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Polychlorodibenzodioxin and -furan (PCDD and PCDF) and Dioxin-like Polychlorobiphenyl (DL-PCB) Congener Levels in Milk of Grazing Sheep as Indicators of the Environmental Quality of Rural Areas

Gianfranco Brambilla,*[,†] Vittorio Abate,[†] Stefania Paola De Filippis,[†] Anna Rita Fulgenzi,[†] Anna Laura Iamiceli,[†] Alessandro Mazzette,[§] Roberto Miniero,[†] and Giuseppe Pulina[§]

[†]Environment Department, Istituto Superiore di sanità, Viale Regina Elena 299, I-00161 Rome, Italy

[§]Animal Science Department, Università degli Studi di Sassari, Via Enrico de Nicola 1, I-07100 Sassari, Italy

ABSTRACT: An observational study was set up to evaluate how the quality of the environment may influence the levels of of PCDDs, PCDFs, and DL-PCBs in sheep's milk. Seven farms under natural and anthropogenic pressures were considered, along with an inventory of the surrounding regular and natural sources of emissions. Analysis by HRGC-HRMS revealed the highest cumulative levels (2.1 pg of WHO_{1998}-TE/g fat) in one organic and one conventional farm, each close to a relevant bushfire. Their pattern was characterized by a noticeable contribution (24%) from *mono-ortho*-PCB congeners to the cumulative WHO-TE. For the other farms, close to potential anthropogenic sources, the levels recorded in milk ranged from 0.7 to 1.3 pg of WHO-TE/g fat. The health and reproductive indicators were in all herds within the physiological range. Results suggest the environmental quality in extensive farming system should be eligible as a food safety factor, also for organic productions.

KEYWORDS: dioxin-like substances, sheep, milk, environment, organic farming

INTRODUCTION

Recently, intensive animal production has suffered dioxin scares related to the use of feed materials heavily contaminated with polychlorodibenzodioxins and -furans (PCDDs and PCDFs) and dioxin-like polychlorobiphenyls (DL-PCBs) as a possible matter of the rendering of industrial byproduct in animal nutrition.[1−3] This fact prompted the development of specific legislation, for instance, at the European Union level, to monitor the contamination in food and feed placed on the market, with the target to bring the intake of such persistent organic pollutants below the Tolerable Daily Intake in 95% of the general population, as stated in EC Regulation 1881 in 2006.[4] Along with the implementation of food safety monitoring plans, the contribution from environmental matrices, such as top soil,[5,6] bedding materials,[7] and particulate matter associated with roughage surface,[8,9] to the levels of the aforesaid contaminants in animal products has been also become more evident. This overall environmental contribution, not framed yet within food safety legislation, may play a pivotal role in determining milk contamination, especially in rural, extensive, and outdoor-based farming systems, such as those represented by sheep reared in the Mediterranean region. In the present work we considered the "dioxins and dioxin-like" contamination in bulk milk from seven sheep farms of the Mediterranean island of Sardinia, Italy, selected as representative of most relevant rural areas, as a paradigm to verify the influence of the environmental quality within such a Mediterranean ecosystem and its subsistence farming context.[10] Such context accounts both for a strong link between the animals produced and the territory aimed to the preservation of natural landscapes and biodiversity[11] and for food safety issues[4] that could preclude a wider market to products with a certified geographical origin.

MATERIALS AND METHODS

Materials, chemicals, standards for analysis, and solvents were of HPLC or pesticide grade high purity, obtained from Merck (Darmstadt, Germany), Riedel-de Haen (Seelze, Germany), or J. T. Baker (Deventer, The Netherlands). All other materials and chemicals were obtained from Merck. The quality of solvents, chemicals, and the other materials was analytically assayed in the laboratory prior to use. All laboratory glassware, tools, and utensils were also preventively checked for analytical integrity. Native and ^{13}C-labeled PCDD, PCDF, and DL-PCB congeners were certified standards (purity = 99%) provided by Cambridge Isotope Laboratories (CIL) (Andover, MA).

