Report for the Community Reference Laboratory for Residues of growth promoters, sedatives and mycotoxins

RIVM, Bilthoven, The Netherlands
Period: 1 July 2001 - 30 June 2002

The duties and operating conditions of the CRL for residues are laid down in Annex V Chapter 2 of Council Directive 96/23/EC.

GENERAL TASKS

i. “EC - 4 CRL for residues” management (co-ordination, co-operation and administration).
   (Annex V Chapter 2, all paragraphs)
   This activity is ongoing

ii. Analyses of samples submitted by EU Member States in case of dispute between Member States or in case of analytical problems within a responsible NRL.
    (Provided separate funding is guaranteed on a case to case basis!)
    (Annex V Chapter 2, section 1, paragraph h)
    Status: In the last contract period assistance with analytical problems was given to one NRL of an EU Member State and one NRL of a Candidate Country. This activity is ongoing.

iii. Identification of new and unknown compounds illegally used for growth promoting purposes (Group A substances).
    (Annex V Chapter 2, section 1, paragraphs a & j)
    Status. This activity is ongoing. No new compounds were identified during the contract period. On request of the Commission the CRL updated the present status for alpha-boldenone as a natural occurring anabolic steroid.

iv. Maintenance of the in-house QA/QC activities in consequence of the accreditation and GLP compliance of all analytical work done within the CRL.
    (Annex V Chapter 2, section 1, paragraph b; section 2, paragraph g)
    Status: Ongoing, the in-house QA/AC system remained fully operational. The formal accreditation of the CRL was continued, now on the basis of ISO 17025. Activities necessary for maintaining the GLP-status were terminated.

v. EC - CRL –NRLs for residues establishment of reference methods and minimum quality criteria (co-ordination, co-operation and harmonisation).
    (Annex V Chapter 2, all paragraphs)
    Further support was given to the revision of Commission Decision 93/256/EEC with respect to performance criteria for analytical methods. Formal procedures for establishing Reference methods and Minimum Required Performance Limits (MRPL) were put on hold, pending the final publication of the revised Commission decision.

1 Official Journal of the European Communities (1996) L125, 10 - 31
vi. EC/CRL – related International Bodies (e.g. AOAC, Eurachem, IUPAC, Codex, JECFA, IOC ) performance quality criteria (communication, co-ordination, co-operation and harmonisation).

(Annex V Chapter 2, all paragraphs)

The efforts to actively introduce the EU concept of performance based criteria for analytical methods was continued, The unfortunate circumstance that the draft document is not yet published as official Commission Decision is seriously hampering this process. The Director of the CRL has been appointed as o-chairman of the methods working party of the Codex Committee Residues of Veterinary Drugs in Food (CCRVDF).

vii. EC/CRL – related EC Bodies (e.g. CVMP, EMEA, FVO, TAIEX, JRCs ) performance quality criteria (communication, co-ordination, co-operation and harmonisation).

(Annex V Chapter 2, all paragraphs)

The CRL contributed to 5th FWPs like MeQualAn and RADAR. Co-operation with several EC related bodies continued. The CRL participates in programs of the JRC Institute for Health and Consumer Protection and TAIEX on technical assistance of candidate Countries. On invitation several introductions and overviews have been presented. The Legal Service has been assisted in a court case.

viii. Acquisition EC/CRL – related EC DGs programmes (e.g. 5th FWP, AIR).

(Annex V Chapter 2, all paragraphs)

In close co-operation with the other CRLs for residues and a number of NRLs, an EOI was prepared in response to call EOLFP6.2002 (FaFoRescontrol). Activities include; Supporting scientific research, quality assurance, analytical methods, training and consumer exposure data.

ix. Preparation of technical and financial reports on the activities of the CRL.

(Annex V Chapter 2, section 1, paragraph k)

Status: The technical and financial reports of the previous contract period (2000.07.01 – 2001.06.30) were prepared and submitted. This activity is ongoing.

