.13 ± 0.04	0.10 ± 0.03	0.11 ± 0.05	0.14 ± 0.04	0.88 (0.5%)
1,2,3,7,8-PeCDD	0.19 ± 0.11	0.21 ± 0.06	0.13 ± 0.05	0.10 ± 0.02	0.22 ± 0.10	0.23 ± 0.04	1.1 (0.6%)
1,2,3,4,7,8-HxCDD	0.93 ± 0.30	1.0 ± 0.11	0.53 ± 0.22	0.41 ± 0.32	1.1 ± 0.28	0.17 ± 0.20	5.2 (2.9%)
1,2,3,6,7,8-HxCDD	1.5 ± 0.22	1.6 ± 0.36	1.2 ± 0.32	0.97 ± 0.24	0.89 ± 0.20	1.0 ± 0.12	7.1 (4.0%)
1,2,3,7,8,9-HxCDD	1.1 ± 0.23	1.4 ± 0.28	0.67 ± 0.16	0.55 ± 0.16	1.1 ± 0.22	1.2 ± 0.16	5.9 (3.4%)
1,2,3,4,6,7,8-HpCDD	5.4 ± 1.22	5.6 ± 1.70	5.1 ± 1.06	4.0 ± 0.80	14.4 ± 2.57	15.2 ± 2.20	49.7 (28.1%)
OCDD	8.5 ± 1.85	9.1 ± 1.04	6.3 ± 1.07	5.8 ± 1.47	37.9 ± 5.35	39.1 ± 7.02	106.6 (60.4%)
Σ PCDDs	17.8 ± 2.65	19.0 ± 1.48	14.0 ± 1.59	11.9 ± 2.47	55.8 ± 7.46	58.0 ± 6.49	176.5 (100%)
Furans							
2,3,7,8-TCDF	0.56 ± 0.27	0.61 ± 0.14	0.13 ± 0.03	0.10 ± 0.02	0.67 ± 0.23	0.71 ± 0.22	2.8 (2.5%)
1,2,3,7,8-PeCDF	0.19 ± 0.09	0.22 ± 0.05	0.14 ± 0.07	0.20 ± 0.04	2.2 ± 0.55	2.3 ± 0.92	5.3 (4.8%)
2,3,4,7,8-PeCDF	0.93 ± 0.29	1.1 ± 0.09	0.80 ± 0.13	0.73 ± 0.34	0.33 ± 0.09	0.45 ± 0.10	4.3 (3.9%)
1,2,3,4,7,8-HxCDF	1.5 ± 0.26	1.5 ± 0.23	1.2 ± 0.14	0.20 ± 0.04	0.78 ± 0.18	0.87 ± 0.09	6.0 (5.4%)
1,2,3,6,7,8-HxCDF	0.93 ± 0.29	1.1 ± 0.17	0.67 ± 0.16	0.23 ± 0.05	1.1 ± 0.26	1.2 ± 0.15	5.2 (4.7%)
1,2,3,7,8,9-HxCDF	0.74 ± 0.21	0.88 ± 0.10	0.53 ± 0.21	0.45 ± 0.18	1.1 ± 0.24	1.0 ± 0.12	4.7 (4.3%)
2,3,4,6,7,8-HxCDF	0.93 ± 0.27	1.2 ± 0.20	0.40 ± 0.15	0.22 ± 0.04	1.1 ± 0.18	1.1 ± 0.12	5.0 (4.5%)
1,2,3,4,6,7,8-HpCDF	4.1 ± 0.93	4.3 ± 0.89	4.7 ± 1.29	3.8 ± 0.64	12.2 ± 1.55	14.1 ± 2.42	43.1 (38.8%)
1,2,3,4,7,8,9-HpCDF	0.93 ± 0.21	1.1 ± 0.18	0.41 ± 0.12	0.38 ± 0.07	1.1 ± 0.16	1.0 ± 0.06	5.0 (4.5%)
OCDF	2.4 ± 0.80	2.7 ± 0.46	2.0 ± 0.58	1.9 ± 0.29	10.1 ± 2.06	10.3 ± 1.53	29.5 (26.6%)
Σ PCDFs	13.1 ± 1.83	14.7 ± 1.70	11.0 ± 2.10	8.2 ± 0.82	30.8 ± 3.52	33.2 ± 4.42	110.9 (100%)
Σ PCDD/Fs	31.0 ± 3.39	33.7 ± 3.17	25.0 ± 3.47	20.1 ± 3.03	86.6 ± 9.64	91.1 ± 7.49	287.5

