



Editorial

I am pleased to present the 3rd issue of the LABERCA newsletter. Previous issues have been well appreciated, considering your reactions and regular sending requests.

The present issue presents some of our research projects having in common to improve the knowledge regarding environmental contaminants in food and feed.

I wish you a pleasant reading.

François André

The LABERCA and the 6th FP



The official launching meeting of the BIOCOP project was held in Brussels the 15th and 16th march 2005. As announced in our last newsletter issue, this integrated project of the 6th FP, coordinated by the Queen University of Belfast (Dr. Chris Elliott) is planned on 5 years and involves around 36 partners. Its main objective is the development of new analytical strategies for residue and contaminant in food, based on emerging techniques such as transcriptomic or proteomic. As responsible of the WP7 "endocrine disruptors", the LABERCA is working on the development of confirmatory methods (based on LC-MS/MS) for phytoestrogens in milk, cereals, and baby-food samples. The laboratory also participates actively to the WP8 "growth promoters", especially through the development of a direct identification method for peptidic biomarkers in plasma, susceptible to reveal the administration of anabolic steroids or corticosteroids. Finally, as responsible of the training activities organisation, the LABERCA will use its official teaching structure, i.e. SARAF (School for Advanced Residue Analysis in Food) in order to harmonise the content and the organisation of the different training courses and workshops proposed during the all project duration.

In Brief

• Continuous Education

The 6th session of the continuous education course SARAF (School for Advanced Residue Analysis in Food) will be organised from the 3rd to the 14th October 2005 (<http://www.saraf-educ.org>).

• Quality assurance

The certification of the quality management system developed by the laboratory for its research activities (according to the ISO 9001:2000 standard) was recognised and prolonged for the third time following the last audit the 28th April.

The isotopic approach (IRMS): new results for a 5th FP European project

The LABERCA is involved since January 2002 in one of the last 5th FP project – ISOSTER – dedicated to the development of innovative analytical methods for the demonstration of the illegal administration of steroid hormones in meat producing animals. Nine partners participate to this project, including laboratories in charge of anti-doping in sport (for example from Cologne, one World reference in this field) or in horseracing (from Newmarket, HFL), some national reference laboratories (NRL) specialised in chemical food safety (BfR, Berlin – TNO, Zeist – QUB, Belfast – CSL, York), as well as two of the main mass spectrometry companies providing IRMS instruments.

The measurement tools and the analytical strategy are now developed, and their robustness was demonstrated by a proficiency test involving ten invited laboratories. This method authorises the unambiguous discrimination in term of isotopic ratio (¹³C/¹²C) between precursors (or endogenous reference compounds such as DHEA or 5-androstenediol) and metabolites (etiocholanolone, androstanediol or 17 α -estradiol) of the administered anabolic steroid.

This approach permits the unambiguous demonstration of steroid misuse up-to several weeks after the administration. Results have also demonstrated the influence of animal feed on the ¹³C/¹²C ratio of the monitored substances, but without affecting the diagnostic capability of the method. These results represent a major advance in the field of control, especially for the "double status" steroids, i.e. both endogenously secreted by the organism and potentially exogenously administered.

Persistent organic pollutants (POP's): a large scale exposure assessment study in seafood

The LABERCA participates to a research project – POPINRA – coordinated by J-C Leblanc (National Institute for Agronomic research), which major objective was the determination of the occurrence of main POPs in various fish species and other seafood products. Because of their position in the food chain and their relatively high fat content (at least for certain fish species), these products are indeed recognised as particularly sensitive to these category of lipophilic environmental contaminants. Moreover, the investigation of the contamination profiles (relative proportions of the different monitored substances and congeners) may sometime be helpful in order to determine the contamination sources and the associated type of pollution.

For each sample, the monitored POPs included 35 molecules: 17 PCDD/PCDF congeners, 18 PCB congeners (12 characterised by toxicological properties similar to dioxins – "dioxin-like" PCB and 6 other presenting distinct effects – "indicator PCB"). The quantification of 7 brominated flame retardants (PBDE-28, 47, 99, 100, 153, 154 and 183) will be also performed on several samples.

This large scale and innovative study, coupled to simultaneous measurements for heavy metals, will undoubtedly lead to a very informative interpretation and a solid basis for further discussion. On the other hand, through the collection of 5565 data (number of measurement results estimated on the basis of 159 samples and 35 monitored analytes), this study will give a response to a need largely underlined at the European level regarding the risk assessment of these substances in France.

Growth hormone and Secretagogues: a new competence now well established

The anabolic properties of the growth hormone (GH, somatotropin) are potentially used in cattle or horseracing in order to improve performances. The LABERCA and the horseracing laboratory (LCH, Verrière le Buisson, France) are then concerned by this problematic. Since September 2004, a Cifre PdD thesis was initiated between the two laboratories. The main purpose of this shared research project is the demonstration of the illegal administration of recombinant growth hormones, especially in horse.

