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USE OF THE HRGC-HRMS APPROACH IN THE MANAGEMENT OF THE PCDD, PCDF AND DL-PCB CONTAMINATION IN BUFFALO SOFT CHEESE, IN ITALY

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Abstract

During 2008, an extraordinary dioxin survey was carried-out in Italy, to prevent the possible unacceptable presence of dioxins and dioxin-like compounds in mozzarella cheese. To reduce the huge number of samples to be investigated, pooled buffalo milk were analysed along to the adoption of guidance limits below the maximum regulatory tolerance levels. An a posteriori evaluation of the HRGC-HRMS results achieved indicate that, when using a bioassay method to screen cumulative dioxins (PCDD/Fs) and dioxin-like PCBs (dl-PCBs), on 314 pooled samples drawn at processing milk plants level the recorded percentage of suspected non-compliances would be 46.2. The testing of the separate PCDD/Fs and dl-PCBs fractions on such bioassay would lead to a percentage of suspected non-compliances of 25.8 for pooled milk. The HRGC-HRMS approach allowed to achieve the following main goals: 1) to avoid a large number of confirmatory analysis, mandatory in the case of a screening approach; 2) to achieve an exhaustive data-set useful for the identification and the evaluation of the possible sources of exposure, and effective for the definition of contamination target levels in buffalo milk.

Introduction

In the period 2008-2009, a monitoring plan was developed by the Italian Ministry of Health in cooperation with the European Commission in order to determine the contamination levels of PCDD/Fs and dl-PCBs in buffalo milk produced in the Campania Region used for the preparation of mozzarella cheese1. The monitoring plan was divided into three phases:

- ***Phase I:*** analysis of bulk milk samples taken at the processing milk plants located in the provinces of Avellino, Caserta and Naples (high frequency of non-compliant samples expected);
- ***Phase II:*** analysis of bulk milk samples taken at the processing milk plants located in the provinces of Salerno and Benevento (low fre quency of non-compliant samples expected);
- ***Phase III:*** analysis of buffalo milk and farm forages/feed samples drawn on suspect at farm level as consequence of non-compliant results obtained in the Phase I and Phase II. In addition, the survey was extended to buffalo, bovine and sheep/goats livestock located in the area of 3 km from the centre of a "positive" farm.

Materials and Methods

The Phase I and Phase II had to be completed within one month; taking into account the large number of samples to be analysed. It was decided to adopt a "pooled milk sampling" approach whenever applicable for a maximum of four different milk samples constituting a pool. The chosen approach permitted to analyse 381 milk samples instead of 959 (the total number of farms delivering milk to processing milk plants subjected to the official control).
To account for the possible dilution of the contamination that could compromise the trace-back of the on-farm contamination, the guidance values for the compliance/non-compliance statement were redrafted according to the following "emergency" grid:

- if the milk was from a single farm, the maximum levels of 3.0 pg WHO-TEQ/g fat for PCDD/Fs and of 6.0 pg WHO-TEQ/g fat for PCDD/Fs + dl-PCBs were adopted, according to the Regulation (EC) 1881/2006;
- if the case of pooled milk samples, the action levels of 2.0 pg WHO-TEQ/g fat for PCDD/Fs and of 2.0 pg WHO-TEQ/g fat for dl-PCBs were adopted, according to the Recommendation 2006/88/EC.

In the Phase III, only individual samples were analysed. As a significant number of samples was expected to exceed the tolerance/action levels2, all samples were analysed using HRGC-HRMS in four different ISO 17025 accredited laboratories and the measurement uncertainty was set at a standard ±20%. This decision was also made in order to prevent delays by confirmation of samples which could have been pre-analysed with bioassay screening methods with additional time and costs.

Results and Discussions

The distribution of the levels of contamination recorded by HRGC-HRMS in pooled and individual milk samples from Phase I, II and III is shown in Figure 1.
According to the Regulation (EC) 1883/2006, the monitoring for the presence of dioxins in foodstuffs may be performed by a strategy involving a screening method in order to select those samples with levels of dioxins and dioxin-like PCBs that are less than 25% below or exceed the maximum level (suspect non-compliances). For such samples, the determination of the cumulative dioxins and dioxins and dioxin-like PCBs is mandatory, by the HRGC-HRMS confirmatory analysis.
In this study two different scenarios regarding screening methods application were designed: the first implied the use of a screening analysis for the cumulative dioxins and dl-PCBs determination while the second one considered the adoption of a screening assay capable to separately analyse dioxins and dl-PCBs.

