NDL-POLYCHLORINATED BIPHENYLS (PCB) IN FOOD: TREE YEARS DATA FROM MARCHE AND UMBRIA REGIONS (ITALY).

Piersanti A[1]*, Tavoloni T[1], Lestingi C[1], Bardeggia V[1], Bastari E[1], Perugini M[2]

[1] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Via Cupa di Posatora 3, 60131 Ancona, Italy
[2] Facoltà di Bioscienze e Tecnologie Agroalimentari e Ambientali, Viale F. Crispi, 212, 64100 Teramo, Italy

Introduction

Although PCBs have been banned in many countries for over thirty years, they are still a threat for the environment and human health because of their persistence. In the last years, several were the RASFF (Rapid Alert System for Food and Feed)[1] alerts dealing with PCBs contamination in food and feed[2].
About 90-98% of the average exposure of humans to PCBs results from dietary intake, with food of animal origin as predominant source. Commission Regulation (EU) 1259/2011 of December 2011 sets for the first time the non dioxin-like-PCB (NDL-PCBs) maximum levels in foodstuffs[3]. Six PCB congeners (28, 52, 101, 138, 153 and 180) were identified as indicators to assess the human exposure upon food consumption and their sum accounts for about half of the NDL-PCB contamination in food. Laboratories in charged of official control are requested to monitor these contaminants in food and feed and check the compliance to regulatory limits. The samples analysed from the Istituto Zooprofilattico dell'Umbria e delle Marche were collected in Umbria and Marche Italian regions in the frame of official monitoring plans issued by the Ministry of Health, to fulfill the requests of the European Union. The authorities incharged of sampling are the Local Health Institutions (ASL), the borther authorities and other official bodies like NAS (Nuclei Antisofisticazioni e Sanità). The reported data on PCBs contamination represents, therefore, the levels measured in food items (mainly meat, eggs, milk and dairy products and fish and fishery products) sold in the Umbria and Marche regions during the years 2011-2013.

Materials and methods

Chemicals and materials

The six PCBs (28, 52, 101, 153, 138 and 180) mixture (>98,5% purity) in *c*-hexane at 10 ng μL^{-1} and the PCB 155 and 198 (99% purity) in isooctane at 10 ng μL^{-1} were purchased from Dr. Ehrenstofer reference materials (Ausgburg, Germany). Pesticide grade *c*-hexane, dichloromethane, analytical grade acetone, and *n*-hexane were supplied by Carlo Erba Reagents (Rodano, Milano, Italia). Fluka pesticide grade isooctane was from Sigma–Aldrich (Steinheim, Germany). Hydromatrix and Extrelut NT3 were supplied by Agilent Techonolgies (Santa Clara, CA, USA) and Merck (Extrelut® NT, Darmstadt, Germany) respectively; 96% concentrated sulfuric acid was analytical grade (Carlo Erba); silica SPE cartridges Isolute SI (1g/6mL) were purchased from Biotage(Uppsala,Sweden). Bio-Beads SX-3 polystyrene resin was from R-Biopharm (Darmstadt, Germany).

Analytical method

The edible part of the food commodities were truly homogenized and weighted on Petri glass plates. An amount of sample yielding around 1 g of fat has to be treated. The samples were frozen at -80 °C overnight and freeze dried (Heto Lyolab 3000, Analitica De Mori, Milan, Italy) from 4 to 6 hrs depending on the moisture content. About 5g of diatomaceous earth were added to the milk samples and then they were oven dried at 45 °C for 8 hours. The dried samples were extracted with *n*-hexane/acetone (1:1) by means of an Accelerated Solvent Extractor (ASE 200 Dionex Corporation, Sunnyvale, CA) and the solvent removed under reduced pressure. The obtained fat extracts were spiked with circa 5 ng of H_6CB-155 and O_8CB-198 internal standards and submitted to clean-up on Extrelut NT-3 column acidified with 3 ml H_2SO_4 connected on top of a silica cartridge 1 g/6 ml. After loading, the analytes were directly eluted with 13 ml *n*-hexane. Removed the solvent, the sample was submitted to further purification by Gel Permeation Chromatography (Bio-Beads SX-3 polystyrene swollen in *c*-hexane/DCM 1:1 and loaded on a 50cm x 1mm Ø column). Finally the sample was dissolved in 250 µL of *iso*-octane and injected in an Agilent-Technologies Gaschromatograph (6890 N) coupled to a 5973 inert MS (Agilent Technologies, Santa Clara, CA, USA). The

