4 Development of a Reference Material in animal feeding stuffs for dioxins and related compounds

4.1 Summary

The episode that occurred in Belgium in May 1999 involving the contamination of fat used as a feedstuff component highlighted the vulnerability of the food chain and the lack of appropriate monitoring. Apart from this acute event, the environmental contamination by dioxin-like compounds may result in an accumulation along the food chain that can reach non-acceptable levels. As food contamination is also directly related to feed contamination, an integrated approach must be adopted to reduce dioxin and dioxin-like PCBs incidence throughout the food chain, i.e. from products intended for animal feed through food-producing animals to humans. Thus, in 2002, the European Union (EU) implemented comprehensive regulations for foods but also for animal feedingstuffs. These regulations set maximum limits at different levels of decision in an effort to control the food chain as well as to integrate this approach into the global issue of reducing the release of these compounds into the environment.

However, the main problems encountered by analysts at that time were the lack of dioxin reference materials (RMs) for animal feed samples to validate their analytical methods even if some reference materials were available for other food matrices (milk powder, fish or fish oil). As a result, we decided to produce a reference material sufficiently homogeneous and stable to support the first European inter-laboratory study on dioxins, furans and dioxin-like PCBs using the GC/HRMS method in animal feed samples. The multi-aims of this study were to assign values for the 29 toxic congeners and to produce an internal RM for validation purposes but also to assess the performance of the GC/HRMS method close to maximum levels as no data were available at that time. Thirteen selected European laboratories from eight countries with a long standing experience in this field were invited to participate.

The selected material consisted of freeze-dried animal feed powder of plant origin representing naturally contaminated feed samples that were collected during the 2000-2001 monitoring program in Belgium. We decided to use naturally contaminated animal feed in order to avoid the homogeneity difficulties encountered when spiking a solid matrix. A total of 12 kilograms of various lots containing different levels of dioxin were chosen. The proportions of feed used to characterize the material were calculated with the aim of

achieving concentrations in terms of Toxic Equivalences (TEQ) that were close to maximum limits (i.e. in this case, it was roughly twice the maximum level set at 0.75 ng TEQ/kg of product). The material was shipped to the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) to be grounded, sieved, homogenized and freeze-dried before being distributed to the participants. Several homogeneity tests were carried out at the IRMM centre. These analyses included the Karl Fisher moisture determination and particle size measurements. About 95% of the particles were smaller than 0.515 mm with the largest particle size being less than 0.735 mm. PCDD/Fs were also analyzed for 'sufficient homogeneity'. This test was based on a new method developed by Fearn (Fearn et al., 2001). All the PCDD/F congeners passed the test, indicating that the material was sufficiently homogeneous for this inter-laboratory study. Prior to shipment, the stability of the RM had been assessed. Samples were analyzed with a frequency of once a week during a six-month period and values were recorded on a QC chart.

Laboratories were asked to perform the analysis in triplicate under repeatability conditions in order to estimate the within and between laboratory standard deviation. Two different statistical approaches (the classical Standard ISO 5725-2 and a novel model developed by Cofino et al., 2000) were used and compared. After the outliers had been removed, both approaches gave similar and comparable results. The study has shown a good agreement (reproducibility CV of 6.2 % in TEQ calculation using upper bound value) between the laboratories at more than twice the level of interest. Thus, it should probably be possible to comply with the required criteria of CV < 15% at the level of interest (Directive 2002/70/EC). In addition, all the laboratories' mean values were within the ± 20% range around the assigned value for trueness and the difference between upper bound levels and lower bound levels did not exceed 20%. (Directive 2002/70/EC). The performances obtained here at ppt levels were comparable with those achieved during previous dioxins certification RMs exercise with selected laboratories (BCR report). However, one should also notice that Proficiency Testing exercise in the field (Smastuen Haug et al., 2002), grouping more than 50 laboratories, higher CVs are generally observed.

We also observed, in this particular study, that the performances of the HRMS method seem to be congener-independent for repeatability and reproducibility over a range of concentration spanning more than 4 orders of magnitude. The results clearly showed the effect of precision loss of the method below 0.1 ppt per congener for repeatability and below 0.2 ppt per congener for reproducibility. The outcomes allowed for modeling the precision (CV expressed in %) of the method by inverse first order equations at the parts-per-trillion level,

hence providing an interesting tool for laboratories developing their own analytical method. Moreover, promising results were also obtained for D-L PCBs. Except for two congeners (PCB 81 and PCB 123), repeatability and reproducibility were satisfactory, and performances were comparable to those obtained for the dioxin and furan congeners.

The inter-laboratory study designed here with a restricted number of expert laboratories permitted the assignment of values traceable to the gold GC/HRMS standard method itself and hence providing an assessment of the trueness of the method. Later, all of the relevant data from the study have been used to support the validation of our internal method. Parameters such as accuracy (trueness), precision (repeatability and intermediate precision) and measurement uncertainty of our method at a level close to the maximum limit were estimated. Because the RM was sufficiently homogeneous and stable, it was afterwards incorporated into QC program for routine samples for long-term intermediate method precision assessment. Moreover, the RM helped us to support trend analysis for quality assurance. Periodically, results were added in a control chart to check the stability, the trend or the drift of our analytical method according to ISO 17025 requirements (See chapter 7).