



Editorial

During its last direction council in January 2004, our laboratory highlighted a need for an improved internal and external communication, especially regarding its research activities.

Therefore, the idea of a new document was proposed to present briefly some of the running research projects and main results, in addition to the existing activity reports and publications.

So it is a real pleasure for me to open the first of these bi-annual newsletters. I thank its authors and designers, and I wish you a good and pleasant lecture.

François André

The LABERCA recognised at the European level



From the 10th to the 12th May at Noordwijkerhout (Netherlands), was held the 5th EuroResidue conference. Organised each 4 years by a committee linked to the Utrecht university, it includes all aspects of residues in food. The analytical, pharmacological, toxicological and regulation topics are especially covered. The contribution of the LABERCA was significant and remarked, through 1 plenary lecture, 2 oral communications and 8 posters. These presentations concerned both hyphenated technical aspects (ion suppression, isotope ratio mass spectrometry), and various applications (proteic hormones, endogenous and exogenous steroids, corticosteroids). An intense discussion was also engaged regarding the minimal required performance limits (MRPL) actually proposed in order to harmonise the methods of control in the EC.

For the first time since its first edition in 1990, this conference was closed with an award ceremony, with the objective to distinguish scientists who have marked the domain through a particularly significant contribution. The first award was then attributed to the Dr. Bruno Le BIZEC, head deputy of the LABERCA, for his reference work especially in the field of steroid hormones.

In Brief

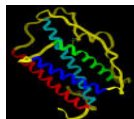
• Continuous Education

The fourth session of the continuous education course "School for Advanced Residue Analysis in Food" (SARAF) will be organised from the 4th to the 15th October. All information on the Web site (<http://www.saraf-educ.org>).

• Quality assurance

The 21th April 2004, the quality assurance system developed for the "project management of research: organisation, control and valorisation", based on the ISO 9001 standard, was successfully audited for the second time.

Proteic hormones analysis: an on-going challenge



Growth hormones, also known as somatotropins, are proteins produced by the anterior pituitary gland. Biological effects of somatotropins are numerous and associated with growth, development and reproductive functions. Growth hormones are widely used outside Europe to stimulate milk production in dairy cows and as a general growth promoter in pigs. Human and equine growth hormones are thought to be widely abused in sports since the increase in muscle size and strength make them a viable alternative to anabolic steroids.

Recombinant DNA techniques allow the production of large quantities of recombinant growth hormones which may exhibit slightly different chemical structures from the pituitary somatotropin, by adding a number of amino acids on the N-terminal side (1 to 8). Recombinant bovine and porcine somatotropins from these sources, legally used in USA, are banned in EU. In order to anticipate a potential illegal use of these substances, specific and sensible methods are required to detect these molecules in biological fluids.

In this context, researches are carried out in LABERCA to detect the illegal administration of recombinant hormones. Identification of natural somatotropins and recombinant ones from different sources and species as well as discrimination in-between the different forms is now overcome through peptide-mapping method (LC-MS/MS). Present work is focused on the extraction and purification steps of these molecules from biological fluids in which they are found at the ppb level. Different approaches are developed which, for example, deal with immuno-affinity chromatography as well as two dimensional electrophoresis.

Endocrine disruptors: new exposure assessment studies



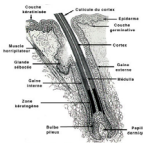
The endocrine disruption has been a research topic of growing concern for several years, both for scientists and public authorities, especially because of a close relation with food safety and consumer health. The LABERCA has been conducting for 2 years some projects dedicated to the development of efficient analytical methods for the identification of substances recognised as endocrine disruptors. A main objective of such developments is to ensure a robust basis for any further exposure assessment, and more generally risk assessment studies, that are actually missing at the national level. This first studies concerned compounds of natural (phytoestrogens) or synthetic (flame retardants) origin.

A first study was already devoted to a method development based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), permitting the identification of 12 isoflavones, lignans and coumestans in milk samples, at concentration as low as 0.1-0.5 ng.mL⁻¹ (ppb). Extended data collection will be now engaged, the purpose being to be interested in the potential beneficial or negative effects of these ubiquitous molecules, especially for young children.

A second project, supported by the Environmental Sanitary Safety French Agency (AFSSE) and coordinated by the UMR INRA 3089 Xenobiotics laboratory, was initiated to evaluate the foetal exposure to the polybromodiphenylethers (PBDEs) and tetrabromobisphenol-A (TBBP-A). The LABERCA will ensure the analytical basis of this study, through a development based on high resolution mass spectrometry (GC-HRMS) realised through a university PhD thesis. This development will be also exploited in order to identify eventual metabolites of these compounds.