Sheep Farms and Milk. Seven farms were selected from the regional register of organic and conventional farms under local milk quality control program and were representative of different management and environmental situations. After an on-site visit, records about the welfare and health status of the herd, animal production data (forage intake and average milk production), and the environmental and geographical characterization of the farm with respect to the presence of natural and anthropogenic, regular or occasional, sources of contaminations were taken (Figure 1; Table 1). During the autumn of 2008, samples of bulk milk were collected from each of the selected farms and stored in glass bottles, at −20 °C, until analyzed for PCDD, PCDF, and DL-PCB congeners. The analytical procedure, based on high-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC-HRMS), was adapted from U.S. EPA Method 1613[12] and validated in-house. Briefly, after spiking of the milk samples (100 g each) with ^{13}C-labeled congeners, samples were allowed to rest at 4 °C for 24 h, under gentle stirring. Then, after addition of sodium oxalate, each

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sample underwent a liquid−liquid extraction with a 150 mL mixture of methanol, diethyl ether, and *n*-hexane. (1:1:1 v/v). After concentration, each extract was then loaded and eluted on a column of Extrelut impregnated with concentrated sulfuric acid (50% by weight). Selective fractionation of PCDDs, PCDFs, and *mono-* and *non-ortho*-PCBs was then achieved with a cleanup step on a Power-Prep unit on columns packed with silica gel, alumina, and graphitic carbon, respectively. The *mono-ortho*-substituted DL-PCBs were eluted from the alumina column, whereas PCDDs, PCDFs, and *non-ortho*-DL-PCBs were eluted from the graphitic carbon column. Analytes were quantified with a Micromass VG Autospec used in the selected ion monitoring mode (SIM). The results reported are the mean of two independent analyses for each sample, and WHO-TE values were calculated using WHO 1998 TEF,[13] in the upper bound mode, in agreement with the prescription of Regulation 1883/2006/EC.[14]

Statistics. A Factor Analysis (Statgraphics, Statpoint Technologies Inc.) was adopted to explore the data set to screen for the influence of possible contamination sources. To this end, to maximize the ratio n/v, four groups of variables were taken into consideration (PCDDs, PCDFs, *non-ortho*-DL-PCBs, and *mono-ortho*-DL-PCBs, on a WHO-TE basis.

