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Polychlorodibenzodioxin and -furan and Dioxin-like Polychlorobiphenyl Distribution in Tissues and Dairy Products of Dairy Buffaloes

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Supporting Information

ABSTRACT: A pilot study was performed on three different dairy buffalo herds exposed without exposure control conditions to Polychlorodibenzodioxins and -furans (PCDDs, PCDFs) and Dioxin-like Polychlorobiphenyls (DL-PCBs). This study dealt with the relationship between the contamination levels (pg WHO_{2005}-TE/g fat) in individual raw milk and those in edible tissues and with the contamination transfer from farm bulk milk to dairy products. On a cumulative basis, kidney (41, 67, and 21 pg WHO-TE/g fat) resulted more in equilibrium with milk (48, 42, and 20) than did muscle (25, 31, and 9), while liver showed a large bioaccumulation (221, 304, and 75), with marked differences of the congener profile. Mozzarella cheese contamination (23, 42, and 29 pg WHO-TE/g fat) was higher than that of bulk milk (20, 36, and 21), which suggested a role of casein precipitation in congener transfer. The above information could improve the effectiveness of risk management during a "dioxin" crisis.

KEYWORDS: *dioxins, buffaloes, milk, dairy products, meat, liver, fat, food safety, food security*

INTRODUCTION

During 2008, the dairy production system in Italy suffered from a "dioxins crisis" for noncompliance with the maximum levels of PCDDs, PCDFs, and DL-PCBs prescribed[1] in buffalo dairy products. Even if confined to clustered areas within the Campania Region, and with a limited percentage of noncompliant results,[2] the crisis had a strong impact on economics and social infrastructure, mainly related to the perception of quality associated with Mediterranean production systems and dietary habits. During such a crisis, extensive investigations were focused on the source(s) of contamination, which involved analyses on agricultural soils and farm forages and the follow-up of noncompliances in buffalo milk farms.[3,4] It is now acknowledged that the PCDD, PCDF, and DL-PCB fallouts from occasional sources of contamination in corn and pasture determined the prolonged uptake of the aforementioned contaminants through locally produced forages, such as silages and grass hays, thus enabling the transfer of the environmental contaminant to the food of animal origin.[5] In the risk management of this crisis, the following knowledge gaps were highlighted concerning the distribution of the aforementioned contaminants in animal tissues and dairy products: (a) the correlation between the contamination in milk and that in edible tissues, including liver, considering milk as a matrix suitable to predict the compliance of the carcass; (b) the correlation between the contamination in milk and that in dairy products (mozzarella cheese, whey, and whey cheese). These data were mandatory to grasp how the native milk contamination could affect the different dairy products and how the possible mitigation or enhancement effects could impact the compliance of the food. Previous studies carried out mainly in long-term-exposed meat-producing animals (steady state) highlighted a possible correlation between WHO-TE levels and congener patterns in well-perfused organs, such as metabolic and muscle fat, thus suggesting the use of small biopsies as a noninvasive sampling procedure able to predict meat safety compliance.[6] However, such studies may not be directly applicable to dairy animals, due to the changes of lipid metabolism associated with the energy balance during lactation, especially in the 90-day period after calving. Such changes could in fact alter the profile of the congeners and the related WHO-TE level of the contamination.[7] Schultz et al.[8] reported PCDD, PCDF, and DL-PCB concentrations in muscles of dairy animals lower than those found in milk. The presence of an efficient clearance of lipophilic contaminants via milk may influence the concentration of the aforesaid contaminants in the different edible parts of the carcass (in a dairy buffalo, on average 1 kg of fat is excreted daily with milk). This point is of considerable importance, because the present European Union legislation sets the cumulative WHO-TE values on a lipid base in edible tissues and milk from food-producing terrestrial animals.[9] In the scientific literature, more attention has been paid to report the occurrence of PCDDs, PCDFs, DL-PCBs, and other

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Table 1. Analytical (pg/g fat) and Cumulative (WHO_{2005}-TE/g fat) Contamination Recorded in Milk, Muscle, Perirenal Fat (K), Retrobulbar Fat (E), and Liver from Three Dairy Buffaloes (A, B, and C)[a]

