National Institute for Public Health and the Environment

2006 Annual CRL workshop

Conclusions and recommendations

18 - 20 October 2006
Bilthoven
The Netherlands
Participants

Participants represented not only the current EU Member States, but through the assistance of TAIEX also from acceding and (future) candidate EU Members:

Conclusions and recommendations CRL-workshop

Based on the discussions on 18 and 19 October, the following conclusions and recommendations are made:

Making available reference standards for difficult to obtain analytes like metabolites and isotope enriched standards remains a priority for the CRLs.

Similarly, making available blank and incurred sample materials is of great value in order to support method validation in NRLs

Information gathering, coordination of stability information for standards and incurred residues could be intensified

The new CRL-website should possible contain a discussion forum.
The set of analytical methods as available through the CRL-website should be further completed, amongst others with (multi-residue) methods for muscle tissue.

There is a need for instruction and training in several (future) Member States.

There is a need for further research on analytical methods for detecting abuse of natural hormones. In addition, an updated overview of the natural occurrence of hormones in biological materials, possibly supported by a web based database, should be prepared. By the CRL.

A paper further clarifying the (legal) status of lists of MRPLs and a clarification about their relation with Annual Residue Control programmes should be prepared by the Commission and the CRLs.

Priorities for proficiency testing

The following suggestions were made for future (2008 and later) proficiency tests.

1. Corticosteroids in bovine urine and liver
2. Chlorpromazine in porcine kidney
3. Methyltestosterone and metabolites and testosterone in bovine urine
4. Trenbolone in urine and muscle
5. Zeranol, taleranol in bovine urine
6. Methylboldenone in urine
7. PTs in ovine and porcine urine
8. Chicken muscle and liver for RALs and Stilbenes

New and improved website with information on CRL-activities and methods database. Available starting Monday 4 December 2006

www.rivm.nl/residues
The last day of the workshop was devoted to boldenone and nortestosterone. The major conclusions from the presentations made are summarized in a small report.

Conclusions and recommendations CRL-workshop
Friday 20 October 2006. : “Boldenone and Nortestosterone issues”

The work presented by Marco Blokland (NL-CRL, RIVM, Bilthoven) demonstrated a correlation between free alpha-boldenone and coprostanol, a marker for faecal contamination. Further, detailed studies on a large population of samples screened positive for boldenone revealed that: none of the samples containing more than 2 ppb alpha-boldenone conjugate, contained detectable amounts of 17beta-boldenone conjugate or 6OH-boldenone.

From this work it was concluded that conjugated 17beta-boldenone and 6OH-17alpha-boldenone are good markers for a treatment with boldenone. The influence of fecal contamination was clearly demonstrated. The population studied included both calves and older bovine animals.

Bruno LeBizec (F-NRL, LABERCA, Nantes) reported detailed studies into the metabolism of boldenone in search for a marker that can be used for detecting abuse. The use of ADD (a feed additive) was included in these studies. A total of 9 individual metabolites were detected which were tested against three different conditions:

- present whatever the treatment
- sufficient abundant
- not found in non-treated animals.

The 6OH-boldenone metabolites were also detected during this study, but none of the metabolites fulfilled all conditions.

Another of the study focused also on phase II metabolites (conjugates) which were determined directly with LC-neg(ESI)-MSMS. From this part of the work it was concluded that conjugated 17β-boldenone (17β-boldenone-17sulphate) is a good marker for abuse.

From the study with ADD as feed additive it was concluded that changes in C12/C13 ratio are significant for 17α-boldenone, demonstrating the usefulness of this GC-C-IRMS technique for discriminating “natural” and exogenous sources.

Hubert the Brabander (UG, Belgium) presented details on biosynthesis and biotransformation processes and the role phytosterols and phytostanols can play. Phytosterols can be present in animal feed. Due to the very low absorption in the intestines, concentrations can be very high. The presence of microorganisms can cause bioconversions, possibly having a relation with the findings of boldenone in faeces. One of the interesting observations studied currently is the role maggots play in bioconversion in faeces.

The studies presented by Giovanni Ferretti (I-NRL, ISS, Rome) confirmed the fact that the 6OH-metabolites of boldenone are useful marker analytes for the detection of abuse of boldenone in Cattle. Detailed kinetic studies showed the presence of this metabolite during several weeks after treatment. False positive results have not been reported.
The presentation by Glenn Kennedy (UK-NRL, AFBI, Belfast) was on a slightly different, but related topic. Analysis of urine obtained from casualty animals, (steers and bulls) revealed the presence in 17α-Nortestosterone. Until now, it was generally accepted that this compound does not occur naturally in these animal which is only proven for pregnant (bovine) animals and very young calves. Detailed studies on the farms and in several EU laboratories, however, lead to the conclusion that:

- the analytical observations are beyond doubt
- there are serious indications that injuries (mainly skeleton) in (male) bovine animals can cause the endogenous formation of 17α-Nortestosterone
- precise mechanisms and conditions remain to be studied further.

Conclusions from these studies are:

- 17beta-boldenone, determined as conjugate, remains a good marker for abuse of 17β-boldenone(esters).
- The previous conclusion (September 2003) with respect to veal calves, is also applicable for older animals.
- 6-hydroxy-17α-boldenone is a suitable alternative marker for boldenone abuse, but more research is necessary
- There is no correlation between (conjugated) 17α-boldenone and any other parameter indicating the use of 17β-boldenone(ester).
- Unconjugated forms of 17α-boldenone present in urine are correlated with faecal contamination.
- Maggots can play a significant role in biotransformation of phytosterols and phytostanols.
- 17α-nortestosterone can be present in samples of urine of bulls and steers after injury (casualty animals). This finding needs further study and communication.