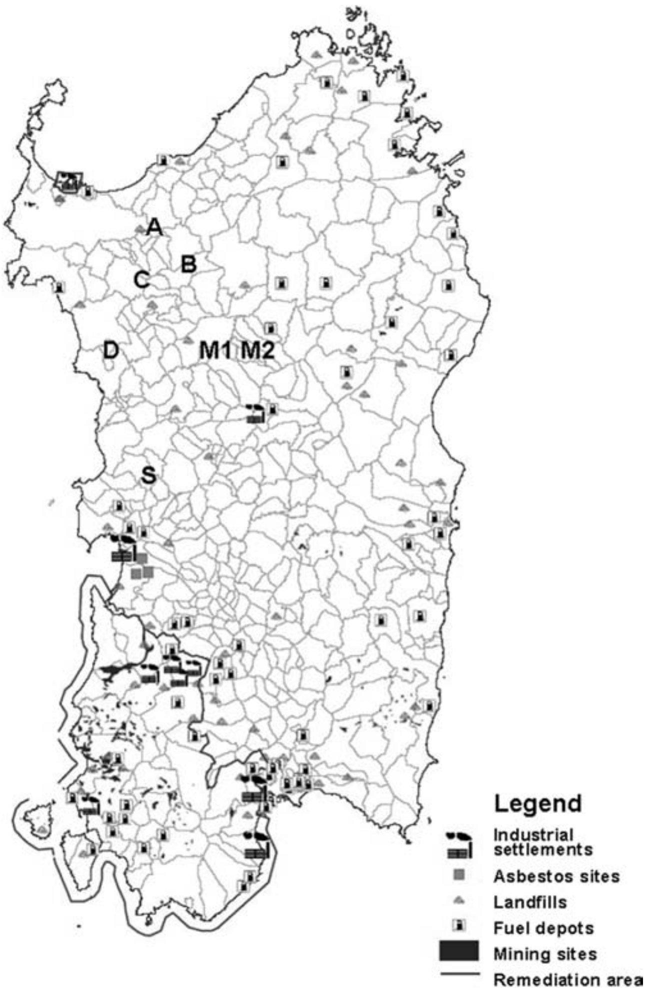

Figure 1. Location of selected sheep farms with respect to the inventoried potential anthropogenic sources of contamination, within the Sardinia region, in Italy.

■ RESULTS AND DISCUSSION

The records of selected health and reproductive indicators were within physiological parameters for all herds considered in this study (Table 2), thus indicating the absence of possible toxicological outcomes related to the exposure to the considered persistent pollutants. Productive parameters were all in line with averaged values recorded at regional level (Table 3). The absence of undercurrent diseases minimized possible interferences in the toxicokinetics of such compounds in milk, such as those caused by a starvation period that could determine a more intensive lipid mobilization from adipose tissues, along with expected qualitative and quantitative changes of the dioxin-like congener profile in milk.[2]

Table 2. Reproductive Parameters Recorded at Farm Level as Possible Bioindicators of POP Exposure in Sheep Herds

	farm						
parameter	A	B	C	D	S	M1	M2
prolific index	1.4	1.5	1.4	1.3	1.2	1.3	1.3
single birth (%)	88.2	86.3	87.6	88.5	88.4	86.7	86.2
twin birth (%)	11.8	13.7	12.4	11.5	11.6	13.3	13.1
fertility (%)	96.7	97.5	98.2	97.6	98.4	99.1	98.8
abortion (%)	2.5	1.9	1.7	2.3	2.7	1.4	1.3
birth defects (hypospadias, cleft palate)	nr[a]	nr	nr	nr	nr	nr	nr

[a] nr = no recorded data.

Table 3. Quality Parameters in Bulk Milk from the Sheep Farms Considered

	farm						
parameter	A	B	C	D	S	M1	M2
milk (kg/head × day)	1.35	1.89	1.56	1.62	1.95	1.78	1.71
fat (%)	7.69	5.81	6.58	6.27	5.66	6.10	5.88
proteins (%)	6.25	5.49	6.20	6.12	6.27	7.21	6.24
lactose (%)	5.07	5.03	4.65	4.40	4.90	4.42	4.30
SCC[a] (10^3/mL)	1177	1061	1857	1056	845	1012	980

[a] SSC = somatic cell counts.

Table 1. Main Descriptors of the Selected Farms Representative of Rural Sheep Farming Practices

farm	type	hectares (ha)	*N* (sheep)	feeding regimen	potential sources inventoried
A	conventional	100	750	local hays and grass, exhausted beet pulps	vicinity to a municipal landfill, use of licensed pesticides, improper disposal of tires
B	conventional	94	1100	local hays and grass, exhausted beet pulps	vicinity to a four-lane road, use of licensed pesticides, improper disposal of tires
C	conventional	30	150	local hays and grass, exhausted beet pulps	use of fire as agricultural practice, no relevant anthropogenic sources
D	organic	55	250	local hays and grass	no relevant anthropogenic sources
S	organic	55	270	local hays and grass	presence of disposed engines and oils
M1	organic	105	750	local hays and grass	recent bushfires in the grazing area
M2	conventional	75	450	local hays and grass, exhausted beet pulps	recent bushfires in the grazing area

