COMMUNITY REFERENCE LABORATORIES IN THE FIELD OF VETERINARY PUBLIC HEALTH WITHIN THE EUROPEAN UNION

CRL for residues RIVM-ARO at Bilthoven, NL

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Annual Report

January 2003 – December 2003
ANNUAL REPORT OF THE
COMMUNITY REFERENCE LABORATORY
FOR RESIDUE TESTING, RIVM, Bilthoven,

January 2003 – December 2003

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

A. General tasks

1) Management of EC CRL for residues (co-ordination, co-operation and administration, inclusive the preparation of technical and financial reports) Annex V, chapter 2, section 1 (k)

The cost statement and summary report for the period 1 January 2002 – 31 December 2002 were prepared and submitted for review by the Commission. A contribution to the combined four CRL report 2001-2002 was prepared. Finalisation of this combined report, however, is delayed.

2) EC/CRL related EC and International Bodies (e.g. AOACi, Eurachem, Codex, CVMP, EMEA, EFSA, JRCs) performance quality criteria (communication, co-ordination, Cupertino and harmonisation) Annex V, chapter 2, section 1 (c and d). Explicit co-ordination of CCRVDF EU/EC involvement.

The efforts to seek international consensus with respect to the performance criteria and validation strategies as layed down in CD 657/2002/EC, were continued. Presentations were made during the AOAC-International meeting in Atlanta (USA, September 2003). In general, the EU approach is much appreciated by the laboratories involved. However, regulatory agencies and analytical organisations remain reluctant.

Specific product

- A report on “World-wide regulations for mycotoxins in products of animal origin” was prepared on initiative of FAO. This report will be published as FAO-document in 2004.

3) Documentation services Annex V, chapter 2, section 1 (f and i)

The CRL internet site was further optimised and now includes an on line possibility for ordering Reference Standards and samples. Results of proficiency tests can be viewed, prior to their official publication and CRL –SOPs can be downloaded.

The database “CB/METHODS” with methods and contact points is currently being redesigned. Progress was limited, but the activities are ongoing, focussing on the implementation of analytical modules during 2004.

1 Official Journal of the European Communities (1996) L125, 10-31
B: Development and validation of analytical methodology

4) Identification of new and unknown compounds illegally used for growth promoting purposes. *Annex V, chapter 2, section 1 (a,j)*

From a variety of sources information and samples were received from which it can, amongst others, be concluded that there is a shift to the use of precursor hormones. For this reason a start was made with setting up databases with reference values for these hormones in samples of urine obtained from untreated animals. This will become a topic of additional interest during 2004 and later.

5) Development and validation of analytical methods necessary for effective inspection and control *Annex V, chapter 2, section 1 (e and l), focussing on improving methodology for relevant metabolites, the analyses of alternative matrices and the analyses of meat.* Studies on several analytical methods for (metabolites of) steroids were continued. In part these included the identification of the relevant metabolites, e.g. in the case of chlortestosterone and nor-chlortestosterone. Studies on these two compounds will be continued. Studies on boldenone, methylboldenone, norethandrolone, normethandrolone will be continued in a special program of ringtests that will be performed next to the proficiency testing program with expert laboratories. This approach is a direct follow up of the 2003-workshop.

Specific products:

Methods for methyltestosterone and metabolites, methylboldenone and metabolites and norethandrolone were developed and validated. A validation report with method description is currently being drafted. Expected publication date early 2004.

- Publication of method validation report: "Analytical methods for the detection of metabolites of androgenic anabolic steroids in urine". This report is ready for print

- The second manual of analytical modules was used during the technical program of the workshop. The individual modules will be included in the CRL-website.

- A publication of a method for the detection of Zeranol, *fusarium* toxins and related metabolites in biological matrices is currently in the submission process, inclusive full validation data. The method as such is available for NRLs. Additional studies on LC-MS are ongoing.

- An animal experiment in order to acquire test and reference materials was performed. A female bovine animal was treated with Somatotropine, clobetasol propionate and norclostebol.

6) Supportive research in the mandate of the CRL and the acquisition/participation in EC/CRL – related ECs DGs programmes *Annex V, chapter 2, section 1 (a)*

- Analytical methods for the detection of illegal use of natural hormones by GC-IRMS, as developed within the fifth framework programme were not tested within the CRL since the equipment, supplied by the industrial partner, was not operational for this purpose. In order to stay involved it was decided to participate further in the ISOSTER project. Several methods developed

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within this project are currently being validated. The CRL is one of the participants in these validation studies.

- The RIVM(CRL) is one of the partners within the project co-ordinated by Dr. C. Elliot (acronym BIOCOP)

C: Quality Assurance and Quality control activities, inclusive the development of incurred test materials and the organisation of proficiency tests

7) Maintenance of in-house QA/QC activities in consequence of the ISO 17025 accreditation of all analytical work done within the CRL Annex V, chapter 2, section 1 (b)

All necessary activities to maintain the in house QA-system were undertaken. An inspection visit by the Dutch Council for Accreditation on 4 September 2003 was concluded favourable .