ND Not Detected.

method. Electron energy was 35 eV and detector voltage was 350 V. The detector resolving power was > 10.000 (10% valley definition) and the two most intense ions were monitored for the determination of the single congeners. Perfluorokerosene (PFK) was the mass reference used. The quantification was performed by isotope dilution method. Multilevel calibration curves ($r^2 > 0.999$) in the linear response interval of the detector were created for the quantification. The calibration curves were prepared to result in a range of 0.5–800 ng ml^{-1} for PCB congeners and in a range of 0.025–2.00 pg/μL for PCDD/Fs.

The oven temperature programme for dl-PCBs was: initial hold at 100 °C for 2 min, increased to 230 °C in 6 min, held at 230 °C for 20 min, then increased to 310 °C at 6 °C/min and maintained for 5 min. For PCDD/Fs instead, the initial oven temperature was fixed at 90 °C and held for 7 min, hence ramped to 220 °C in 10.5 min and maintained for 10 min; increased to 235 °C in 5 min and held for 7 min then ramped again to 300 °C in 16 min and held for 5 min.

2.5. Quality control and quality assurance

QA/QC was performed through the analysis of procedural blanks, quantitative control sample for each batch of samples, duplicate sample and a standard reference material for each set of samples. For the replicate, standard reference materials and recovery of labelled compounds, the relative standard deviations (RSD) were < 10% for all the detected compounds. The recovery rates of labelled standards were between 85 and 120%. Additionally, the PCB method performance was assessed through participation to interlaboratory studies organized by QUASIMEME (Laboratory Performance Studies). Obtained values were deviating with less than 20% from the consensus values. The limits of detection (LODs) were calculated as three times the signal-to-noise ratio and varied amongst analyte groups (0.00038–0.16 pg g^{-1} for PCDD/Fs and 0.04–1.40 ng g^{-1} for PCBs). The limits of quantification (LOQs) were the followings: 0.004–1.0 pg g^{-1} for PCDD/Fs and 4×10^{-6} – 0.04 ng g^{-1} for PCBs. Concentrations of PCBs and PCDD/Fs are presented as ng g^{-1} on a lipid weight basis and pg g^{-1} on a lipid weight basis, respectively.

2.6. Statistical analysis

Kruskal–Wallis test was used to test hypothesis about differences in the levels of contaminant accumulation and to determine whether there were concentration differences in the accumulation pattern as a function of the different groups of meat. The level of significance was set at $P < 0.05$. A simple linear regression coefficient was used to examine the relationship between the sum of six iPCBs and total PCBs.

2.7. Exposure assessment

The dietary intakes of PCDD/Fs plus dl-PCBs were calculated via the deterministic approach. Point estimates were obtained by multiplying the meat consumption data (bovine and poultry: 50.97 g day^{-1}; pork: 110.37 g day^{-1}) (FAO, 2013) by mean concentrations in each meat category and then dividing by the body weight (70 kg) using the following equation:

$$Y = C \times X/b.w$$

where C is the sum of PCDD/F plus dl-PCB levels express as TEQ (pg TEQ g^{-1} wet weight); X is the consumption quantity of that particular item by individual (g wet weight); b.w. is the body weight of the individual. TEQ concentrations were calculated by multiplying the individual congener concentrations by their respective toxic equivalency factors (TEFs), as established by the World Health Organization (WHO) in 2005 (Van den Berg et al., 2006) and subsequently summed up to give the total concentrations. For exposure calculations, the contamination level of each sample expressed in lipid weight was converted into wet weight using the lipid content of the samples. Non detected congeners were considered as equal to zero (lower bound estimates).