Table 4. PCDD, PCDF, *non-ortho*-PCB, and *mono-ortho*-PCB Congeners Found in Bulk Milk from Seven Different Sheep Farms[a]

	farm A		farm B		farm C		farm D		farm S		farm M1		farm M2	
congener	analyte	WHO-TE	analyte	WHO-TE	analyte	WHO-TE	analyte	WHO-TE	analyte	WHO-TE	analyte	WHO-TE	analyte	WHO-TE
2,3,7,8-TCDD	<0.037	3.7×10^{-2}	<0.068	6.8×10^{-2}	0.065	6.5×10^{-2}	<0.049	4.9×10^{-2}	0.07	7.0×10^{-2}	0.088	8.8×10^{-2}	<0.045	4.5×10^{-2}
1,2,3,7,8-PeCDD	0.13	1.3×10^{-1}	0.25	2.5×10^{-1}	0.089	8.9×10^{-2}	0.103	1.0×10^{-1}	0.236	2.4×10^{-1}	0.251	2.5×10^{-1}	0.254	2.5×10^{-1}
1,2,3,4,7,8-HxCDD	<0.06	6.0×10^{-3}	<0.17	1.7×10^{-2}	0.1	1.0×10^{-2}	<0.12	1.2×10^{-2}	0.09	9.0×10^{-3}	0.19	1.9×10^{-2}	0.16	1.6×10^{-2}
1,2,3,6,7,8-HxCDD	0.49	4.9×10^{-2}	1.09	1.1×10^{-1}	0.23	2.3×10^{-2}	0.29	2.9×10^{-2}	0.57	5.7×10^{-2}	0.58	5.8×10^{-2}	0.52	5.2×10^{-2}
1,2,3,7,8,9-HxCDD	0.28	2.8×10^{-2}	1.08	1.1×10^{-1}	0.08	8.0×10^{-3}	0.26	2.6×10^{-2}	0.36	3.6×10^{-2}	0.27	2.7×10^{-2}	0.28	2.8×10^{-2}
1,2,3,4,6,7,8-HpCDD	0.11	1.1×10^{-3}	<0.17	1.7×10^{-3}	0.22	2.2×10^{-3}	0.15	1.5×10^{-3}	0.12	1.2×10^{-3}	0.28	2.8×10^{-3}	0.32	3.2×10^{-3}
OCDD	<0.21	2.1×10^{-5}	<0.57	5.7×10^{-5}	<0.44	4.4×10^{-5}	<0.74	7.4×10^{-5}	<0.30	3.0×10^{-5}	<0.34	3.4×10^{-5}	<0.39	3.9×10^{-5}
2,3,7,8-TCDF	<0.03	3.0×10^{-3}	<0.07	7.0×10^{-3}	0.04	4.0×10^{-3}	0.17	1.7×10^{-2}	0.1	1.0×10^{-2}	0.23	2.3×10^{-2}	0.21	2.1×10^{-2}
1,2,3,7,8-PeCDF	<0.03	1.5×10^{-3}	<0.06	3.0×10^{-3}	<0.03	1.5×10^{-3}	<0.04	2.0×10^{-3}	0.04	2.0×10^{-3}	0.06	3.0×10^{-3}	0.11	5.5×10^{-3}
2,3,4,7,8-PeCDF	0.08	4.0×10^{-2}	0.09	4.5×10^{-2}	0.23	1.2×10^{-1}	0.17	8.5×10^{-2}	0.38	1.9×10^{-1}	0.66	3.3×10^{-1}	0.63	3.2×10^{-1}
1,2,3,4,7,8-HxCDF	0.11	1.1×10^{-2}	<0.08	8.0×10^{-3}	0.32	3.2×10^{-2}	0.26	2.6×10^{-2}	0.18	1.8×10^{-2}	0.45	4.5×10^{-2}	0.5	5.0×10^{-2}
1,2,3,6,7,8-HxCDF	0.07	7.0×10^{-3}	0.12	1.2×10^{-2}	0.18	1.8×10^{-2}	0.09	9.0×10^{-3}	0.18	1.8×10^{-2}	0.32	3.2×10^{-2}	0.36	3.6×10^{-2}
1,2,3,7,8,9-HxCDF	<0.05	5.0×10^{-3}	0.220	2.2×10^{-2}	<0.06	6.0×10^{-3}	<0.10	1.0×10^{-2}	0.18	1.8×10^{-2}	<0.10	1.0×10^{-2}	<0.08	8.0×10^{-3}
2,3,4,6,7,8-HxCDF	<0.06	6.0×10^{-3}	<0.11	1.1×10^{-2}	0.2	2.0×10^{-2}	<0.11	1.1×10^{-2}	0.18	1.8×10^{-2}	0.33	3.3×10^{-2}	0.38	3.8×10^{-2}