In co-operation with the CRL-Berlin and on behalf of TAIEX, a workshop for Candidate Countries was organised on 3 and 4 June4.

8) Assistance with the implementation of Quality Assurance and Quality Control systems in NRLs Annex V, chapter 2, section 1 (b). Active participation in projects focussing on Candidate Member States.

Specific products scheduled:

- A letter-report “Homogeneity and stability of milkpowder reference materials certified for their aflatoxin M1 content” was prepared. This activity was performed partly under contract with JRC-IRMM.

9) Organisation of proficiency tests of methylboldenone and norethandrolone. Preparation of materials for proficiency test on chlortestosterone and nor-chlortestosterone. Annex V, chapter 2, section 1 (e and g).

Specific products scheduled:

- CRL reports in the corticosteroids5 and the methyltestosterone proficiency6 were published. In the corticosteroids study eleven laboratories participated. Samples of urine (three) and liver (one) were distributed. In general, good results were obtained for urine. However, only a limited number of laboratories were able to analyse samples of liver. In the other study, six different samples of lyophilised bovine urine, containing different concentrations of methyltestosterone and its metabolites 17α-methyl-5α-androstan-3β, 17β-diol (5α,3β-mead) and 17α-methyl-5β-androstan-3α (5β,3α-mead). In total 17 laboratories participated. Eleven laboratories reported acceptable results for the parent compound methyltestosterone. Only four to seven laboratories reported results for the metabolites.

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4 TAIEX Office, Workshop on “Inter-laboratory testing of certain residues”

5 Results RIVM/CRL proficiency study of corticosteroids in lyophilised samples of urine and liver. Report 310309001/2003.

6 Results RIVM/CRL proficiency test methyltestosterone and metabolites in lyophilised bovine urine. RIVM report 310309002/2003
D: Technical and scientific support to Member States and the Commission, inclusive arbitration and training activities.

10) 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 Annex V, chapter 1, section 1 (h).

There has been an exchange of samples between laboratories within the EU in relation to the studies on the natural occurrence of Boldenone. Disputes between Member States or technical problems with NRLs were not reported during this year.

11) Providing the Commission Services, National Reference Laboratories, the European Food Safety Authority (EFSA), the European Agency for the Evaluation of Medicinal Products (EMEA) and Third Countries with technical and scientific assistance (j,f)

The Commission was assisted with respect to the problems associated with the detection of residues of 17alpha-boldenone and 17beta-boldenone. Contributions were made to the Expert report and follow-up studies. Studies, performed together with a group of NRLs included studies on the influence of animal feed composition, faecal contamination and the conjugation status and the study of possible other related metabolite. From the studies, which are still ongoing, it was preliminary concluded that 17beta-boldenone can be present in faeces and can be the source of 17beta-boldenone in urine. However, in such cases the compound is not conjugated. The value for the MRPL was set at 1 µg/l for both 17beta- and 17alpha-boldenone, but an action level was set at 2 µg/l for 17alpha-Boldenone. No limits were set for 17alpha boldenone. However, the finding of conjugated 17beta-boldenone in urine still is proof for illegal administration.

An evaluation of the ANPs for 2001 was prepared and submitted to the Commission (July 2003) A similar report on 2002 was prepared. This evaluation will be combined with the current evaluation of the plans for 2003 and an overview report will be prepared.

Requests for information were received on a regular basis. In total 51 request were received from EU Member States and 53 from other countries. In total 746 ampoules with reference standards were distributed to NRLs or, in some cases, directly to routine laboratories. There is a continued interest in vials with reference samples, either blank or with incurred residues. In total 104 specific requests for information were received and answered. With the TAIEX office there was a close co-operation in relation to a number of activities directed to new Member States.

12) EC-CRL-NRLs for residues establishment of confirmatory methods for arbitration and minimum quality criteria (co-ordination, co-operation and harmonisation) Annex V, chapter 2, section 1 (c and e).

Contributions were made to a paper on alternative procedures for estimating the reliability of qualitative analytical methods. These discussions are still ongoing and will have to be included in future revisions of CD 2002/657.

13) Organisation of annual workshop on residue analysis, tentatively entitled “MRPLs and validation procedures, setting criteria and procedures” Annex V, chapter 2, section 1 (i)

A workshop was organised from 20-25 October 2003. NRLs from both current Member States and Candidate Member States were invited to participate. The proceedings were published in
November 2003. One of the topics discussed was the organisation of proficiency test. The participants agreed that the CRL will set up two programmes. The first will be a truly proficiency testing programme for compounds currently included in the ANPs, the second will have a strong research character, focussing on new compounds or metabolites. Further conclusions were made with respect to several MRPL values and time necessary for NRLs to fully comply with CD 2002/657 with respect to finalising validation studies for group A compounds before 1 August 2004. The CRL will undertake all necessary actions to actively discuss these issues with the Commission.

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