SPECIFIC TASKS

In addition to the general tasks, the following specific work program was undertaken:

1. Development and validation of analytical methods necessary for effective inspection and control.

2. Supportive research in the mandate of the CRL.

3. Quality Assurance and Quality Control.
4. Documentation services.

**PROGRESS DURING THE PERIOD FROM 1 July 2001 - 30 June 2002.**

**Ad 1. Development and validation of analytical methods necessary for effective inspection and control**

1.1 A protocol and validation report on MS-methods for thyreostatic compounds.  
(Annex V Chapter 2, section 1, paragraph f & l).  
*Status: Continuation of this activity was postponed in view of other priorities.*

1.3 Extension of existing multi residue methods of analysis of tissues with new compounds and new tissues from different species, including poultry, eggs and fish.  
(Annex V Chapter 2, section 1, paragraph f & l)  
*Status: The existing multi residue method was validated for the screening and confirmation of muscle tissues (bovine and porcine animals) for a range of natural anabolic compounds, inclusive nortestosterone and boldenone.*

1.4 Implementation of automated Supercritical Fluid Extraction (SFE) equipment in existing multi-residue methods of analysis.  
(Annex V Chapter 2, section 1, paragraph f)  
*Status: This activity is ongoing, focusing on a method for natural hormones based on the combination of SFE with LC-MS.  
A method for the determination of low levels of Stanozolol in muscle tissues, based on SFE and LC-MS was validated and submitted for publication.*

1.4 Implementation of automated Supercritical Fluid Extraction (SFE) equipment for the analyses of kidney fat 6 for gestagens. *The results of these studies were demonstrated during the annual workshop and published in the open literature.*  
(Annex V Chapter 2, section 1, paragraph f)  


1.5 A protocol and optimisation/validation report on methods for the identification and quantification of anabolic steroids (a.o. chlorotestosterone, methylboldenone, algestone acetophenide, norethynodrel, delmadinone and metabolites) in samples of urine and faeces.  
(Annex V Chapter 2, section 1, paragraph f)  
*Status: This work was finished for methyltestosterone, methylboldenone and norethandrolone.. New scientific data with respect to suitable target metabolites were obtained and used in improved proficiency testing programs.*
1.7 A protocol for testing biological samples of the presence of (recombinant) somatotropines.
(Annex V Chapter 2, section 1, paragraph f)
Status: Ongoing. The results of the studies were published during an international symposium and will be published in the open literature in the near future. Activities are put on hold temporarily in order to evaluate the general scientific progress in this area.

Ad 2. Supportive research within the mandate of the CRL

2.1 Participation in a work programme for zeranol metabolism study (EC DG6 / FAIR related milestone).
(Annex V Chapter 2, section 1, paragraph a & f)
Status: Analytical work was ended, the final report is being prepared by the co-ordinator. As a result of this project a range of GC-MS and LC-MS for Zeranol, Fusarium toxins and related metabolites has become available at the CRL. Comparative validation studies are ongoing and the outcome will be published in the near future.

2.2 Animal experiments into metabolism of different anabolic compounds and in support of the development of test and reference materials.
(Annex V Chapter 2, section 1, paragraph a & f & j)
Status: Ongoing. During this contract period one animal experiment was performed. Metabolism and biotransformation studies on methyltestosterone, methylboldenone and norethandrolon were performed. A study into possible natural occurrence of 17alpha- and/or 17beta-boldenone is ongoing.

2.5 Participation in European Consortium for Continuing Education for Advances in Meat Science and Technology (ECCE-AMST) project in co-operation with University of Utrecht, NL.
(EC DG12 /5th Framework proposal in Thematic Networks).
(Annex V Chapter 2, section 1, paragraph a)
Status: The activities are pending,

2.6 Participation in ISOTRACE project for the detection of illegal drugs by isotope ratio MS.
(EC DG12 /5th Framework Measurement & Testing proposal)
(Annex V Chapter 2, section 1, paragraph a & f)
Status: Ongoing. Project is ongoing. Equipment was installed during the summer 2002.

2.7 Measurement of natural steroids, especially WTO dispute related ones, in various food commodities to establish the total daily dietary intake of such steroids.
(Annex V Chapter 2, section 1, paragraph I)
Status: Measurements with respect to the presence of natural hormones in meat are, at a limited scale, being performed. This activity is ongoing.
2.8 Investigation of residues of endogenous and/or exogenous anabolic compounds in meat products after preparation for consumption. Risk estimate related task.  
(Annex V Chapter 2, section 1, paragraph I)  
Status: A series of samples of liver from retail shops was analysed for nortestosterone. The results were presented during a scientific symposium and will be discussed during the 2002 workshop. This activity is ongoing.