chromatographic separation was achieved injecting 1 μL in split-splitless mode at 270°C on a SGE-HT8 PCB capillary column (60m x 0.25mm x 0.25μm) in the following conditions: oven temperature program: 120°C, ramp to 200°C at 20°C/min, ramp to 270°C at 3°C/min, ramp to 300°C at 15°C/min, hold 4.67 min. The MS parameters and the transitions chosen as target and qualifiers are summarised in table 1.

Table 1: Selected ions for quantification and confirmation of target PCB

Transfer line T 280 °C	**EI-Ion Source T** 230 °C		**Quadrupole T** 150 °C	
Time segment	**Analyte**	**Molecular Weight**	**Target Ion**	**Qualifier Ion**
1	PCB28	257.54	256	258, 260,186
	PCB52	291.99	292	290, 255, 220
2	PCB 155	360.88	360	362, 290, 288
	PCB101	326.43	326	328, 256, 254
3	PCB153	360.88	360	362, 290, 288,
	PCB138	360.88	360	362, 290, 288,
4	PCB180	395.32	394	396, 324, 398
	PCB198	429.78	430	428, 393, 360

Method validation

The method validation was started in 2010 when the maximum limits for NDL-PCBs were not yet defined. At that time a working document issued by an organic contaminants panel of the European Commission defined the foreseeable limits for PCBs in food matrices.[4] This document was taken as reference to plan the validation study. Replicated analysis (n=7) were performed in intra-laboratory reproducibility conditions at four different concentration levels (1.0, 3.3, 6.6, 13 ng/g fat) for pork muscle and hen eggs. For milk the spiked concentrations were 1.0, 2.1, 4.2, 8.4 ng/g fat in consideration of the suggested limit of 25 ng/g fat. Good recoveries and precision for all the six analytes in the tested matrices were obtained (table 2). The multipoint calibration technique was used to study the linear response interval of the detector. Standard mixtures of the 6 PCB congeners (1, 2, 5, 10, 15, 50, 150, 250 ng/mL in *iso*-octane) and of the two internal standards PCB-155 and PCB 198 (both at 15 ng/mL) were injected in the GC-MS in three different days. Good determination coefficients r^2 (>0.995) were obtained for each congener applying the least square method to two subintervals of concentrations (***a***- 1-15 ng/mL; ***b***-15-250 ng/mL). The compliance to linearity was also investigated performing the residues analysis and verifying that the values obtained for each congener were included in the mean value ± 20 %.

Samples

The reported results are from official control of food commodities marketed in Umbria and Marche regions in Italy. The sampling involves both breeding and manufactory plants, but also items sold at the retail market and food commodities imported from abroad.

Results and discussion

The contamination levels for the different food groups taken into account in this survey are shown in table 3 and 4. Mean meat contamination reaches 6.5 ng/g fat and the highest PCB levels are observed in beef. Eggs and dairy products show PCB concentrations comparable to meat .

Much higher are the levels measured in fish and seafood, irrespective of whether they originate from sea or freshwater (table 3). In order to compare the contamination data in the different food matrices, in table3 the PCBs concentrations in fish were reported on fat basis even if generally the results are on fresh weight to be coherent with the Commission Regulation limits[3]. As expected, lean fish (<2% fat) are less contaminated than oily fish and shellfish (clams and mussels) are far less contaminated than fish.

