



## Editorial

The first issue of the LABERCA research newsletter was favourably received. Here is the second issue; some aspects not treated before are presented.

It summarises some research results included in the official area of the laboratory (MAAPAR/DGAL), but also other ones around this scope, for example regarding PAH or pesticides.

With all my best wishes for the new year 2005, I hope you will have a pleasant lecture.

François André

## The LABERCA involved in the 6<sup>th</sup> FP



In the 6<sup>th</sup> FP, the laboratory will participate to a European research project (IP) since January 2005. This project called "BIOCOOP", will start next January and will run over 5 years, involving about 40 partners. Its main objective is the development of new analytical strategies, based on the emerging techniques such as transcriptomic and proteomic, for screening of residue and contaminants in food. The current controls, which are based on a limited number of selected target analytes, today appears clearly limited in term of large scale screening.

Therefore, the proposed systemic and innovative new approach will consist to study the global effect of the concerned substances, rather than looking for their identification. Thus, the challenge will be to identify some relevant biomarkers (RNA or proteins) characteristic of these compounds. This project will concern various classes of molecules including toxins, pesticides, steroid hormones, quinolones, phytoestrogens, and heavy metals. The LABERCA will be responsible for the workpackage dedicated to phytoestrogens, and will be also actively involved in the workpackage related to growth promoting factors. The laboratory will be finally in charge of the scientific dissemination.

## In Brief

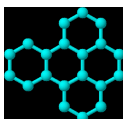
### • Continuous Education

In answer to a request of the European Commission, a special SARAF session (School for Advanced Residue Analysis in Food) was organised from the 22<sup>nd</sup> November to the 3<sup>rd</sup> December 2004 (20 participants/15 nationalities)

### • Quality assurance

LABERCA was invited to present its quality management system dedicated to its research activities (ISO 9001) through an oral communication in a congress organised by the Belgium accreditation body (Melle, 20<sup>th</sup> October 2004).

## Polycyclic aromatic hydrocarbons: characterisation of the food intake



The Polycyclic Aromatic Hydrocarbons (PAH) are organic compounds constituted by two to seven benzene rings. For humans, the main ways of exposure to these substances are the ambient air (depending on various factors including urban traffic and industrialisation), as well as food intake through products contaminated by environmental sources and/or specific food processes such as smoking, roasting or torrefaction. For a non smoker individual, food remains the most important exposure vector to PAH, with global intake estimated to 3 µg/day. For a smoker, this value may be increased by a factor 2.

The French Agency for Food Safety (AFSSA) established in 2003 a list of 11 indicator PAH to evaluate the risk associated to these compounds through food intake. The National Institute for Industrial Environment and Risk (INERIS) also published in 2003 a report validating the AFSSA recommendations with the addition of a proposed virtually safe dose equal to 5 ng/kg/bw/day, based on Toxic Equivalent Factors (TEF).

An analytical method dedicated to the identification and quantification of these contaminants in various food products was developed, using gas chromatography coupled to mass spectrometry (GC-MS). This study is accompanied by a more basic study led in collaboration with the Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires (ENSAIA), with the objective to assess the transfer of PAH in ruminant from feed to milk. This project is expected to improve the knowledge regarding the metabolism of PAH and especially determine PAH markers in milk.

## Phytosanitary products: some projects for a regional concern



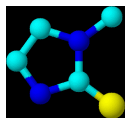
Since 1999, the laboratory participated to 3 research projects involving regional partners (Bretagne and Pays de la Loire) in the field of phytosanitary products. The objective of the first project was to evaluate the transfer of some phytosanitary products especially used at the regional level, to food products from vegetal (carrots, spinach, apples, baby-food) and animal (milk, meat) origins. It has been demonstrated that all these samples were compliant according to the current regulation (observed concentrations far below the maximal residue limit).

The second project consisted to study the eventual contamination of marine molluscs by 3 herbicides families widely used at the regional level (phenylurea, triazines, aminophosphates). After an important analytical development, assays were realised on samples collected in various locations, supposed to be particularly exposed; no trace of contamination was observed. A complementary study realised on oysters incubated with diuron permitted to show that the concentration retrieved in oysters was 5 to 10 times higher than the concentration measured in water.

The last project actually running is dedicated to the two insecticides highlighted by bee keepers: fipronil and imidaclopride. The aim is on one hand to evaluate the contamination of surface water in South Vendée, and on the other hand to study the eventual transfer of fipronil from the maize seed to the milk. This last study is realised in collaboration with an experimental farm "Les Trinottières" in Maine et Loire.