Behind the study of the growth hormones as such, the interest of the LABERCA is also focused on substances susceptible to induce and promote the natural secretion of somatotropins : the secretagogues. A master student was then in charge of this subject, with main objective to develop efficient analytical tool authorising the identification of these substances in biological matrices. The final report of this study was defended the 4th July 2005.

This "growth hormone" thematic of the LABERCA appears more and more recognised; a review presentation is planned to be prepared for the next International Symposium on Hormone and veterinary Drug Residue Analysis which will be held in Antwerpen (Belgium) next 16-19 May 2006.

Insecticides (Fipronil and Imidaclopride) : toward the evaluation of human exposure

In front of the abnormal mortality of bees observed by the beekeepers professional organisations, a study was initiated by the LABERCA in order to determine the presence of Fipronil and Imidaclopride residues in honey. With main objective to anticipate potential phytosanitary crisis, the developed method was extended to various food matrices.

The first work focused on honey and milk samples, as representative of natural and baby-food products, respectively. Limits of quantification obtained after validation of the methods were found to be fit-for-purpose regarding the expected concentration, i.e. around 25 ng.L⁻¹ for fipronil and lower than 1 µg.L⁻¹ for chloronicotinic residues (imidaclopride, nitenpyram, thiamethoxam, acetamipride, thiaclopride and clothianidine) in milk. For honey, limits of quantification were found to be lower than 5 µg.kg⁻¹.

These methods were applied on a preliminary sample set consisting in various commercially collected products. Honey samples have been analysed, some of them (from importation) showed the presence of imidaclopride and thiaclopride residues.

Endocrine disruptors: Three classes of compounds particularly surveyed

Endocrine disruption is a growing concern for the scientific community for several years. Indeed, an increasing number of chemical substances are regularly pointed out because of their potential negative impact on human health, especially for the reproduction and development functions and particularly sensitive populations (foetus, young children). For this kind or residue and contaminants, it is now well established that food intake represent, with the environment, a major route of exposure to these substances. In this context, and according to his contract with the AlimH department of the National Institute for Agronomic Research (INRA), the LABERCA is interested since 2002 by three families of compounds for natural (steroid hormones, phytoestrogens) or synthetic origin (brominated flame retardants). Current researches focus on one hand to (re)-evaluate the endogenous production of natural sexual estrogens in the concerned population (prepubertal children), on the other hand to characterize the exposure to these substances (direct intake in case of foetus or food intake for young children), and finally to determine the hormonal activity associated to this intake. The expected results would probably permit both to a data set clearly missing at the national and European level, and to reach a better characterisation of the eventual danger associated to these compounds.

Boldenone : a new control strategy under investigation

Boldenone is an anabolic steroid characterised by its androgenic activity; its use is strictly forbidden in cattle within the EU. The control of its illegal use was based on the identification of 17 α -boldenone in urine. Recent studies showed a possible but non systematic endogenous production of this steroid in bovine. A research project dedicated to the metabolism study of boldenone in cattle was started in LABERCA. Potential marker phase I metabolites were identified in urine (5 β -androst-1-en-17 α -ol-3-one (M2) and 5 β -androst-1-en-17 β -ol-3-one (M4)) as well as phase II metabolites such as 17 β -boldenone sulphate and glucuronide.

Two analytical methods were developed in LABERCA, the first relying onto specific separation of free, glucuro- and sulphoconjugates, specific hydrolysis and GC-MS/MS (EI) analysis, the second onto the direct measurement of conjugates by LC-MS/MS (ESI). This last approach would constitute a major improvement and a definitive analytical tool for the demonstration of the illegal use of this anabolic steroid in cattle.

Last Publications

- Antignac J-P., Brosseau A., Gaudin I., André F. and Le Bizec B. Analytical strategies for the direct mass spectrometric analysis of steroid and corticosteroid phase II metabolites. *Steroids*, 2005;70:205-216.
- Le Bizec B., Antignac J-P., Bertrand D., Qannari El M. and André F. Multidimensional statistical analysis applied to electron ionization mass spectra to determine steroid stereochemistry. *Rapid Communication in Mass Spectrometry*, 2005;19(4):509-518.
- Pinel G, Bichon E, Poupponeau K, Maume D, Andre F, Le Bizec B. Multi-residue method for the determination of thyrostats in urine samples using LC-electrospray-MS/MS after derivatisation with 3-iodo-benzyl-bromide. *Journal of Chromatography A*, 2005, *sous presse*.
- Laurent C, Marchand P, Feidt C, Le Bizec B, Rychen G. Tissue distribution and bio-concentration factors of PCDD/Fs in the liver and adipose tissue following chronic ingestion of contaminated milk in rats. *Chemosphere*, 2005, 60(7):929-938.
- Debrauwer L, Riu A, Jouahri M, Rathahao E, Jouanin I, Antignac JP, Cariou R, Le Bizec B, Zalko D. Probing new approaches using atmospheric pressure photoionization for the analysis of brominated flame retardants and their related degradation products by LC-MS. *Journal of Chromatography A*, 2005, 1082(1):98-109.