	farm A					farm B					farm C				
	milk	muscle	K fat	E fat	liver	milk	muscle	K fat	E fat	liver	milk	muscle	K fat	E fat	liver
congener, % fat	25.6	1.50	N.A.[b]	N.A.	3.42	9.27	1.55	N.A.	N.A.	4.60	10.9	1.30	N.A.	N.A.	4.82
2,3,7,8-TCDD	3.15	2.42	3.07	2.16	4.15	6.15	5.12	7.78	8.45	10.9	2.05	1.06	2.21	1.73	3.74
1,2,3,7,8-PeCDD	9.79	5.76	9.90	5.43	36.4	10.6	7.90	17.0	13.5	61.7	3.74	1.48	4.20	3.36	11.0
1,2,3,4,7,8-HxCDD	4.22	1.99	3.16	1.53	47.4	2.15	2.42	5.34	2.67	69.7	0.79	0.52	1.32	0.86	12.1
1,2,3,6,7,8-HxCDD	33.2	13.3	29.1	8.80	96.2	16.0	12.7	46.1	18.7	122	3.78	1.80	<6.44	4.57	16.1
1,2,3,7,8,9-HxCDD	5.16	1.61	3.73	1.04	24.9	2.13	1.44	4.83	2.58	34.7	0.89	0.43	<0.96	0.60	4.96
1,2,3,4,6,7,8-HpCDD	13.9	7.50	15.4	2.80	262	5.71	7.75	28.5	4.89	412.7	2.18	1.83	6.08	1.98	88.7
OCDD	1.97	4.43	7.40	1.38	213	0.85	4.81	16.7	2.3	402	0.88	4.03	3.41	<0.97	108
total PCDDs UB	17.3	9.95	16.7	8.76	60.0	18.8	14.8	30.7	24.4	99.5	6.36	2.83	7.34	5.71	19.0
2,3,7,8-TCDF	0.30	0.19	0.33	0.18	<1.80	0.23	0.34	0.23	0.39	<1.22	<0.22	<0.20	0.10	0.27	1.46
1,2,3,7,8-PeCDF	0.88	0.49	0.96	0.50	2.92	0.47	0.52	0.98	0.80	2.35	<0.45	<0.19	0.35	0.56	2.08
2,3,4,7,8-PeCDF	24.5	15.9	22.6	12.0	167	21.1	14.9	28.5	22.7	188	8.03	4.10	10.4	8.26	52.6
1,2,3,4,7,8-HxCDF	34.5	14.9	24.4	11.2	389	29.0	22.8	48.0	26.2	655	8.19	4.11	10.5	6.45	120
1,2,3,6,7,8-HxCDF	33.6	13.7	25.2	8.27	220	19.2	12.0	37.4	15.9	245	6.08	2.21	7.49	4.67	50.3
1,2,3,7,8,9-HxCDF	<0.62	<0.15	<0.09	<0.09	<3.31	<0.39	<0.25	<0.10	<0.09	<2.51	<0.35	<0.19	<0.09	<0.11	2.36
2,3,4,6,7,8-HxCDF	30.7	10.1	21.0	6.76	140	17.1	10.0	32.9	14.3	170	5.56	2.02	6.58	4.10	34.8
1,2,3,4,6,7,8-HpCDF	33.4	9.92	25.9	5.00	263	14.7	12.3	56.0	12.9	399	4.14	2.13	<8.00	3.13	77.8
1,2,3,4,7,8,9-HpCDF	0.98	0.39	0.86	0.29	21.5	0.66	0.76	1.09	0.69	51.8	0.35	<0.23	<0.47	0.28	15.7
octaCDF	<0.23	0.9	1.39	0.32	13.9	0.13	0.98	4.38	0.92	72.7	0.30	<0.47	0.62	<0.28	17.4
total PCDFs UB	17.7	8.79	14.2	6.32	128	13.1	9.16	21.0	12.7	168	4.51	2.13	5.69	4.09	37.6
PCB 77	<0.17	<6.10	3.77	3.11	<2.07	0.98	<5.24	4.34	3.95	<2.38	<0.40	<7.72	2.25	4.03	2.37
PCB 81	5.38	2.53	4.09	2.49	8.95	4.90	3.19	8.25	5.67	11.8	5.20	2.08	3.37	3.74	5.87
PCB 126	94.9	49.9	71.3	44.6	311	69.7	51.5	88.5	73.0	327	72.3	28.4	57.1	43.3	179
PCB 169	111	50.0	91.8	35.2	56.0	94.7	73.6	203	72.9	95.5	42.2	23.5	65.9	33.2	25.4
total non-*ortho*-PCBs UB	12.8	6.49	9.89	5.52	32.7	9.81	7.36	14.9	9.49	35.6	8.50	3.55	7.69	5.33	18.6
PCB 105	1209	598	948	640	1850	1210	820	1410	1030	2497	786	419	884	709	991
PCB 114	282	140	214	129	206	663	386	752	527	472	226	113	247	153	159
PCB 118	3525	1830	3030	2290	2623	3880	2650	4610	3260	3920	2395	1300	3250	2520	1891
PCB 123	72.6	36.8	43.0	36.4	68.1	130	74.2	129	95.3	124	59.4	30.1	60.7	41.5	63.3
PCB 156	1035	480	978	436	847	1545	947	2010	1050	1491	578	307	794	511	475
PCB 157	388	155	287	146	347	457	290	648	344	523	159	79.7	219	139	266
PCB 167	324	92.3	178	174	385	575	312	602	387	509	227	100	299	140	173
PCB 189	408	234	577	156	299	201	467	1560	432	998	126	109	404	145	153
total mono-*ortho*-PCBs UB	0.22	0.11	0.19	0.12	0.20	0.26	0.18	0.35	0.21	0.32	0.14	0.07	0.18	0.13	0.13
total PCDDs + PCDFs UB	35.0	18.7	30.9	15.1	188	31.9	23.9	51.7	37.1	268	10.9	4.97	13.0	9.80	56.6
total DL-PCBs UB	13.0	6.60	10.1	5.64	32.9	10.1	7.54	15.3	9.70	35.9	8.63	3.62	7.87	5.46	18.8
cumulative UB	48.1	25.3	41.0	20.7	221	42.0	31.4	67.0	46.8	304	19.5	8.59	20.9	15.3	75.4

[a]Values expressed on a lipid base in upper bound (UB) mode. [b]Not analyzed.

lipophilic contaminants in cheese,[10] while the few studies on the milk-to-cheese transfer basically dealt with veterinary drug residues.[11] Since toxicokinetics and toxicodynamics factors are not relevant to this issue, and because of the standardization of both temperatures and microbial fermentations during the cheese production process, it is supposed that both distribution patterns and concentration on a lipid basis of PCDD, PCDF, and DL-PCB congeners should not change during the production of a particular type of cheese. The precipitates of casein and lactoglobulins may, however, show absorption of hydrophobic organic chemicals,[12] thus influencing their repartition between the liquid and the solid phases of the curd, during milk processing. Therefore, this study aims to fill the above-mentioned gaps of knowledge and to improve in the future the cost/effectiveness of the management measures taken and the food safety/security aspects related to "dioxin crises" in dairy animals.

MATERIALS AND METHODS

Three dairy buffalo farms (A, B, and C) whose bulk milk was noncompliant to PCDDs, PCDFs, and DL-PCBs also in the follow-up investigations were selected for the study. Three lactating animals were randomly chosen, and individual milk from each animal was collected before slaughter and official veterinary inspection. Subsequently, during the post-mortem inspection, the following parts were sampled from the condemned carcasses: skeletal muscle from gastrocnemius, renal fat, retrobulbar fat, and liver (Spigelius' lobe). The bulk milk from the three contaminated farms was sampled as well and then *ad hoc* processed in a dedicated area of a dairy establishment, according to the following procedure. The milk temperature was brought to 38.8−39.0 °C, and, after the addition of liquid rennet (1:15 000), coagulation took place in 10−12 min. The curd was cut with a knife