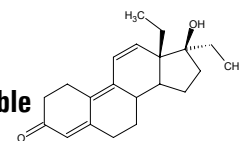
## Thyrostats: a new strategy for the control of their illegal use



With their property to inhibit the natural production of thyroid hormones, thyrostats disturb the metabolism pathways under their direct dependence. As a result, an extracellular water retention as well as a reduction of the tractus transit may be expected through their illegal use in breeding animals, with alteration of the muscle quality. Moreover, the presence of thyrostat residues represent a carcinogenic and teratogenic risk. The extraction of these small, highly polar, and amphoteric molecules from biological sample, as well as their measurement represent a real analytical challenge.

Therefore, a innovative derivatisation reaction was developed. The interest of this chemical modification is first to stabilise all possible tautomeric forms characteristic of these molecules under a unique and less polar structure, which facilitate the extraction. Then, the addition of the derivatisation agent led to an increase of the molecular weight of the molecule, permitting to improve significantly the signal specificity. Finally, the obtained derivative presents a diagnostic signal with higher sensitivity and the possibility to be fragmented in tandem mass spectrometry (that was impossible on the original molecule). A validation is currently running on urine sample according to 2002/657/EC Decision.

## Tetrahydrogestrinone (THG): the new anabolic steroid now detectable



Despite the intensity of the controls realised within the EU to survey the illegal use of steroid growth promoters, the number of non compliant samples reported in the main species of breeding animals is clearly decreasing since the end of the 90's. This observation may be explained by various factor, including the existence of new compounds belonging to the classical families of growth promoters, or the apparition of new classes of compounds. Tetrahydrogestrinone (THG) is one of these new substances recently discovered, revealed in 2003 by the USADA (U.S. Antidoping Agency).

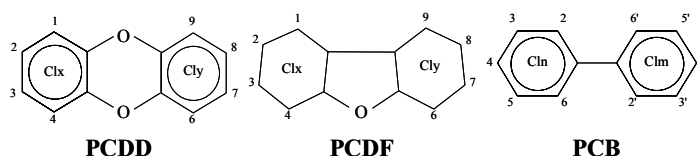
The LABERCA immediately launched a research project dedicated to the development of efficient method permitting the identification of this molecule based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The metabolism of this drug in bovine was also investigated after intra-muscular or oral administration. The results demonstrated that the more appropriate biomarker remains the non-modified parent drug, with minor proportion of conjugated forms, both in urine and faeces. The method was then applied to hundred urine samples (80% bovine and 20% porcine) coming from the French monitoring plan. No trace of THG was detected in these sample, at a decision limit (CC $\alpha$ ) estimated to 0.1 ng.mL<sup>-1</sup> (ppb).

## Persistent organohalogenated contaminants: the analytical tool developed by the national reference laboratory today permits large scale risk exposure studies

The LABERCA was designed in 2000 as National Reference Laboratory for the control and analysis of dioxins and related compounds in food. The competence acquired in the field of gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) permitted to develop efficiently and rapidly some analytical methods for which it is today accredited (ISO 17025 standard). The missions of this unit (official survey programs, organisation of ring tests, training,...) are realised by a team of 5 individuals. Some research project were initiated since the creation of the unit. One of them, funded by Bretagne and Pays de la Loire and realised in collaboration with the Ecole de Chimie de Rennes (LARCIP) is now finalised.

The objective of this study was to improve the analytical competences at the regional level, as well as to collect data regarding the occurrence of these contaminants both in the sources of emission and in food products. Four industrial sites were monitored in 2003, in term of emission and evaluation of environmental rejections around the sites.

The collected samples included soil (as marker of long term exposure), grass (as marker of short term exposure), and milk from 10 farms selected considering their location from the source and position compared to winds. The results demonstrated than no sample presented a dioxin content higher than the authorised limit during the year, even if a clear seasonality phenomenon was revealed whatever the considered site. The examination of the full patterns given by the concentrations of the different analysed congeners demonstrated that an isolated measurement is insufficient to identify a source of pollution.



### Last Publications

- Antignac J-P., De Wasch K., Monteau F., De Brabander H., André F. and Le Bizec B. The ion suppression phenomenon in liquid chromatography-mass spectrometry and its consequences in the field of residue analysis. *Analytica Chimica Acta*, *in press*.
- Meunier-Solère V, Maume D, André F and Le Bizec B. Pitfalls in trimethylsilylation of anabolic steroids: new derivatisation approach for residue at ultra-trace level. *Journal of Chromatography B*, *in press*.
- Pinel G, Buon R, Aviat F, Larre C, André-Fontaine G, André F, Le Bizec B. Assessment of Western blotting and 2D-electrophoresis methods on biological samples for the demonstration of its administration. *Analytica Chimica Acta*, *in press*.
- Rambaud L, Bichon E, Cesbron N, André F and Le Bizec B. Study of 17 $\beta$ -estradiol-3-benzoate, 17 $\alpha$ -methyltestosterone and medroxyprogesterone acetate fixation in bovine hair. *Analytica Chimica Acta*, *in press*.