

Editorial

Our 6th Newsletter not only summarizes some of the projects which we finalised at the end of this year but also introduces the research actions which will be initiated in 2008 in order to follow up on the same topics.

Our objectives remain the same and are perfectly in line with new partnerships and the integration of our team in the 7th FP.

I wish you a pleasant reading and a festive end of year.

Bruno LE BIZEC

SARAF and the European Commission

In 2006, LABERCA answered a call for tender from the European Commission for the organisation of engineering training cycles in eight countries (Ghana, The Philippines, Vietnam, Costa Rica, Papua New Guinea, Thailand, Honduras and Gambia). LABERCA was granted the contracts and contacted the European delegations in the countries concerned in order to define a training programme dedicated to their specific needs.

This programme is now being established and about ten experts in the analysis of residues and contaminants in food will be commissioned to these countries in 2008.

In 2007, LABERCA also won the call for tender from the European Commission for the organisation and implementation of a training programme on the analysis of residues and contaminants in food, dedicated to the reference laboratories of about twenty third country selected by the DG-Trade.

In this respect, a new SARAF Training Session will be organised in June 2008 in the National Veterinary School of Nantes.

In Short

▪ Retirement

Mr Daniel Maume will be retiring on 31 December 2007 after working for 17 years for LABERCA.

We wish him a long and happy retirement, which will no doubt be very active in the pursuit of his favorite pastimes.

Exposure characterisation of the prepubertal children to gonadal steroid hormones (PhD defended by Frédérique COURANT on 22/10/2007)

Global concerns have been raised in recent years over the potential adverse effects that may result from exposure to chemicals that have the capacity to interfere with the endocrine system. Our main purpose was to investigate to what extent food intake of steroid hormones can represent a risk for prepubertal children.

At first, a new analytical strategy for the measurement of steroid hormones at ultra-trace level in food and complex biological matrices was developed. Subsequently, steroid measurements in various food products (milk, egg and meat) were performed in order to evaluate the food intake in steroid hormones for this population.

Then, the quantification of the main androgens and estrogens in more than 120 serum samples from children aged 6-16 years gave access to the steroid plasmatic hormonal rates and consequently to the daily endogenous production of this population.

A first interpretation of these data in terms of risk assessment is also provided, in connection with existing CODEX, JECFA and FDA recommendations regarding the maximal acceptable daily intake for estradiol.

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DEER or the risks of endocrine disruption induced by certain residues and contaminants in Man

In 2008, the LABERCA will become part of a European Research Project from the 7th FP entitled « Developmental effects of environment on reproductive health » (DEER).

This project, coordinated by the University of Turku (Pf. J. Topari) gathers 9 partners coming from the most advanced teams on the subject (Pf. N. Skakkebaeck, Pf. R. Sharpe, Pf. S. Swan, Pf. B. Jegou, ...). Its general objective is to study the potential links between a chemical exposure at various early development stages (early puberty, infertility, « testicular dysgenesis syndrome ») and the LABERCA will be in charge of a work package whose task will be to develop and apply metabolomic approaches in order to characterise in a global manner the samples taken in the various population groups concerned, then to identify several relevant biomarkers for the pathologies studied.

The LABERCA will also take part in the characterisation of the contents of the various chemical residues and contaminants present in the biological samples of these populations.

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Detection of recombinant equine growth hormone administration: antidoping application in horseraces

(PhD defended on 23 November 2007 by Ludovic BAILLY-CHOURIBERRY)

The growth hormone is a peptidic molecule with anabolic activity potentially used to improve horse athletic efficiency and to increase the production profitability of farm animals. However, the use of this molecule is strictly forbidden by the races code and the European directives in force.

The control of its potential use constitutes an analytical challenge which many laboratories have attempted to tackle. The difficulty of this analysis lies mainly in the fact that the hormone of interest is present at trace level ($\mu\text{g} \cdot \text{L}^{-1}$, $\text{fmol} \cdot \text{L}^{-1}$) in complex biological matrices such as blood, with a short half-life estimated to a few hours.