Scenario 1: screening method for cumulative dioxins and dl-PCBs analysis
The screening requirements, when transposed to the already mentioned guidance values set for the management of the crisis, would result in the following decision limits for suspect non compliant samples: 2.25 pg WHO-TEQ/g fat for individual milk samples and 1.50 pg WHO-TEQ/g fat for pooled milk samples.
By applying the aforesaid criteria to the results achieved in HRGC-HRMS the screening outcome would result as follows:

- Phase I and II (381 milk samples):
  - 21 out of 67 (31.3%) individual milk samples ≥ 2.25 pg WHO-TEQ/g fat
  - 145 out of 314 (46.2%) pooled milk samples ≥ 1.50 pg WHO-TEQ/g fat
- Phase III (433 milk samples):
  - 306 out of 433 (70.7%) individual milk samples ≥ 2.25 pg WHO-TEQ/g fat

To summarise, 166 out of 381 (43.6%) samples would have been confirmed by HRGC-HRMS during the Phase I and Phase II (1 month time frame). On a whole, 472 out of 814 (58.0%) samples would have been confirmed by HRGC-HRMS.

Scenario 2: screening method for separate determination of dioxins and dl-PCBs
In this case, the decision limits for suspect non-compliant samples would be 2.25 pg WHO-TEQ/g fat (PCDD/Fs) and 4.50 pg WHO-TEQ/g fat (PCDD/Fs + dl-PCBs) for individual milk samples, and 1.50 pg WHO-TEQ/g fat (PCDD/Fs and dl-PCBs) for pooled milk samples.
When applying the above-cited criteria to the data obtained from HRGC-HRMS analysis the screening results would be as follows:

- Phase I and II (381 milk samples):
  - 11 out of 67 (16.4%) individual milk samples ≥ 2.25 pg WHO-TEQ/g fat for PCDD/Fs and/or ≥ 4.50 pg WHO-TEQ/g fat for PCDD/Fs + dl-PCBs
  - 81 out of 314 (25.8%) pooled milk samples ≥ 1.50 pg WHO-TEQ/g fat for PCDD/Fs and/or dl-PCBs
- Phase III (433 milk samples):
  - 219 out of 433 (50.6%) individual milk samples ≥ 2.25 pg WHO-TEQ/g fat for PCDD/Fs and/or ≥ 4.50 pg WHO-TEQ/g fat for PCDD/Fs + dl-PCBs

In summary, 92 out of 381 (24.1%) samples analysed during Phase I and II, and 311 out of 814 (38.2%) total samples would have been subjected to HRGC-HRMS confirmatory analysis.

Therefore, even if the separate determination of PCDD/Fs and dl-PCBs was performed as screening, nearly 40% of samples would have been confirmed by HRGC-HRMS. With higher measurement uncertainties for screening methods, this proportion would have been higher than calculated on a basis of ± 20 % for confirmatory methods. The use of a conservative cut-off level to reduce the false compliant rate (less than 1% according to the Regulation 1883/2006/EC for dioxins and dl-PCBs analysis in foodstuffs by screening methods) could result in a lower specificity thus affecting the overall cost-effectiveness3.
The a posteriori evaluation of the data set confirmed the validity of the direct HRGC-HRMS approach when a relevant percentage of non-compliant results is expected, and when the sources of contaminations may vary (regular vs occasional, punctual vs diffuse), thus highlighting the need for a full characterisation of the environment along with the toxicokinetics evaluation of the carry-over rate. At the same time, most of Phase II results felt below the determination limits of the screening results, usually targeted close to the legislative action levels; also in this case the screening approach would be ineffective to identify the possible target levels to be quoted as example of good farming practice in open and free-range farmed animals.

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Figure 1. Distributions of contamination levels (pg WHO-TEQ/g fat) recorded by HRGC-HRMS for cumulative PCDD/Fs and dl-PCBs in the milk samples collected in Phase I, II and III (concentrations are ordered according to crescent values).