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## PCDD and PCDF depletion in milk from dairy cows according to the herd metabolic scenario

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#### **ABSTRACT**

High level of PCDD + PCDF contamination in bulk milk (9.7 pgWHO-TE  $g^{-1}$  fat) from 1604 Holstein Fresian lactacting cows was observed just four weeks after the beginning of their exposure to a feed supplement contaminated at 10.4 ngWHO-TE  $kg^{-1}$  dry matter. In-farm produced hay and silage showed levels not exceeding 0.2 ng WHO-TE kg<sup>-1</sup> dry matter. After the supplement withdrawal, it was possible to monitor the depletion phase for a following 75-day period in milk, until the levels dropped well below 3.0 pgWHO-TE  $g^{-1}$  fat, the EU regulatory Maximum Residue Level for PCDD + PCDF. During this phase, the half-life was calculated as  $17 \pm 3$  days, on WHO-TEQ basis. The full availability of farm data on both cow nutrition and milk production allowed the calculation of the carry-over rate (COR) (PCDD + PCDF milk excretion vs. feed), which was 46% at the end of the exposure. This COR value is justified from the main TE contribution of Penta-CDD and -CDF congeners (63%), and the half-life is among the shortest of all those described in the literature both for experimental and naturally-exposed dairy cows. A fugacity-based model predicts a bulk milk contamination of 5 pgWHO-TE  $g^{-1}$  fat, compared to the 10 pgWHO-TE  $g^{-1}$  fat level observed. Such findings are discussed in light of the lactation and metabolic status of the herd for which the transition period, characterised by a negative metabolic energy balance and a consequent adipose tissue mobilization, could play a relevant role.

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### 1. Introduction

The European Union has recently established provisional maximum and alert levels for PCDDs and PCFDs in cow milk at 3.0 and 2.0 pgWHO-TE  $g^{-1}$  fat, respectively, to prevent unacceptable high human exposures ([Council Regulation, 2001](#page--1-0)). During routine monitoring activity, when non compliant samples for PCDD/F contamination are found in large milk production farms, one of the more pressing questions is how much time is needed to restore the soundness of the milk after the identification and removal of the source of PCDD/Fs. Much effort has been made in the past for modelling the bioaccumulation and carry-over of persistent organic pollutants from contaminated feeds to cows' milk. Nevertheless, some models are derived from experimental trials and possibly biased due to single-dose exposures [\(Olling et al., 1991; Slob](#page--1-0) [et al., 1995](#page--1-0)) or to the reduced number of animals [\(McLachlan](#page--1-0) [and Richter, 1998; Fries et al., 1999; Huwe and Smith, 2005\)](#page--1-0). On the contrary, the data arising from bulk milk collected from longterm naturally-exposed herds, i.e. as in the case of contaminated

\* Corresponding author. E-mail address: [g.brambi@iss.it](mailto:g.brambi@iss.it) (G. Brambilla). citrus pulp [\(Traag et al., 1999; Malisch, 2000\)](#page--1-0), could be more informative as it is representative of field situations, for both animal welfare and food safety management purposes. It may be envisaged that lactating cows may not reach a steady state due to metabolic changes occurring during the milking period after labour ([Sweetman et al., 1999](#page--1-0)). For instance, during the first three months of lactation, the metabolic difference between the energetic intake from feeds and the needs represented by body weight maintenance and the quantity of milk produced determines a status of ''transition" cow, where up to 7% of the body weight can be lost mainly due to adipose tissue mobilization ([Dechow et al., 2004\)](#page--1-0) ([Fig. 1\)](#page-1-0). In this work, we report a case where the carry-over and the depletion of PCDDs and PCDFs were observed in a Holstein Fresian herd, fed on a contaminated feed supplement for 28 days, along with the description of its feed regimen, metabolic state and composition.

#### 2. Materials and methods (case report)

In late autumn 2005, a dairy farm, during periodical internal quality milk checking, found unacceptable levels of PCDD/F in the bulk milk (9.7 pgWHO-TE  $g^{-1}$  fat). The herd consisted of 1604

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Fig. 1. Typical metabolic balance of an Holstein Fresian cow during lactation period. Time reported in days from parturition. Energy calculated as net energy intake (NEI).

lactating animals (32% at the first calving) for a total 37598 l/day production (26.4 kg cow/day as an average) of milk with 3.8% fat. The detailed composition of the herd is reported in Table 1.

In-farm produced hay and silage were given at  $24 \pm 2$  kg dry matter per head per day and the feed supplement purchased from the market was administered only to lactacting cows at 1.5 kg per head per day. Both feeds were sampled and analysed for PCDD + PCDF content. The analyses revealed that the contaminated feed supplement was introduced just 28 days before the first non compliant finding. Bulk milk samples representative of the exposed group were then sub sequentially drawn at 14, 21, 28, 35, and 50 days from the removal of the exposure. A milk sample from 40 non-exposed dairy cows belonging to the same farm and receiving the same feed regimen was considered as an indicator of the natural environmental background levels. The local Public Health Veterinary unit followed the case and collected all the analytical reports both coming from inter calibrated private and officially-appointed public laboratories (extended uncertainty within 25%, upper bound approach), according to international standardised criteria ([Commission Directive, 2002](#page--1-0)). Feed intake (in kg day<sup>-1</sup> of dry matter) and the daily milk yield were recorded as basis to calculate the carry-over ratio (COR) and the Bio-concentration factors (BCF) ([Huwe and Smith, 2005](#page--1-0)), along with the herd composition data. Contamination half-life was estimated by a logarithmic (ln) plot of concentrations (pgWHO-TE  $g^{-1}$  fat) vs. time (days).

