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

CRL for residues RIVM-ARO at Bilthoven, NL

Annual Report

January 2004 – December 2004
COMMUNITY REFERENCE LABORATORY FOR RESIDUE TESTING, RIVM, BILTHOVEN, NL
REPORT OF IMPLEMENTATION OF THE WORK PROGRAMME

JANUARY 2004 – DECEMBER 2004

This report describes the activities performed by the CRL in Bilthoven during the 2004 contract period. The report is based on the work programme as agreed upon by the Commission at the start of the 2004 period. This layout allows the reader to directly relate the activity report to the corresponding planning. This planning is given in italics.

The report has four individual chapters (A – D), reflecting the four area’s of CRL activities.

LEGAL AND FINANCIAL BASIS

The powers and operating conditions of the Community Reference Laboratory for the detection of residues in live animals, their excrement and body fluids and in tissue, animal products, animal feed and drinking water are laid down in Annex V of Council Directive 96/23/EC (Official Journal of the European Communities No L 125 of 23.5.1996).

The financial contribution of the Commission was decided on as laid down in Commission Decision 2004/142/EC. This contribution of Euro 415000 covered in the 2004 contract period 46% of the total operating costs of the CRL. The complementary 54% of the costs was covered by the Dutch Ministry of Public Health, Welfare and Sports (VWS).

OBJECTIVES AND INDICATIVE PERCENTAGE OF THE TOTAL OF ACTIVITIES FOR THE PERIOD JANUARY 2004 – DECEMBER 2004

A: General Tasks. Annex V, chapter 2, section 1 (c,d,f,i,k)

The percentage of staff costs for this activity was estimated as 25% of the total staff costs. Based on the figures presented in the cost statement, this figure actually was 28% in 2004.

B: Development and validation analytical methodology. Annex V, chapter 2, section 1 (a,e,h,j,l)

The percentage of staff costs for this activity was estimated as 40% of the total staff costs. Based on the figures presented in the cost statement, this figure actually was 37% in 2004.

C: Quality Assurance and Quality control activities, inclusive the development of incurred test materials and the organisation of proficiency tests. Annex V, chapter 2, section 1 (b,e,g).

The percentage of staff costs for this activity was estimated as 20% of the total staff costs. Based on the figures presented in the cost statement, this figure actually was 21% in 2004.
D: Technical and scientific support to Member States and the Commission, inclusive arbitration and training activities. *Annex V, chapter 2, section 1 (c,e,f,h,i)*

The percentage of staff costs for this activity was estimated as 15% of the total staff costs. Based on the figures presented in the cost statement, this figure actually was 15% in 2004

**Work programme and implementation for the period 2004**

**A: General Tasks**

1) *EC-4 CRL for residues management (co-ordination, co-operation and administration, inclusive the preparation of technical and financial reports) Annex V, chapter 2, section 1 (k), inclusive a contribution to Joint 4 CRL Report 2003.*

The summary report and cost-statement covering the 2003 contract period were prepared and submitted in March 2004. During the Commission-4CRL coordination meeting (Brussels, 27 January 2005) it was agreed that a new joint 4CRL report shall be prepared including activities for 2003 and 2004. For this new joint report information will be submitted after 30 March 2005 to Dr. Caroli (Director CRL / ISS in Rome) who shall act as coordinator – editor for the report.

2) *EC/CRL related co-operation with International Bodies (e.g. AOACi, Eurachem, Codex, CVMP, EMEA, EFSA, JRCs) on method validation, analytical methodology and performance quality criteria (communication, co-ordination, 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 laid down in Commission Decision 657/2002/EC, were continued. World-wide activities currently focus on single laboratory validation protocols, e.g. within AOAC-International, CODEX and WADA. In general progress, however, is slow. The same applies to the refinement of criteria for qualitative residue testing.

On 20 –21 January 2004 in Brussels the Commission was assisted in a CCRVDF document drafting group. During the 15th session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) on 25-29 October 2004 at Alexandria, VA, USA the Director of the CRL co-chaired (with Canada) the *ad hoc* Working Party on Methods of Analyses and Sampling. The CRL was also continuously involved in drafting and editing of new underpinning CCRVDF documents.