Table 2: Method validation results for replicated analysis (n=6 for each spiking level of each matrix) in meat muscle, eggs and milk (R%: recovery)

	MUSCLE				EGGS				MILK			
PCB	**Spiking Level (ng/g fat)**	**Mean (ng/g fat)**	**RSD_r%**	**R%**	**Spiking Level (ng/g fat)**	**Mean (ng/g fat)**	**RSD_r%**	**R%**	**Spiking Level (ng/g fat)**	**Mean (ng/g fat)**	**RSD_r%**	**R%**
28	**1,0**	1,1	16	120	**1,0**	0,8	8,8	72	**1,0**	0,8	23	77
	3,3	2,8	9,1	93	**3,3**	3,3	6,2	80	**2,1**	1,6	10	73
	6,5	3,7	23	63	**6,5**	6,3	4,7	78	**4,2**	2,8	22	72
	13	11	9,9	92	**13**	12	6,6	80	**8,4**	4,9	16	60
52	**1,0**	0,8	9,7	93	**1,0**	1,0	8,2	84	**1,0**	0,8	1,7	73
	3,3	3,0	3,8	98	**3,3**	3,6	10	86	**2,1**	1,8	11	83
	6,5	4,4	15	75	**6,5**	6,3	7,6	79	**4,2**	2,9	11	74
	13	10	11	89	**13**	13	6,1	85	**8,4**	5,1	21	61
101	**1,0**	0,9	7,9	99	**1,0**	1,1	18	98	**1,0**	1,1	11	106
	3,3	3,3	6,0	108	**3,3**	3,9	3,1	94	**2,1**	2,3	4,6	109
	6,5	6,4	3,9	109	**6,5**	7,6	1,1	95	**4,2**	3,8	3,3	97
	13	13	5,2	11	**13**	15	4,1	96	**8,4**	8,0	3,5	96
153	**1,0**	0,5	33	60	**1,0**	1,1	18	98	**1,0**	0,5	33	44
	3,3	2,9	5,5	97	**3,3**	4,6	9,7	110	**2,1**	2,0	21	95
	6,5	7,0	9,5	119	**6,5**	8,3	3,3	105	**4,2**	3,4	8,3	87
	13	14	5,5	120	**13**	15	4,4	98	**8,4**	8,9	11	106
138	**1,0**	1,0	13	109	**1,0**	0,9	17	83	**1,0**	1,1	14	102
	3,3	3,2	13	105	**3,3**	4,4	13	104	**2,1**	2,4	6,9	110
	6,5	7,1	8,0	122	**6,5**	8,0	3,6	100	**4,2**	3,9	5,4	100
	13	13	4,8	115	**13**	14	4,8	92	**8,4**	9,1	10	109
180	**1,0**	1,0	15	106	**1,0**	0,6	35	52	**1,0**	0,9	9,4	88
	3,3	3,2	1,6	104	**3,3**	4,8	23	115	**2,1**	2,0	7,5	94
	6,5	5,4	17	92	**6,5**	7,5	11	93	**4,2**	3,8	6,4	97
	13	11	15	90	**13**	13	15	83	**8,4**	6,9	7,0	82

Commission Regulation 1259/2011/EU (enforced since January 1th 2012) has set *de novo* maximum tolerable levels (MLs) for the sum of the six "indicators" NDL-PCBs 28, 52, 101, 138, 153 and 180 (Σ_6 NDL-PCBs) also in fish flesh. Among the fish samples analysed the highest levels were found in Picked dogfishes (*Squalus acanthias*) caught in the north Atlantic ocean. NDL-PCBs mean values were 69.0 ng/g fresh weight with maximum values of 139.5 ng/g fresh weight. Three samples out of ten overhead the limit of 75 ng/g fresh weight above any reasonable doubt, while in two other samples PCB values were very close to the limit (74.3 and 75.1 ng/g fresh weight). This species is very long-lived and lives usually near the bottom, but also in midwater and at the surface. This shark, located at the top of food chain (trophic level of about 4.3) is a voracious predator that feeds primarily on bony fish as herring, sardines and other clupeids and hake, cod and other gadoids. It seems evident that the highest PCBs levels were found in this top predatory fish.