#### Table 1

Structure of the herd exposed to the contaminated feed supplement and relative milk yields

Days from labour	Cows (N)	Milk (1) per head per day	Total amount (1) per day	% on the total daily production
$16 - 30$	78	23	1.794	4.7
$31 - 60$	163	27	4.401	11.7
61-90	160	29	4.640	12.3
$91 - 120$	147	29	4.263	11.3
$121 - 150$	111	27	2.997	7.9
$151 - 180$	123	25	3.075	8.2
$181 - 210$	95	24	2.280	6.0
211-240	73	23	1.679	4.6
241-270	73	23	1.679	4.6
271-300	101	21	2.121	5.6
$301 - 330$	122	21	2.562	6.8
331-360	108	19	2.052	5.4
361-390	55	17	935	2.6
>390	195	16	3.120	8.3

$$
C_M=Q_F C_F K_{0W}(1.0\times 10^{-9})
$$

where  $C_M$  is the PCDD and PCDF contamination, expressed in pgWHO-TE  $g^{-1}$  whole milk,  $Q_F$  is the amount of feed ingested as dry matter,  $C_F$  is the feed contamination, in pgWHO-TE  $g^{-1}$  dry matter;  $K_{\text{OW}}$  was averaged at 10<sup>7</sup>.

#### 3. Results and discussion

Feed supplement was found contaminated at 10.4 pgWHO-TE  $g^{-1}$  dry matter, while in-farm produced feeds revealed 0.2 pgWHO-TE  $g^{-1}$  dry matter, respectively. The depletion curve for PCDDs and PCDFs in milk is reported in [Fig. 2.](#page--1-0) The linear regression was characterised by high significance ( $R^2 = 0.9712$  and  $F = 136$  with  $p > 99.9%$ ). Plotted half-life on WHO-TEQ basis was estimated as  $17 \pm 3$  day. The milk from non-exposed cows showed a 0.7 pgWHO-TE  $g^{-1}$  fat contamination, in line with the previous internal checks, thus representative of the overall environmental situation of the farm. The congener profile of milk samples from exposed and non-exposed cows and of the feed supplement are summarised in [Fig. 3](#page--1-0). The CORs calculated as ratio between total milk excretion of PCDDs and PCDFs and overall intake of PCDDs and PCDFs in mineral feed was 46% in exposed, and 13% in nonexposed cows, on WHO-TE basis. The COR and the BCF of most representative congeners are reported in [Table 2](#page--1-0), on analytical basis, along with their estimated half-lives. Extrapolated milk contamination, calculated according to the fugacity-based model of [Travis](#page--1-0) [and Hattemer-Frey \(1991\)](#page--1-0) gave 5.0 pgWHO-TE  $g^{-1}$  fat and 1.3 pgWHO-TE  $g^{-1}$  fat for the two groups, respectively.

It is well recognised that, in dairy cows, the steady state for PCDD and PCDF in milk and body fat could be reached only after long-term exposures, in conditions of energetic homeostasis, and substantial equivalence of quality and quantity of feed intake. In this case, steady state was not reached due to the relatively short exposure (28 days). Moreover, the herd composition revealed a quite relevant number of transition cows (cows within the 90th day of lactation characterised by a negative energetic balance) (Fig. 1), accounting for the 25% of the herd and contributing 29% to the overall daily milk production (Table 1). According to the fugacity-based model at steady state, the predicted milk contamination should be around 5.0 pgWHO-TE  $g^{-1}$  fat, just half of that observed in field conditions (10 pgWHO-TE  $g^{-1}$  fat) [\(Fig. 2](#page--1-0)).

Further evidence of possible body burden contribution from mobilised adipose tissues arises from the observed mass balance between the PCDD and PCDF milk excretion and the feed intake at the end of the exposure. The calculated COR value of 46% is in line with those already reviewed ([Hoogenboom, 2004](#page--1-0)), according to the pattern of the milk contamination, where contribution of penta and hexa congeners accounted for 63% and 32% of the total WHO/TEQ value ([Table 2\)](#page--1-0) ([Fig. 3](#page--1-0)), respectively. The body burdens of fresh first calving cows may have represented an unexpected contribution of higher chlorinated molecules to the observed milk contamination, as far as hexa and hepta congeners are more prone to bioaccumulate in adipose tissues in non-lactating animals [\(Tuin](#page--1-0)[stra et al., 1992\)](#page--1-0) as consequence of long-term exposures (two years before the first calving and lactation). On the contrary, the value of 13% found in the non-exposed group seems more in line with the scientific literature documenting the lower oral bioavailability (<11%) of those PCDD and PCDF congeners with  $K_{\rm ow} > 6.5$ , as in the case of hexa and heptaCCD/CCF ([McLachlan and Richter, 1998\)](#page--1-0).

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