During the Olympic Summer Games on 13 – 29 August 2004 at Athens, Greece the Director of the CRL was on duty in the Doping Control Laboratory in its capacity of Independent Observer for the World Anti-Doping Agency (WADA) gaining a deep insight in the practice of the residue testing related field of doping testing in sports.

3) *Documentation services Annex V, chapter 2, section 1 (f and i). Developments with respect to analytical methodology and (EU) legislation are constantly monitored. In addition, information on the use of new compounds or alternative approaches to improve the growth of livestock will be collected and use as input for future studies.*

The documentation services of the CRL are more and more focussed on the exchange of information through the Internet. The website now includes information on analytical methods and is actively used in support of the proficiency testing program. The documentation centre maintained its advisory role and was responsible for the evaluation of
the National Residue Plans of the EU Member States for 2003 and their results submitted for 2002.

An overview of the relevant publications and documents CRL authored or co-authored is included in Annex I.

**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). There is a trend observed into the use of e.g. pro-hormones in animal feed as an alternative approach to supplying growth promoting compounds to livestock. Selected samples will be analysed and reference values for biological materials will be determined.

Several sets of samples have been analysed for a number of specific pro-hormones in order to get a first impression of the levels of these compounds in urine of bovine, porcine and ovine animals. For this purpose a new multi-residue method was developed. The method was validated for samples of animal feed but is in need for further optimization in 2005.

A new possible growth promoting compound, glyburide, was detected in a non-declared veterinary preparation. An *in vitro* metabolism study was conducted and a method for the detection of the parent compound and its metabolites in urine was developed. The results of this study were published in the Proceedings of the fifth EuroResidue Conference. The Commission and all EU NRLs were immediately alerted and fully informed, but up to the present no similar findings were reported to the CRL.

From one NRL a preparation containing several anabolic compounds was received. The compounds present were identified, but were all known from previous occasions.

5) **Development and validation of analytical methods necessary for effective inspection and control Annex V, chapter 2, section 1 (e and l).** Supporting studies will be conducted on the identification of metabolites of norclostebol and clobetasole propionate in bovine urine. Previously developed methods for bovine Somatotropine (bST) will be evaluated on biological materials obtained from a treated animal.

**Specific products scheduled:**

- **Publication of method validation report: analytical methods for the detection of norclostebol and metabolites in bovine urine.**

This part of the work programme is directly linked to the Proficiency Testing (PT) programme. As explained in the relevant chapter, part of this programme focuses on innovating residue control programmes by including new analytes, especially metabolites of compounds which prolong the duration of the period during which abuse can be detected. Norchlorlortestosterone acetate was included in the animal experiment and studies were undertaken to elucidate the metabolism. Studies at the CRL tentatively identified 17α-Norclostebol and 4-chloro-17α-hydroxy-19-norandrostane-5ξ-3one as the main metabolites. Excretion curves prepared for these two compounds showed that the latter compound can be detected during a significant longer period after administration. In order to confirm the identity of the metabolites detected and to further study the precise stereochemistry a reference standard 4-chloro-17β-hydroxy-19-norandrostane-5ξ-3one was synthesized by pressurized hydrogenation of 17β-Norclostebol. This compound was used to quantify the two metabolites.
Based on the materials obtained from the animal experiment and on the information available on the metabolism, a study was organised among 10 NRLs, representing 9 Member States. The results of this study currently are under evaluation. The analyses for this analyte is included in the general multi residue screening procedure used at the CRL.

*Publication of method validation report: analytical methods for the detection of clobetasol and/or metabolites in bovine urine.*

A treatment with clobetasol propionate was included in the animal experiment. However, extensive studies of excreta did not reveal any compounds related to clobetasol. A special procedure, based on neutral loss mass spectroscopy was used. The presence of a fluor group in the molecule makes this approach in principle very effective. The reason for this result is unknown until now. Two possible explanations exist though: no release from the injected compound from the injection site or extensive metabolism.

As a consequence, no further studies were undertaken with this compounds. However, within another activity samples of hair will be analysed for clobetasol (propionate).