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Table 2. Analytical (pg/g fat) and Cumulative (WHO_{2005}-TE/g fat) Contamination Recorded in Bulk Buffalo Milk, Mozzarella (M) Cheese, Whey, And Whey (W) Cheese from Three Farms (A, B, and C)[a]

	farm A				farm B				farm C		
	milk	M cheese	whey	W cheese	milk	M cheese	whey	W cheese	milk	M cheese	whey
congener, % fat	7.19	33.0	1.50	28.8	5.36	28.0	0.70	34.5	7.97	28.0	1.05
2,3,7,8-TCDD	1.56	2.06	1.95	1.52	6.18	6.64	7.32	5.26	2.00	2.99	3.30
1,2,3,7,8-PeCDD	4.78	4.88	4.80	3.60	8.38	10.7	10.5	7.77	4.13	5.14	5.40
1,2,3,4,7,8-HxCDD	1.68	1.71	1.63	1.25	1.84	2.45	2.84	1.99	1.00	1.18	1.28
1,2,3,6,7,8-HxCDD	5.99	5.49	6.00	4.70	10.88	14.5	14.7	11.8	4.35	5.05	5.90
1,2,3,7,8,9-HxCDD	0.91	1.59	0.83	0.77	2.37	2.45	2.63	1.61	0.98	1.45	1.21
1,2,3,4,6,7,8-HpCDD	2.70	2.61	2.77	1.84	4.81	5.31	5.69	3.98	2.33	2.63	3.02
OCDD	0.56	0.78	1.90	0.90	0.58	1.09	8.10	1.09	0.44	0.74	3.70
total PCDDs UB	7.23	7.84	7.62	5.81	16.1	19.4	19.9	14.6	6.8	8.92	9.57
2,3,7,8-TCDF	0.22	<0.40	0.21	0.11	0.23	<0.39	0.48	0.20	0.27	<0.64	0.55
1,2,3,7,8-PeCDF	0.31	<0.39	0.32	0.19	1.02	0.83	0.97	0.55	0.86	0.83	0.93
2,3,4,7,8-PeCDF	10.6	11.5	11.7	9.00	18.9	18.9	24.2	16.5	8.55	10.2	12.4
1,2,3,4,7,8-HxCDF	12.8	14.1	14.1	10.0	19.1	21.7	28.6	20.8	9.38	9.88	11.7
1,2,3,6,7,8-HxCDF	8.14	8.48	6.90	4.90	14.0	16.6	14.1	11.2	7.20	8.13	7.10
1,2,3,7,8,9-HxCDF	<0.29	<0.30	<0.16	<0.08	<0.29	<0.69	<0.37	<0.06	<0.63	<0.52	<0.25
2,3,4,6,7,8-HxCDF	6.79	6.96	6.70	5.00	12.0	12.9	14.1	10.7	6.16	7.23	7.90
1,2,3,4,6,7,8-HpCDF	4.36	4.34	3.50	2.50	8.54	9.85	8.90	6.51	4.23	4.34	4.10
1,2,3,4,7,8,9-HpCDF	0.29	0.36	0.34	0.09	0.50	0.49	0.65	0.20	0.28	0.28	0.38
octaCDF	<0.14	0.18	0.74	0.18	<0.24	0.30	0.95	0.34	<0.20	0.23	0.62
total PCDFs UB	6.05	6.54	6.37	4.74	10.3	11.0	13.1	9.33	5.00	5.76	6.54
PCB 77	1.17	<0.39	<5.39	<2.58	1.78	<0.56	<11.9	<2.52	2.26	<0.39	<6.88
PCB 81	4.27	5.38	1.35	1.51	8.87	11.5	6.82	5.43	16.3	25.8	7.54
PCB 126	59.8	76.1	46.5	36.6	80.9	101	65.8	59.8	79.3	133	76.2
PCB 169	31.6	31.0	32.2	26.2	48.2	53.6	52.9	41.7	46.8	42.5	41.7
total non-*ortho*-PCBs UB	6.93	8.54	5.62	4.45	9.54	11.7	8.17	7.23	9.34	14.6	8.87
PCB 105	682	676	633	563	699	731	708	714	807	837	898
PCB 114	126	159	123	106	326	424	348	321	195	232	221
PCB 118	1863	2245	2180	1980	1975	2107	2480	2330	2538	2818	3130
PCB 123	38.4	45.3	41.3	35.5	73.3	77.2	80.1	71.3	70.1	70.0	62.7
PCB 156	410	369	362	290	666	709	703	558	560	535	561
PCB 157	141	118	121	93.7	235	243	242	196	160	133	167
PCB 167	165	187	152	103	250	274	272	229	233	293	194
PCB 189	90.1	81.8	91.8	85.3	154	195	218	204	107	117	125
total mono-*ortho*-PCBs UB	0.11	0.12	0.11	0.10	0.13	0.14	0.15	0.14	0.14	0.15	0.16
total PCDDs + PCDFs UB	13.3	14.4	14.0	10.6	26.5	30.4	33.0	23.9	11.8	14.7	16.1
total DL-PCBs UB	7.00	8.66	5.73	4.54	9.67	11.8	8.32	7.37	9.48	14.7	9.03
cumulative UB	20.3	23.0	19.7	15.1	36.1	42.2	41.4	31.3	21.3	29.4	25.1

[a]Values expressed on a lipid base in upper bound (UB) mode.

into four segments 60 min later and into approximately 1 cm pieces after an additional 30 min. Then, 70% of the whey was removed and the curd was allowed to ripen for 90−120 min, until a pH of 5.05−5.10 was reached. After the addition of boiling water, the curd was stretched with a wooden stick into a smooth, plastic mass, manually molded into balls weighing approximatively 100 g, cooled in water at +8−12 °C for 20 min, and then stored at +6 °C in a brine (13.0−14.0 Soxhlet−Henkel degrees,[13] °SH) containing 3% NaCl (w:v), thus obtaining mozzarella cheese as the final product. Whey cheese was recovered after boiling the whey and collected into plastic sieves. Analysis was performed on the lipid extracts from such matrices, which were spiked with internal ^{13}C-labeled standards before extraction. Cheese and whey cheese were previously subjected to acidic hydrolisis, and then, as milk and whey, their fat was extracted with a mixture of organic solvents of different polarity (methanol, diethyl ether, and *n*-hexane) to efficiently extract also phospholipids and short-chain free fatty acids.[14,15] Perirenal and retrobulbar adipose tissues and liver were analyzed as already described.[16] Muscle was homogenized and freeze-dried; then it underwent an instrument-aided extraction by ASE (accelerated solvent extraction), carried out with *n*-hexane at a temperature of 100 °C and a pressure of 100 atm before being subjected to the same cleanup steps of the other specimen matrices.[15,16] Analysis of PCDD, PCDF, and non-*ortho*-DL-PCB congeners was carried out by high-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC-HRMS) techniques. Mono-*ortho*-DL-PCBs were determined by high-resolution gas chromatography coupled to low-resolution mass spectrometry (HRGC-LRMS), negative chemical ionization (NCI), operating in the single ion monitoring (SIM) mode. The method fulfilled the analytical requirements set by the Commission Regulation 252/2012/EC.[17] Method repeatability did not exceed 15% on each congener. Trueness of quantification ranged between −19% and +18%. Results were expressed on the WHO_{2005}-TE scale on a fat basis, following the upper bound (UB) approach. Differences with results computed on lower bound (LB) estimates did not exceed 20%, thus complying with Regulation 252/2012/EU.[17] Quality control was assured by the use of butter oil as candidate reference material,[18] with consensus TE values of 3.1 pg WHO_{1998}-TE/g fat (PCDDs + PCDFs) and 6.1 pg WHO_{1998}-TE/g fat (PCDDs + PCDFs + DL-PCBs); quality assurance was guaranteed by the regular participation of the laboratory in proficiency tests, under accreditation conditions. The congener profile was computed separately for PCDD + PCDF, non-*ortho*-DL-PCB, and