1,2,3,4,6,7,8-HpCDF	0.05	5.0×10^{-4}	<0.11	1.1×10^{-3}	0.14	1.4×10^{-3}	<0.17	1.7×10^{-3}	0.06	6.0×10^{-4}	0.09	9.0×10^{-4}	0.18	1.8×10^{-3}
1,2,3,4,7,8,9-HpCDF	<0.09	9.0×10^{-4}	<0.19	1.9×10^{-3}	<0.09	9.0×10^{-4}	<0.14	1.4×10^{-3}	<0.09	9.0×10^{-4}	<0.14	1.4×10^{-3}	<0.11	1.1×10^{-3}
OCDF	<0.24	2.4×10^{-5}	<0.56	5.6×10^{-5}	<0.23	2.3×10^{-5}	<0.62	6.2×10^{-5}	<0.23	2.3×10^{-5}	<0.38	3.8×10^{-5}	<0.27	2.7×10^{-5}
PCB 77	<10	1.0×10^{-3}	54	5.4×10^{-3}	<10	1.0×10^{-3}	24	2.4×10^{-3}	<7	7.0×10^{-4}	<12	1.2×10^{-3}	<10	1.0×10^{-3}
PCB 81	0.40	4.0×10^{-5}	3	3.0×10^{-4}	0.5	5.0×10^{-5}	1	1.0×10^{-4}	0.4	4.0×10^{-5}	0.5	5.0×10^{-5}	0.2	2.0×10^{-5}
PCB 126	2.13	2.1×10^{-1}	5.45	5.5×10^{-1}	7.2	7.2×10^{-1}	4.56	4.6×10^{-1}	4.12	4.1×10^{-1}	6.4	6.4×10^{-1}	5.75	5.8×10^{-1}
PCB 169	1.10	1.1×10^{-2}	1.1	1.1×10^{-2}	4.3	4.3×10^{-2}	2	2.0×10^{-2}	3.8	3.8×10^{-2}	3.6	3.6×10^{-2}	3.9	3.9×10^{-2}
PCB 105	71.00	7.1×10^{-3}	112	1.1×10^{-2}	103	1.0×10^{-2}	94	9.4×10^{-3}	106	1.1×10^{-2}	1069	1.1×10^{-1}	1077	1.1×10^{-1}
PCB 114	<10	5.0×10^{-3}	6	3.0×10^{-3}	<12	6.0×10^{-3}	10	5.0×10^{-3}	14	7.0×10^{-3}	110	5.5×10^{-2}	90	4.5×10^{-2}
PCB 118	175.00	1.8×10^{-2}	321	3.2×10^{-2}	227	2.3×10^{-2}	247	2.5×10^{-2}	216	2.2×10^{-2}	1856	1.9×10^{-1}	1877	1.9×10^{-1}
PCB 123	<13	1.3×10^{-3}	<3	3.0×10^{-4}	<17	1.7×10^{-3}	<3	3.0×10^{-4}	<10	1.0×10^{-3}	35	3.5×10^{-3}	30	3.0×10^{-3}
PCB 156	53.00	2.7×10^{-2}	41	2.1×10^{-2}	131	6.6×10^{-2}	58	2.9×10^{-2}	78	3.9×10^{-2}	180	9.0×10^{-2}	183	9.2×10^{-2}
PCB 157	<13	6.5×10^{-3}	9	4.5×10^{-3}	27	1.4×10^{-2}	16	8.0×10^{-3}	21	1.1×10^{-2}	51	2.6×10^{-2}	48	2.4×10^{-2}
PCB 167	<13	1.3×10^{-4}	15	1.5×10^{-4}	24	2.4×10^{-4}	15	1.5×10^{-4}	22	2.2×10^{-4}	39	3.9×10^{-4}	37	3.7×10^{-4}
PCB 189	<24	2.4×10^{-3}	6	6.0×10^{-4}	<34	3.4×10^{-3}	10	1.0×10^{-3}	<21	2.1×10^{-3}	<31	3.1×10^{-3}	<30	3.0×10^{-3}
total PCDDs UB		0.251		0.554		0.197		0.221		0.409		0.446		0.398
total PCDFs UB		0.075		0.111		0.199		0.163		0.276		0.478		0.476
total *non-ortho*-PCBs UB		0.225		0.562		0.764		0.479		0.451		0.677		0.615
total *mono- ortho*-PCBs UB		0.066		0.072		0.123		0.078		0.092		0.470		0.462
PCDDs + PCDFs UB		0.326		0.665		0.396		0.384		0.685		0.924		0.875
cumulative LB		0.542		1.180		1.263		0.852		1.223		2.056		1.894
cumulative UB		0.618		1.299		1.283		0.940		1.228		2.071		1.952