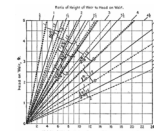
Analysis of Residues in hair: a promising improvement for the control



Urine, faeces, or tissue, are biological matrices used for many years in order to control the utilisation of xenobiotic agents in cattle. Hair represent an interesting alternative strategical matrix for revealing a misuse of steroids, due to an easy sample collection and a usual extended period of detectability. However, the mechanisms and incorporation kinetic of xenobiotics in the hair remained not fully understood and characterised. In this context, a project was initiated to study the fixation of médroxyprogesterone acetate (MPA), estradiol benzoate, and 17 α -methyltestosterone in bovine hair.

An analytical development based on a specific purification associated to selective and sensitive measurement techniques (GC-MS/MS and LC-MS/MS) permitted to clearly decrease the limits of detection. The elimination kinetic was monitored during several months after intramuscular administration. Steroid residues were then detected during several months. Residues of estradiol esterified forms were also identified during several weeks. This observation permits to propose this matrix as a promising candidate to demonstrate an illegal misuse of natural hormones in cattle, the detection of the injected ester form revealing unambiguously an exogenous origin.

Mass spectrometry and statistics: a new analytical approach



If the high interest of multivariate statistical techniques (MANOVA, PCA, LDA, classification,...) for the treatment of large data set is known for a long time, their utilisation in the field of analytical chemistry remains more recent. For several years, the LABERCA has been progressively introducing these techniques to analyse spectrometric data. The first applications were found very promising, and concerned the differentiation of stereoisomers (corticosteroids betamethasone and dexamethasone, androstan-diol steroids), on the basis of a global (MS in full scan mode) or specific (MS² in MRM mode) mass spectrometric fingerprint.

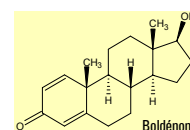
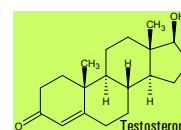
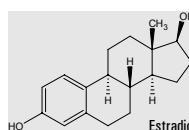
New projects are currently running using these techniques, with the objective to exploit their descriptive and explicative power, in order to investigate and explain the variability observed in term of contamination profile for the contaminant families constituted by different congeners such as dioxins.

This thematic is today the opportunity to collaborate with the UMR INRA Chemometry and Sensometry of the National School of Engineers for Agriculture and Food Industries (ENITIAA), Nantes, France.

The difficult case of endogenous hormones: new results for a European need

The research of xenobiotic anabolic steroids in biological matrices such as urine, tissue, or hair is today usually well controlled, in a context of food safety, human antidoping or horseracing control. The situation is clearly different when the purpose is to control the misuse of natural steroid hormones, in other words to distinguish the residue origin and ask the question: production by the organism or consequence of an exogenous compound administration? Indeed, the administration of these substances induces only minor change in their residual concentrations in tissue or excreta's. These variations are therefore usually considered as non significant compared to the natural physiological variations between and within specimen.

The research projects conducted at the LABERCA since 1997 in this field today lead to very promising results. The actual approach developed by the laboratory consists in the differentiation of the origin – endogenous or exogenous – on the basis of the ¹³C/¹²C ratio of metabolites and



precursors of these steroids in bovine urine, using gas chromatography-isotope ratio mass spectrometry (GC-C-IRMS). The precursors, also named endogenous reference compounds (ERC), are characterised by an isotopic composition non modified by an administration of gonadic steroids such as testosterone, estradiol, progesterone, or nandrolone, that appears at the end of the metabolic pathways. These synthetic anabolic steroids have the particularity to present an isotopic composition decreased compared to the endogenous sources. At the opposite, the ¹³C/¹²C ratio of DHEA and 5-androstene-3 β ,17 α -diol (ERC) reflects the endogenous isotopic composition in carbon, directly depending on the alimentation specific of each animal. Thus, the observation of a difference between precursors and metabolites in term of ¹³C/¹²C ratio permits to suspect an exogenous administration of gonadic steroid hormones in cattle, over several weeks after the treatment. These results are currently obtained in a European project of the 5th FP and by a university PhD thesis.

Last Publications

- Pinel G, André F and Le Bizec B. Discrimination of recombinant and pituitary-derived bovine, porcine and human growth hormones by peptide mass-mapping. *Journal of Agricultural and Food Chemistry* 2004;52(3):407-414.
- Antignac J-P., Cariou R., Le Bizec B. and André F. New data regarding phytoestrogens content in bovine milk. *Food Chemistry* 2004;87(2):275-281.
- Antignac J-P., Monteau F., Négriloli J., André F. and Le Bizec B. Application of hyphenated mass spectrometric techniques applied to the determination of corticosteroid residue in biological matrices. *Chromatographia* 2004;59:S13-S22.
- Le Bizec B, Maume D, Marchand P, Monteau F, Bichon E, André F. Review: control of anabolic steroid in breeding animals: mass spectrometry, a powerful analytical tool. *Chromatographia*, 2004;59:S3-S11.