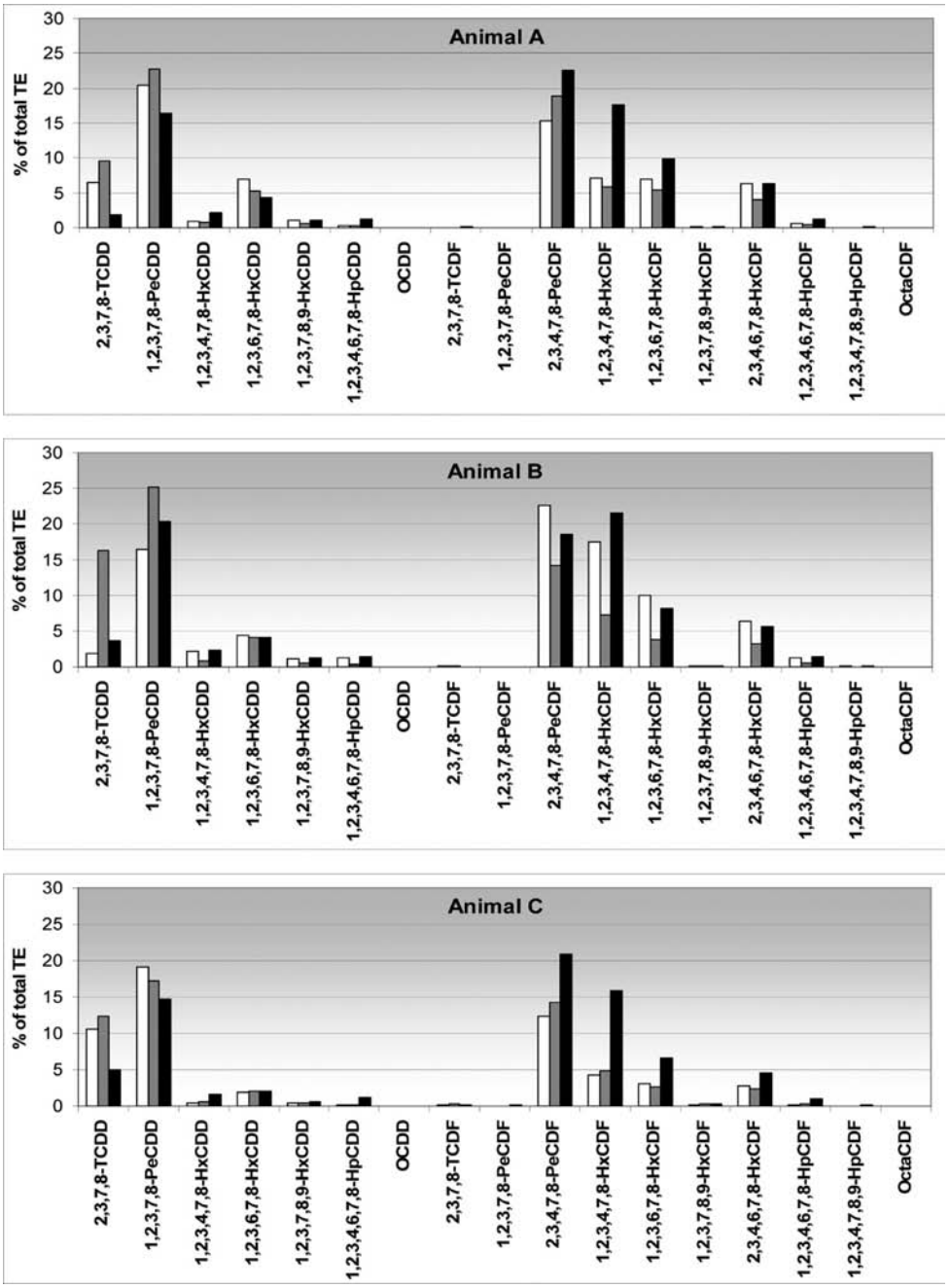

Figure 1. Relative contribution of PCDD and PCDF congeners to the total TE in milk (white bar), muscle (grey bar), and liver (black bar) from animals A, B, and C, respectively.

mono-*ortho* DL-PCB groups: each analytical value was normalized to that of the most abundant congener belonging to the same group, taken as 100%.

RESULTS AND DISCUSSION

Cheese Production. In the cheese-making process, mozzarella yields of 19, 16, and 27%, (w/w) were obtained from the bulk milk of farms A, B, and C, respectively, reflecting probable differences in the composition of the milk, with respect to the κ-casein content.

Analysis. Recovery of internal standards was quantitative in all the matrices considered (>75%). Fat extraction in dairy products, for PCDD, PCDF, and DL-PCB determination, was performed according to the Röse–Gottlieb method.[19]

Differences between analytical and commercial procedures fell within 0.5% (w/w), thus indicating that the recorded differences in the contamination could not be traced back to poor lipid extraction of short-chain free fatty acids and phospolipids, usually generated by the enzymatic activities of lipase, chemotrypsin, and pepsin present in native calf rennet. An incomplete lipid extraction could bring a bias in the expression of the contamination on a lipid weight basis, as requested by the EU legislation,[17] rather than a whole weight basis. The analytical results referred to the milk and organs from individual buffaloes, expressed on a fat basis for PCDD, PCDF, and DL-PCB congeners, along with the cumulative WHO_{2005}-TE values are reported in Table 1, while Table 2 shows the same results in bulk milk and milk-derived products.