Table 3: Contamination levels for the sum of 6 NDL-PCB in animal products (ng/g of fat) and maximum tolerable limits (MLs).

Products	**n**						
		Min	**Max**	**Mean**	**SD**	**Median**	**MLs**
Egg	28	6.0	9.1	6.3	0.8	6.0	40
Milk and dairy products	26	6.0	9.6	6.6	0.9	6.1	40
All types of meat*	49	6.0	24.1	6.5	2.6	6.0	40
Poultry meat	40	6.0	24.1	6.5	2.8	6.0	
Beef	6	6.0	21.0	6.9	1.1	6.5	
Pork	3	6.0	7.10	6.4	0.6	6.0	
All types of fish and shellfish	190	20.0	1057	207.4	188.8	154	

Table 4: Contamination levels for the sum of 6 NDL-PCB in fish and seafood (ng/g of fresh weight) and maximum tolerable limits (MLs).

Products	n						
		Min	Max	Mean	SD	Median	MLs
Shellfish	128	0.1	5.0	1.4	1.4	0.6	75
Fish	50	0.8	139.5	22.6	29.7	10	75
Aquaculture fish	6	1.0	2.0	1.3	0.4	1.2	75
Eels	6	3.6	30.6	18	9.6	19.2	300
Picked dogfish	10	18.6	139.5	69.0	33.3	79.1	75

Eating these fish predators expose the consumers to PCB levels even more than hundred times higher than meat, eggs and milk. In human fish is, considered the major source of organic contaminants.
Considering the pattern distribution of the single NDL-PCB congeners in fish and their contribution to the total sum, it is clear that PCB 153 is predominant (abundance 40-47% of the sum), followed by the congeners 138 (22-27%), 101 (6-17%), 180 (7-18%) and finally by 52 (3-7%) and 28 (1-4%). This particular food-finger-print (food of animal origin) is a result of both, Aroclor mixtures composition, environmental persistence and metabolic pathway. These results are consistent with those reported in other studies showing that PCB-153 has an average contribution of more than one third to the sum of the six PCBs indicator[5,6]

Conclusions

The obtained results confirm those reported in the recent report of the European Food Safety Authority (EFSA) showing particularly high levels of NDL-PCBs in fish and fishery products[7]. The fish consumption is one of the most relevant pathways for the transfer of PCBs from the environment to humans. It is well known that fish is a key source of fatty acids, essential not only for the development of the nervous system but also for their protecting effect on cardiovascular functions (particularly omega 3 polyunsaturated fatty acids), of proteins, vitamins and trace elements (such as selenium). Considering such nutritional benefits, a relevant number of national and international regulatory bodies have established fish consumption guidelines, in order to minimize PCBs intake from oily fish of the most contaminated areas. The European Union has also provided recommendations of alternative diets in order to avoid consumption of contaminated products.

References

1. Rapid Alert System for Food and Feed portal: http://ec.europa.eu/food/food/rapidalert/ index_en.htm.
2. Malisch R, et al. (2014); *Sci Total Environ* http://dx.doi.org/10.1016/j.scitotenv.2014.03.022
3. Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006. *Off J Eur Union* L 320/18.
4. Working Document. Accessed on 14 May 2014 http://www.slv.se/upload/dokument/remisser/remisser_2008/Remiss_23_08_proposals-NDLPCBs-March-2008.pdf
5. EFSA (European Food Safety Agency) (2005). Opinion of the Scientific Panel on Contaminants in the Food chain on a Request from the Commission related to the Presence of non dioxin-like Polychlorinated Biphenyls (PCB) in Feed and Food, 284, 1-137.
6. Jursa S, Chovancova J, Petrik J, Loksa J. 2006. Dioxin-like and non-dioxin–like PCBs in human serum of Slovak population. Chemosphere 64:686–691.
7. EFSA (European Food Safety Agency) (2010). Results of the monitoring of dioxin levels in food and feed. EFSA Journal 2010; 8(3):1385[35 pp].