In this context, this PhD thesis work enabled the development of two innovative methods, one direct and one indirect, for the detection of recombinant equine growth hormone (reGH) illegal use in horses. A protocol of extraction/purification of the hormone in plasma leading to an enzyme digestion and the detection by LC-MS/MS of a specific peptide has enabled for the first time to reveal growth hormone in samples from animals treated with a preparation of hormones and to build a kinetic of elimination over 48 hours.

As for the indirect detection method, it was developed by ELISA to detect the production of anti-reGH antibodies consecutive to a treatment with reGH up to 5 months after treatment. This method thus allows to envisage immediate applications in the field of antidoping in horseraces and more generally in farm animals.

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Growth hormone and secretagogues: ... Continued

The regulations forbidding this molecule in the world of horseracing have now been very recently followed by sensitive and specific screening and confirmatory analytical methods. Nevertheless, the performance of the methods is limited by the short half-life span of this type of proteic substance, which does not allow an optimal detection in time of the fraudulent use of these molecules. It now seems obvious that a more global approach has to be implemented. Facing this very same problem and after several years of collaboration on the subject, the Laboratoire des Courses Hippiques (LCH) and the LABERCA have launched a new research work together by the means of a co-financed PhD. This innovative strategy will allow to set and compare the urine and blood biological prints of two groups of animals, one treated with equine recombinant growth hormone and the other consisting of control animals. The setting up of such a study requires the use of specific and adequate tools such as high performance Liquid Chromatography coupled to High Resolution Mass Spectrometry on a brand new generation hybrid instrument (LTQ-Orbitrap).

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NUPEM

Starting in 2008, the LABERCA will participate in a regional research project coordinated by the Centre Régional en Nutrition Humaine (CRNH), whose subject is perinatal nutrition and the notion of metabolite profiling. The laboratory will be mostly involved in the development and application of general solutions of metabolomic-type aimed at characterising the potential impact of certain food constituents (polyunsaturated fatty acids, oligosaccharides, proteins and chemical pollutants) on newborns.

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Latest Publications

- G. PINEL, L. RAMBAUD, G. CACCIATORE, A. BERGWEFF, C. ELLIOTT, M. NIELEN and B. LE BIZEC. Elimination kinetic of 17β -estradiol 3-benzoate and 17β -nandrolone laurate esters metabolites in calves urine. *Journal of Steroid Biochemistry and Molecular Biology* 2008. Under press.
- E. BICHON, F. KIEKEN, N. CESBRON, F. MONTEAU, S. PREVOST, F. ANDRE and B. LE BIZEC. Development and application of stable carbon isotope analysis to the detection of cortisol administration in cattle. *Rapid communications in Mass Spectrometry* 2007;21:2613-2620.
- J.P. ANTIGNAC, R. CARIOU, D. MAUME, P. MARCHAND, F. MONTEAU, D. ZALCO, A. BERREBI, J.P. CRAVEDI, F. ANDRE and B. LE BIZEC. Exposure assessment of foetus and newborn to brominated flame retardants in France: preliminary date. *Molecular Nutrition and Food Research* 2008. Under press.
- L. BAILLY-CHOURIBERRY, E. CHU-VAN, G. PINEL, P. GARCIA, M.A. POPOT, G. ANDRE-FONTAINE, Y. BONNAIRE and B. LE BIZEC. Detection of a secondary biomarker of met-eGH as a strategy to screen for somatotropin misuse in horseracing. *The Analyst* 2008; 133: 270-276.
- H. NOPPE, B., B. LE BIZEC, K. VERHEYDEN and H. DE BRABANDER. Review: novel analytical methods for the determination of steroid hormones in edible matrices. *Journal of Mass Spectrometry*, 2008. Under press.

HDR

Dr. Jean-Philippe ANTIGNAC has passed his « Habilitation à Diriger des Recherches » examination on 5th October 2007. Jean-Philippe has been working in LABERCA since 1998 and his research activities deal mainly with chemical food safety. Since 2003, the main focus of his work has been research topics aimed at the study of some specific chemical pollutants (endocrine disruptors), on the angle of their potentially adverse effects on man and his descendants, especially by developing new global approaches such as Mass-Spectrometry based metabolomics.