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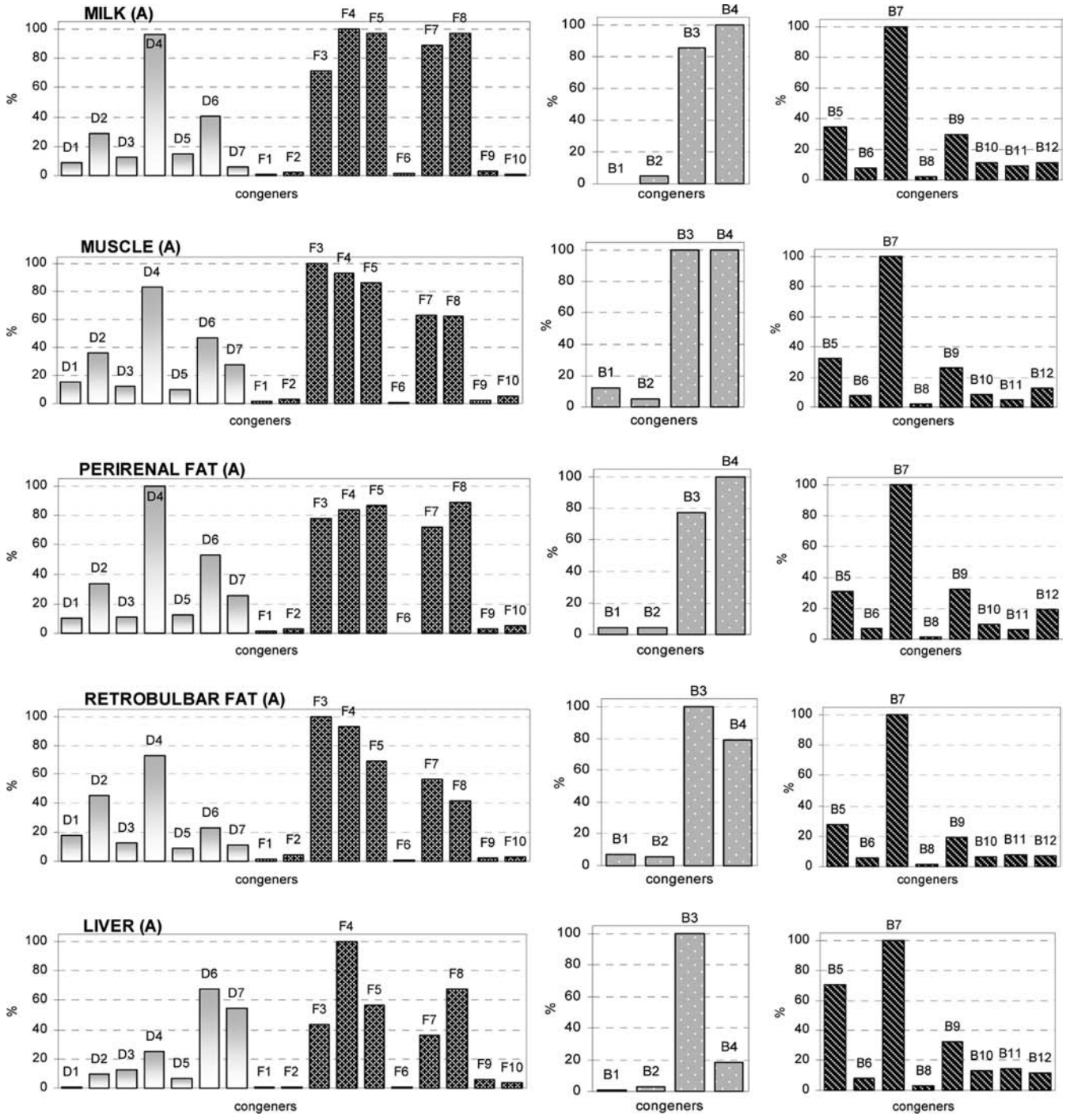

Figure 2. PCDD, PCDF (left), non-*ortho*-PCB (middle), and mono-*ortho*-PCB (right) congener profiles in milk, muscle, perirenal fat, retrobulbar fat, and liver from animal A (values expressed as percentage with respect to the congener with the maximum value).

Figure 1 shows the relative contribution of PCDD and PCDF congeners to total TE in milk, muscle, and liver from animal A, B, and C. The congener pattern on an analytical basis in milk and organs is exemplified in Figures 2, 3, and 4. Figure 5 reports PCDD and PCDF, DL-PCB, and PCDD + PCDF + DL-PCB cumulative differences recorded in dairy products with respect to bulk milk.

Milk and Edible Tissues. Apart from the analytical uncertainties, already discussed, our results might have been influenced by the small (N = 3) number of animals utilized. The individual milk from farm A showed a fat percentage notably higher than the average value (26 vs 10%), possibly influenced by the low milk yeld (<1−3 kg/per head/day). Anyway, buffaloes selected from each farm (a) were representative of the most abundant animal class (parity 4−5); (b) were not pregnant and beyond month six of lactation; the feeding regimen and the related exposure was almost the same for the herd to which the individual buffalo belonged. Despite the above-mentioned sampling uncertainties, clear trends were noted in the cumulative contamination distribution among the three buffaloes. The WHO-TE contamination in the muscle was always lower and in liver always higher than that in