*Publication of method validation report: analytical methods for the detection of thyreostats in bovine urine by LC-MSMS.*

The development and validation of a new analytical method for thyreostatic compounds in bovine urine was finalised. A full method description and validation reports are available.

*Publication of interim report on analytical methods for detection, identification and quantification of Somatotropine in biological matrices.*

Several studies on the development of analytical methods for bST were undertaken. The studies focused on:

- Applicability of Biosensor techniques for detection
- Characterization of different antibodies against bST with biosensors
- Preparation of affinity columns with a selected antibody
- Detection of endogenous and recombinant bST in biological materials, inclusive milk.

Several antibodies were tested and the binding conditions were optimized. Binding and elution conditions for bST were determined. Currently, an analytical method based on ultra-filtration, affinity chromatography and LC-MS(MS) is studied further.

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

Additional studies on metabolism and natural occurrence of boldenone in different species. These studies will focus on the conjugation status of boldenone and its major metabolites in a variety of biological materials.

A method specifically determining beta-boldenone and beta-boldenone conjugates was developed and validated. This method was discussed and demonstrated during the annual workshop at Bilthoven in October 2004 and currently is available through the CRL-website.
Activities within DG RESEARCH sixth Framework Projects, participation in BIOCOP (PM).

Contract negotiations between the co-ordinator and work-package and the EC were finalized by the end of 2004. The involvement of the CRL has been limited during this phase, but will increase during the duration of the project.

Support and activities within the follow up of the workshop on “The impact of quantitative chemical analysis in the 6th Framework program” (IQualAN-NAS project, contract 96MA-CT-2002-04043).

Up to CRL knowledge the co-ordinator of this activity did not acquire a project for this purpose within the 6th Framework program as a follow up of the successful 5th FP MeQualAn for qualitative testing of residues and contaminants. Consequently the CRL had no further significant involvement.

Support and consultation within the RADAR project “Biosensors for androgenic growth promotors in cattle”.

The project was assisted during its plenary project meetings in Barcelona, Spain on 8-9 March and in Leipzig, Germany on 13-14 September 2004. The experimental activities were near to completion at the end of 2004. The CRL provided advise and performed part of the confirmatory analyses that were necessary. The project suffered from the absence of Scientific Commission Officers of DG Research. In recognition a slight time extension of the project was granted. The co-ordinator will organize a final “show & tell” meeting in Cork, Ireland in April 2005 and the final report will become available mid 2005.

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)

Activities necessary to maintain the QA-system continued. The accreditation of the laboratory was fully re-assessed on the bases of ISO 17025 and granted for a new period of 4 years. As in 2003 also in 2004 formal GLP compliance of the CRL was discontinued due to financial constraints.

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.

EU PHARE TWINS Project

12-13 January 2004, Bratislava, Slovakia

Workshop on Quality and Validation of analytical Methods
LASER Enlargement Unit
Food and Consumer Product Safety Authority (Dutch VWA)
Representatives of the CRL participated in a number of TAIEX DG ENLARGEMENT events for new and candidate member states:

**Workshop on validation**

17 – 19 March 2004, Bilthoven, NL

In co-operation with TAIEX, the CRL organised a workshop “Laboratory staff training, Validation of analytical methods in residue analyses” to provide laboratory staff of the Acceding / Candidate Countries (CC) with the necessary theoretical and practical tools for method validation in residue analyses

**4th Meeting of the CC CVO Subgroup on Laboratories**

26 May 2004, Brussels, AGR 9668, co-chaired by the CRL

The aim of this meeting was to discuss the results of the TAIEX activities carried out since the last meeting in June 2003, such as advisory visits to the Candidate Countries, accreditation of laboratories, workshops on inter-laboratory ring trials, a seminar on tasks and duties of National Reference Laboratories and furthermore to discuss future assistance of TAIEX in this domain

**Veterinary assessment mission to the Northern part of Cyprus**

Meeting with the Turkish Cypriot Community of Cyprus and Seminar on EU legislation

12-16 July 2004, Nicosia, PEER 10320

The Commission proposed on July 7th a package of aid and trade measures which aim to put an end to the isolation of the Turkish Cypriot community and to facilitate the reunification of Cyprus. These measures will facilitate trade from the northern part of the island and strengthen its economic integration through financial assistance of €259 million. They also set specific rules for goods crossing the green line separating the Greek Cypriot and the Turkish Cypriot communities. This was the first time a delegation from the EU visited the northern part of Cyprus in order to investigate the needs of the region in order to fulfil EU legislation.