3. Results and discussion

3.1. Concentration and congener profile of PCBs and PCDD/Fs in meat samples

Analyses results of PCBs and PCDD/Fs in meat samples are illustrated in Table 2. Among the chemicals tested, PCBs were dominant (range: 41.8–77.7 ng g^{-1} lipid weight) respect to PCDD/Fs (range: 20.1–91.1 pg g^{-1} lipid weight) ($P < 0.004$), whereas between PCDDs (range: 11.9–58.0 pg g^{-1} lipid weight) and PCDFs (range: 8.2–33.2 pg g^{-1} lipid weight) no difference in contamination level was found ($P > 0.05$). With respect to detection frequency, PCBs 81, 126 and 169 were absent in all samples; PCB 77 was present only in chicken breast (40%) and hamburger samples (40%), while PCB 114 was encountered solely in pork sausage (80%) and hamburger samples (60%). PCB 157 and PCB 167 were found in all meat types with a detection frequency varying from 40% to 80% and from 40% to 60%, respectively. The remaining PCBs and PCDD/Fs were encountered at detectable levels in all the samples analyzed. Within the meat samples tested, PCB contamination levels appeared to be rather dissimilar, with hamburger (77.7 ng g^{-1} lipid weight) and chicken breast (41.8 ng g^{-1} lipid weight) samples showing relatively higher and lower concentrations, respectively ($P < 0.02$). In contrast, PCB congener distribution remained more or less unchanged with concentrations generally tending to decrease from the lowest to the highest chlorinated congeners. In fact, the low chlorinated congeners such as PCBs 28, 52 and 101, collectively accounting 39.1% of the total PCBs, were most prominent respect to the higher chlorinated congeners, PCBs 180 and 189, which together gave a contribution percentage of 12.7%. However, nevertheless the comparable profile, differences relatively to PCBs 118, 156 and 189 were observed in pork sausage samples having concentrations higher than other meats ($P < 0.03$). More, in chicken breast PCB 156 showed concentrations extremely lower than the other products considered ($P < 0.003$). Within the group of iPCBs, the concentrations varied from a minimum of 33.4 ng g^{-1} lipid weight encountered in pork loin to a maximum of 63.0 ng g^{-1} lipid weight in hamburger samples. With percentages of 21.1% and 21.9%, the congeners 138 and 153 dominated, followed by PCB 101 (19.3%), PCB 52 (15.4%) and PCB 28 (14.5%) showing also consistent contributions, while PCB 180 (7.8%) constituted the smallest percentage quota of total sum of the six iPCBs. Among dl-PCBs, the concentrations ranged between 5.3 ng g^{-1} lipid weight in chicken breast and 19.3 ng g^{-1} lipid weight in pork sausage samples. With regard to mono-*ortho*, the highest percentage contribution to the total dl-PCBs corresponded to PCB 189 (32.0%), followed by PCB 118 (18.9%), PCB 123 (17.0%), PCB 105 (17.2%) and PCB 156 (14.6%), while PCBs 114, 157 and 167 collectively constituted a negligible percentage (0.1%). Among non-*ortho* congeners, PCB 77, solely compound detected, constituted a very little percentage (0.2%) of the total dl-PCBs. With regard to PCDD/Fs, the maximum values were recorded in white meat, especially in turkey breast (91.1 pg g^{-1} lipid weight), whereas the lowest levels were in pork sausage samples (20.1 pg g^{-1} lipid weight) ($P < 0.0001$). OCDD and HpCDD were the most prominent congeners collectively constituting 54.4% of total PCDD/Fs, followed by 1,2,3,4,6,7,8-HpCDF and OCDF showing percentage contributions of 15.0% and 10.2%, respectively. However, a detailed analysis of data revealed significant differences between red and white meat relatively to some congeners. Within PCDDs, HpCDD and OCDD had much higher concentrations in white meat than in the other type of meats ($P < 0.001$). Also for furans, concentrations of 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF together to 1,2,3,4,6,7,8-HpCDF and OCDF were higher in white meats ($P < 0.001$). This variability might be mainly related to contaminated feed (Waegeneers et al., 2009), thought several studies have shown that also soil ingestion by these animals have a strong influence on pollutant accumulation (Waegeneers et al., 2009). Fragment of tiles, concrete located in the farmyard, preserved wood used to build coops, farm paint exc., are other factors influencing birds' exposure (Piskorka-Pliszczynska et al., 2017). It is complicated to compare the results obtained with literature data because multiple reasons can hamper their interpretation. For example, besides to kind and number of meat products analyzed, there are differences in the number of congeners tested as well as differences in the approach for the calculation (lower, medium and upper bound) and presentation of concentrations (wet, dry or lipid weight). Nonetheless, a clear similarity emerges regarding the prevalence of PCB congeners with low chlorination grade respect to higher-chlorinated congeners (Bordajandi et al., 2004; Kim et al., 2004; Llobet et al., 2008) and a concentration profile dominated by the presence of highly chlorinated dioxins and furans (Llobet et al., 2003; Marin et al., 2011; Perelló et al., 2012).