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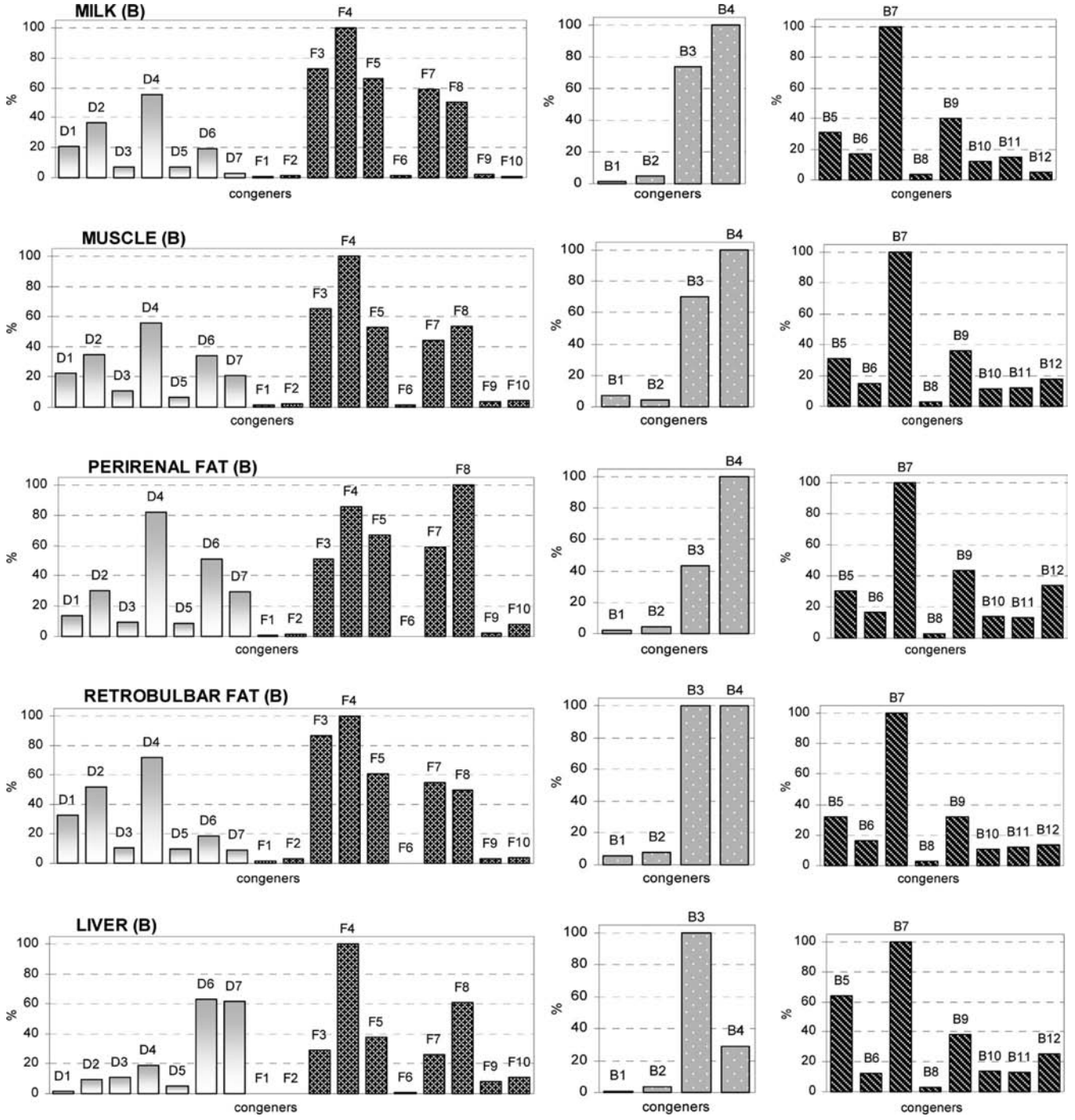

Figure 3. PCDD, PCDF (left), non-*ortho*-PCB (middle), and mono-*ortho*-PCB (right) congener profiles in milk, muscle, perirenal fat, retrobulbar fat, and liver from animal B (values expressed as percentage with respect to the congener with the maximum value).

milk (Table 1). For the gastrocnemius muscle from the hind quarter, the very low fat content (in the range 1.30−1.55%) may have resulted in a reduced bioaccumulation of such contaminants, since its fat can be reasonably considered subjected to continuous depletion as a well-perfused district. Milk represents the main route of excretion of such liphopilic contaminants in dairy animals: the amount of fat excreted daily with buffalo milk (on average, 1000 g/day/per head) could therefore be equivalent to that present in about 67 kg of muscle mass. In this respect, our results in dairy buffaloes are in good agreement with the findings reported by Schultz et al.[8] in dairy cows and sheep. Liver analysis indicated again this organ as the main bioaccumulating site in dairy animals, as already reported,[20,21] with a congener selectivity influenced by both the abundance of aryl hydrocarbon receptors (AhR) expressed on liver cells and their different selectivity for the congeners, along with the related induced metabolism.[22] In Figure 1, it may be noticed that PCDFs' contribution to the cumulative TE values in liver is higher than that recorded in milk and muscle. The latter result is in good agreement with previous comparative studies in liver and muscle in pigs and sheep.[16,20] On an analytical basis the liver profiles indicate a relative reduction of tetra-, penta-, and hexa-PCDDs and of DL-PCB 169 in all three animals (Figures 2, 3, and 4). With respect

