The quality of food is an increasingly important matter of concern in Europe. The feeding stuffs poisoning episode that occurred in Belgium in May 1999 pointed out the vulnerability of the food chain and the lack of appropriate monitoring. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and polychlorobiphenyls (PCBs) were the key contaminants involved. This has triggered new EU legislation, including maximum and action limits for relevant food and feed products as well as requirements for analytical method used to verify compliance. Large monitoring programs to test food and feed have been launched and, in many countries, efforts to monitor dioxins and related compounds strongly increased. To cope with the large number of samples statistically required for monitoring, the recommanded strategy involves the use of screening methods based on low resolution mass spectrometry (LRMS) and/or bio-assays, and high resolution mass spectrometry (HRMS) method, used to bear out their presence. Major analytical challenges had to be met to face with the large number of samples including the authority's requests on developing screening and alternative methods for monitoring programs of PCDD/Fs and PCBs in food and feed.

The first part of this document is devoted to the development of an alternative LRMS-based method for PCDD/Fs and PCBs measurement in food and feed.

The second part of this thesis is an answer to the basic questions commonly addressed to all analytical chemists developing method but here in a particular context due to very specific family of compounds involved:

- How to make sure that my method is able to achieve sufficient accuracy on results?
- Are there any analytical benchmarks available for validation purposes?
- How to evaluate measurement uncertainty expressed in toxic equivalent (TEQ) units?
- How to report results right?
- How can the proficiency of my laboratory be measured?

To ensure the quality of data obtained, laboratories in charge of the food control on PCDD/Fs and dioxin-like PCBs encounter a number of severe problems. One has to mention the lack of sufficient and reliable certified reference materials that are necessary to validate methods, too scarce data available on current analytical method performances, and the absence of quality criteria approach for analytical method.

During the course of this work, I contributed to answer these questions to the general analytical effort by providing useful tools and methodologies.

At that time, straightforward answers could not be found in the scientific literature. One of the reasons was the scarce data of dioxins in food and feed available. One can also mention the

unusual part the dioxins play in chemical analysis. Indeed, the main features that characterize a dioxin measurement are the low levels at which these compounds occur in biological samples (sub parts-per-trillion), levels that are currently not explored by any other applications in chemical analysis in the food sector and therefore the difficulty to cope with precision models available; the reporting of results expressed in total tetrachloro dibenzo-*p*-dioxin (TCDD) toxic equivalent concentration for compliance assessment with statutory limits; and, what necessarily follows from the decision-making: the statement of the uncertainty interval also expressed in toxic units.

To answer the foregoing questions in an international frame, notions such as validation of analytical procedures, fitness for purpose, internal quality control, interlaboratory studies, proficiency testing, measurement uncertainty, traceability had to be introduced. They are all encompassed in the analytical quality assurance management a laboratory should implement. These concepts are strongly connected to statistical techniques. This branch of analytical chemistry that consists in extracting relevant information from data using statistical and mathematical methods adapted to the specific needs for the chemists is called chemometrics. Quality is an essential preoccupation of chemometrics but it cannot be only limited to these aspects. Chemometrics relates also to other topics such as experiments and experimental design methodologies, (new) knowledge about chemical systems.

Chemometrics and quality

This thesis treats several aspects and new approaches of quality assurance for an ultra-trace contaminant laboratory: external method validation through interlaboratory studies and estimation of repeatability, reproducibility and trueness using simple statistics based on normal distributions (ISO 5725) but also more complex statistical tests for heavily tailed, skewed or even bimodal distributions; the production and the use of a reference material for internal validation and internal quality control (QC) purposes; advanced statistics in quality control chart and multi-level control charts for sensitive detection of bias; proposal of quality criteria for assessment of proficiency of dioxin laboratories; proposal of benchmark precision for internal validation purposes; estimation of measurement uncertainty.

The thesis is divided in the following chapters:

Chapter 1 is a general introduction for dioxins and related compounds. It consists of a brief introduction to general characterization, mechanism of toxicity, human exposure and European legislation in food and feed.

Chapter 2 provides an overview of the analytical procedures for mass spectrometry based methods. It gives a brief summary of the most frequently used techniques to extract and purify PCDDs, PCDFs and dioxin-like PCBs (DL-PCBs) from food and feed matrices. Regarding detection, special attention of the principles of detection and quantification by HRMS in selected ion monitoring mode (SIM) and the quadrupole ion storage low resolution mass spectrometer in MS/MS mode is addressed.