**Meeting of the Chief Veterinary Officers of the Western Balkan.**

Seminar on EU legislation, TAIEX AGR 11391

15 - 16 December 2004, Brussels, BE

The aim of the meeting was to discuss the TAIEX activities and potential assistance in the veterinary domain as well as a common approach for the working programme in 2005

**Individual training course**

29 November – 2 December 2004, Bilthoven, NL

In the last years, several requests came from Candidate Countries and new EU Member States to attend an individual training course at the Laboratory for Food and Residue Analyses. A first training of this type was organised with participants from Poland and Turkey.
9) Organisation of proficiency tests Annex V, chapter 2, section 1 (e and g).

Annually, the CRL conducts an animal experiment in order to obtained incurred biological materials to be used in its Proficiency Testing (PT) programme. When possible, these experiments are combined with metabolism studies. In 2003 a female bovine animal was treated with bovine Somatotropine, clobetasol propionate and norclostebol and the resulting materials used for specific studies. In 2004 a similar animal was treated by intramuscular injection with Diethylstilbestrol (DES), 17β-Oestradiol and Ethynyleoestradiol (EE2). In addition, one of the pro-hormones, Androstanedione (AAD) was included in the feed. For two other proficiency tests the CRL had suitable materials available from other sources.

The organisation of proficiency tests (PT) is one of the most important tasks of the CRLs. During annual discussions with representatives of the NRLs the importance of this activity is reaffirmed. The nature of the PT-programme, however, has evolved. The number of participating laboratories has grown with the inclusion of the new Member States and the priorities have changed. Currently two different programmes run alongside. The first program is the basic PT-programme focussing on compounds included in all Annual Residue Plans of the Member States, the second is more innovative in the sense that it includes new compounds or metabolites not regularly tested for. For 2004 originally two tests were scheduled, Trenbolone and DES in bovine urine. Follow-up activities of 2003 PT for norethandrolone and Zeranol and the research study on methylboldenone had to be undertaken as well. With the exception of the PT for DES all activities were finalized as scheduled. The PT had to be postponed until early 2005. In practice, the large group of participating laboratories make it difficult to have two PT each year. This topic was discussed during the October 2004 workshop at Bilthoven and for 2005 again two PT have been announced with optimized planning and strict reporting guidelines.

Specific activity reports

The PT norethandrolone and metabolites was finalised and a full report was published.

The report describes the results of the proficiency study organised in 2003. The samples that were distributes contained both the parent compounds norethandrolone and its major metabolite 17α-ethyl-5β-androstane, 3α(17β)-dil. Metabolism studies showed that the metabolite can be detected for a significant longer period after treatment. In total 12 laboratories, representing 10 Member States (2003!) participated in this study. In total six different materials were distributed. These materials had demonstrated excellent homogeneity and stability prior to distribution. Only prolonged storage at 37°C results in a significant decline of the mass concentration of 17α-ethyl-5β-androstane,3α(17β)-dil. Finally six to nine laboratories provided adequate analytical results for both the parent compound and the target metabolite at the levels tested (1- 5 µg/l).

A PT “Zeranol and Taleranol in lyophilised bovine urine” was undertaken early 2004. The evaluation report was made available to the participants after summer and the full report will be ready for publication during the first quarter of 2005. Based on the results it is concluded that routine testing for the illegal use of Zeranol, either by monitoring Zeranol or its metabolite Taleranol, is performed with excellent quality at a level of 3 µg/l. The current MRPL of 2 µg/l, though slightly less, reflects this situation adequately. In total 27 laboratories participated in this study. The majority of laboratories was able to confirm the identity at the level of 3 µg/l based on the criteria described in CD 2002/657/EC.
Samples for the PT for Trenbolone and its metabolite 17α-Trenbolone were distributed among the participants. The data received are currently under evaluation and will be available for comments by the participants in 2005.