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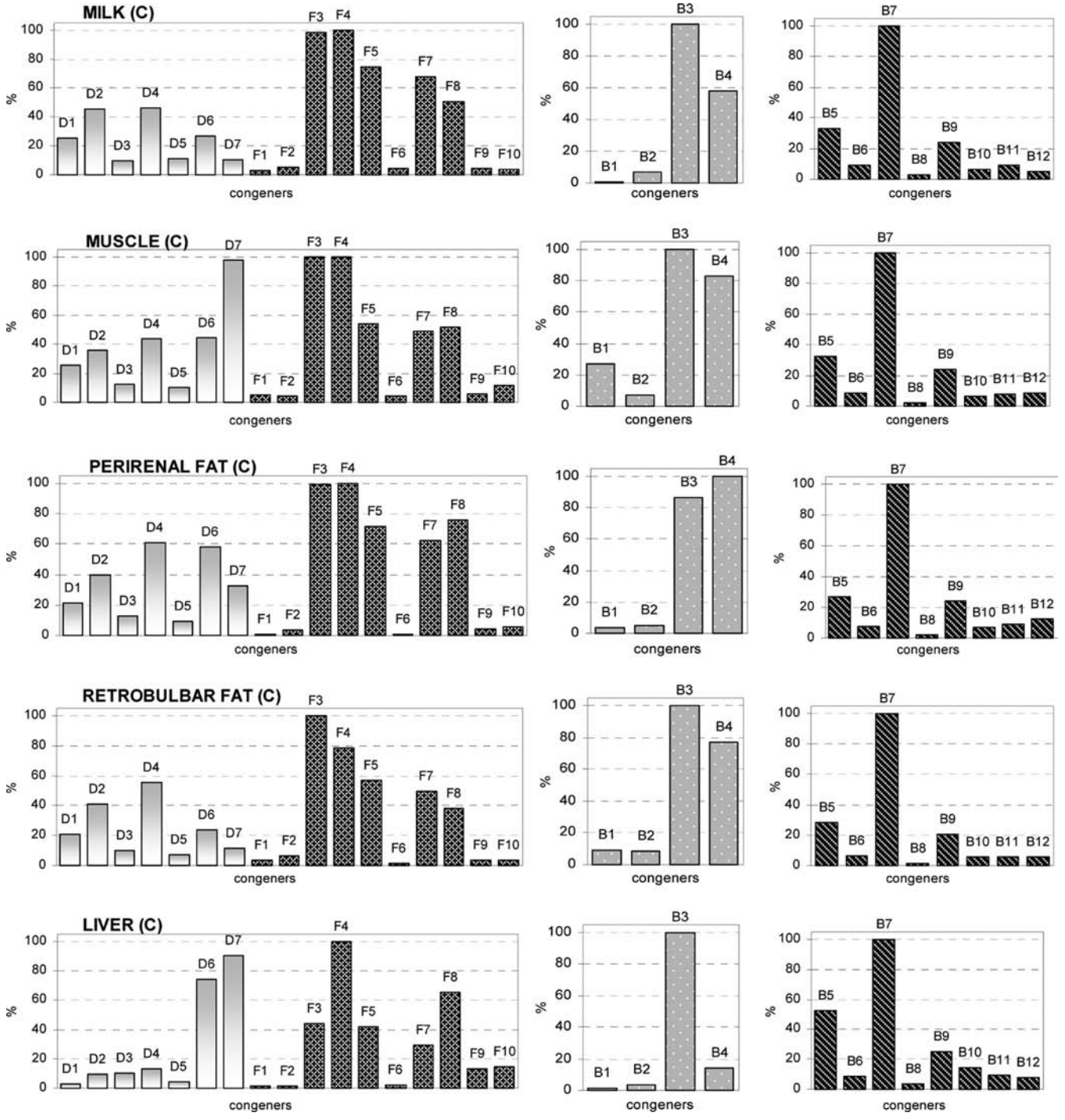

Figure 4. PCDD, PCDF (left), non-*ortho*-PCB (middle), and mono-*ortho*-PCB (right) congener profiles in milk, muscle, perirenal fat, retrobulbar fat, and liver from animal C (values expressed as percentage with respect to the congener with the maximum value).

to tissues other than liver, the perirenal fat and the retrobulbar fat gave contradictory information: in two cases out of three both perirenal fat (animals A and C) and retrobulbar fat (animals B and C) showed cumulative values in line with that recorded in the corresponding milk (Table 1). The cumulative mismatches noted in the retrobulbar (animal A) and perirenal (animal B) fats with respect to milk fat may reflect temporal differences in the respective herd exposure. It is known that the physical–chemical properties of PCDD, PCDF, and DL-PCB congeners, such as their K_{ow} and degree and position of chlorine substitutions on the ring, influence the toxicodynamics and toxicokinetics of such compounds.[23] Generally, highly chlorinated congeners show a lower bioavailability and a stronger bioaccumulative behavior. Their diffusion from the primary compartment, such as blood, milk, and well-perfused organs, to secondary compartments may reflect long-term exposures to reach a steady state, with respect to low-chlorinated congeners. Consequently, during the depletion phase, their clearance from secondary to primary compartments is also slower, thus possibly causing a different contamination pattern on an analytical basis, which also affects cumulative WHO-TE values. From Figures 2, 3, and 4 the main variation in

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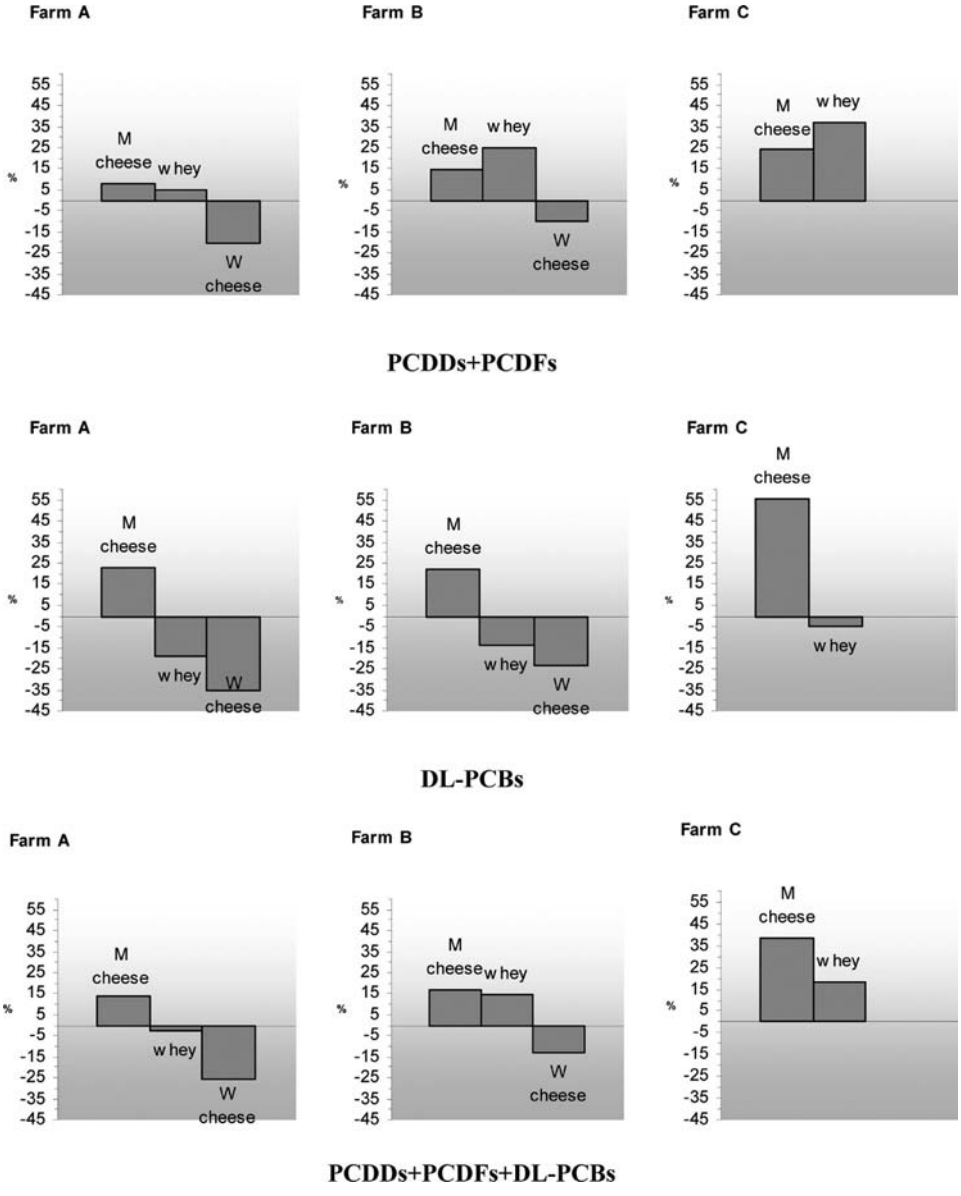

