DIOXIN-LIKE CONGENERS DISTRIBUTION IN MILK AND EDIBLE TISSUES OF NATURALLY EXPOSED DAIRY BUFFALOES

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Introduction

The study of the distribution of dioxin-like substances (PCDDs, PCDFs and DL-PCBs) in the edible tissues of dairy animals may acknowledge some important issues such as: a) to establish the correlation between the contamination recovered in milk from that of possible presence in different body compartments; b) to recover informations about possible long-term exposures; c) to predict the possible compliance of any of the edible parts of the carcass on the basis of a non invasive sampling practice, such as milk drawing [1]. During the management of the 2008 dioxin crisis in buffalo milk, in Italy [2, 3,] there was the opportunity to deepen such toxicodinamics aspect. Animals were selected in those farms where milk resulted still non compliant (above the maximum EU regulatory limit of 6 pgWHO-TE/ g on lipid base), after three subsequent checks within a 3 months period. As consequence, such buffaloes were abated along with the the condemnation of their carcasses.

Materials and methods

Three non primiparous lactacting buffalo animals (A, B, and C) belonging to different farms were selected for the study. Under official veterinary inspection,, at slaughter, during the ante-mortem visit, individual milk was withdrawn. Subsequently, during the post-mortem inspection, under derogation for scientific purposes, the following parts of the animal were sampled from condemned carcasses: a) scheletal muscle from gastrocnemius, b) renal and c) retroocular fats, and e) liver (Spigelius' lobe). Analysis were performed on the lipid extracts from such matrices, spiked with internal ^{13}C labelled standards, before the freeze-drying process. The procedure was based on the HRGC-HRMS approach on a TRACE GC 2000, Mat 95 XP Instrument, for PCDD, PCDF and *non ortho* DL-PCB congeners; *mono-ortho* DL-PCBs were determined on a HRGC-LRMS (NCI), in SIM mode, as already described [4, 5]. Values were reported on pgWHO-TE_{1998} /g , and expressed on lipid base (*lb*), with the upper-bound approach. Quality control was assured by the use of a bovine fat (butter) as certified reference material; quality assurance was guaranteed by the regular participation of the lab to proficiency tests, under the accreditation procedure.

Results and discussion

The cumulative results on the selected matrices, are reported in Table 1, while Figure 1 shows the PCDD/F congener pattern found in milk and liver, of the same animal**.** On fat basis, the contamination levels found in milk did not show to be in equilibrium with those found in the muscle, even if mammary gland and gastrocnemius muscle can be ranked among the most perfused organs. In muscle, the WHO-TEQ levels were about 43% lower on average, than those found in milk from all the A, B, and C animals. even if the congeners profile resulted almost similar. Such findings may be explained as milk represents the main route of excretion of such liphopilic contaminants in dairy buffaloes, where the intramuscolar fat is very scarce (1% of the tissue mass). So, muscle fat is not representative of the adipose mass involved in lipid mobilisation during the lactation, as other tissues, such as perirenal fat [6]. This latter, considered as one of the main metabolic fat depot in dairy animals, resulted in good equilibrium with milk, in buffaloes A and C. The higher ratio milk/ perirenal fat recorded in buffalo B, suggests a previous long term and higher exposure in such animal, with respect to A and C buffaloes; this last consideration is reinforced from the higher contamination found in a very low perfused district, such its retro-ocular fat. On the contrary, animal A seems exposed more recently to dioxin-like sources as far as the contamination recorded in the retroocular fat accounted only to the 44% of that present in milk. Liver analysis indicated again such tissue to be the main bio-accumulating organ [7] (Table 1), with a congeners selectivity influenced both from the abundances of AhR receptors expressed on liver cells and from the induced