The study on methylboldenone and metabolites was be performed within a small group laboratories with experience in metabolism studies. The data received are currently under evaluation and will be available for comments by the participants in 2005.

The CRL contributed to a carry over experiment on the mycotoxin deoxynivalenol in dairy cattle. The study was conducted by the Institute of Animal Nutrition, Federal Agricultural Centre Braunschweig, Germany. CRL tasks included the confirmatory analysis of milk samples for DON, DON metabolites and DON conjugates with GC-MS. The results of the study will be published in 2005 in Food Additives and Contaminants.

**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 2, section 1 (h).

No samples within this framework were received during 2004

11) Providing the Commission Services (e.g. SANCO/FVO, JRC, legal 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).

Evaluation reports were prepared on the Annual National Residue Control Plans of 2003 and of the results reported for 2002. From the evaluation of the National Plans it was concluded that these are very static. The positive (non compliant) results obtained in 2002 were to a large degree related to the boldenone issue, which was ongoing at that time. Further, several reports were made on medroxyprogesterone acetate (MPA) in animal feed and kidney. No new information on non compliant results originated from this evaluation.

The CRL was strongly involved in the organization and implementation of the fifth EuroResidue Conference in Noordwijkerhout, The Netherlands on 10 - 12 May 2004. This very successful conference with 389 participants from all over the world covers the CRL field of activities strongly and belongs to one of the two largest scientific events in this field. The Proceedings (2 volumes; in total 1024 pages) were available during the conference. Besides the contributions in the Proceedings (see Annex I: CRL Products 2004), in the field of the CRL activities a number of lectures was presented as well as 6 posters. These posters are available on request as PDF files.

On invitation the CRL presented at various scientific events and training courses updates about the EU approach in veterinary residue testing and worldwide regulations for mycotoxins: in Geel, Belgium (JRC-CRL cooperation 15 March), in Noordwijk aan Zee, the Netherlands (Symposium on Rapid Test Methods in Europe, 25-26 March), in Parma, Italy (IDF/ISO/OAOC Millenium Conference, 21 April), in Bethesda, USA (XIth IUPAC Symposium on Mycotoxins and Phycotoxins, 17 – 21 May), in Ghent, Belgium (workshop on residues EU law enforcement, 8 October), in Santiago, Chile (IAEA/FAO South America
training, 18-22 October), in Alexandria, USA (CODEX CCRVDF-MAS, 25 October) and in Geel, Belgium (JRC-CRL inauguration, 9 November).

Requests for information were received on a regular basis. Again, there was a strong increase of the number of ampoules distributed to NRLs. Details are included in Annex II.

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)

The CRL participated in a meeting in Brussels (15 July 2004) with the objective of clarifying a number of specific issues related to Commission Decision 2002/657/EC.

13) **Organisation of annual workshop on residue analysis. Annex V, chapter 2, section 1 (i) tentatively titled “Evaluation Results Proficiency Tests and Technical Training”**

The annual workshop was organised from 11 - 13 October 2004 at RIVM in Bilthoven, followed by a specific technical laboratory training on 14 and 15 October. Proceedings were prepared, inclusive a CD-ROM with the full Powerpoint presentations and supporting documents.
ANNEX I: CRL PRODUCTS 2004

I. REPORTS


II. ARTICLES PUBLISHED IN SCIENTIFIC PAPERS.


Stephany RW. The EU system of reference laboratories for residues in food of animal origin. Accreditation and Quality Assurance 2004; 9: 578-582

Stolker AAM, Linders SHMA, Ginkel LA van, Brinkman UAT. Application of the revised EU criteria for the confirmation of anabolic steroids in meat using GC-MS. Analytical and Bioanalytical Chemistry 2004; 378: 1313-1321

III. OTHER (CO)-PRODUCTS (E.G PROCEEDINGS, BOOK CHAPTERS)


### ANNEX II: OVERVIEW OF CRL ACTIONS WITH RESPECT TO REFERENCE MATERIALS

**Period: 2004.01.01 – 2004.12.31**

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