[a] Results expressed both on analytical (pg/g fat) and on pg WHO(1998)-TE/g fat bases, in upper bound (UB) and lower bound (LB) mode for cumulative WHO-TE values.

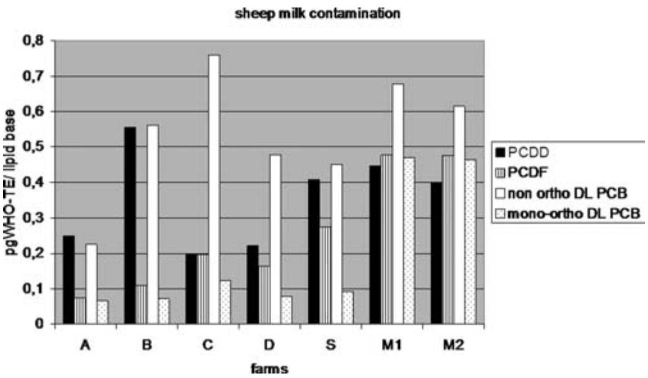

Figure 2. Contamination pattern recorded in the milk from the selected farms, expressed on WHO-TE basis, according to the different classes of dioxin-like substances.

The contamination levels for each of the congeners considered in the different bulk milks are reported in Table 4 on both analytical and WHO-TE bases, whereas Figure 2 details the PCDD, PCDF, non-ortho, and mono-ortho congener contributions to the cumulative WHO-TE. None of the samples analyzed exceeded the maximum levels for PCDDs, PCDFs, and DL-PCBs, set at a cumulative 6 pg WHO-TE/g fat[4] within the European Union.