Chapter 3 discusses the development and optimization of a large volume injection (LVI)-gas chromatography (GC)-ion trap MS/MS method as an alternative to GC-HRMS for the measurement of PCDD/Fs in food and feed. Instrumental detection limits were lowered by a factor 2 to 3 with the development, in collaboration with the manufacturer, of a system of damping gas pressure inside the trap that improves precursor ions trapping efficiency. We achieved 5:1 signal to noise with the injection of 200 fg of 2,3,7,8 TCDD. With slight adjustments to sample size and final extract volume, we demonstrated on QC samples the good agreement between this method and the reference GC/HRMS method for PCDD/Fs and DL-PCBs in food and feed.

In **chapter 4**, the first European inter-laboratory study on dioxins, furans and dioxin-like PCBs using the HRGC/HRMS method in animal feed samples is described with two main objectives. The first objective was to produce a reference material for internal validation and QC purposes. The second objective was to assess the analytical performances of the GC-HRMS method close to maximum levels as no data were available at that time and to check whether EU directives requirements were met.

Chapter 5 is a general discussion on the capability of the state-of-the-art HRGC/HRMS method to provide reliable results at decreasing maximum levels. Levels have to decrease according to EU policy regarding human exposure to those contaminants. In this case, we present the issue from a different angle, i.e. the analytical point of view for the future establishment of target levels. Based on the results of PCDD/Fs and DL-PCBs interlaboratory

study in animal feedingstuffs described in chapter 4, we demonstrated for the sum of the 17 PCCD/Fs toxic congeners that reliable results can be easily provided up to a value of 0.17 ng WHO-TEQ/kg. The ability to reliably quantify a minute trace of these contaminants has been pointed out with the aim of providing an analytical benchmark for the future establishment of target dioxin levels in animal feedingstuffs. Hence, both analytical and toxicological aspects should be examined together to set realistic target levels achievable for most dioxin laboratories involved in monitoring programs.

One of the central themes of this thesis is the establishment of an empirical relationship between reproducibility standard deviation and the dioxin congeners level in food and feed. **Chapter 6** deals with raw data from numerous performances interlaboratory studies of PCDD/Fs and DL-PCBs in food and feed. Striking linear functions in log scale between reproducibility standard deviation and congener's level over a concentration range of 10^{-8} to 10^{-14} g per g fresh weight were observed. The data fit very well to a Horwitz-type function of the form $s_R = 0.153c^{0.904}$, where s_R and c are dimensionless mass ratios expressed in pg/g on fresh weight, regardless of the nature of the toxic congeners, food and feed matrices, or sample preparation methods. I called this relationship the *'dioxin function'*. 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 proficiency testing (PT) exercises. We illustrated its use with practical example with data from the largest international PT in this field. Another application is its use as benchmark precision criteria for internal validation.

Chapter 7 discusses the role of internal quality control (IQC) to monitor analytical processes. Introducing new QC methods derived from the industrial practice to analytical chemistry, improving data evaluation and allowing to detect shifts or trends, are elements that are difficult to point out with classical approach (Shewhart chart). The importance of ARL (average run length) as a key-criteria of the efficiency of a quality control procedure will be emphasized. The introduction of the multivariate approach of multilevel control with the Hotelling's T^2-test will lead to a better detection of random errors than the independently managed conventional Shewhart charts. Moreover, the Exponentially Weighted Moving Average (EWMA) will offer a flexible tool for detecting the 'inacurracy' of a method, especially where small shifts or bias are of interest. All these concepts, recently introduced in clinical chemistry, were applied here for the monitoring of PCDD/Fs and DL-PCBs in food and feed.

Chapter 8 introduces the concept of measurement uncertainty (MU). Three 'top-down' approaches for uncertainty estimation are proposed on the example of the GC-HRMS method for PCDD/Fs and DL-PCBs in various food and feed matrices: the approach which combines long-term precision and trueness data to obtain an estimate of MU (Barwick and Ellision method); the approach which uses the reproducibility estimate from interlaboratory-studies as uncertainty estimate; the concept of accuracy profile used in the context of validation and internal quality control to assess MU.

Chapter 9 presents a general conclusion