3.2. Compliance with EU regulation and exposure assessment

European regulation No 1259/2011 (Official Journal of the European Union, 2011) sets maximum permissible levels (MPLs) in many foods. As shown in Table 3, the limits for PCDD/Fs plus dl-PCBs, expressed as TEQ value, are different in relation to various meat types, whereas for the sum of the six i-PCBs has been set a solely value, which cannot exceed 40 ng g^{-1} lipid weight. Following these legislative measures, the sample percentage with PCDD/F plus dl-PCB residues exceeding MPLs was 23.3% attributable to pork loin and pork sausage (1.4 pg WHO-TEQ g^{-1} lipid weight). With regard to the iPCBs, the sum of concentrations was high, compared with the MPL fixed in Regulation No 1259/2011 (Official Journal of the European Union, 2011) in 53.3% of the analyzed samples, with exception of pork loin (33.4 ng g^{-1} lipid weight) and chicken breast (36.5 ng g^{-1} lipid weight) showing values slightly lower than those considered acceptable for human consumption. Together to these legal limits adopted to prevent exposure of the human population, the Scientific Committee on Food of the European Commission (SCF, 2000) has established a tolerable daily intake (TDI) of 2 pg WHO-TEQ kg^{-1} body weight for the most potent 2,3,7,8-substituted PCDD/Fs and the dl-PCBs, expressed as WHO-TEQ (van den Berg et al., 2006). Recently the EFSA's expert panel has lowered of

Table 3
Concentration of dl-PCBs, PCDD/Fs expressed as WHO-TEQ (pg g^{-1} lipid wt) and iPCBs (ng g^{-1} lipid wt) and comparison with maximum permissible levels (Official Journal of the European Union, 2011).

	Beef steak	Hamburger	Pork loin	Pork sausage	Chicken breast	Turkey breast
	This study					
iPCBs (ng g^{-1} lipid wt)	52.66	62.98	33.43	40.60	36.48	43.15
dl-PCBs (pg WHO-TEQ g^{-1} lipid wt)	0.26	0.44	0.28	0.58	0.16	0.34
PCDD/Fs (pg WHO-TEQ g^{-1} lipid wt)	1.58	1.78	1.14	0.82	1.58	1.72
PCDD/Fs + dl-PCBs (pg WHO-TEQ g^{-1} lipid wt)	1.84	2.22	1.42	1.40	1.75	2.06
	Maximum permissible levels of meat and meat products					
iPCBs (ng g^{-1} lipid wt)	40	40	40	40	40	40
PCDD/Fs (pg WHO-TEQ g^{-1} lipid wt)	2.5	2.5	1.0	1.0	1.75	1.75
PCDD/Fs + dl-PCBs (pg WHO-TEQ g^{-1} lipid wt)	4.0	4.0	1.25	1.25	3.0	3.0

Table 4
Estimates of PCDD/Fs plus dl-PCBs (pg WHO-TEQ kg^{-1} b.w. $week^{-1}$) and iPCBs (ng kg^{-1} b.w. day^{-1}) intakes through meat consumption.