Ad 3. Quality Assurance & Quality Control

3.1 EU-NRL Inventory of infrastructure and QA systems for mycotoxin analysis  
(Annex V Chapter 2, section 1, paragraph b, section 2, paragraph h)  
Status: This inventory is finalised and the report is in preparation.

3.2 Organisation of “Analyses of Gestagens: an analytical update”  
(15-18 October)  
(Annex V Chapter 2, section 1, paragraph i)  
Status: Ready. The workshop was organised. An extensive documentation set was made available to the participants and has been widely disseminated as PDF-file.


3.3 Training of staff of NRLs (EU Member States and Third Countries).  
(Annex V Chapter 2, section 1, paragraph i)  
Several trainees visited the laboratory and received training on specific topics related to residue analysis.

3.4 Audits and visits of NRLs in different Member States.  
(Annex V Chapter 2, section 1, paragraph b & j)  
Status: Ongoing. No NRLs were visited during the last contract period due to budgetary and time constrains.

3.5 Extension of Bank of Certified Blank Samples and Test samples, with samples of urine, liver, muscle, and faeces. (In co-operation with JRC Geel & EC DG12 SMT (former BCR) program).  
(Annex V Chapter 2, section 1, paragraph e & g)  
Status: Activities with respect to Reference Materials during this period were focussed on incurred samples for methylboldenone, methyltestosterone and norethandrolone.

3.6 Extension of Bank of Reference Standards with more compounds like metabolites of non-authorised substances, conjugates of steroids, isotope labelled internal standards and pure compounds. (In co-operation with JRC Geel & EC DG12 SMT (former BCR) program, specified in separate proposals.)
Status: No new compounds were added during this period but for several compounds new batches were prepared. Again, more than one thousand units were supplied to NRLs.

3.7 Evaluation report of ongoing 3th QA inventory for NRLs.
(Annex V Chapter 2, section 1, paragraph b & j, section 2, paragraph h)
Status: This activity is ongoing, now also focussing, in co-operation with TAIEX, on the Candidate Countries.

3.8 Organisation of proficiency test
Organisation of comparative tests for the benefit of the national reference laboratories
(Annex V Chapter 2, section 1, paragraph (g)
Status: Samples for a proficiency test on corticosteroids were distributed and a preliminary report was send to the participants.
A proficiency test methyltestosterone and metabolites was organised, samples were distributed in May.


Ad 4. Documentation services.

4.1 Collection, evaluation, digitalisation and stock keeping of the analytical method standard operating protocols (SOPs) requested from the NRLs.
(Annex V Chapter 2, section 1, paragraph a & j & l, section 2, paragraph h)
Status: This activity is ongoing, focussing on new approaches for dissemination of information through the internet. The RIVM/CRL website is under development.

4.2 Preparation of a first manual of analytical methods from the NRLs which are proposed to be reference methods (Paper report and PDF electronic version).
(in view of revision of 93/256/EC guideline)
(Annex V Chapter 2, section 1, paragraph a & j & l, section 2, paragraph h)
Status: Waiting for final version of the revised Commission Decision 93\256EC. The possibilities of distributing analytical methods through the internet was postponed too, but are currently being studied.

4.3 Literature study on residues in meat of animals treated with growth promoters.
(Annex V Chapter 2, section 1, paragraph j)
Status: This activity is ongoing However, due to other priorities and the lack of additional funding, its priority is low.
4.4 Continuous update of methods, regulations & reference materials catalogue CB\METHODS and distribution of 2nd CD-ROM version of the data base to NRLs.

(Annex V Chapter 2, section 1, paragraph a & f, section 2, paragraph h)

Status: This activity is ongoing, distribution of the second CD-ROM has been delayed.

4.5 Continuous screening of Internet sites for information relevant to the mandate of the CRL and its related NRLs.

(Annex V Chapter 2, section 1, paragraph a & f, section 2, paragraph h)

Status: This activity is ongoing.