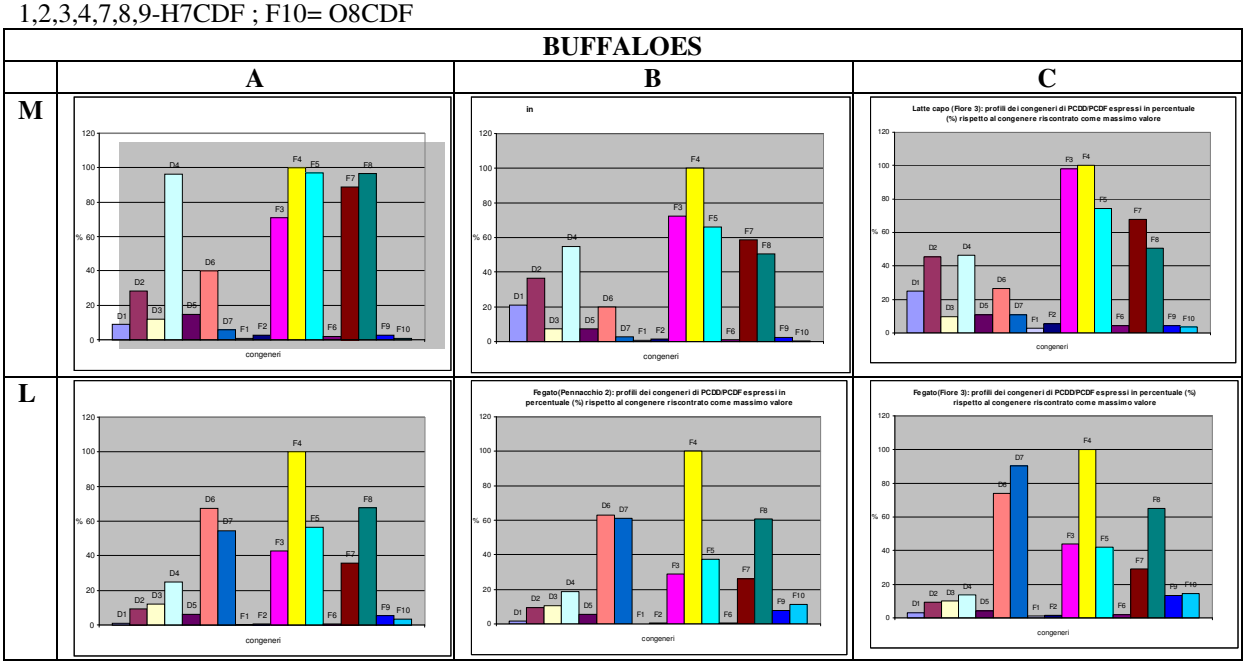
metabolism of some congeners [8]. Such findings are in good agreement with previous reports [9]; showing the contaminants metabolism resulted basically directed towards DL-PCBs and low-chlorinated PCDD/F congeners, with a subsequent change both of the WHO-TE PCDD/F vs DL-PCB ratio, and of the single PCDD/F congeners profile with respect to the cumulative PCDD/F WHO-TE (Table 1; Figure 1).

Despite the "dinamic" steady state in dairy animals, strongly influenced from the lipid mobilisation, due to the energy unbalance period during each lactation, from our study we could recover useful information about the congeners levels in meat (muscle) and liver, as well to draft a possible strategy to discriminate between exposures periods, through the comparison of dioxin like compounds distribution in different adipose depots of the same animal.

Animal	Milk pgWHO-TE/g *lb*	Muscle	Kidney*	Eye*	Liver
A	51.93	55%	98%	44%	490
B	45.92	73%	151%	111%	792
C	20.95	44%	107%	80%	410
WHO-TE PCDD+F *vs* DL-PCB					
A	3.3	3.6	3.8	3.2	6.7
B	3.7	3.8	4.3	4.4	8.5
C	1.5	1.6	1.9	2.1	3.5

Table 1. Above: contamination found in milk and in the other considered matrices, expressed as percentage of that found in milk. Below: cumulative PCDD/F vs DL-PCB congeners ratio; * referred to perirenal and retrobulbar fat

Figure 1. Normalised WHO-TE profile in Milk (M) and Liver (L) for PCDD/F congeners, in the three differnt animals (A, B, and C) considered: from left to right D1= 2,3,7,8-T4CDD; D2= 1,2,3,7,8-P5CDD; D3= 1,2,3,4,7,8-H6CDD; D4= 1,2,3,6,7,8-H6CDD ; D5= 1,2,3,7,8,9-H6CDD; D6= 1,2,3,4,6,7,8-H7CDD ; D7= O8CDD; F1= 2,3,7,8-T4CDF ; F2= 1,2,3,7,8-P5CDF ; F3= 2,3,4,7,8-P5CDF ; F4= 1,2,3,4,7,8-H6CDF; F5= 1,2,3,6,7,8-H6CDF ; F6= 1,2,3,7,8,9-H6CDF; F7= 2,3,4,6,7,8-H6CDF F8= 1,2,3,4,6,7,8-H7CDF; F9= 1,2,3,4,7,8,9-H7CDF ; F10= O8CDF



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