No abundant data are available about dioxin-like compound milk contamination in dairy sheep in the Mediterranean region, despite extensive sheep farming having a strong social and economic relevance both for the quality of dairy products and for the ability to preserve the landscape and local heritage. Highest levels (on average, cumulative 22 pg WHO-TE/g fat) were recorded in milk from sheep reared in the surroundings of one of the largest industrial areas in Europe.[15] Sheep reared in the vicinity of an incinerator plant showed a PCDD, PCDF, and DL-PCB bulk milk contamination ranging from 0.71 to 2.9 pg WHO-TE/g fat, against a recorded background level from 0.52 to 0.59 pg WHO-TE/g fat in milk from control farms.[16] After an accidental fire in a municipal landfill, a maximum 1.65 pg WHO-TE/g fat level for PCDD/F congeners in milk was recorded against a background value of 0.5−0.7 pg WHO-TE/g fat.[17] In our case, the most relevant hotspot was found to be a natural bushfire, where milk analysis from the exposed farms M1 and M2 revealed the highest level of PCDDs, PCDFs, and DL-PCBs (2.1 pg WHO-TE/g fat), which definitively cannot be ranked among background values, in milk. In these two farms close each other, the levels of contaminations recorded seem largely irrespective of the management system (organic vs conventional). The cumulative levels recorded in sheep milks from the other farms span from 0.7 to 1.3 pg WHO-TE/g fat, with the lowest contamination found at farm A, close to a municipal landfill (Table 2). This demonstrates that, when properly managed, potential anthropogenic sources of contamination close to the grazing area do not affect dioxin-like compound levels in sheep milk. Such consideration is reinforced from the scientific evidence that such animals can be ranked among the most susceptible species for their anatomy and grazing behavior,[18,19] which makes more relevant the uptake of the persistent organic pollutants from the topsoil.

Two components were extracted from the factor analysis, which takes into account a cumulative percentage of 96% of variance of the database. Sample B shows the highest loading for component 1, which is characterized by PCDDs, PCDFs, and

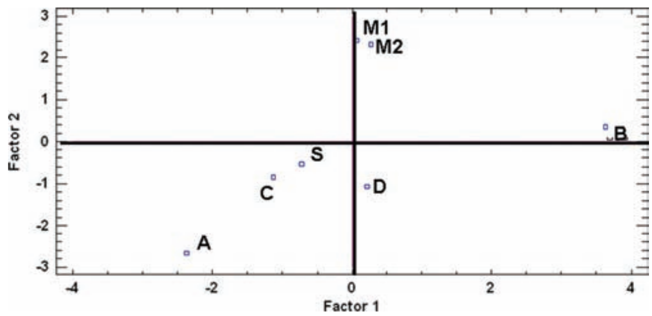

Figure 3. Factor analysis plot of the contamination profile found in the milk of the selected farms.

non-ortho-DL-PCBs. Samples M1 and M2, showing the highest loadings mainly for component 2, are characterized by PCDFs and *mono-ortho*-DL-PCBs. Samples A, C, D, and S do not show any specific influence from the indicated sources (Figure 3). Among them, the influences on samples B and M1/M2, respectively, are differentiated: on the first one, emissions from the four-lane roads appear to affect it, whereas the last two samples, although differentiated in terms of *mono-ortho*-PCB contribution, appear to be under the same impact type of inventoried bushfire (Table 1). Such congener contribution is consistent with the profile recorded in the fat of a wild pig population within a natural preserved area of Sicily affected by bushfires.[20]

Within the Mediterranean area, natural fires can represent a relevant source of emission, the consequence of which could be at least the doubling of the PCDD, PCDF, and DL-PCB background levels in sheep milk. Under such an impact, congener pattern is characterized by a noticeable WHO-TE contribution of *mono-ortho*-PCB congeners, despite their low WHO-TEF (Figure 2). Such evidence may endorse fire prevention in natural areas, encourage the proper disposal of agricultural wastes at the farm level, and prompt regulatory authorities to establish appropriate environmental requirements linked both to landscape preservation and possible food safety issues for free-grazing animals. Within this framework, our study could give scientific support to set target (background) contamination levels in sheep's milk, as a tool to link agriculture practices and environmental quality. To this purpose, the chemical−toxicological characterization of agricultural soil for DL-PCB congeners should not be overlooked, even if such a chemical family is not framed within the International Toxicity Equivalency (I-TE) scale, considered as the benchmark for the analysis of PCDDs and PCDFs in environmental matrices.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +39(0)6 49902764. Fax: +39(0)6 49902836. E-mail: gianfranco.brambilla@iss.it.

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