9 General conclusion

The work presented in this thesis finds its origin in the multiple crises in the food sector that were impossible to control due to the lack of *ad hoc* monitoring strategies. Our work intended not only to provide new faster and lower cost methods but also to elaborate the frame for a confident use of the data.

The first aim of this study was to develop a ^{13}C-labelled isotope dilution PTV-LV-GC-MS/MS method for the measurement of the 17 PCDD/Fs and the 12 DL-PCBs in foodstuffs. We demonstrated its feasibility. The method fulfils the European Commission Regulation 1883/2006 analytical requirements regarding screening approaches. Consequently, PTV-LV-GC/MS/MS is an attractive technique and can be used as a cost effective complementary method to HRMS for dioxin levels monitoring in food and feed. However, we have underlined two major drawbacks which may not be sustainable in routine practice. One is the high frequency of maintenance and cleaning steps to keep the instrument at its maximum sensitivity performances, the other one is associated to data handling and processing time for quantification. They do not fit with high throughput screening approach.

The second aim of this study was to provide answers to the basic questions addressed to analytical chemists dealing with trace analysis of complex samples and concerning the quality of the data. By answering these questions, we placed the validation of analytical method in a broader context of quality assurance including single-laboratory validation, interlaboratory study, proficiency testing, internal quality control, measurement uncertainty and accreditation. The following questions were raised:

- How to make sure that my method is able to achieve sufficient accuracy on results?

As reported in chapter 4, we produced a reference material for PCDD/Fs and DL-PCBs in animal feedingstuffs, sufficiently homogenized and stable to be used for validation and for internal quality control purposes. The precision (repeatability and within-lab reproducibility) could therefore be estimated. However, little was gained without knowledge of the trueness of the method. The interlaboratory study designed here, with a restricted number of expert laboratories, permitted to assign values traceable to the standard method itself and hence providing an assessment of the trueness of the method. All these relevant data have been used to support the method validation.

-Are there any analytical benchmarks available for validation purposes?

Guidelines dealing with validation methods explain that ideally the laboratory should agree with the customer an analytical requirement which defines the performance that the method must have to solve the analytical problem. The reality is somewhat different and rarely agreed in such a formal way. In chapter 6, data gathered from interlaboratory studies allowed us to revisit the application range of the Horwitz equation for PCDD/Fs and DL-PCBs in food and feed and we proposed a new empirical function called the 'the Dioxin function' adapted to ultra-trace analysis of these contaminants in biological samples. As the levels of the 29 PCDD/Fs and DL-PCBs are generally quite different in naturally contaminated biological samples (i.e. spanning several orders of magnitude depending on the congener), adapted target RSDs according to the dioxin function are therefore available. Calculated RSDs performed during the internal validation in repeatability and intermediate precision should be lower or equal to target RSDs provided by the equation as acceptance criteria.

-How to evaluate measurement uncertainty expressed in toxic equivalent (TEQ) units?

We reported in chapter 8 three practical examples of measurement uncertainty assessment by top-down approaches. The Barwick and Ellison approach can be easily developed with both matrix CRMs or spiked materials. When available, matrix CRMs provide the most reliable estimate of laboratory and method bias and together with intermediate precision give an absolute uncertainty assessment. Precision and trueness studies were carried out separately for PCDD/Fs and DL-PCBs on different food and feed samples. Expanded uncertainties between 15.0% and 23.7% were reported. A second top-down approach also based on trueness and precision studies has been evaluated. Measurement uncertainty can be easily related to the trueness and precision of the data collected when building the method accuracy profile. We showed that internal quality control data can also be used to check or to refine the measurement uncertainty estimated in a predictive way from validation data. Expanded uncertainties between 13.0% and 19.4% were reported for PCCD/Fs in beef fat in the range from 1.69 to 10.75 pgTEQ g^{-1} fat. The last top-down approach dealt with the reproducibility uncertainty estimated for each congener. As congener patterns vary from sample to sample, the method takes into account the influence of the different profiles on measurement uncertainty assessment. We demonstrate that a linear combination of congener uncertainties is a better approximation than root sum of squares to express the total uncertainty in TEQ.

- How to report results right?

In chapter 7, we implemented new internal quality control approaches to improve data evaluation. A set of procedures using multi-levels QC and Exponentially Weighted Moving Average (EWMA) for the continuous monitoring of these compounds were undertaken. Several criteria are tested to decide whether results are reliable enough to be released and reported. In chapter 5, we discussed different approaches for detection and quantification limits. These parameters are considered as key issues for the determination of PCDD/Fs and DL-PCBs at sub-parts per trillion levels in food and feed, particularly since the EU legislation introduced the concept of lower, middle and upper bound values for reporting data.

- How can the proficiency of my laboratory be measured?

The specificities that characterize a dioxin measurement are the low levels, the wide range of congener's concentration and the expression of the result in a unique toxic equivalent (TEQ) value. EU documents set analytical performance criteria in TEQ. Proficiency testing is a useful way to assess the quality of a laboratory's results. Assessment of results expressed in TEQ is mandatory and laboratories should demonstrate their competence with satisfactory z-scores. We propose to go further and to analyse, inside the data set, the performances of all relevant congeners that significantly contribute to the TEQ. In chapter 6, we demonstrated that one of the main features of the dioxin function could be its use as a suitable fitness-for-purpose criterion for dioxins and related compounds in PT exercises. A European expert committee recently recognizes its usefulness and will assess its use in the network of national reference laboratories (NRLs) participating at PTs for dioxin and related compounds in food.

In conclusion, our contribution helped in filling a real gap in the scientific approach for ultra-traces analysis in food control. This lack of global analytical strategy had in 1999 a large impact on the Belgian external trade of animal based food products. We contributed to assess the quality of the data obtained since the implementation of the European strategy. The monitoring programs have shown a real efficiency, allowing early warning and preventing a widespread of subsequent contaminations of the food chain. Part of this work is now referenced as a working document in Europe and in United-States.