Figure 5. Relative differences of PCDD and PCDF, DL-PCB, and PCDD + PCDF + DL-PCB cumulative WHO_{2005}-TE contamination in mozzarella (M) cheese, whey, and whey (W) cheese with respect to bulk milk from farms A, B, and C, respectively.

the congener analytical profile can be considered within each animal, accounting for an analytical uncertainty of ≤20%. In the specimens tested, the lower concentrations of certain congeners (e.g., 1,2,3,4,6,7,8-HpCDD, OCDD, 1,2,3,4,6,7,8-HpCDF, and OCDF) in retrobulbar fat relative to those measured in perirenal fat suggest that a steady state has not been fully reached in such nonmetabolic districts for some highly chlorinated, more hydrophobic compounds. Also in the non-*ortho*-PCB profile, the ratio between the pentachlorinated PCB 126 and the hexachlorinated PCB 169 in the eye and kidney fat may support the above consideration. In farm B, a depletion phase characterized by the mobilization of low-chlorinated congeners from the metabolic perirenal fat to milk may be envisaged since 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and 1,2,3,4,6,7,8-HpCDF congener concentrations were greater in perirenal fat than in milk. The differences recorded in the cumulative WHO-TE values (Table 1) between the considered districts also indicate a situation far from equilibrium.

Milk and Dairy Products. The cumulative contaminations found in the raw milk from farms A, B, and C exceeded by a factor of 4 (A, C) and by a factor of 6 (B) the current WHO_{2005}-TE EU legislative limit of 5.5 pg/g fat in dairy products, thus representing the worst case situation monitored during 2008 under field conditions.[2] On a fat basis, the mozzarella cheese was always more contaminated than the corresponding raw milk processed (Figure 5). Even if the extended analytical uncertainty of the method (±20% on WHO-TE basis) does not support sound evidence of a PCDD, PCDF, and DL-PCB enrichment in mozzarella cheese in two of the three cases described, nevertheless there seems to be a trend associated with an increased hydrophobicity of the cheese, due to the precipitation of caseins. The amount of protein precipitates (cheese yields during the process) may be a factor underlying an increase of the contamination: in farm C mozzarella cheese, the higher yield of 27% was associated with a 38% increase of the cumulative WHO-TE contamination originally present in the milk, as opposed to a 13−17%

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increase recorded in farm A and B cheese (19 and 16% yields, respectively) (Figure 5). The other consideration supporting a possible influence of protein precipitates in the contamination transfer from milk is that the WHO-TE increase observed in cheese is always higher for DL-PCBs (23, 22, and 55%) compared with PCDDs and PCDFs (8, 15, and 25%) for farms A, B, and C, respectively: this suggests that casein precipitates could interact better with the less hydrophobic PCBs, compared with PCDD and PCDF congeners. No significant differences were recorded in the congener profile between bulk milk and its products (see Supporting Information). The observed variability in the transfer of the WHO-TE contamination in farms A, B, and C can be reasonably taken as representative of incurred situations in small cheese plants, such as those usually processing buffalo milk, as a consequence of different batch-to-batch consignment and of seasonal variation (winter vs summer) of the quality of bulk milk. In conclusion, this study seems to indicate that liver from dairy buffaloes may be found to be noncompliant when the levels in milk are compliant but close to regulatory limits. On the contrary, noncompliant levels in milk, close to regulatory limits, may not be automatically applied to hind quarter muscles. In the buffalo cheese-making process, which accounts for possible differences in the PCDD + PCDF and DL-PCB contributions to the total WHO-TE, it could be safe to use milk with a bulk contamination well below the prescribed maximum level to prevent potential noncompliances in cheese leading also to potential food security problems. In the case of EU legislation for dairy products, this would roughly correspond to a threshold of 4 against 5.5 pg WHO(2005)-TE/g fat in the case of a balanced contribution to the cumulative TE of PCDD, PCDF, and DL-PCB congeners. Due to the large variety of cheese products and their processing, and the wide panel of persistent organic pollutants (POPs) potentially present in milk, it may be appropriate to set up dedicated studies on their transfer to prevent potential noncompliances in those food items with a high added value.

■ ASSOCIATED CONTENT

Supporting Information

The analytical congener profile of bulk milk and its products from the three different farms. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

PCDD, polychlorodibenzodioxin; PCDF, polychlorodibenzofuran; DL-PCB, dioxin-like polychlorobiphenyl; °SH, Soxhlet—Henkel degrees; ASE, accelerated solvent extraction; HRGC-HRMS, high-resolution gas chromatography coupled to high-resolution mass spectrometry; HRGC-LRMS, high-resolution gas chromatography coupled to low-resolution mass spectrometry; NCI, negative chemical ionization; SIM, single-ion monitoring; UB, upper bound; LB, lower bound; AhR, aryl hydrocarbon receptors; POPs, persistent organic pollutants

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