Meat and meat products	PCDD/F plus dl-PCB Estimates Weekly Intake				
	Mean	P50	P75	P95	P97.5
Beef steak	1.08	1.12	1.13	1.15	1.16
Hamburger	1.70	1.59	1.80	2.09	2.12
Pork loin	1.18	1.17	1.29	1.31	1.31
Pork sausage	4.16	4.23	4.79	4.88	4.89
Chicken breast	0.08	0.08	0.09	0.09	0.09
Turkey breast	0.13	0.12	0.12	0.14	0.15
	iPCB Estimates Daily Intake				
	Mean	P50	P75	P95	P97.5
Beef steak	4.41	4.42	4.48	4.69	4.71
Hamburger	6.88	6.78	7.61	9.67	9.93
Pork loin	3.95	4.22	4.51	5.21	5.30
Pork sausage	17.22	16.39	19.54	20.74	20.89
Chicken breast	0.24	0.24	0.26	0.26	0.26
Turkey breast	0.38	0.40	0.44	0.50	0.50

seven-fold the PCDD/F plus dl-PCB tolerable weekly intake (TWI) fixing it at 2 pg WHO- TEQ kg^{1} body weight (EFSA, 2018). Examining our results in the light of this new criterion, the mean weekly intakes estimated were between 0.08 and 4.16 pg WHO-TEQ kg^{-1} b.w. $weekly^{-1}$ (Table 4). In particular, all the estimates of the intake were within the new limit, except pork sausage showing a twofold higher value than the limit, probably in relation to the fact that, often, the processed meat contains high quantities of minced fatty tissues (Domingo and Nadal, 2017). For the remaining meats, the exposure via hamburger consumption, accounting for 85.0% of TWI, was rather high, even though, also the consumption of pork loin and beef steak gave an appreciable contribution to TWI (pork loin: 59.0%, beef steak: 54.0%). By contrast, the exposure from the consumption of chicken (4.0%) and turkey breast (6.5%) contributed with a small extent to TWI, due to the lower consumption amount and lower concentration of PCBs and dioxin compounds. Also at the 97.5th percentile dietary estimates were low respect to TWI, indicating that the exposure of consumer to these toxicants is not likely to be of human health concern, except for consumption of pork sausage and hamburger. However, it must be highlighted that in every dietary exposure assessment, several sources of under- and/or over estimation should be considered. For example, in our case it is important to take in account that the expression of the results with lower bound approach can be a cause of an under-estimation of the exposure level. Also the effect of cooking leading to leakage of fat or further contamination can determine an exposure under- or overestimation (Domingo, 2011). For example, Hori et al. (2005) report a reduction from 14 to 40% of dioxin concentration in fish and meat in dependence on the cooking method used, while an other study shows an increase of dioxin concentrations with the smoking of food matrices (Van Leeuwen et al., 2007). Uncertainties emerge also relatively to comparison with intake estimates from scientific literature, because several factors including differences in the sampling strategies, number and type of meat, in the methodologies adopted as the applied TEF system (1998 or 2005), ways to express the contaminant concentrations and intakes, but above all differences in the consumption habits are an obstacle to the interpretation of data. In spite of this, our daily intake estimates from the sum of PCDD/Fs plus dl-PCBs via meat consumption appear to be reasonably coherent with the findings from other European Union member states surveys. In detail,

Table 5
Overview of dietary intake reported for European countries.

Location	Mean Daily intake (pg WHO-TEQ kg^{-1} bw day^{-1}) via meat consumption	Daily intake (pg WHO-TEQ kg^{-1} bw day^{-1}) via total diet	Percentage meat contribution	References
Italy	0.19	–	–	This study
Italy	–	2.40	7	Fattore et al. (2006)
Spain	0.10	–	–	Marin et al. (2011)
Spain	0.89	–	–	Gonzáles et al., 2018
Spain	0.04	0.74	5.5	Perelló et al. (2012)
Austria	0.06–0.12	–	–	Rauscher-Gabernig et al. (2013)
Netherlands	–	0.80	17	De Mul et al. (2008)
Germany	0.49	–	–	Schwarz et al. (2014)
Denmark	–	0.80–1.1	13–18	Cederberg et al. (2010)
Sweden	0.18	–	–	Ankarberg et al. (2007)
Ireland	0.07–0.08	–	–	Tlustos et al. (2014)
France	–	1.80	8	Volatier et al. (2006)
Belgium	–	0.61	22	Windal et al. (2010)

accordance has been observed with previous studies from Italy (Fattore et al., 2006), Spain (Marin et al., 2011), Austria (Rauscher-Gabernig et al., 2013), Netherlands (De Mul et al., 2008), Denmark (Cederberg et al., 2010), Sweden (Ankarberg et al., 2007), Ireland (Tlustos et al., 2014), Belgium (Windal et al., 2010) and France (Volatier et al., 2006) (Table 5). Concerning ndl-PCBs, in its Scientific Opinion the CONTAM Panel underlines that the sum of the six iPCBs represents about 50% of the total PCBs in food (EFSA, 2005). According to this, a linear regression model showing a highly significant relationship ($R = 0.94$; $P < 0.006$) between the sum of six iPCBs and total PCBs was encountered, confirming the assumption above mentioned. Nevertheless, until now no health – based guidance value has been adopted because the combined exposure to ndl-PCB and dl-PCB congeners may result in misleading interpretations of the toxicological and epidemiological data. However, a tolerable daily intake of 10 ng kg^{-1} b.w. day^{-1}, formerly set for iPCBs at the national level in Netherland and than adopted also by the French Agency Food Safety (AFSSA, 2007), may constitute a reference value. In our case mean exposure to iPCBs was between 0.24 and 17.22 ng kg^{-1} b.w. day^{-1} and between 0.26 and 20.89 ng kg^{-1} b.w. day^{-1} at the 97.5th percentile (Table 4). A high exposure level up at about to two times the guideline limit was ascribed solely to pork sausage consumption (mean: 17.22 ng kg^{-1} b.w. day^{-1}), though also exposure level derived from hamburger intake (mean: 6.88 ng kg^{-1} b.w. day^{-1}) merited attention being close to above mentioned limit.

4. Conclusions

PCBs were the dominant chemicals followed by PCDD/Fs in all samples examined. The concentrations of these substances changed according to meat type. In particular, hamburger samples were the most contaminated relatively to PCBs, while for PCDD/Fs the highest concentrations were encountered in turkey breast. Concentrations of PCDD/Fs plus dl-PCBs and iPCBs exceeding the European Commission maximum permissible levels were found in 23.3% (pork loin and pork sausage) and 53.3% (beef steak, hamburger, pork sausage and turkey breast) of the samples analyzed, respectively. The health risk of PCDD/Fs plus dl-PCBs for consumer via meat is within the new total diet intake level except for pork sausage, whose consumption determines a two fold higher value (4.16 pg WHO-TEQs kg^{-1} b.w. $weekly^{-1}$). Concerning the exposure levels derived from iPCBs the maximum level set by different European countries (10 ng kg^{-1} b.w. day^{-1}) was surpassed solely via consumption pork sausage (17.22 ng kg^{-1} b.w. day^{-1}), thought also the hamburger consumption gave a relatively high exposure (6.88 ng kg^{-1} b.w. day^{-1}) being close to guideline limit. Results of this study need consideration by risk assessors and organizations especially for processed meat (e.g. pork sausage and hamburger) whose consumption determines a consistent exposure, not only for PCDD/Fs plus dl-PCBs but also for iPCBs, for which do not exist till now toxicological reference values.

Transparency document

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